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Actions of the phytopharmacon Iberogast® in different regions of the gut

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To the soul of my Father

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Original manuscript

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Effects of hyoscine butylbromide (Buscopan®) on cholinergic pathways in the human intestine

Neurogastroenterol Motil 2013, 25 (8), 530-9.

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Allam S, Krueger D, Kelber O, Demir IE, Ceyhan GO, Zeller F, Schemann M.

Identification of pro-secretory components in STW 5, a fixed herbal combination medicine to treat functional gut disorders

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Allam S, Krueger D, Kelber O, Demir IE, Ceyhan GO, Zeller F, Schemann M.

Region-specific effects of the herbal medicine STW 5 (Iberogast®) and its individual extracts on human intestinal motility

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Zusammenfassung

Iberogast® (STW 5) ist ein hydroethanolisches Arzneimittel bestehend aus neun Pflanzenextrakten: Bittere Schleifenblume, Angelikawurzel, Kamillenblüte, Kümmelfrüchte, Mariendistelfrüchte, Melissenblätter, Pfefferminzblätter, Schöllkraut und Süßholzwurzeln. STW 5 wird erfolgreich zur Behandlung von zwei Arten gastrointestinaler Funktionsstörungen eingesetzt: funktionale Dyspepsie und Reizdarmsyndrom. Allerdings ist der Wirkmechanismus bislang noch nicht bekannt. Das Ziel dieser Studie war die *in vitro* Wirkung von STW 5 und seiner Einzelkomponenten in Bezug auf Motilität und Sekretion im Magen, Dünndarm und Dickdarm zu untersuchen.

Vorhergehende Studien haben gezeigt, dass STW 5 eine relaxierende Wirkung auf den proximalen Teil des Magens im Menschen und Meerschweinchen hat. Es konnte ebenso gezeigt werden, dass diese Wirkung weder nerval vermittelt wird noch abhängig ist von Stickstoffmonoxid . Ein Ziel dieser Studie war es den Wirkmechanismus, durch den STW 5 den Muskeltonus herabsetzt, zu untersuchen. Muskelpräparate des Meerschweinchenmagens wurden in gepaarten Experimenten mit unterschiedlichen Blockern inkubiert. Die Änderung des Muskeltonus nach Verabreichung von STW 5 wurde anschließend mit isometrischen Kraftaufnehmern aufgezeichnet. STW 5 induzierte eine stabile und anhaltende Relaxation der Magenmuskelpräparate. Dieser Effekt war signifikant höher in Muskelpräparaten der Longitudinalmuskulatur im Vergleich zu Zirkulärmuskulatur. Durch pharmakologische Studien konnten folgende Wirkmechanismen ausgeschlossen werden: Calcium sensitive Kaliumkanäle, Calcium aktivierte Chloridkanäle, spannungsabhängige Calcium Kanäle, durch Calcium Freisetzung aktivierte Kanäle, veränderte Freisetzung aus intrazellulären Calcium Speichern, Proteinkinase A, Proteinkinase G und Calmodulin Kinase II. Stattdessen konnte gezeigt werden, dass die durch STW 5 induzierte Muskelrelaxation durch die Inhibierung des extrazellulären Calciumeinstroms ausgelöst wird. Verantwortlich dafür ist ein funktioneller Antagonismus der TRPA1 (transient receptor potential ankyrin 1) und TRPC (transient receptor potential canonical), insbesondere TRPC3, Kanäle. Diese Kanäle scheinen während der Ruhephase der glatten Magenmuskulatur geöffnet zu sein. Ein weiteres Ziel in der Wirkung von STW 5 scheint die Proteinkinase C zu sein.

Der zweite Teil dieser Studie beschäftigt sich damit, die Wirkung von STW 5 und seiner Einzelkomponenten auf die tonische und phasische Kontraktionsaktivität der Zirkulär- und Longitudinalmuskulatur des humanen Dünn- und Dickdarms zu beschreiben. Die Ergebnisse machen deutlich, dass STW 5 eine Dosis abhängige inhibitorische Wirkung auf die glatte

Muskulatur des menschlichen Darms besitzt. Diese Wirkung war regionenspezifisch und auch abhängig von der betrachteten Muskelschicht. Der Haupteffekt von STW 5 war in allen Regionen eine Muskelentspannung. Andererseits reduzierte STW 5 phasische Kontraktionen im Duodenum und Jejunum, nicht aber im Ileum. Im Dickdarm induzierte STW 5 wiederum gebündelte Kontraktionen. Den inhibitorischen Effekt von STW 5 ahmten Pfefferminz, Süßholz und Angelika nach. Der Mechanismus durch den die Inhibierung des Muskeltonus ausgelöst wird, ist beim Menschen ähnlich wie im Meerschweinchenmagen. Dieser Wirkmechanismus beinhaltet die Reduktion des intrazellulären Calcium Levels durch eine antagonistische Wirkung auf TRPA1 und TRPC3.

Der dritte Teil der Studie behandelt die sekretorische Wirkung der neun Pflanzenextrakte aus denen STW 5 besteht. Experimente wurden mit Mukosa / Submukosa Präparaten des humanen Dünn- und Dickdarms durchgeführt sowie mit der Epithelzelllinie T84. Die Ergebnisse deuten darauf hin, dass von diesen neun Pflanzenextrakten vor allem Angelika und zu einem kleineren Anteil auch Pfefferminz und Melisse für die sekretionsfördernde Wirkung von STW 5 verantwortlich sind. Diese sekretionsfördernde Wirkung war vergleichbar in Dünndarm und Dickdarm. Nur in T84 Zellen führte Kamille zu einer zusätzlichen Stimulation der Sekretion. Der prosekretorische Effekt involviert die Aktivierung von cAMP- und Calcium aktivierten Chlorid Kanälen im Epithel des Darms. Nur im Dünndarm hatte Süßholz eine sekretionshemmende Wirkung, die durch Amiloride inhibiert werden konnte. Dieser Effekt deutet darauf hin, dass sie durch eine verstärkte Natriumabsorption ausgelöst wurde.

Zusammengefasst hat diese Arbeit zum ersten Mal gezeigt, dass die Relaxierende Wirkung von STW 5 und seinen Komponenten auf eine Beteiligung von TRP Kanälen zurückzuführen ist. Angelika ist sowohl in die spasmolytische (krampflösende) als auch sekretionsfördernde Wirkung von STW 5 involviert. Die Wirkweise von STW 5 kann zur Aufklärung der Pathologie der funktionellen Dyspepsie und Reizdarmsyndrom beitragen. Dies kann dabei helfen Therapeutika zu entwickeln, die genauere Wirkziele haben, um bestimmte Darmfunktionen zu normalisieren.

Summary

Iberogast® (STW 5) is a hydroethanolic multi-herbal drug combination from nine plant extracts which are: angelica roots, peppermint leaves, lemon balm leaves, chamomile flowers, liquorice roots, caraway fruits, milk thistle fruits, greater celandine herb and fresh bitter candytuft plant. STW 5 is successfully used to treat two functional gastrointestinal disorders, functional dyspepsia and irritable bowel syndrome. However, its mode of action is still unclear. This study aimed to investigate the *in vitro* actions of STW 5 and its individual components on motility and secretion of gastric, small and large intestinal preparations.

In previous studies, it was shown that STW 5 has a relaxing effect on human and guinea pig proximal stomach. It was also shown that this effect is neither nerve mediated nor nitric oxide dependent. In the present study, one of the aims was to determine the mechanism of action by which STW 5 relaxes the muscle tone. In a paired design, guinea-pig gastric muscle preparations were incubated with several blockers and the *in vitro* tone changes in STW 5 responses were recorded by the isometric tension transducer method. STW 5 evoked robust and sustained relaxation of gastric corpus muscle strips. This effect is significantly higher in longitudinal than in circular muscle. Pharmacological studies showed that STW 5 induced muscle relaxation was not dependent on calcium-sensitive potassium channels, calcium-activated chloride channels, voltage dependent calcium channels, calcium release activated channels, altered release from intracellular Ca^{2+} stores, protein kinase A, protein kinase G or calmodulin kinase II. Instead, pharmacological intervention revealed that muscle relaxation was due to inhibition of extracellular calcium influx by functional antagonism of transient receptor potential ankyrin 1 (TRPA1) and transient receptor potential canonical (TRPC), in particular TRPC3, channels. These channels seemed to be open in the resting state of the gastric smooth muscle. Furthermore, protein kinase C might be involved as an additional target of STW 5.

The second part of the study aimed to investigate the effects of STW 5 and its individual extracts on tonic and phasic contractile activity of circular and longitudinal muscle strips of human small and large intestine. The results revealed that STW 5 exerted dose-dependent inhibitory effects on human intestinal smooth muscle. These effects were region-dependent and layer-specific. The main effect of STW 5 in all intestinal regions was a reliable muscle relaxation. On the other hand, STW 5 significantly inhibited the phasic contractility of duodenum and jejunum without affecting ileum contractions whereas in large intestine, STW 5 provoked clustered contractions. Peppermint, liquorice and angelica mimicked the

inhibitory effects of STW 5. The mechanism of muscle tone inhibition is similar to that found in guinea-pig gastric smooth muscle strips and involved decreased intracellular calcium level via TRPA1 and TRPC3 antagonism.

The third part of the thesis dealt with the prosecretory profile of the nine herbal extracts present in STW 5. Experiments were performed in human small and large intestinal mucosal / submucosal preparations as well as in the epithelial cell line T84. The results provided evidence that out of the nine herbal extracts mainly angelica is responsible for the prosecretory action of STW 5 in human intestine with some more minor contribution of peppermint and lemon balm. The prosecretory potentials were comparable in small and large intestine. Only in T84 cells, chamomile showed additional secretagogue activity. The prosecretory effects involved activation of cAMP- and calcium-activated chloride channels in intestinal epithelium. Only in the small intestine, liquorice exerted anti-secretory effect which was inhibited by amiloride and therefore due to enhanced sodium absorption.

In summary, this thesis revealed for the first time the crucial role of TRP channels in the muscle relaxing effects of STW 5 and its components. Angelica is significantly involved in both, the spasmolytic as well as the pro-secretory action of STW 5. The mode of action of STW 5 may shed some light on the pathology of functional dyspepsia and irritable bowel syndrome and may help to design drugs that are more selectively targeted to normalise particular gut functions.

1. Introduction

Functional gastrointestinal disorders (FGIDs) are common disorders characterized by persistent and recurring gastrointestinal symptoms as a result of abnormal functioning of the GI tract. In most cases, routine medical diagnostic investigations revealed normal results so that FGIDs are frequently perceived as psychological or psychosomatic disorders (Ottillinger *et al.*, 2013).

There are more than twenty functional GI disorders that have been identified affecting various parts of the gut including the oesophagus, stomach, bile duct and/or intestines. The most common, highly prevalent (~10-20 % in western countries) and best studied FGIDs are functional dyspepsia (FD) and irritable Bowel Syndrome (IBS). The symptoms of FD and IBS are overlapped (Thompson, 2006).

1.1 Functional dyspepsia (FD)

According to Rome Criteria, dyspepsia has been categorized in three different subgroups; ulcer-like dyspepsia, dysmotility-like dyspepsia, unspecified dyspepsia. Dyspepsia can be caused by many diseases. When the upper endoscopy and normal diagnostic tests failed to identify definite pathological, biochemical or structural lesion, physicians label it as functional (non-ulcer) dyspepsia (Agreus & Talley, 1998).

Functional dyspepsia (FD) is one of the most common symptomatic FGIDs (Koloski *et al.*, 2002). People suffering from upper gastrointestinal discomfort, heartburn, dyspepsia, early satiety, postprandial fullness, epigastric pain, nausea, vomiting, belching and bloating for more than three months are categorized as FD patients (Thompson, 2006).

Etiology

Many causes were proposed to be involved in the pathophysiology of dyspepsia, including gastric hyperacidity, peptic ulcer, esophagitis (gastro-esophogeal reflux disorder; GERD), biliary tract disorders (as gall bladder stones), gastric cancer, gastric/pre-pyloric erosion and duodenitis (Agreus & Talley, 1998). However, less if any is known about the etiology of FD subtype. Several factors and mechanisms have been postulated in FD development such as visceral hypersensitivity, hyperacidity, helicobacter pylori infection, psychological disorder, stress, genetic predisposition and motility disorders (Allescher, 2006). Dysmotility-like dyspepsia was believed to be associated with gastric accommodation disorder, delayed gastric emptying, impaired proximal gastric relaxation, attenuated gastric antral contractility, altered duodenal motility, impaired duodenal mucosal integrity and low-grade inflammation

(Hausken & Berstad, 1992;Troncon *et al.*, 1994;Troncon *et al.*, 1995;Stanghellini *et al.*, 1996;Gilja *et al.*, 1996;Tack *et al.*, 1998;Kim *et al.*, 2001;Schwartz *et al.*, 2001;Vanheel *et al.*, 2014). There is also higher sensitivity to chemical and mechanical stimuli (Bradette *et al.*, 1991;Barbera *et al.*, 1995). However, fat modulates the responses of the gut to various stimuli, and some of these modulatory mechanisms are abnormal in patients with FGIDs (Feinle-Bisset & Azpiroz, 2013).

There is no standard medication for the treatment of dyspepsia. Most effective treatments showed small superiorities over placebo (~10-20 %), where placebo effect is ranging from (~30-70 %). For peptic ulcer-like dyspepsia, treatments include antacids, H_2-receptor antagonist, proton pump inhibitor or Helicobacter pylori eradication (Allescher, 2006). In dysmotility-like dyspepsia, sometimes also referred to as non-ulcer or functional dyspepsia, prokinetic drugs, such as domperidone (peripheral dopamine D_2 antagonists), metoclopramide (dopamine D_2 blocker, 5-HT_3 antagonist and 5-HT_4 agonist), cisapride and tegaserode (5-HT_4 agonists), mosapride and renzapride (5-HT_3 antagonist/5-HT_4 agonist) or the most recent highly selective 5-HT_4 agonist drugs; prucalopride, noranopride are used in the clinic (Sahyoun *et al.*, 1982;Schuurkes & van Nueten, 1984;Camilleri *et al.*, 2006;Allescher, 2006;McCullough *et al.*, 2006;Camilleri, 2014). Because of serious adverse effects, some of these drugs were withdrawn from the market (Pasricha, 2007). Drugs that didn´t primarily target motility, such as tricyclic antidepressants or serotonin reuptake inhibitors, also showed symptom improvement (Braak *et al.*, 2011;Talley *et al.*, 2012).

1.2 Irritable bowel syndrome (IBS)

Irritable bowel syndrome (IBS) is a functional disorder of the lower GI tract with higher prevalence in females than in males (~2:1). It is characterized by chronic or recurrent abdominal pain, discomfort, bloating and altered bowel habit (Rahimi & Abdollahi, 2012). Based on the Rome III Criteria and according to the bowel habit, IBS is classified into four groups; IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), mixed IBS (IBS-M; fluctuation between IBS-D and IBS-C) and unsubtyped (IBS-U; the bowel habit does not meet the criteria of IBS-D, IBS-C or IBS-M) (Thompson, 2006;Yao *et al.*, 2012).

The pathophysiology of IBS is uncertain, and it is unlikely that a single unifying mechanism explains the condition but IBS is most likely multifactorial. It involves enhanced visceral perception, altered gut motility, abnormal intestinal secretion, altered intestinal microbiota, inflammation and immune disturbance, genetic factors, abnormal gas handling, psychosocial

factors, intestinal infections, central nervous system and post-infectious plasticity (Andresen *et al.*, 2011;Rahimi & Abdollahi, 2012).

There are several treatment options for IBS:

(a) 5-HT_3 antagonists showed efficacy in IBS-D via reducing the intestinal motility, secretion and visceral sensitivity (De Ponti, 2013).

(b) 5-HT4 agonists have been demonstrated to be effective in IBS-C and chronic constipation through activation of acetyl choline release and hence muscle contraction and increased bowel frequency (Camilleri, 2014).

(c) Guanylate cyclase C has been reported in treatment of IBS-C and chronic constipation by increasing the intestinal secretion and accelerating transit (Thomas & Allmond, 2013).

(d) Chloride channel activator provokes intestinal secretagogue activity and increases small intestinal circular smooth muscle contraction and is effective in the treatment of constipation and IBS-C (Ao *et al.*, 2011;Chan & Mashimo, 2013).

(e) Glutamine restores intestinal permeability in IBS-D patients as well as improves abdominal pain, bloating and diarrhea. IBS-D is characterized by higher intestinal permeability and reduced expression of the tight junction protein, claudin-1 (Camilleri, 2014).

(f) Histamine H_1 receptor antagonists and mast cell stabilizers considerably relieve IBS-associated symptoms. Histamine, serotonin and proteases are mediators released from mucosal biopsies IBS-patients (regardless its subtype). There are evidences of increased histamine release from mast cells in IBS patients which excites submucosal plexus neurons, mesenteric afferents and sensitizes dorsal root ganglion neurons (Buhner *et al.*, 2009;Buhner *et al.*, 2012;De Ponti, 2013;Camilleri, 2014;Buhner *et al.*, 2014).

(g) There are some newly developed drugs, most of which are still in the early phases of clinical trials:

  (i) Tachykinin NK-2 receptor antagonists: Because of the ubiquitous distribution of tachykinin receptors in intrinsic nerves, extrinsic nerves, inflammatory cells, and smooth muscle, their blockade has the potential to inhibit motility, sensitivity, secretion, and inflammation in the gastrointestinal, which could improve targets for visceral pain, IBS-D and diarrhea (De Ponti, 2013).

  (ii) Opioid receptor activation reduces visceral pain through peripheral (spinal afferents) and central mechanisms and inhibits motility through decreased

acetylcholine release. Preliminary data showed the efficacy of μ-opioid agonist (loperamide) in patients with diarrhea and IBS-D (Camilleri, 2014).

(iii) Gehrlin agonist accelerated gastric, small bowel, colonic transit, and improved stool consistency in patients with functional constipation. Ghrelin receptors are identified throughout the gastrointestinal tract, including the colon. Ghrelin hyperpolarized resting membrane potential of human colon circular smooth muscle cells, suggesting that it has also functional effects on colonic motility (Camilleri, 2014).

In practice, upper and lower GI symptoms often coexist and hence FD and IBS clinically overlap. The rate of overlap could be in the range of 11-27 % (Suzuki & Hibi, 2011). In both diseases, as well as all FGIDs, non-pharmacological therapy is recommended as first line of treatment including patient education, dietary recommendations, physical exercise, relaxation and stress management (Ottillinger *et al.*, 2013) however the efficacy is highly debated. If this is not successful, pharmacologic interventions become an option for the treatment. In addition to the above listed medications, proton pump inhibitors (PPI), antibiotics for Helicobacter pylori eradication, probiotics, melatonin, dietary fibres, bulking agents or anticholinergic spasmolytic are prescribed (Rahimi & Abdollahi, 2012;Ottillinger *et al.*, 2013).

However, all treatments have been rather disappointing. There could be several reasons for the unsuccessful treatment, these include:

(a) Involvement of numerous factors in pathophysiology and in some cases significant placebo effect cause therapy of this disease to be more complex (Duracinsky & Chassany, 2009).

(b) Most of the drugs treat only one symptom at a time and thus cannot address overlapping and variable complaints (Ottillinger *et al.*, 2013).

(c) There are no complete remedies, only symptoms improvement (Rahimi & Abdollahi, 2012).

(d) Patients discontinue drug treatment due to serious side effects (Glessner & Heller, 2002;Pasricha, 2007;Brierley & Kelber, 2011).

Considering all these limitations of conventional drugs and specifications of optimal ones could give to complementary and alternative medicines a chance to become attractive options for many clinicians and patients (Wu, 2010). There are several phytochemical drugs used to treat FD and IBS. They are used either as monotherapy like peppermint oil and curcumin or in

combined herbal preparations such as Dai-kenchu-to, Hangeshasin-to, Padma® Lax, Tong-Xie-Yao-Fang, Tong-xie-ning and Rikkunnshi-to (Brierley & Kelber, 2011). One of these herbal medicines is Iberogast®.

Iberogast® is the trading name of STW 5, a fixed hydroethanolic multiherbal combination of a fresh plant extract from bitter candytuft (*Iberis amara*) and eight drug extracts from angelica roots (*Angelica archangelica*), peppermint leaves (*Mentha piperita*), lemon balm leaves (*Melissa officinalis*), liquorice root (*Glycyrrhiza glabra*), chamomile flowers (*Matricaria recrutita*), greater celandine herbs (*Chelidonium majus*), caraway fruits (*Carum carvi*) and milk thistle fruits (*Silybum marianum*) (Wegener & Wagner, 2006).

For more than five decades Iberogast® was successfully used for treatment of functional gastrointestinal disorders. Since 1987, six clinical trials showed the efficacy of STW 5 in the treatment of patients with functional dyspepsia and irritable bowel syndrome (Brierley & Kelber, 2011;Ottillinger *et al.*, 2013).

Controlled and randomized clinical studies showed the efficacy of STW 5 in the treatment of dysmotility type of functional dyspepsia (Madisch *et al.*, 2001;Madisch *et al.*, 2004a;von Arnim *et al.*, 2007). A double blind study showed non-inferiority of STW 5 compared to the prokinetic drug cisapride which is not available anymore (Rosch *et al.*, 2002b). Cisapride acted as a 5-HT_3 antagonist (Schemann, 1991). Another clinical study showed the comparable efficacy of STW 5 versus metoclopramide in patients with functional dyspepsia (Raedsch *et al.*, 2007). This efficacy in functional dyspeptic patients is independent on acceleration of gastric emptying (Braden *et al.*, 2009). Similar efficacy has been demonstrated in a double-blind, randomized, placebo-controlled multi-centre clinical study for treatment of irritable bowel syndrome (Madisch *et al.*, 2004b). In meta-analysis studies, STW 5 has also been shown to be effective in relieving symptoms of functional dyspepsia and irritable bowel syndrome (Melzer *et al.*, 2004;Liu *et al.*, 2006). Because of its proven tolerability and safety, STW 5 was also used in children (Brierley & Kelber, 2011;Ottillinger *et al.*, 2013).

Although classical medicine is sceptic towards alternative medicines, there are strong evidences demonstrated for biological actions of components in natural products with specific effects on ion channels and receptors. These findings may provide novel therapeutic targets for mainstream drug development (Brierley & Kelber, 2011).

Various pre-clinical studies have been carried out in recent years to understand the mode of action of STW 5. STW 5 suppressed intestinal afferent sensitivities to mechanical and chemical stimuli (Muller *et al.*, 2006) which may explain its pain relieving property in FD and IBS. STW 5 binds to 5-HT_4, to less extent to 5-HT_3, M3 and opioid receptors (Simmen *et al.*,

2006). It is not known whether this binding represent agonistic or antagonistic properties or no biological activity. In a rat model, STW 5 and its individual components had been shown to have gastro-protective effect against ulcers and rebound acidity and their activities were comparable to H_2 blockers or antacids (Khayyal *et al.,* 2001;Khayyal *et al.,* 2006). This involved enhanced synthesis of prostaglandins. In the same context, Abdel Aziz *et al* (2010) reported the efficacy of STW 5 in treatment of reflux esophagitis in rats. STW 5 has been shown to possesses anti-inflammatory activity on colon adenocarcinoma (HT29) cells and isolated rat small intestine treated with 2,4,6-trinitrobenzene sulfonic acid (Michael *et al.,* 2012;Bonaterra *et al.,* 2013). This anti-inflammatory effect was mediated via adenosine A2a receptors (Michael *et al.,* 2012). Moreover, STW 5 protected against dextran sulphate sodium-induced colitis in rats *in vivo* by normalising inflammatory mediator profiles (Wadie *et al.,* 2012). Additionally, its strong antioxidant and free radical scavenging activities had been reported (Schempp *et al.,* 2006;Germann *et al.,* 2006). Recently, the protective effects of STW 5 against 5-Fluorouracil- and radiation-induced intestinal damage was reported (Wright *et al.,* 2009;el Ghazaly *et al.,* 2014). Its effect on motility is likely relevant for its clinical use. Based on in *vitro data* in guinea pig and rat intestine, STW 5 had spasmolytic activity on histamine/acetyl choline pre-contracted muscles (Ammon *et al.,* 2006;Heinle *et al.,* 2006). Additionally, its effects in modulating the intestinal electrophysiology was reported (Sibaev *et al.,* 2006).

In 2004, Hohenester *et al,* showed region-specific effects of STW 5 on guinea pig gastric smooth muscle strips (Hohenester *et al.,* 2004). STW 5 significantly and dose-dependently relaxed the proximal stomach (fundus and corpus) and increased phasic contractility in the antrum. They also demonstrated that this relaxation is neither nerve mediated nor nitric oxide dependent. Similar region specific effects in the stomach were reported for its individual extracts (Schemann *et al.,* 2006). This study further demonstrated that the region-dependent effects were not due to region specific effects of the individual components but rather due to region-specific properties of smooth muscle. The relevance of these findings were further confirmed in healthy volunteers (Pilichiewicz *et al.,* 2007) and in isolated human gastric muscle strips (Schemann *et al.,* 2006). In isolated human mucosal/submucosal preparations, STW 5 enhanced chloride secretion via release of pro-secretory neurotransmitters and activation of epithelial CFTR and Ca^{2+} activated chloride channels (Krueger *et al* 2009).

1.3 Gastrointestinal motility

The gastrointestinal tract is divided into upper and lower tracts. The former consists of esophagus and stomach whilst the latter consists of small and large intestine. The gastrointestinal tract is the organ that accomplishes a remarkable variety of functions like transport of luminal content, secretion and absorption of ions, water, and nutrients, blood flow, defense against pathogens and elimination of waste and/or noxious substances (Schemann, 2005). The transit of the gut contents is achieved by contraction and relaxation of the smooth muscle and tissues.

Smooth muscle

Muscle fibres are called 'smooth' if they lack the characteristic striations formed by the organized groups of contractile filaments, actin and myosin. The gastrointestinal tract is composed of single-unit muscles. The muscle cells are embedded in a connective tissue matrix containing glial cells, fibroblasts, and a distinctive population of cells, the interstitial cells of Cajal (ICC) (Hansen, 2003). The individual smooth muscle cell is small and spindle-shaped and coupled with one another through electrically conducting gap junctions. The excitation is passed through these gap junctions. As a result, contraction generates and moves throughout larger parts of the intestine in a wave. The smooth muscle of the gut exhibits two distinct types of contractions: tonic contractions and rhythmic phasic contractions which cause mixing and propulsive movements (Hansen, 2003). Tonic contractions are slow, lasting from few seconds to minutes whilst phasic contractions are rapid with duration of few seconds (Scratcherd & Grundy, 1984). Tonic contractions are maintained by neurogenic and myogenic mechanisms. The neurogenic tone results from a constant low discharge of excitatory innervation, while the myogenic tone results from a property of the muscle itself (Gregersen, 2000). The activity of the smooth muscle is involuntary and controlled by intrinsic myogenic activity, intrinsic and extrinsic neurons and hormonal action (Scratcherd & Grundy, 1984;Hansen, 2003).

1.3.1 Neural regulation

The neuronal regulation of GI motility involves intrinsic and extrinsic nerves. The intrinsic innervation involves the enteric nervous system (ENS), whereas the extrinsic innervation involves the vagus nerve and splanchnic nerves to the stomach and upper intestine, while the pelvic nerves supply the distal intestines (Hansen, 2003). Unlike other organs, the gut is able

to contract and relax even when completely isolated from the body. The main reason for this unique ability is that the gut has its own nervous system, which is referred to as the enteric nervous system (ENS) (Schemann, 2005). The ENS contains about 100 million nerve cell bodies. This is second to the brain, the largest accumulation of nerve cells in the body. The ENS has structural and functional resemblance to the brain and is in its true sense an autonomic nervous system. It accomplishes these functions because it contains neurons encoding stimuli, interneurons and motor neurons (Wood, 1984). Complex interactions between these populations are the basis for the regulation of gastrointestinal motility. The ENS is usually formed from two major plexus: the myenteric plexus mainly regulating muscle activity and the submucous plexus mainly regulating mucosal functions (Schemann & Neunlist, 2004;Schemann, 2005;Furness, 2006). The ENS contains hardwired circuits that consist of ascending excitatory motor neurons that release acetyl choline, which contracts smooth muscle through muscarinic receptors and descending inhibitory neurons that release a cocktail of transmitters like nitric oxide (NO), adenosine triphosphate (ATP), vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP), all of which evoke relaxation via non-adrenergic non-cholinergic pathway (Schemann, 2005).

The extrinsic innervation is either vagal or sympathetic. The vagal nerve arises from the dorsal motor nucleus of the vagus in the medulla and supply the stomach, small and large intestine as far as the middle of transverse colon. The vagal efferent preganglionic fibres innervate the postganglionic intrinsic neurons (Scratcherd & Grundy, 1984). The sympathetic supply arises from the anterolateral column of the spinal cord and reaches the gut through splanchnic nerves after relay in the coeliac and mesenteric ganglia and terminates in myenteric and submucous plexus (Scratcherd & Grundy, 1984). Extrinsic neurons of the sympathetic and parasympathetic systems influence smooth muscle indirectly by acting on neurons of the myenteric plexus. However, also neurons from the submucous plexus innervate the innermost layers of circular muscle, at least in large species (Hansen, 2003;Furness *et al.*, 2004).

Throughout the gut the innervation of the circular muscle layer depends on polarized projections of neurochemically distinct populations (Neunlist *et al.*, 1999). Peristaltic waves are circular constrictions propagating aborally. In agreement with the peristaltic reflex the sequential activation of polarized circuits in the ENS is responsible for aboral transport of luminal content and mediates relaxation below and contraction above a stimulus. Initiation or inhibition of the peristaltic reflex will result in peristaltic or segmental activity, respectively. The redundancy of inhibitory transmitters supports the functional importance of inhibitory

tone to the muscle which usually assures that the gut lumen is not occluded, thereby avoiding mechanical obstruction. Although there is a profound knowledge on the basic circuits in the ENS that regulate peristaltic reflex activity, much less is known about the changes in ENS circuitry that are responsible to initiate different motility patterns, like propulsion, clustered contractions or segmental activity (Schemann, 2005). Neurons synapse onto smooth muscle and can modulate the frequency and the strength of the contraction however the neuronal input is not required for contraction.

1.3.2 Electrical activity of smooth muscle

The resting membrane potential of smooth muscle cells of the gut is in the range of –40 to –80 mV and is largely determined by activity of the Na^+/K^+ pump, voltage-gated Ca^{2+} channel and several types of K^+ channels including Ca^{2+}-activated K^+ channels. High conductance channels with mixed selectivity for K^+ and Na^+ carry an inward depolarizing current and are activated at membrane potentials negative to –70 mV, known as the pacemaker potential (Hansen, 2003).

Smooth muscle shows two different electrical activities: action potentials which initiate muscle contraction and slow waves. The slow waves are cyclic changes in the membrane potential unaccompanied by mechanical activity and generated by interstitial cells of cajal (ICC). The slow waves are the electrophysiological basis for peristaltic and segmental smooth muscular contraction in the gastrointestinal tract (Scratcherd & Grundy, 1984;Sanders, 1996). ICC are responsible for spontaneous pacemaker activity, remain rhythmic in culture and generate voltage-independent inward currents via a non-selective cation conductance. Ca^{2+} release from sarcoplasmic reticulum and uptake by mitochondria initiates pacemaker currents (Sanders *et al.*, 2000). The ionic determinant of the spike potential appears to be membrane Ca^{2+} flux. Excitatory agonists such as acetylcholine stimulate intestinal phasic motor activity by enhancing spike potential activity resulting in a contractile wave passing down the gut. Contraction occurs when spike activity superimposes on the slow wave and depolarization reaches a critical threshold (Hansen, 2003).

1.3.3 Contractile filament

There are three contractile filaments, thin actin, thick myosin and intermediate desmin. These filaments are responsible for excitation-contraction coupling in smooth muscle which controls contraction and relaxation (Hansen, 2003).

Ca^{2+} binds to calmodulin forming Ca^{2+}/calmodulin complex. This complex binds to an elongated protein, caldesmon. In absence of Ca^{2+}, caldesmon binds to actin filament restricting myosin-actin interaction and inhibiting contraction. On the other hand, phosphorylated caldesmon by protein kinase C (PKC) cannot bind to actin, therefore phosphorylation of caldesmon or its binding to Ca^{2+}/calmodulin activates smooth muscle contraction. There are three other mechanisms that involve the regulatory light chains of myosin: Firstly, direct Ca^{2+} binding induces conformational changes in myosin that allows it to bind to actin resulting in muscle contraction. Secondly, myosin phosphorylation by myosin light chain (LC) kinase that also causes contractions. The phosphorylation is Ca^{2+}-dependent as the myosin LC kinase is activated by Ca^{2+}/calmodulin. Thirdly, phosphorylation of another site in myosin by PKC induces conformational changes however these changes inhibit binding to actin and lead to relaxation (Murthy, 2006).

1.3.4 Hormonal regulation

Hormones and neurotransmitters are the dominating components which act and interact directly and indirectly on smooth muscle cells. The hormonal influence and the interplay with the ENS takes place after and in between meals. The postprandial endocrine response includes release of insulin, neurotensin, cholecystokinin (CCK), gastrin, glucagon-like-peptides (GLP-1 and GLP-2) and glucose-dependent insulinotropic polypeptide but not motilin nor somatostatin (Hansen, 2003). These released hormones have all been demonstrated to have functional importance for digestion. For example, CCK is released into the circulation from the upper small intestine, causing direct contraction of muscle cells in the gall bladder and neutrally-mediated relaxation of muscle cells in the sphincter Oddi, which is mediated by VIP at the neuromuscular junction (Hansen, 2003).

There are hormones which are released locally from endocrine cells in the mucosal lining like serotonin (5-HT) from enterochromaffin cells that modulates motility by activating receptors on the extrinsic sensory fibers (e.g. vagal) and intrinsic primary afferent neurons (IPANs), and again back on the endocrine cells in an autoregulatory fashion. This system conveys sensory information, so the CNS can evaluate GI activity and modulate motility accordingly (Hansen, 2003).

There are two types of adenosine receptors coexist on intestinal smooth muscle cells: A1 receptors mediate contraction due to decrease in cAMP and mobilization of Ca^{2+} and A2 receptors which mediate relaxation through the increase of cAMP (Ukena *et al.*, 1986;Strohmeier *et al.*, 1995;Murthy, 2006).

Other stimulatory neurohumoral substances in the gut include: bombesin, gastrin releasing polypeptide (GRP), histamine, motilin, Neurokinin A, Opioids, prostaglandin (PGE2), substance P (SP) and thyrotropin-releasing hormone (TRH). Whereas inhibitory neuro-transmitters include: calcitonin gene-regulated peptide (CGRP), gamma butyric acid (GABA), galanin, glucagon, nitric oxide (NO), neuropeptide Y (NPY), peptide YY (PYY), Neurotensin, peptide histidine isoleucine (PHI), somatostatin and secretin (Hansen, 2003).

1.3.5 Intrinsic myogenic activity

Calcium is a fundamental second messenger in smooth muscle cells. Increasing cytoplasmic Ca^{2+} concentration $[Ca^{2+}]_i$ and binding to calmodulin and activation of myosin light chain kinase is the primary stimulus for contraction. Elevations in global cytoplasmic Ca^{2+} resulting in contraction are accomplished by Ca^{2+} entry from extracellular pool and Ca^{2+} release from intracellular stores (Sanders, 2001). These events are accomplished by at least a dozen of specialized Ca^{2+} transporters and ion channels which are arranged in membranes separating at least five distinct compartments and capable of facilitating Ca^{2+} movements up and down significant electrochemical gradients (Sanders, 2001). Ca^{2+} entry is the major access route to elevate $[Ca^{2+}]_i$. Ca^{2+} utilized in contractile apparatus enters the cytoplasmic compartment during periods of membrane depolarization, mechanical distortion or stimulation by agonists (Sanders, 2001). Ca^{2+} influx can be achieved via voltage dependent calcium channels (VDCC), non-selective cation channels, store-operated calcium channel (SOC), receptor-operated calcium channel (ROC), stretch-sensitive non-selective cation channels and adenosine receptors (Sanders, 2001;Chalmers *et al.*, 2007). The second mean of increasing $[Ca^{2+}]_i$ is release of Ca^{2+} from its intracellular stores. Calcium is stored intracellulary in sarcoplasmic reticulum (SR) and released through ryanodine receptors (RyRs) and inositol triphosphate receptors (IP_3-Rs) (Iino, 1990). After an excitatory event, Ca^{2+} homeostasis, reducing $[Ca^{2+}]_i$ and hence relaxation take place. Decreasing $[Ca^{2+}]_i$ can be achieved by calcium uptake in SR via sarcoplasmic endoplasmic reticulum calcium ATPase pump (SERCA) (Chalmers *et al.*, 2007) and calcium extrusion through plasma membrane calcium ATPase pump (PMCA) and Na^+/Ca^{2+} exchanger (Sanders, 2001) (Figure 1).

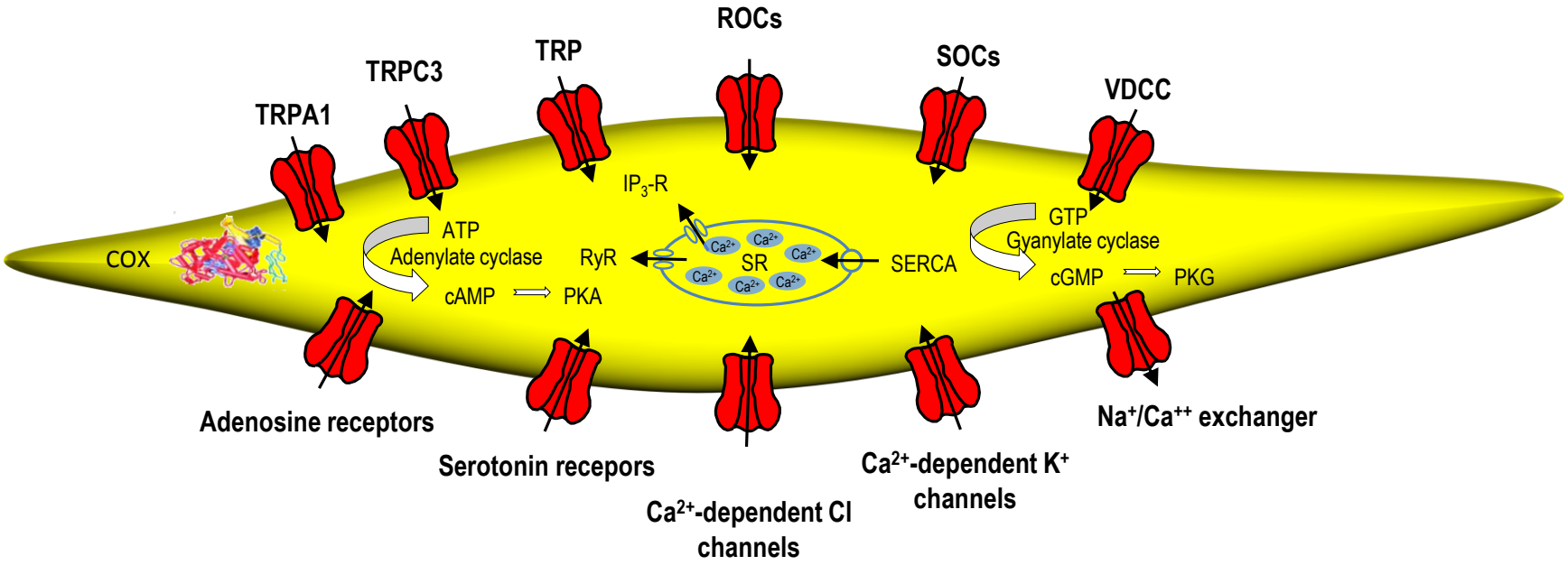

Figure 1: Illustration of smooth muscle cell. Smooth muscle contraction and relaxation are controlled by several factors. Calcium homeostasis is the key feature in this process. Ca^{2+} entry provides the major access route to elevate $[Ca^{2+}]_i$ and can be achieved via store-operated (**SOC**s), voltage-dependent (**VDCC**s) and/or receptor operated calcium channels (**ROC**s). **TRP** channels like **TRPA1** and **TRPC3** could also function as SOC channels. The second pathway to increase $[Ca^{2+}]_i$ is release of Ca^{2+} from intracellular store (sarcoplasmic reticulum, **SR**) via ryanodine receptors (**RyRs**) and inositol triphosphate (**IP_3-R**).on the other hand $[Ca^{2+}]_i$ level can be decreased by Ca^{2+} uptake into stores via **SERCA** or calcium extrusion via **Na^+/ Ca^{2+}** exchanger. There are other neurotransmitters that regulate muscle contraction through acting on the membrane channel like **adenosine** and **serotonin**. In gastrointestinal smooth muscle there are two types of adenosine receptors A1 and A2. Both interact with cAMP however in different ways; the former induces contraction by decreasing **cAMP** whereas the latter relaxes the muscle by increasing the cAMP. As well, different types of **calcium-dependent potassium channels** (SK,IK and BK) affect the contractile machinery of smooth muscle. calcium-sensitive chloride channels are also involved.

There are a number of open questions related to the mode of action of STW 5 on motility and secretion. This thesis was intended to address the following topics:

(1) Identify the individual component(s) responsible for the prosecretory effect of STW 5 in human intestine. After identification, it was important to investigate their mechanism of action.

(2) Explore the effect of STW 5 and its individual components on human intestinal circular muscle, as well as its mechanism of action.

(3) Investigate the mechanism(s) of action by which STW 5 relaxes the gastric smooth muscle.

2. Material and Methods

2.1 Tissue samples and tissue preparations

All procedures regarding human tissue samples were approved by the ethics committee of the Technische Universität München (1748/07 and 2595/09) or according to the guidelines of the charitable state controlled foundation (Human Tissue and Cell Research foundation; HTCR), with the informed patient's consent.

2.1.1 Human samples for recording secretion with Ussing Chambers

Human tissue samples of large and small intestine were obtained from 119 patients (57 female and 62 male) with an age of 64 ± 1.4 years (mean ± SEM) ranging from 14 to 93 years undergoing surgeries at the Medical Clinics in Freising and *Rechts der Isar* in Munich. Surgical resectates were taken from macroscopically unaffected, non-damaged areas as determined by pathologists. Diagnoses that led to surgeries were: stomach carcinoma (15), pancreatic carcinoma (10), colorectal carcinoma (55), diverticulitis (7), ovarian carcinoma (3), polyp (3), allergic eosinophilic gastroenteritis (AEG) (3), large intestinal stenosis (2), elongated sigma (2), ileostoma reversal (3), gall bladder carcinoma (2), small intestine carcinoma (2), chronic ileus (1), esophageal shift (1), motility disorder after gastrectomy (1), fistula (1), small intestine twist (1), small intestine perforation (1), large intestinal stoma (1), intestinal obstruction (1) or unspecified reason (4). Experiments were performed in 543 mucosal/submucosal preparations (264 small intestine and 279 large intestine).

Immediately after resection, samples were transferred to the institute under aseptic conditions in cold oxygenated sterile Krebs solution. Transferred specimens were washed 3 times with ice-cold Krebs solution (containing in mmol: 1.2 $MgCl_2$, 2.5 $CaCl_2$, 1.2 NaH_2PO_4. 120.9 NaCl, 14.4 $NaHCO_3$, 11.5 Glucose, 5.9 KCl; pH = 7.4), oxygenated with 95 % O_2 and 5 % CO_2 (Carbogen, *Westfalen Gas AG, Münster, Germany*). Segments were dissected carefully in such conditions to obtain mucosal/submucosal preparations containing the submucous plexus. Experiments were performed only with freshly dissected tissues (at the same day of operation). In the following sections, these mucosal/submucosal preparations will be referred to as ''human intestinal preparations''.

2.1.2 Human samples for recording motility with organ baths

Freshly isolated tissues were obtained from 114 patients (55 female and 59 male) with an age of 68 ± 1.5 years (mean ± SEM) ranging from 15 to 88 years. Fifty samples were taken from

the small intestine and 64 from the large intestine. Diagnoses that led to surgeries were: stomach carcinoma (11), pancreatic carcinoma (11), colorectal carcinoma (51), caecum carcinoma (7), polyp (4), mesenterial tumour (1), ovarian carcinoma (2), diverticulitis (3), allergic eosinophilic gastroenteritis (AEG) (3), elongated sigma (1), elongated colon (1), megacolon (1), oesophagus carcinoma (1), sigma perforation (1), small intestine twist (1), stoma correction (2), pancreatic cyst (2), rectocele (1), acute abdomen (1), Hartmann situation (1), stenosing flexor carcinoma (1) or unspecified reason (7). The tissues were dissected under the same conditions as described in 2.1.1. Tissue dissection allowed preparation of muscle strips containing the myenteric plexus. The strips were cut either along their circular or their longitudinal axis in about 0.5 cm^2 pieces (1 cm in length and 0.5 cm in width). Experiments were performed with 575 muscle strip preparations (137 longitudinal and 438 circular). Experiments were done using both fresh and overnight-stored muscle strips (56% vs. 44%, respectively). Dissected muscle strips were stored in cold oxygenated Krebs solution.

2.1.3 Human intestinal epithelial T84 cell line to record secretion

Experiments using human intestinal T84 cell line were performed on 760 T84 filter discs. T84 cells were purchased from European Collection of Cell Cultures (*88021101; ECACC; Salisbury, UK*). The adherent epithelial cell line was derived from a lung metastasis of colon carcinoma in a 72 year old male (Murakami & Masui, 1980;Dharmsathaphorn *et al.*, 1984). This cell line has served as an excellent model system for the study of vectorial electrolytes transport processes and their regulation by peptide hormones, neurotransmitters, secretagogues and antisecretagogues similar and behaves similar to isolated intestinal mucosa. The cells grew to confluence as a polarized monolayer with the basolateral membrane attached to the surface of the culture filter and the microvillus-studded apical membrane facing the media. Tight junctions and desmosomes were demonstrated between adjacent cells. The T84 cell line is an established model system for studying (patho-)-physiology of intestinal epithelial cells (Madara *et al.*, 1987).

2.1.4 Guinea-pig tissue samples to record gastric motility

Tissue samples were obtained from 288 male guinea-pigs (*Dunkin Hartley, Harlan Winkelmann, Borchen, Germany*). The animals were kept under standardised conditions in the animal facility at the institute at least one week before the start of the experiments (isolated airflow unit, *Uni-Protect, Ehret, Emmendingen, Germany*). Animals received standard pellet diet (*Altromin Spezialfutter GmbH & Co. KG, Lage, Germany*) and drinking water *ad libitum*.

Guinea-pigs were kept at 20-24°C, 60% humidity and a 14:10 h light/dark cycle. The weights of the guinea-pigs at the day of experiments ranged from 220-400 g. Animals were killed by concussion and exsanguination from their cervical blood vessels. The stomach was removed and immediately placed in ice-cold oxygenated Krebs solution (as described in 2.1.1). The stomach was opened along the greater curvature; mucosa and submucosa were dissected carefully from the corpus region in order to obtain the muscle layers with the myenteric plexus. Muscle strips were cut along their longitudinal or circular axis from both parietal and visceral sides with the use of micro scissors (*FST #14058-11, Fine Science Tools (FST)*) and forceps (*Dumostar #5, Dumont, Switzerland*). All procedures were conducted according to the German ethical guidelines for animal care and welfare.

2.2 Cell culture techniques

2.2.1 Cell culture medium

T84 cells were cultured in Dulbecco's modified Eagle's medium/nutrient mixture F-12 Ham (DMEM/F12; *Sigma-Aldrich, Schnelldorf, Germany*) supplemented with 10% (v/v) heat inactivated foetal bovine serum (FBS; *Invitrogen, Karlsruhe, Germany*) and 1% (v/v) antibiotic-antimycotic solution (AA; *CC-Pro, Neustadt, Germany*) containing 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 ng/ml amphotericin B. In the following sections, this mixture will be referred to as ''culture medium''.

2.2.2 Resuscitation of frozen cells

Cryopreserved T84 cells kept at a -80 °C in a deep freezer (*GFL-Gesellschaft für Labortechnik, Burgwedel, Germany*) were thawed in a shaking water bath (1083; *GFL-Gesellschaft für Labortechnik, Burgwedel, Germany*) at 37 °C. Since dimethyl sulphoxide (DMSO) is a toxic component of the freezing medium, cryotubes containing the cells were quickly transferred in an ice container to a class II biological safety cabinet (*Holten Lamin Air S 2010, type 1.2; Heto-Holten, Allerød, Denmark*). There, the transferred cells were immediately pipetted into a sterile 15 ml centrifuge tube (*Greiner Bio-One, Frickenhausen, Germany*) prepared with 5 ml of pre-warmed (37 °C) culture medium using a 5 ml serological pipette (*Costar® stripette, New York, USA*). Then the tube was centrifuged at 1200 rpm for 5 minutes at 4 °C (*Z 513 K; Hermle Labortechnik, Wehingen, Germany*). Afterwards, the supernatant was carefully removed with a sterile glass Pasteur pipette (*Brand, Wertheim, Germany*) attached to a vacuum pump (*KNF Neuberger, Freiburg, Germany*). The cell pellet

was carefully resuspended in 3 ml pre-warmed culture medium. The cell suspension was transferred into a 75 cm^3 cell culture flask (*Greiner Bio-One, Frickenhausen, Germany*) filled with 4 ml pre-warmed culture medium. The flask was cultured under standard cell culture conditions in a humidified CO_2 incubator (37°C, 5% CO_2, *Binder, Tuttlingen, Germany*). Twenty four hours later, culture media were changed and the cells were examined with a phase contrast microscope (*DM IL; Leica Microsystems, Wetzlar, Germany*). Then, media were changed every second day and the cultures were checked for confluency and subcultured if needed.

2.2.3 Subculture of adherent cells

The cells were examined daily with a phase contrast microscope to check the general appearance of the culture and to detect possible microbial contamination. When cells reached 80-90 % confluency, they were subcultured using enzymatic dissociation. Culture medium was removed from 75 cm^2 cell culture flasks and the cells were gently rinsed with 5 ml pre-warmed sterile phosphate buffer saline (PBS). PBS was then removed and discarded. Trypsin (0.5%)/EDTA (0.2%) 10x (*CC-Pro, Neustadt, Germany*) was diluted with deionized sterilized H_2O (*NANO pure Diamond; Barnstead Int., Dubuque, IA, USA*) to 1x and pipetted onto the washed cells using 1 ml per 25 cm^2 of surface area. The flask was carefully rotated to cover the monolayer with trypsin/EDTA and placed in the incubator for 5 min. After the incubation period, the flasks were gently shook under microscopic control or tapped on the side of the flask to ensure that all cells were detached and floating. The enzymatic dissociation was immediately stopped by addition of 7 ml fresh culture medium supplemented with 10% (v/v) FBS to inactivate the trypsin. The cells were firmly washed from the bottom of the cell culture vessel with a serological pipette and transferred into a 15 ml centrifuge tube. For counting the cells an aliquot of 100 µl was transferred into a 0.5 µl tube (*Eppendorf, Hamburg, Germany*). The enzymatic dissociation solution was separated from the cell suspension by centrifugation (1200 rpm for 5 min at 4 °C) and removal of the supernatant. Subsequently, the cells were resuspended in pre-warmed culture medium and the required number of cells was seeded in newly labelled 75 cm^2 cell culture flasks containing 7 ml pre-warmed medium. Alternatively, the cell suspension was split among a number of culture flasks of equivalent surface area with a ratio of 1:2 to 1:10 depending on the growth characteristics of the cell culture. The culture vessels contained a final volume of about 0.2 ml per square centimetre of growth area. The cells were maintained in the incubator (*Binder*). Culture medium was changed 24 hours after

seeding and then three times a week until a confluency of 80-90% was reached. With this confluency cells were ready for further culturing or cryopreservation.

2.2.4 Cell Quantification

In order to use defined cell numbers for culture experiments, cryopreservation or subculture, it was necessary to quantify the number of cells prior to use. An adherent cell culture was brought into suspension using trypsin/EDTA as described before and resuspended in fresh medium. A haemacytometer (*Sigma-Aldrich, Schnelldorf, Germany*) was cleaned with 70% ethanol. The coverslip was moistened by breathing onto it and then slid over the counting chamber back and forth using slight pressure until Newton's refractions rings appeared. 20 µl of a 100 µl aliquot taken from the cell suspension was carefully filled into both sides of the haemocytometer. The number of viable cells which was seen as bright dots was counted under a phase contrast microscope. In detail, all viable cells in the 1 mm centre square and the four 1 mm corner squares were counted and an average value was calculated. Only cells on top and right touching the middle line of the perimeter of each square were included in the cell counts. The number of viable cells per ml and the total cell number were calculated using the following equations:

Cells/ml = (average count/1 mm^2) x 10^4 (*chamber conversion factor*)

Total cell number = (cells/ml) x (original or pre-dilution volume).

NB: The chamber conversion factor (10^4) was derived from the total volume of chamber square; since each 1 mm^2 had a depth of 0.1 mm, the total volume was 0.1 mm^3 or 10^{-4} mm^3.

2.2.5 Cryopreservation of Cells

Cryogenic preservation was used to maintain backups and preserve cells for experiments to start with always identical passage numbers, which is an important indicator for the age of a cell culture. Passage number refers to the number of times the cells have been subcultured; ideally taking 1:2 splits into account. Cells which had to be frozen were detached from the cell culture flask when they had reached 80-90 % confluency using enzymatic dissociation, and a cell count was performed. After the dissociation, solution were removed by a centrifugation step, the supernatant was discarded and the cell pellet at the bottom of the tube was resuspended in a calculated volume of frozen medium (DMEM/F12 containing 20% (v/v) FBS and 10% (v/v) DMSO). A final concentration of 1-2x10^6 cells per ml was obtained. Aliquots of 1 ml of the cell suspension were stored in 2 ml cryo tubes (*Greiner Bio-One,*

Frickenhausen, Germany) at -80 °C, which were labelled with the cell culture name, passage number, cell concentration and date. All procedures involving DMSO were performed quickly and on ice to minimize toxic effects and ensure cell viability.

2.2.6 Culture procedures

T84 cells were seeded in sterile MF-Millipore Membrane Filters (*Merck Millipore* ^{TM}, *Ireland*). The filter discs are hydrophilic with a diameter of 25 mm and 0.45 µM pore size. Filters were placed in sterile 6-well cell culture plates with a lid (*Greiner Bio-One, Frickenhausen, Germany*) and pre-moistened with 2 ml sterile culture medium. Cells were seeded at a density of 50000 cells/insert. The plates were kept under standard culture conditions in the incubator (*Binder*). The culture medium was changed first after 24 hours and then every 24-48 h depending on the growth rate. The cells grew as confluent monolayers covering the filter surface and lay down basement membrane-like material within 18 h. Basolateral compartment was attached to the porous membrane whereas the apical one was directed upwards. Experiments were performed at day 14. Passages 20-43 were used.

2.3 Ussing Chamber techniques

2.3.1 Principle of the Ussing Chamber to record mucosal secretion

The Ussing Chamber was invented by the Danish zoologist Hans Ussing in 1951 to study the electrophysiological behaviour of epithelial ion transport mechanisms, like that seen in the gastrointestinal tract (Ussing & Zerahn, 1951;Li *et al.*, 2004) . This technique considers two important characteristics of epithelia: polarity and permeability. The Ussing Chamber is a two chamber system, which connects two chambers separated by an epithelial tissue (Figure 2). The T84 cell filter discs or the mucosal/submucosal preparation containing submucous plexuses from human small or large intestine was used as epithelial tissue, which is polar with a mucosal (apical) and serosal (basolateral) side.

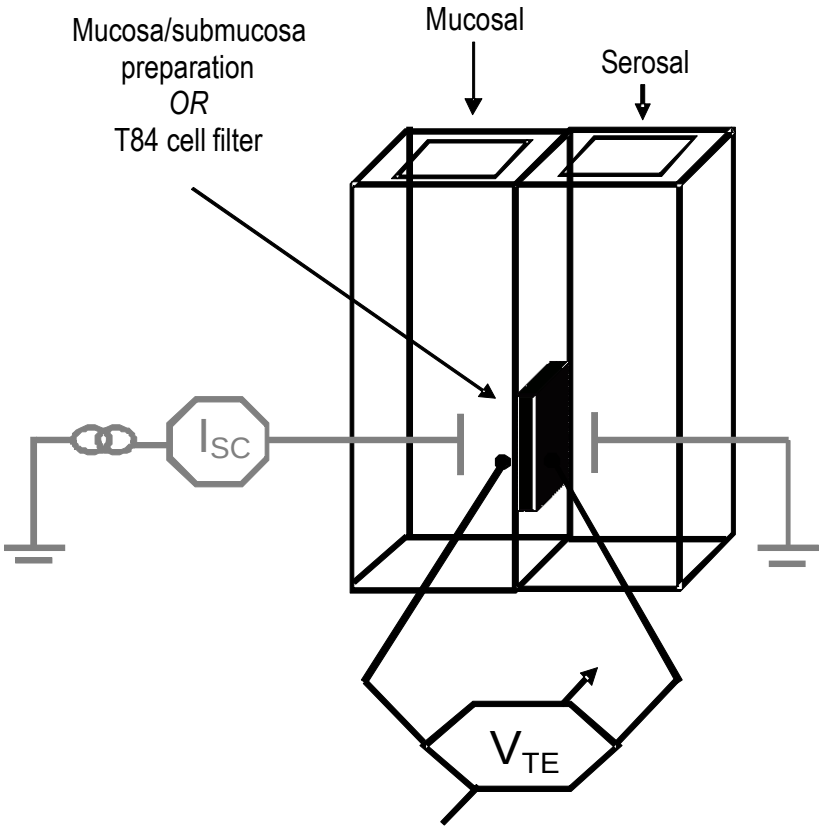

Figure 2: Illustration of the Ussing Chamber set up: The mucosa/submucosa preparation or T84 filter disc (Black) is fixed onto a slider and placed in between two Krebs solution filled chambers (5 mL volume). The black electrodes measure the transepithelial potential (V_{TE}) and the grey electrodes apply the short-circuit-current (I_{SC}). Redrawn from a figure from Prof. M. Schemann.

The vectorial transport of ions over the epithelial tissue creates a potential difference across the tissue, the so called transepithelial potential (V_{TE}). With the short-circuit-current technique, a current (I_{SC}) is applied that clamps the transepithelial potential to 0mV (voltage-

clamp). This current is called the short circuit current and is identical to the absolute value of the current that is generated by active ion transport. Thus by measuring the short-circuit-current, the net secretion or net absorption of the epithelium can be determined indirectly. The short-circuit-current is defined as follows:

$$\mathbf{I_{SC} = V_{TE} / R}$$

$\mathbf{I_{SC}}$: short-circuit-current [µA]

$\mathbf{V_{TE}}$: transepithelial potential [V]

R: electrical resistance [Ω]

The transport of positive charge from mucosal to serosal side or of negative charge from serosa to the lumen induces a positive short-circuit-current. This corresponds to a cation net resorption and/or anion net secretion.

The tightness/permeability of the epithelium and integrity of the human tissue or T84 cells can be determined by measuring the transepithelial resistance. The electrical resistance is defined as follows:

$$\mathbf{R = \rho * L / A}$$

R: electrical resistance [Ω]

ρ: material specific electrical resistance constant [$\Omega * m^2/m$]

A: cross section area of the flow through tissue [m^2]

L: length or size of the flow through tissue [m]

The electrical resistance is experimentally determined indirectly using the Ohm's law:

$$\mathbf{R = \Delta U / \Delta I}$$

R: electrical resistance [Ω]

ΔU: change of the electrical potential over the tissue [V]

ΔI: change of the electrical current over the tissue [A]

The corresponding values for potential and current can be read off the voltage current clamp/pulse generator (*Physiologic Instruments, San Diego, CA, United States*). The current corresponds to the value necessary to clamp the potential to 0 mV. The potential difference over the tissue is determined by suppressing the application of the short-circuit-current.

2.3.2 Calibration and experimental procedure of the Ussing Chamber

Before starting the experiments, the Ussing Chambers (*Easy Mount Chambers, Physiologic Instruments, San Diego, CA, USA*) were calibrated. To calibrate the system a calibration block was put between the two chambers. The calibration block allows the fluid exchange (Krebs solution) between the two chambers. Two potential electrodes were placed close to the tissue to measure the transepithelial potential and two current electrodes, which apply the short-circuit-current, were placed distant to the tissue (*Easy Mount Electrode Set P2020, Physiologic Instruments, San Diego, CA, USA*). These Ag/AgCl electrodes were connected to the Krebs solution via a 3 M KCl-filled agar bridge with the AD-converter (*PowerLab, ADInstruments, Spechbach, Germany*) via the voltage clamp generator (*VCC 600L, Physiologic Instruments, San Diego, CA, USA*). The Software LabChart (*Version 7, Chart Software: AD Instruments, Spechbach, Germany*) detected the short-circuit-currents during the experiment. After inserting the calibration blocks the chambers were filled with Krebs solution and warmed to 37 °C whilst continuously being bubbled by carbogen and then calibrated to 0 mV.

The Ag/AgCl electrodes were prepared by filling them with 30-40 µL of a 3-4% KCl-agar (*Merck, Darmstadt, Germany*). After hardening of the KCl-agar the electrode tips were bubble-free filled with 3 M KCl solution. The electrodes were stored in a 3 M KCl solution at 4 °C.

To study the effect of STW 5, its individual extracts or their combinations on ion secretion in mucosal/submucosal preparations of human intestine or T84 cells, the tissue specimens or T84 cell filters were put onto sliders and mounted into the Ussing Chambers resulting in a recording area of 0.5 cm^2 (Figure 2). Apical and basolateral sides were bathed separately in 5 mL pre-warmed Krebs solution, continuously bubbled with carbogen and maintained at 37 °C. Chambers were covered by caps with small sampling port (*Easy Mount Chambers, Physiologic Instruments, San Diego, CA, USA*) to prevent small droplets splattering caused by aeration and loss of volume. Drugs were applied through these ports. The transepithelial potential difference was measured by a pair of Ag/AgCl electrodes, connected to a voltage clamp apparatus that compensated for the solution resistance between the electrodes. Ion secretion was measured as short-circuit-current (I_{SC}) and expressed in µA/cm^2.
Equilibration periods of 45-60 min (human) / 20 min (T84 cells) were required to adjust the short-circuit-current to a stable basal value before the experimental protocols were started.

Experimental procedure for recording secretion from human tissue

For the human mucosal/submucosal preparation experiments, the electrical resistance was determined as described above (see 2.3.1). Then the tissue was electrically stimulated by silver electrodes placed on either side of the tissue and connected to a constant voltage stimulator (*Grass SD-9; Grass-Telefactor, Middleton, USA*). The neural stimulation of the human tissue was achieved by delivering a train of pulses with supramaximal stimulus parameters (pulse amplitude, 20 V; pulse frequency, 10 Hz; pulse duration, 1 ms; train duration, 10 s). Twenty to thirty minutes later, the test drugs STW 5, its individual extracts or combination of extracts were applied. For pharmacology experiments, blockers were applied 20 min prior to drug application. All drugs and blockers were applied basolaterally (to serosal bathing solution) except $CFTR_{inh}$-172, SITS and amiloride which were applied apically (to mucosal bathing solution). After the response of the test drug, the transepithelial resistance was again determined and the tissue was electrically stimulated.

Experimental procedure for recording secretion from T84 cells

Like for experiments with human tissue, the transepithelial resistance was determined after an initial equilibration period. Then drugs and blockers were applied using the same protocol as described above. After 45 min the electrical resistance was determined again and then 1 µM forskolin or 100 µM bethanechol was applied basolaterally at the end of each experiment.

2.3.3 Data analysis of the Ussing Chamber experiments

The analysis of the Ussing Chamber experiments was done with the following software; LabChart for Windows (*Version 7, Chart Software, AD Instruments, Spechbach, Germany*), Igor Pro for Windows (*Version 6.22A, WaveMetrics, Lake Oswego, United States*) and Sigma Plot 12.5 for Windows (*Systat Software Inc., Erkrath, Germany*).

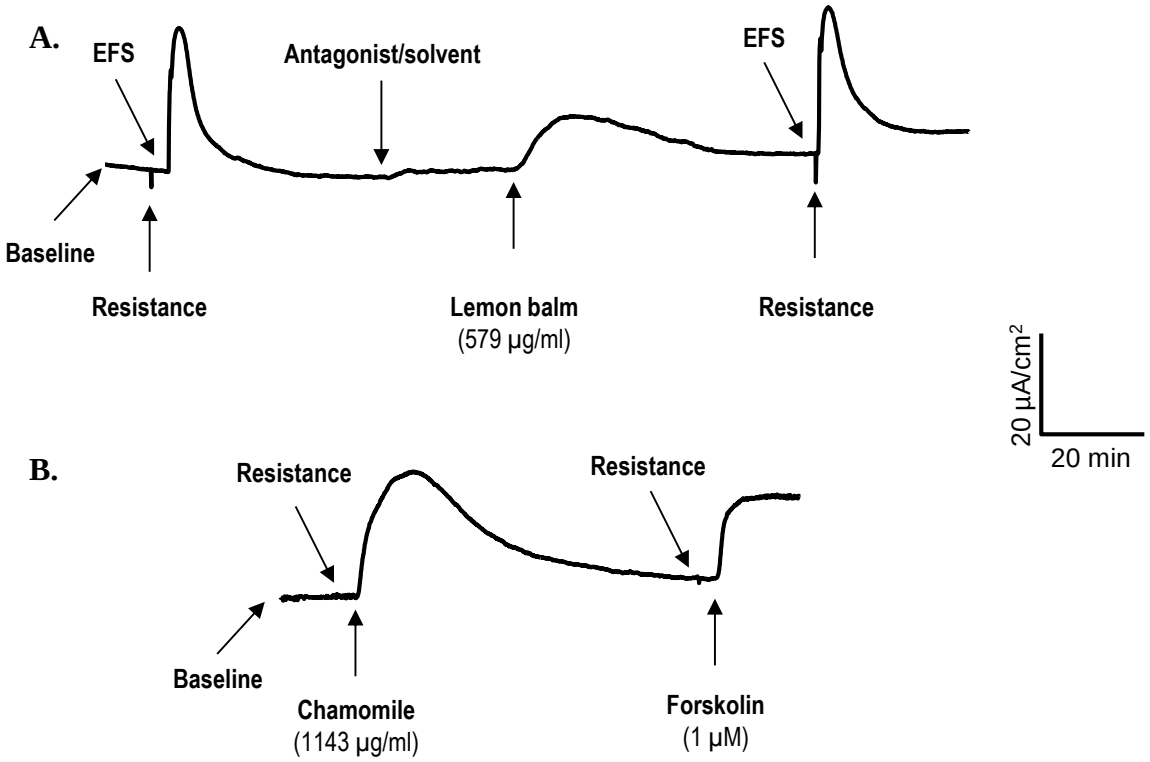

Figure 3: Illustration of the Ussing Chamber experimental procedure and definition of the analysed parameters in human specimens (A) and T84 cells (B): baseline, transepithelial resistance, electrical field stimulation (EFS) (only in A), antagonists and drugs.

Figure 3 illustrates the experimental procedure and a representative experiment in human large intestinal mucosal/submucosal preparation (A) and T84 cells (B), which was recorded by LabChart. The transepithelial resistance [Ω*cm^2], the electrical field stimulation (EFS) induced secretion [µA/cm^2] (only in human tissue) and the extract induced secretion [µA/cm^2] were determined. The absolute delta I_{SC} value was defined as difference between the baseline before stimulation/application and the maximum peak value.

The statistics are based on the number of patients/filter discs. In a paired design, each antagonist was tested in a separate tissue preparation/filter disc from the same patient/batch. Human tissue preparations with no response to EFS or less than 5 µA/cm^2 or filters with lower resistance than 100 Ω*cm^2 were discarded and excluded from analysis (8 % and 12 % respectively were excluded).

2.4 Organ bath techniques

The idea of recording various time-related events like physiological and muscular changes came up for the first time by the German physiologist Carl Ludwig in 1840s who invented the Kymograph to record blood pressure changes on a smoky drum. It was refined in 1934 by Warren to record on a long paper band (Stuart, 1891;Solberg, 1942). Recently, the multi-chamber compact organ baths have been developed offering advanced characteristics that render them suitable for the *in-vitro* study of tissue behaviour.

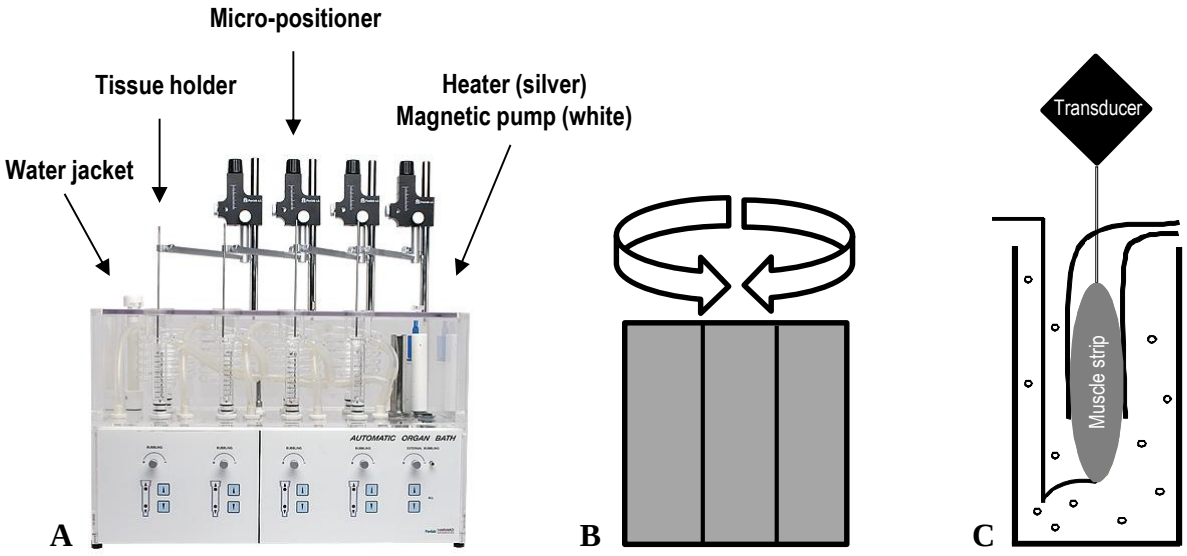

Figure 4: Illustration of Vertical Compact Automatic organ bath set up: (A) Photo of the four chambers organ bath set up. (B) 1.5 cm^2 muscle preparations cut along their circular or longitudinal axis. (C) Diagram of 25 ml vessel filled with carbogen-gassed Krebs solution in which the muscle preparations (grey) are attached to force transducers and a hook by polyamide monofilament. The tissues were placed between two platinum electrodes. Figure (A) copyright and all rights reserved to Panlab (C) shown with permission of Prof. M. Schemann.

The Vertical Compact Automatic organ bath (Panlab, AD Instruments, Spechbach, Germany) consists of four individual tissue chambers each of 25 ml volume jacketed by 8 liter distilled water (Figure 4). The water is warmed via a built in heating rod and maintained at 37 ºC by an external thermostat controller (LE 13206 thermostat, Panlab, AD Instruments, Spechbach, Germany). A highly efficient, magnetic water pump is built into the water bath to provide turbulence-free heated water circulation and ensure even heat distribution throughout the entire system.

The tissue chambers are automatically and individually filled and emptied using electro-valves. All input/output connections are accessible at the rear panel of the bath. Vessels are carbogen-bubbled by means of crystalline diffuser filters situated at the base of each; bubbling

inflow is regulated independently for each chamber by corresponding buttons. Each chamber is connected to a 60 ml pre-heating reservoir coil which is also positioned within the water bath to heat the Krebs solution before entering the tissue chamber.

Each vessel has its own self-made tissue holder, at which the tissue is fixed to a hook from one side and located between two platinum electrodes whereas the other side of the tissue is attached to the force transducer. Micro-positioners allow for fine adjustment of the tension applied to the tissue by the transducers.

2.4.1 Principle of organ bath to record muscle strip contractile activity

The isometric tension transducer method enables recording of contractions from isolated muscle strip preparations. The dissected muscle strip is attached by a polyamide thread (*Gütermann, Italy*) to a force transducer (*Force - Displacement Transducers FT03C, Grass Technology, Middleton, USA*) which converts the force generated by the muscle into an electrical signal that can then be amplified by signal amplifier for bridge-system transducer and detected on a computer based data acquisition system via AD-converter (*PowerLab, AD Instruments*) and the LabChart Software.

The force transducer makes an isometric measurement that measures the change in force (milliNewtons [mN]) produced by muscle relaxation or contraction while the muscle length remains constant.

2.4.2 Experimental procedure for recording motility

Before starting the experiments, each channel (organ bath chamber) was calibrated independently. To calibrate each isometric tension transducer, we used a two point calibration method from the LabChart software: The output of the unloaded transducer was set to 0mN and the output with a calibration weight of 2 grams was set to 20 mN.

A dissected muscle strip cut along its longitudinal or circular axis was tightly tied with double surgical knots from both ends by polyamide thread. One end was fixed to the hook of the holder and the other was attached to the force transducer. After mounting the tissue, it was carefully checked that it did not touch the holder or the bath walls and was in line with the transducer. The holder containing the tissue was then immersed in the tissue chamber filled with 25 ml carbogen-gassed pre-warmed Krebs solution. Pre-tension of mounted muscle strips was adjusted and then bathing Krebs was replaced with a new pre-warmed solution from the reservoir. Tissues were allowed to equilibrate for 45-60 min with 20 min wash out intervals till they had reached nearly steady tone and contraction patterns. With the multi-channel organ bath system, up to 8 muscle strips could be run in parallel.

Experimental procedure for recording intestinal motility from human tissue

Dissected muscles were cut along their longitudinal or circular axis into strips of approximately 1 cm in length and 0.5 cm in width. After mounting, the pre-tension was adjusted to 15 mN and muscle strips were allowed to equilibrate for 60-120 min with 20 min wash out intervals until reaching nearly stable baseline and contractility pattern. Then tissues were electrically stimulated by two platinum electrodes placed on either side. Twenty to thirty minutes later, carbachol in concentrations of 0.05 nM – 1 µM or bethanechol in concentrations of 0.5 µM – 10 µM was applied to increase the muscle tone to 40 mN. After reaching stable tonic and phasic contractions, different concentrations of STW 5 ranging from 64 - 5120 µg/ml or its individual extracts at concentrations corresponded to 512 and 5120 µg/ml STW 5 were applied. Sixty minutes later, a second EFS was carried out.

Due to difficulties to reach the same tone of 40 mN in all tissues with carbachol or bethanechol (see section 3.2 for discussion) another set of experiments was started with preloads of 40 mN rather than using cholinergic agonists to set the tone.

Electrical field stimulation (EFS) was performed with a constant voltage stimulator (*Hugo Sachs D7801, March-Hugstetten, Germany*) using 20 V pulse amplitude at 10 Hz for 10 s with individual pulse duration of 0.5 ms. EFS evoked nerve-mediated contractions resulted in

a biphasic contractile ON and OFF response; the former appears during EFS followed by an off-response immediately after cessation of EFS pulses (Figure 5).

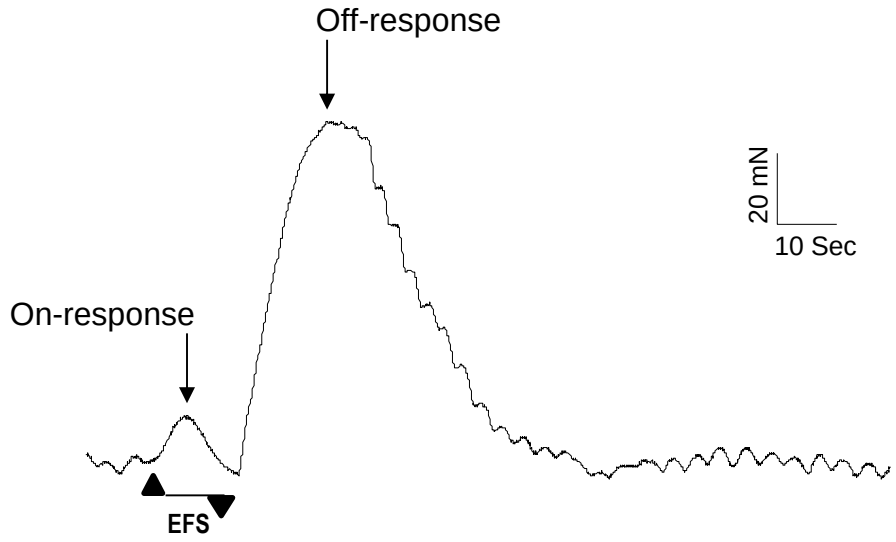

Figure 5: Electrical stimulation of muscle strips induced a biphasic response. The on-response started with the onset of the electrical pulses (shown as lines below the traces with upward and downward triangles); the off-response started after cessation of the electrical stimulation.

For pharmacology experiments, each blocker was applied 20 min before STW 5 in a different muscle strip from the same patient.

Experimental procedure for recording gastric motility from guinea pig tissue

Muscle strips of approximately 1.5 cm^2 were dissected from the parietal or visceral gastric corpus. Muscle strips were mounted in the organ bath along their longitudinal or circular axis (Figure 4, B). The pre-tension was adjusted to 15 mN, and tissues were allowed to equilibrate till nearly stable muscle tone was reached. Then STW 5 in a concentration of 256 µg/ml was applied.

Blockers were applied 20 min prior to STW 5. All experiments were performed in paired designs in which a control muscle strip from the same animal, region (corpus), side (parietal or visceral) and layer (longitudinal or circular) was run parallel to the treated muscle. Control tissues were simultaneously treated with the blockers' solvent. When needed, tissues were treated with carbachol to increase the muscle tone of either tissue to reach similar comparable tones in both muscles (control and treated).

2.4.3 Data analysis of the organ bath experiments

The graphical analyses of the organ bath experiments were performed with the programs: LabChart for Windows, Igor Pro for Windows and Sigma Plot for Windows (for more detailed description see 2.3.3).

Human tissue experiments to record intestinal motility

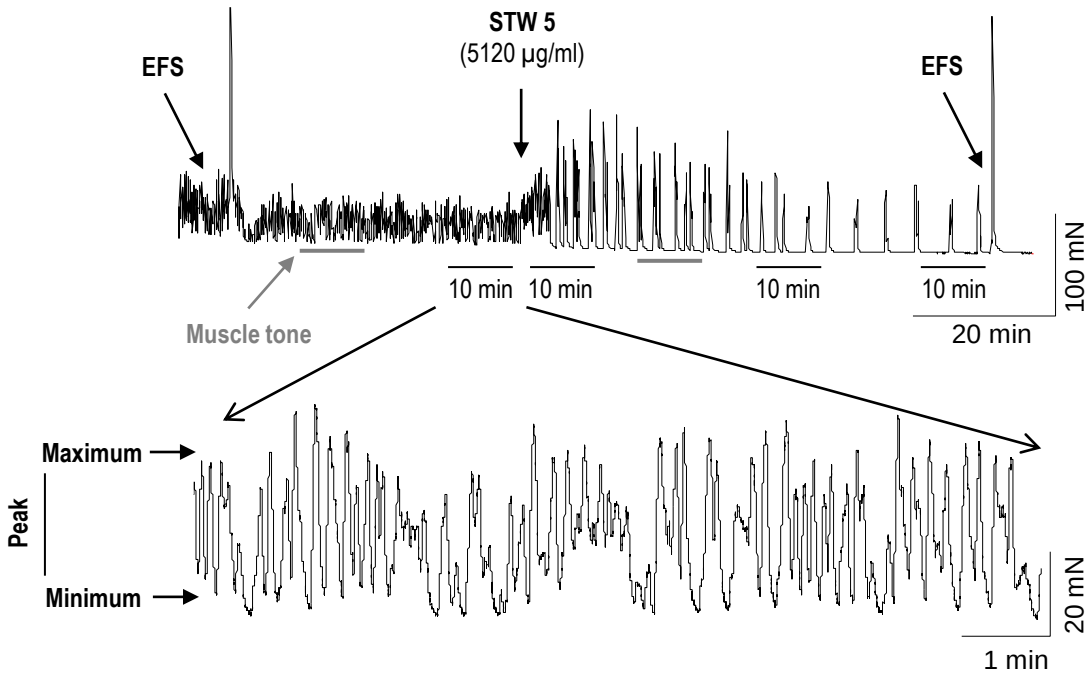

Figure 6: Illustration of the organ bath experimental procedure and definition of the analysed parameters in human large intestinal circular muscle: muscle tone (mean minimum, marked with dark grey lines), contractile amplitude (mean maximum-mean minimum), frequency of contractions (peak per minute) and motility index (contractile amplitude * contractile frequency) and drugs. Four time intervals (each of 10 minutes) were analysed, immediately before and after drug application, after 30 min and at the end of recording time 50 min.

Figure 6 illustrates the experimental procedure and a representative experiment with 5120 µg/ml STW 5 in human large intestinal circular muscle which was recorded by LabChart. The electrical field stimulation (EFS-) evoked contraction and STW 5-/extract-induced changes in tonic and phasic contractions were determined. Four periods were analysed; just before drug application, immediately after as well as 30 min and 50 min after drug application. Each interval was 10 min long. The measured parameters included minimum value (muscle tone), amplitude of contractions (maximum - minimum), frequency

of contractions (peak per minute) and motility index (contractile amplitude * contractile frequency). Values of the four parameters were calculated as mean values during the 10 min period. The absolute delta values of on and off responses were defined as the difference between the baseline before stimulation and the maximum peaks during and after 10 seconds, respectively.

Peak detection was performed automatically using a specific function in Labchart: Cyclic measurements detection settings which allowed detection of small peaks and excluded noise-like peaks.

The statistics are based on the number of patients. Each extract or antagonist was tested in separate preparations from the same patient. Tissues with no response to EFS or those that showed no spontaneous activities were excluded from the statistical analysis (5%).

Guinea pig experiments to record gastric motility

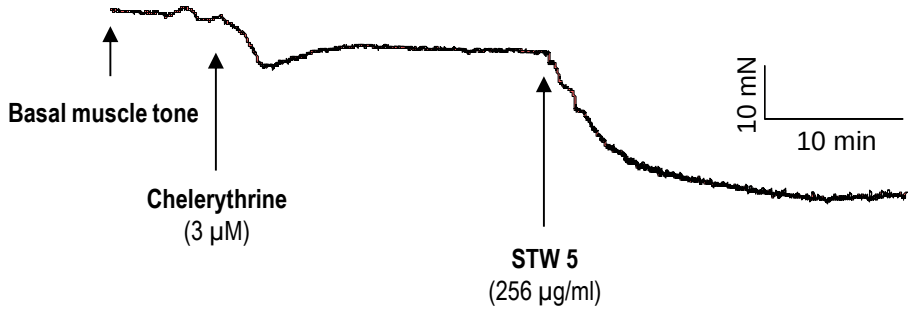

Figure 7: Illustration of the organ bath experimental procedure of longitudinal muscle strip isolated from guinea pig stomach corpus indicating basal muscle tone, chelerythrine and STW 5.

Figure 7 illustrates the experimental procedure and a representative experiment of guinea pig gastric corpus longitudinal muscle strip pre-treated with the protein kinase C inhibitor chelerythrine chloride (3 µM) followed by STW 5 (256 µg/ml) application.

Changes in muscle tone were defined as absolute delta value (mN) representing the difference between the average baseline tone before and the peak muscle tone after application of a drug. Statistics are based on the number of animals. Each agonist or antagonist was tested in a separate tissue preparation from the same animal.

2.5 Statistical analyses of the experiments

In all experiments for recording secretion and motility, the statistical analyses were performed with Sigma Plot 12.5 (*Systat Software Inc., Erkrath, Germany*). According to the design of the

study and the distribution of the data, normally distributed data were expressed as mean ± SEM (standard error of mean) and statistically analysed by using one-sample *t*-test, paired *t*-Test or one way Repeated Measures Analysis of Variance (one way RM ANOVA) followed by Bonferroni's *post-hoc* test. Non-normally distributed data were expressed as median with the 25^{th} and 75^{th} percentiles given in brackets and statistically analysed by one-sample Signed Rank Test, Mann-Whitney Rank Sum Test, Wilcoxon Signed Rank Test or RM ANOVA on Ranks followed by Dunn's *post-hoc* test.

In the following sections, unless otherwise stated, all data and figures are presented as mean ± SEM. The number of tissue preparations (equal to number of patients/animals) or number of T84 cell filters is referred to as "n".

2.6 Drugs and Solutions

Unless otherwise stated, all chemicals were purchased from *Sigma-Aldrich, Schnelldorf, Germany*.

2.6.1 Solutions

Krebs solution for mounting and storage of samples

Krebs solution (pH 7.4, 4 °C, 293 mosmol/L) was used for mounting of tissue preparations and their storage over night at 4 °C. The Krebs solution contained (mM/L): $MgCl_2.6H_2O$, 1.2; $CaCl_2.2H_2O$, 2.5; NaH_2PO_4, 1.2; NaCl, 117; $NaHCO_3$, 25; glucose, 11; KCl, 4.7. Only tissue culture tested substances were used. The pH was adjusted by carbogen-bubbling for approximately 15 min.

Krebs solution for organ bath and Ussing chamber experiments

A Krebs solution different from the one above (pH 7.4, 37 °C, 293 mosmol/L) was used for the Ussing chamber and organ bath experiments. It had the following composition in mM/L: $MgCl_2.6H_20$, 1.2; $CaCl_2.2H_2O$, 2.5; NaH_2PO_4, 1.2; NaCl, 117; $NaHCO_3$, 20; glucose, 11; KCl, 4.7. Only tissue culture tested substances were used. The pH was adjusted by carbogen-bubbling for approximately 15 min.

Hepes-Solution for Human-Tissues

Hepes solution (pH 7.4, 37 °C, 293 mosmol/l) used for the Ussing Chamber experiments had the following composition (mM/litre): $MgCl_2.6H_20$, 1.0; $CaCl_2.2H_2O$, 1.25; NaH_2PO_4, 1.2; NaCl, 135.4; Hepes, 3; glucose, 12.2; KCl, 2. Only tissue culture tested substances were used. The pH was adjusted by carefully adding 1 M NaOH. During these experiments, only air-bubbling was used.

STW 5

STW 5 and its nine individual components were kindly provided as lyophilisates by *Steigerwald Arzneimittelwerk GmbH (Darmstadt, Germany*). The lyophilisates were dissolved in Krebs solution, stored at 4 °C and used for maximally one week. For the motility studies of guinea pig gastric corpus muscle strips STW 5 was applied at a final concentration of 256 µg/ml. For motility experiments in human muscle strips, STW 5 was applied in concentrations of 64, 128, 256, 512, 768, 1024 and 5120 µg/ml. For studying its effect on secretion STW 5 was serosally (basolaterally) applied at concentrations of 512 and 5120 µg/ml in the Ussing Chamber.

Individual extracts of STW 5

The nine individual extracts of STW 5 were tested at concentrations corresponding to their concentrations in 512 or 5120 µg/ml STW 5. Table 1 shows the percentage of each extract in 100 ml STW 5 (Iberogast®) and its concentration corresponding to 512µg/ml STW 5.

Table 1: Concentrations of the nine individual extracts in STW 5

Name	**Code**	**Latin name**	**% in 100 ml Iberogast**	**Concentrations in 512 µg/ml STW 5**
Bitter candytuft	STW 6	Iberis amara	15	27.3 µg/ml
Peppermint	STW 5-K II	Menthae piperitae folium	5	37.2 µg/ml
Chamomile	STW 5-K III	Matricariae flos	20	114.3 µg/ml
Liquorice	STW 5-K IV	Liquiritiae radix	10	80.1 µg/ml
Angelica	STW 5-K V	Angelicae radix	10	89.5 µg/ml
Caraway	STW 5-K VI	Carvi fructus	10	28.4 µg/ml
Milk thistle	STW 5-K VII	Cardui mariae fructus	10	14.4 µg/ml
Lemon balm	STW 5-K VIII	Melissae folium	10	57.9 µg/ml
Greater celandine	STW 5-K IX	Chelidonii herba	10	62.9 µg/ml
Iberogast®	***STW 5***		***100%***	***512 µg/ml***

2.6.2 Drugs used in Ussing Chamber experiments

Tetrodotoxin (TTX)

The neural blocker tetrodotoxin (TTX; *Biozol Diagnostica, Eching, Germany*) was dissolved in distilled H_20 and stored as a 1 mM stock solution at -20 °C. TTX (1 µM) was applied basolaterally to the mucosal/submucosal preparations. TTX is a fast sodium channel blocker.

Forskolin

Forskolin was dissolved in dimethyl sulphoxide (DMSO) and stored as 10 mM stock solution at -20 °C. 1 µM forskolin was applied basolaterally to T84 cells. The bicyclic diterpenoide forskolin (7β-Acetoxy-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one) stimulates the adenylate cyclase, which induces the formation of cAMP and subsequently activates the cAMP-dependent Cl^--secretion (Seamon & Daly, 1981;de Souza *et al.*, 1983;Seamon, 1984).

Amiloride

The epithelial sodium channel (ENaC) blocker Amiloride was dissolved in DMSO. Stock solution of 1 mM was stored at -20 °C and applied apically in the Ussing Chamber at a final concentration of 10 µM.

4-Acetamido-4-isothiocyanato-2,2-stilbenedisulfonic acid disodium salt hydrate (SITS)

The stock solution of 1 M SITS was dissolved in DMSO after sonication and kept at room temperature. SITS was applied apically at a final concentration of 1 mM. SITS is calcium-activated chloride channel blocker.

***cis*-N-(2-Phenylcyclopentyl)-azacyclotridec-1-en-2-amine hydrochloride (MDL-12,330A hydrochloride)**

The adenylyl cyclase inhibitor MDL-12,330A was dissolved in DMSO. Stock solution of 100 mM was kept at 4 °C and basolaterally applied at a final concentration of 10 µM.

5-[(4-Carboxyphenyl)methylene]-2-thioxo-3-[(3-trifluoromethyl)phenyl-4-thiazolidinone ($CFTR_{inh}$-172)

The stock solution of $CFTR_{inh}$-172 was prepared in DMSO and stored at -20 °C. $CFTR_{inh}$-172 is applied apically at a concentration of 20 µM and acts as a selective inhibitor for cystic fibrosis transmembrane conductance regulator which is a cAMP dependent Cl^- channel.

2.5.3 Drugs used in muscle strip organ bath experiments

Adenosine

Adenosine activates the G protein- coupled adenosine receptor. It was freshly prepared in Krebs solution and applied at a final concentration of 10 µM.

Alloxazine

The selective A2B adenosine receptor antagonist (alloxazin) was dissolved in DMSO and stored at -20 °C as 10 mM stock solution. Alloxazin was applied at a concentration of 1 µM.

3,7-Dimethyl-1-propargylxanthine (DMPX)

DMPX is a selective A2A adenosine receptor antagonist and dissolved in DMSO. Stock solution of 100 mM was stored at -20 °C and applied at a final concentration of 1 µM.

8-Cyclopentyl-1,3-dipropylxanthine (DPCPX)

The selective A1 adenosine receptor antagonist DPCPX (*Tocris Bioscience, Bristol, UK*) was dissolved in DMSO. Stock solution of 1 mM was stored at -20 °C and applied at a final concentration of 0.1 µM.

Piroxicam

Piroxicam is a non-selective cyclooxygenase (COX) inhibitor. Stock solution of 100 mM was dissolved in chloroform and applied in a final concentration of 10 µM.

Niflumic acid

Stock solution of niflumic acid 100 mM was dissolved in DMSO, stored at -20 °C and applied at a final concentration of 10 µM. Niflumic acid is cyclooxygenase-2 (COX-2) inhibitor as well as calcium activated chloride channel blocker.

4-(4-Chlorophenyl)-3-methylbut-3-en-2-oxime (AP-18)

The novel selective transient receptor potential ankyrin 1 (TRPA1) receptors blocker AP-18 was dissolved in DMSO. Stock solution of 100 mM was stored at -20 °C and applied at a final concentration of 10 µM.

(2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4 isopropyl-phenyl) acetamide) (HC-030031)

HC-030031 was dissolved in DMSO, stored at -20 °C and applied at a final concentration of 10 µM. HC-030031 is a selective TRPA1 channel blocker.

Cinnamaldehyde (CNA)

The stock solution of CNA was prepared in DMSO at a concentration of 100 mM and applied at a final concentration of 100 µM. CNA was freshly prepared.

Ethyl-1-(4-(2,3,3-trichloroacrylamide)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate (Pyr3)

The stock solution of Pyr3 was dissolved in DMSO, stored as 100 mM at -20 °C and applied at a final concentration of 10 µM. Pyr3 is a pyrazole compound that potently and selectively antagonizes the transient receptor potential cation channel 3 (TRPC3).

1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl] imidazole,1-[β-(3-(4 Methoxyphenyl)propoxy)-4-methoxyphenethyl]-1H-imidazole hydrochloride (SKF-96365)

SKF-96365 (*Biomol, Hamburg, Germany*) was dissolved in deionised bi-distilled water. Stock solution was prepared as 1 mM, stored at -20 °C and applied at a final concentration of 10 µM. SKF-96365 is an inhibitor for receptor-mediated Ca^{2+} entry (ROCs) and TRPC channel.

Nifedipine

The selective L-type calcium channel blocker nifedipine is light sensitive so it was dissolved in absolute ethyl alcohol in a tinted eppendorf cup. The stock solution of 10 mM was stored at 4 °C for maximum one week and applied at a final concentration of 1 µM.

4S-1,4-Dihydro-2,6-dimethyl-5-nitro-4-[2-trifluoromethyl)phenyl]-3 pyridine carboxylic acid methyl ester ((S)-(-)-Bay k 8644)

The selective L-type calcium channel activator (S)-(-)-Bay k 8644 (*Tocris Bioscience, Bristol, UK*) was dissolved in DMSO. The stock solution of 10 mM was stored maximally for 1 month at -20 °C and applied at a final concentration of 1 µM.

Mibefradil

Mibefradil dihydrochloride hydrate is a selective T-type calcium channel blocker. Stock solution of 100 mM was dissolved in deionised bi-distilled water, stored at -20 °C and applied at a final concentration of 0.1 µM.

Phorbol 12-myristate 13-acetate (PMA)

The potent protein kinase C activator; PMA was dissolved in DMSO and stored as 100 mM stock solution in dark at -20 °C. PMA was applied at a final concentration of 10 µM.

1,2-Dimethoxy-N-methyl(1,3)benzodioxolo(5,6-c)phenanthridinium chloride (Chelerythrine chloride)

Chelerythrine chloride was dissolved in deionised bi-distilled water. The stock solution of 10 mM was stored at -20 °C and applied at a final concentration of 3 µM. Chelerythrine chloride is a protein kinase C inhibitor.

N-{4-[3,5-bis(Trifluoromethyl)-1H-pyrazol-1-yl]phenyl}-4-methyl-1,2,3-thiadiazole-5-carboxamide (BTP2)

The stock solution of BTP2 (*Calbiochem, Merck KGaA, Darmstadt, Germany*) was dissolved in 100% DMSO, stored as 100 mM at -20 °C and applied in a final concentration of 1 µM. BTP2 is a potent blocker for CRAC (Ca^{2+} release-activated Ca^{2+}) channel-mediated SOCE (store-operated Ca^{2+} entry).

Lanthanum trichloride

The calcium channel blocker Lanthanum (III) chloride ($LaCl_3$) was dissolved in deionised bi-distilled water, stored as 100 mM in 2-8 °C. $LaCl_3$ was applied in a concentration of 200 µM.

Ryanodine

The stock solution of ryanodine (*Alomone labs, ICS; International Clinical Service GmbH, Munich, Germany*) was dissolved in DMSO, stored as 50 mM stock solution at -20 °C and applied at a concentration of 10 µM. At this concentration it acts as a ryanodine receptor antagonist which inhibits calcium release from sarcoplasmic reticulum.

Ruthenium red

The ryanodine receptor antagonist ruthenium red was dissolved in deionised bi-distilled water, stored as 100 mM stock solution at -20 °C and applied at a final concentration of 10 µM.

2-Aminoethyl diphenyl borinate (2-APB)

2-APB is an IP_3-receptor blocker and also a non-selective TRP channel blocker (primarily TRPC). It was prepared in DMSO and stored as 300 mM stock solution at -20 °C. 2-APB was applied at a final concentration of 30 µM.

Thapsigargin

The stock solution of thapsigargin (*Alomone labs, ICS; International Clinical Service GmbH, Munich, Germany*) was dissolved in 100% DMSO, stored at -20 °C as 1 mM stock solution and applied in a final concentration of 0.3 µM. Thapsigargin is a selective inhibitor for the sarcoplasmic/endoplasmic reticulum calcium ATPase pump (SERCA).

Caffeine

The stock solution of caffeine was dissolved in deionised bi-distilled water, stored at concentration of 1 M at room temperature. Caffeine was applied in a concentration of 1 mM. Caffeine modulates the intracellular calcium concentration via several mechanisms as discussed later.

N-[2-[N-(4-Chlorocinnamyl)-N-methylaminomethyl]phenyl]-N-(2-hydroxyethyl)-4-methoxy benzene sulfon-amide phosphate salt, N-[2-[[[3-(4′-Chlorophenyl)-2-propenyl] methyl-amino] methyl] phenyl]-N-(2-hydroxyethyl)-4′-methoxy-benzenesulfonamide phosphate salt (KN-93)

The selective Ca^{2+}/calmodulin-dependent protein kinase II inhibitor KN-93 was dissolved in deionised bi-distilled water. Stock solution of 10 mM was stored at -20 °C and applied at a final concentration of 10 µM.

Dihydrochloride (N-[2-(p-Bromocinnamyl) amino)ethyl]-5-isoquinoline sulfonamide. dihydrochloride (H -89)

The potent protein kinase A (PKA) inhibitor H-89, (*Calbiochem, Merck KGaA, Darmstadt, Germany*) was dissolved in deionised bi-distilled water. Stock solution of 10 mM was stored at 2-8 °C and applied in a final concentration of 10 µM.

Methylene blue

Methylene blue was dissolved in phosphate buffered saline with sonication. Stock solution of 0.5 mM was stored at 4 °C and applied in a final concentration of 50 µM. Methylene blue is a protein kinase G (PKG) inhibitor.

Piboserod

The stock solution of the 5-HT4 receptor antagonist Piboserod (*Ref. Number: SB207266A, GlaxoSmithKline, Harlow, UK*) was prepared at 10 mM in Krebs and stored in aliquots at 4 °C for a maximum of one week. Piboserod was applied in a final concentration of 10 µM.

Prucalopride

Prucalopride is a novel 5-HT4 receptor agonist (*Ref. Number: GW686036A, GlaxoSmithKline, Harlow, UK*). 100 mM stock solution was prepared in Krebs solution and stored at 4 °C for one week. Working concentration was 1 µM.

Iberiotoxin

The selective inhibitor of high-conductance (BK) Ca^{+2}-activated K^+ channels iberiotoxin was dissolved in deionised bi-distilled water, stored as 1 mM stock solution at -20 °C and applied in a final concentration of 0.1 µM. Iberiotoxin occurs naturally in the venom of the scorpion *Buthus tamulus*.

Apamin

Apamin, which is isolated from bee venom, blocks ATP-type Ca^{+2}-activated K^+ channels. Stock solution of 1 mM was prepared in ultrapure water, stored at -20 °C and applied in a final concentration of 0.3 µM.

Charybdotoxin

The selective inhibitor of high-conductance (BK) and intermediate (IK1) Ca^{+2}-activated K^{+} channels charybdotoxin was dissolved in deionised bi-distilled water, stored as 1 mM stock solution at -20 °C and applied in a final concentration of 0.1 µM. Charybdotoxin is a peptide found in the venom of the scorpion *Leiurus quinquestriatus*.

Carbachol

Carbachol (Carbamoylcholine chloride) was dissolved in deionised bi-distilled water, stored in aliquots as 1 mM stock solution at -20 °C. Carbachol was used to increase the tone of some muscle strips. For this purpose it was applied gradually at concentrations ranging from 0.05 nM - 1 µM.

Bethanechol

Bethanechol (Carbamyl-ß-methylcholine chloride) was dissolved in deionised bi-distilled water, stored in aliquots as 100 mM stock solution at -20 °C and applied in a final concentrations ranging from 0.5 - 10 µM in human motility experiments and 100 µM in T84 cells. Bethanechol is a selective muscarinic receptors agonist.

Dimethyl sulphoxide (DMSO)

Dimethyl sulphoxide (DMSO) was kept at room temperature under hood. DMSO was used as solvent for some drugs.

3. Results

The aim of this study was to investigate the effects of the herbal medicine STW 5 (Iberogast®) and its individual components on human intestinal secretion and motility, the actions on guinea pig gastric corpus motility as well as to study the mechanisms of actions by which STW 5 induces changes in gut functions *in vitro*.

3.1 Mechanism of action of STW 5-induced relaxation in Guinea pig proximal stomach

In a previous study, our group reported that STW 5 relaxes the guinea pig proximal stomach (fundus and corpus) (Hohenester *et al.*, 2004). Similar findings had been shown in human proximal stomach (Pilichiewicz *et al.*, 2007). As well, as described in section 3.2, STW 5 relaxes human intestinal smooth muscle. However the mechanism(s) of action by which STW 5 induces its inhibitory effect on the muscle tone is still unknown. The aim of this part of the study was to address the mechanism(s) underlying the observed STW 5 effects.

3.1.1 Effects of STW 5 on guinea pig proximal stomach

It is most unlikely that effects of Iberogast® are due to the solvent ethanol as the amount of 300 µl in each therapeutic dose is very low. Nevertheless, STW 5 was used as an ethanol-free lyophilisate to ensure that the responses are not due to the action of ethanol on gastrointestinal motility (Wali *et al.*, 1987).

To ensure that neither isolation, extraction nor lyophilisation processes influence STW 5 action, all nine lyophilisates were combined at concentrations corresponding to their concentrations in 256 µg/ml STW 5, this mixture is referred to as sSTW 5. We confirmed that both STW 5 and sSTW 5 have comparable inhibitory effects on muscle tone (STW 5; -20.7±4.7mN vs. sSTW 5; -25.6±3.6 mN; $n = 6$, $P = 0.3$).

Based on previous dose response studies (Hohenester *et al.*, 2004;Schemann *et al.*, 2006), STW 5 was applied at a final concentration of 256 µg/ml, a concentration that evokes reliable robust inhibitory effect in the muscle tone of the guinea pig proximal stomach. In this study, circular (CM) and longitudinal (LM) muscle strips dissected from either parietal or visceral sides of guinea pig stomach corpus were used. Because no significant differences were observed between the responses from muscle strips cut from parietal versus visceral sides

(LM, $P = 0.5$ and CM, $P = 0.7$), results from both sides were pooled for the final analyses (Figure 8).

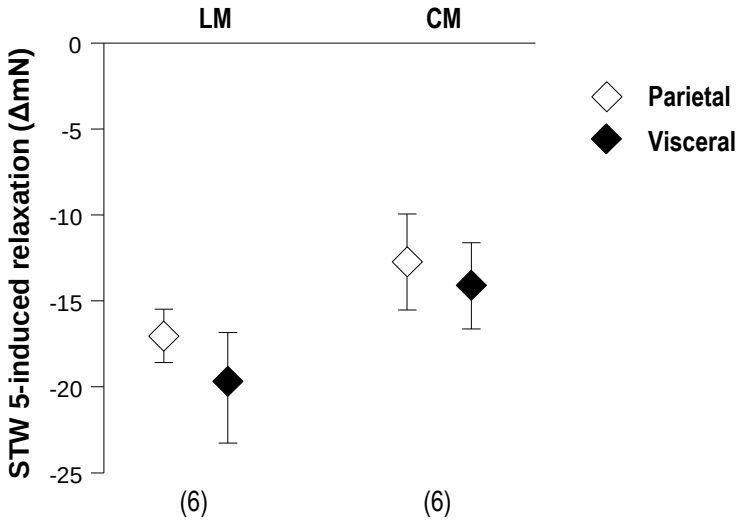

Figure 8: STW 5 induced comparable relaxations on muscle strips from parietal and visceral sides. Longitudinal (LM) and circular (CM) muscle strips of guinea pig stomach corpus dissected from either sides parietal or visceral showed comparable responses to STW 5 (256 µg/ml). Numbers in parentheses indicate the number of muscle strips (equal to number of animals) studied.

Application of STW 5 (256 µg/ml) relaxed both longitudinal and circular muscles. The percentage reduction in muscle tone was significantly higher in longitudinal than in circular muscle: 42.9 % vs. 28.4 % respectively (Figure 9). The pre-STW 5 baselines were comparable in longitudinal and circular muscle 40 [30.6/51.75] vs. 39.4 [34.4/50.1] respectively (Figure 9).

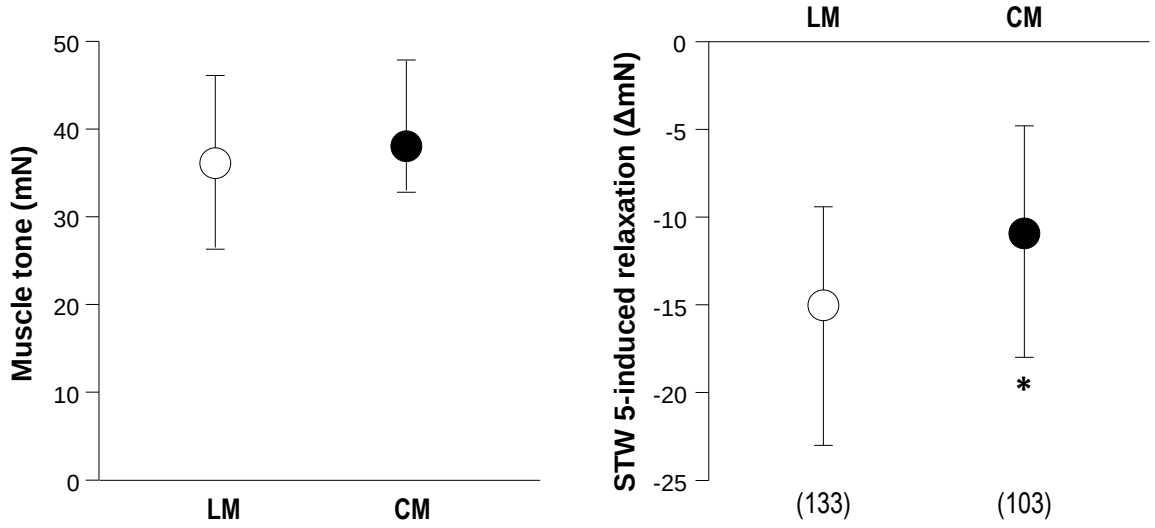

Figure 9: STW 5 evoked higher relaxation in longitudinal than circular muscles. Although starting with comparable muscle tones (left panel), STW 5 (256 µg/ml) evoked significantly higher relaxation in longitudinal (LM) vs. circular (CM) muscle strips of guinea pig gastric corpus (right panel). Numbers in parentheses indicate the number of muscle strips (equal to number of animals) studied. * marks significant difference ($P \leq 0.001$).

There was a correlation between muscle tension and the percentage reduction in tone in response to STW 5 only in circular muscle ($n = 103$, $P = 0.04$) but not in longitudinal muscle ($n = 133$, $P = 0.2$) (Figure 10).

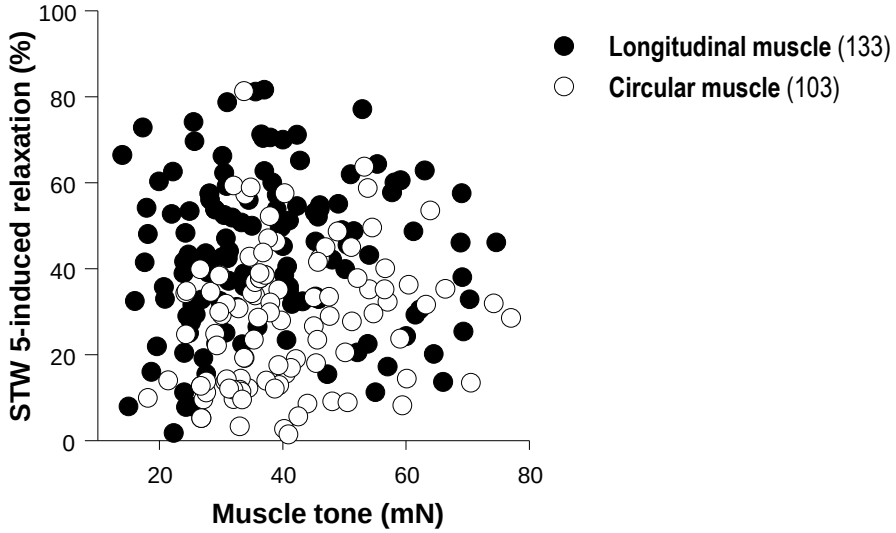

Figure 10: Correlation between STW 5-induced relaxation and pre-treatment muscle tone. Only in circular muscle there is a positive significant correlation between the basal muscle tone and the response to STW 5. Numbers in parentheses indicate the number of muscle strips (equal to number of animals) studied.

Due to this correlation, experiments were performed in paired designs to compare treated muscles strips with respective controls from the same region and the same muscle layer of the same animal. Both muscle strips (treated and control) were adjusted to have approximately

similar muscle tone. When needed, carbachol (0.05 nM) was added to increase the muscle tone. Application of carbachol had no effect on STW 5-induced relaxation (longitudinal muscle: $n = 6$, $P = 0.7$) and (circular muscle: $n = 6$, $P = 0.8$) (Figure 11).

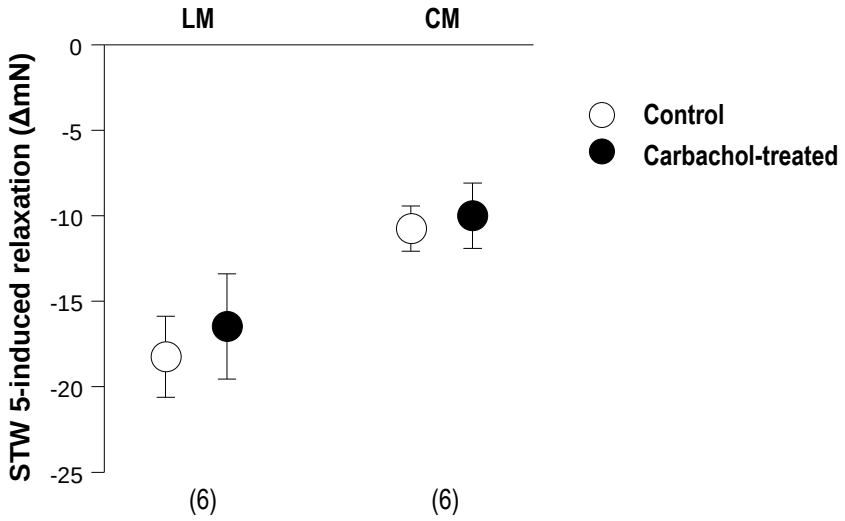

Figure 11: STW 5-induced relaxation in guinea pig proximal stomach is not influenced by carbachol. Pre-treatment of muscle strips with carbachol (0.05 nM) to increase the muscle tone did not affect its response to STW 5. Numbers in parentheses indicate the number of muscle strips (equal to number of animals) studied.

3.1.2 Pharmacology of STW 5

In 2004, Hohenester *et al*., demonstrated that STW 5-induced relaxation in guinea pig proximal stomach is neither nerve mediated nor nitric oxide dependent suggesting a direct myogenic effect of STW 5 on smooth muscle cells (Hohenester *et al.*, 2004). In various cell, tissue and animal models other groups have demonstrated effects of STW5 on a plethora of cell functions. This seemingly involved a number of different receptors and intracellular pathways. Some of these mechanisms also regulate smooth muscle activity. We aimed to test whether these pathways are implicated in the relaxation of gastrointestinal smooth muscle.

To determine the mechanism(s) underlying the observed STW 5 effects, tissues were pre-treated with several agonists and antagonists and changes in STW 5 responses were recorded, analysed and tested against respective controls. Agonists and antagonists were categorized based on their targets and mechanisms of actions.

Adenosine receptors

Adenosine (10 µM) induced significant relaxation only in longitudinal muscle by 38 % ($n = 8$, $P = 0.007$) and slightly increased circular muscle tone by 8 % ($n = 8$, $P = 0.3$). Adenosine effects are basically mediated by four different G-protein coupled receptors. This study focused on involvement of A1, A2A and A2B receptors. These adenosine A1, A2A and A2B receptors can be selectively blocked by DPCPX, DMPX and alloxazin respectively (Ukena *et al.*, 1986;Haleen *et al.*, 1987;Pilitsis & Kimelberg, 1998). None of these antagonists affected the muscle tone by itself (Table 2).

Blockade of adenosine A1 receptors by the xanthine derivative DPCPX (0.1 µM) had no influence on STW 5 inhibitory effects. The responses were non-significantly decreased by 3 % in longitudinal muscle and increased by 17 % in circular muscle (Figure 12).

Similarly, pre-treatment of muscle strips with 1 µM DMPX did not affect STW 5-induced relaxation in longitudinal and circular muscles; both responses were non-significantly increased by 13 % and 21 % respectively (Figure 12).

The relaxing effect of STW 5 was not affected by pre-treatment with the selective A2B receptor blocker alloxazin (1 µM). The effect was non-significantly reduced in longitudinal and circular muscles by 9 % and 21 % respectively (Figure 12).

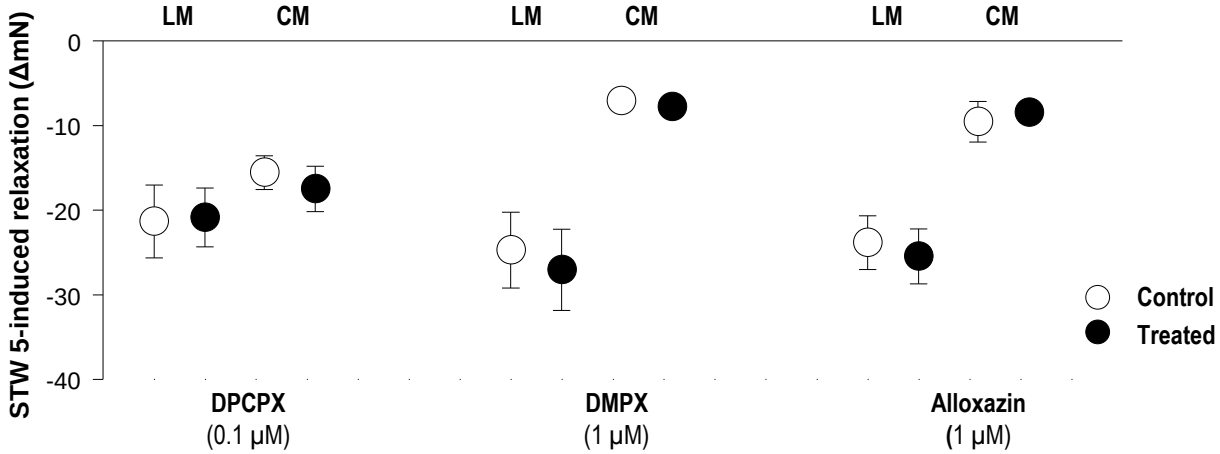

Figure 12: Blockade of adenosine receptors did not influence STW 5-induced relaxation. Pre-treatment of corpus muscle strips with selective adenosine A1 blocker DPCPX, A2A antagonist DMPX or A2B blocker alloxazin did not affect STW 5-induced relaxation in neither longitudinal (LM) nor circular muscle (CM).

Table 2: Effects of adenosine receptor blockers on STW 5-induced relaxation

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	**STW 5-induced relaxation Control vs. treated (ΔmN)**	**Effect of the blocker on muscle tone (mN)**	**STW 5-induced relaxation Control vs. treated (ΔmN)**
DPCPX (0.1 µM)	39.1±7.8 vs. 39.9±7.9	-21.3±4.3 vs. -20.9±3.5	42.5±4.1 vs. 43.4±4.5	-15.6±2.0 vs. -17.5±2.7
A1 antagonist	*n* = 4; *P* = 0.4	*n* = 4; *P* = 0.9	*n* = 4; *P* = 0.1§	*n* = 4; *P* = 0.6
DMPX (1 µM)	45.1±7.7 vs. 45.0±7.6	-24.7±4.5 vs. -27.4±4.8	37.2±3.4 vs. 38.5±2.3	-7.1±1.0 vs. -7.8±0.7
A2A antagonist	*n* = 6; *P* = 0.7	*n* = 6; *P* = 0.4	*n* = 4; *P* = 0.5	*n* = 4; *P* = 0.6
Alloxazin (1 µM)	46.7±3.2 vs. 46.1±3.6	-23.8±3.2 vs. -25.5±3.2	37.9±2.9 vs. 38.4±3.5	-9.6±2.4 vs. -8.5±1.2
A2B antagonist	*n* = 6; *P* = 0.6	*n* = 6; *P* = 0.3	*n* = 6; *P* = 0.7	*n* = 6; *P* = 0.6

(*n*) = number of muscle strips (equal to number of animals) studied. § marks non-normally distributed data.

Calcium-activated potassium channels

Ca^{2+}-sensitive K^+ channels are located on smooth muscle cell membrane and activated by an increase of the intracellular calcium level. Based on their conductance, they are divided into SK, IK and BK channels (small-, intermediate- and big-conductance respectively) and play an important role in smooth muscle contraction and relaxation (Ledoux *et al.*, 2006;Kim *et al.*, 2009b). SK and BK channels can be blocked by apamin and iberiotoxin respectively whereas charybdotoxin antagonised both IK and BK channels (Galvez *et al.*, 1990;Doughty *et al.*, 1999;Hsieh *et al.*, 2013). Again, none of the blockers influenced the basal muscle tone by itself (Table 3).

Pre-treatment with 0.3 µM apamin non-significantly decreased the relaxing effect of STW 5 by 9 % in longitudinal muscle and 14 % in circular muscle (Figure 13 and Table 3).

The results were similar with Iberiotoxin (0.1 µM) where STW 5 effects were non-significantly reduced by 4 % and 8 % in longitudinal and circular muscle respectively (Figure 13 and Table 3).

As well, charybdotoxin (0.1 µM) non-significantly decreased the relaxing effect of STW 5 in longitudinal and circular muscle by 7 % and 17 % respectively (Figure 13 and Table 3).

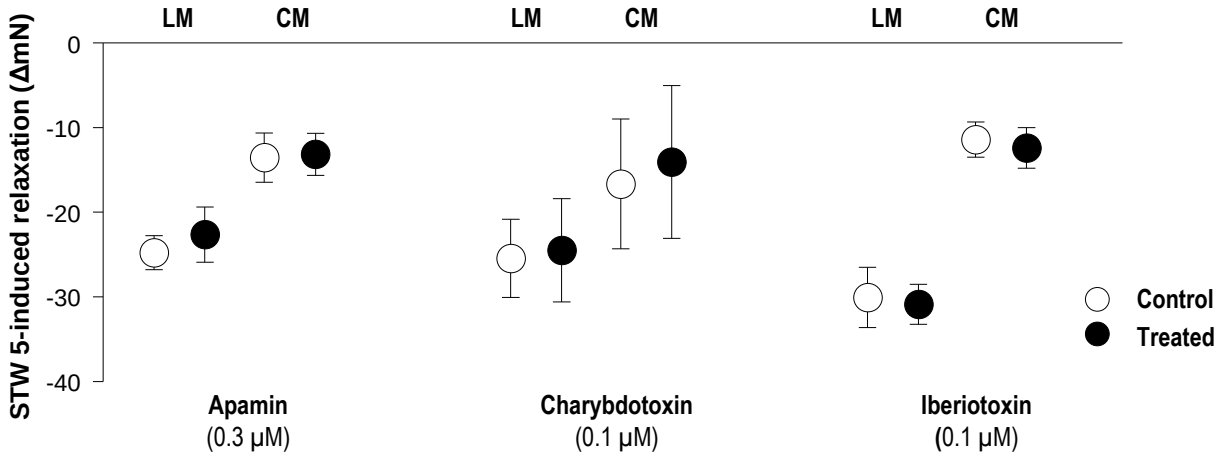

Figure 13: Calcium-activated potassium channels are not involved in the mechanism STW 5-induced relaxation. Pre-treatment of muscle strips with apamin: selective SK channel blocker, charybdotoxin: IK and BK channel antagonist or iberiotoxin: selective IK channel antagonist did not influence STW 5-inhibitory effect neither in longitudinal muscle (LM) nor in circular muscle (CM).

Table 3: Effects of calcium-activated potassium channel blockers on STW 5-induced relaxation

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	STW 5-induced relaxation Control vs. treated (ΔmN)	Effect of the blocker on muscle tone (mN)	STW 5-induced relaxation Control vs. treated (ΔmN)
Apamin (0.3 µM)	36.6±4.1 vs. 38.9±5.8	-24.8±2.0 vs. -22.7±3.3	51.5±2.7 vs. 51.2±2.6	-13.5±2.9 vs. -13.2±2.5
SK channel antagonist	*n* = 6; *P* = 0.3	*n* = 6; *P* = 0.4	*n* = 7; *P* = 0.1	*n* = 7; *P* = 0.9
Charybdotoxin (0.1 µM)	44.9±5.9 vs. 47.6±7.5	-25.5±4.6 vs. -24.5±6.1	42.1±4.0 vs. 42.1±3.9	-16.7±7.7 vs. -14.1±9.0
IK&BK channel antagonist	*n* = 4; *P* = 0.2	*n* = 4; *P* = 0.6	*n* = 3; *P* = 1.0	*n* = 3; *P* = 0.5
Iberiotoxin (0.1 µM)	40.1±3.2 vs. 42.2±4.4	-30.1±3.6 vs. -30.9±2.4	46.5±8.8 vs. 46.1±8.9	-11.4±2.1 vs. -12.4±2.4
BK channel antagonist	*n* = 3; *P* = 0.2	*n* = 3; *P* = 0.8	*n* = 3; *P* = 0.5	*n* = 3; *P* = 0.3

(n) = number of muscle strips (equal to number of animals) studied.

Calcium-activated chloride channels (CaCCs)

Application of the calcium-activated chloride channel blocker niflumic acid (10 µM) relaxed longitudinal and circular muscle tone (Table 4). Niflumic acid did not affect the relaxation evoked by STW 5. The effect was non-significantly increased by 14 % and 10 % in longitudinal and circular muscle respectively (Figure 14 and Table 4). In addition to calcium-

activated chloride channel blockade, niflumic acid is also a COX II inhibitor (Pacaud *et al.*, 1989;Hashitani *et al.*, 2005).

Cyclooxygenase (COX)

Prostaglandins play an important role in smooth muscle relaxation. To elucidate the contribution of this pathway in STW 5-induced relaxation, cyclooxygenase inhibitors were used. Piroxicam is a non-selective COX inhibitor (Reeves & Stables, 1985). Application of piroxicam (10 µM) did not influence STW 5 effects on smooth muscle. The effect was decreased in longitudinal muscle by 14 % and increased in circular muscle by 10 % (Figure 14 and Table 4). Piroxicam itself relaxed both longitudinal and circular muscle tone (Table 4).

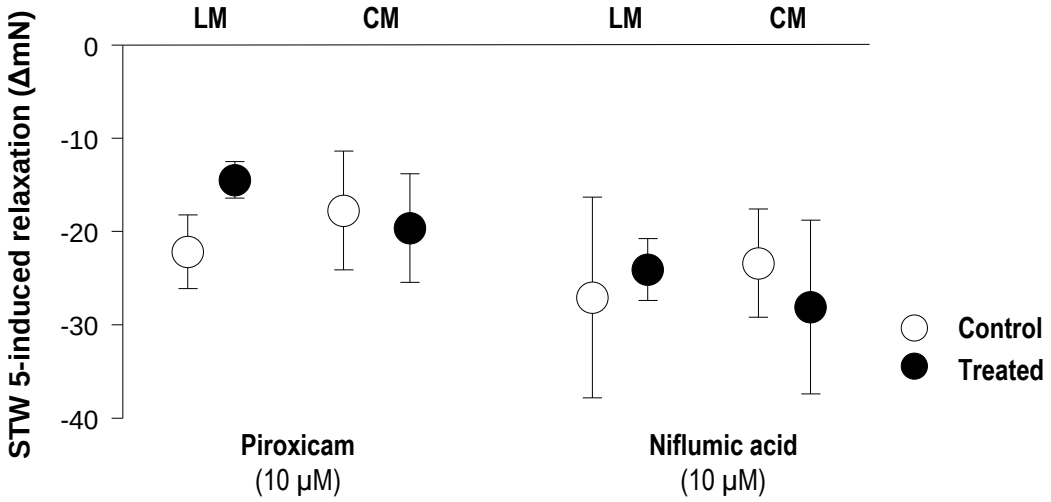

Figure 14: Calcium-activated chloride channels and COX pathways are not involved in the mechanism STW 5-induced relaxation. Pre-incubation of longitudinal (LM) or circular muscle (CM) strips with piroxicam (COX inhibitor) or niflumic acid (COXII inhibitor/CaCCs antagonist) did not affect the inhibitory effect of STW 5.

Table 4: Effects of piroxicam and niflumic acid on STW 5-induced relaxation.

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	**STW 5-induced relaxation Control vs. treated (ΔmN)**	**Effect of the blocker on muscle tone (mN)**	**STW 5-induced relaxation Control vs. treated (ΔmN)**
Niflumic acid (10 µM)	**60.0±8.1 vs. 10.9±2.1***	-27.1±10.8 vs. -24.1±9.3	**45.2±2.9 vs. 26.4±4.2***	-23.4±5.8 vs. -28.1±9.3
CaCCs & COXII inhibitor	***n* = 5; *P* = 0.005***	*n* = 5; *P* = 0.8	***n* = 5; *P* = 0.03***	*n* = 5; *P* = 0.5
Piroxicam (10 µM)	**52.0±6.6 vs. 24.2±3.8***	-22.4±3.5 vs. -15.9±3.1	**37.9±3.5 vs. 20.5±5.5***	-17.8±6.4 vs. -19.6±5.8
COX inhibitor	***n* = 9; *P* = 0.001***	*n* = 9; *P* = 0.1	***n* = 5; *P* = 0.04***	*n* = 5; *P* = 0.8

(n) = number of muscle strips (equal to number of animals) studied. * mark significant differences.

Serotonin 5-HT_4 receptors

Prucalopride and piboserod are selective serotonin 5-HT_4 agonists and antagonists, respectively (Prins *et al.*, 2001;McCullough *et al.*, 2006). Neither prucalopride (1 µM) nor piboserod (1 µM) had any effect on either longitudinal or circular basal muscle tone by themselves (Table 5). Piboserod non-significantly increased the effect of STW 5 in longitudinal muscle by 11 % whilst decreased it in circular muscle by 14 % (Figure 15 and Table 5).

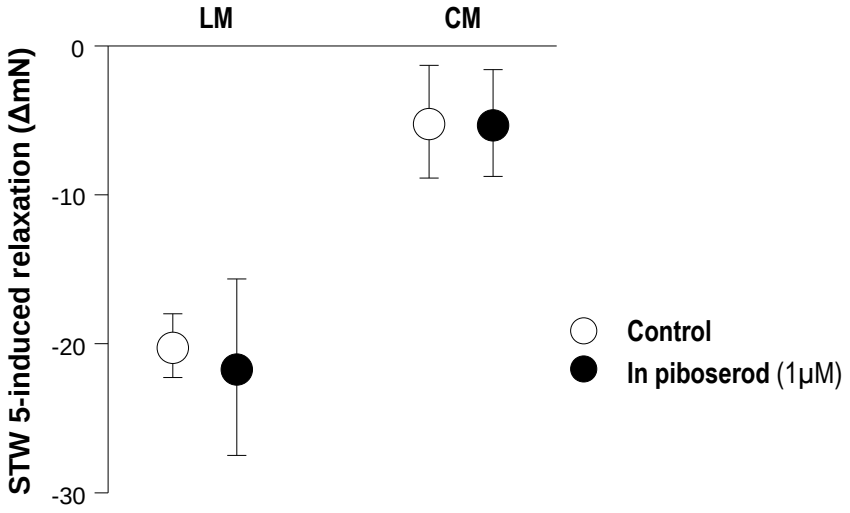

Figure 15: Selective blockade of serotonin 5-HT_4 receptors by piboserod did not affect STW 5-induced relaxation in guinea pig corpus stomach in both longitudinal (LM) and circular muscle strips (CM).

Table 5: Effects of serotonin 5-HT4 receptors on STW 5-induced relaxation

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	STW 5-induced relaxation Control vs. treated (ΔmN)	Effect of the blocker on muscle tone (mN)	STW 5-induced relaxation Control vs. treated (ΔmN)
Piboserod (1 µM)	45.0±4.9 vs. 47.9±5.0	-20.1±2.1 vs. -21.6±5.9	35.4±2.9 vs. 37.1±3.3	-5.1±3.8 vs. -5.2±3.6
5-HT_4 antagonist	*n* = 7; *P* = 0.1	*n* = 7; *P* = 0.8	*n* = 6; *P* = 0.1	*n* = 6; *P* = 0.9
Prucalopride (1 µM)	25.9±2.2 vs. 25.3±2.2		35.4±2.9 vs. 37.1±3.3	
5-HT_4 agonist	*n* = 6; *P* = 0.5		*n* = 6; *P* = 0.1	

(n) = number of muscle strips (equal to number of animals) studied.

Protein kinases

Among the intracellular signalling cascades, protein kinases such as protein kinase A (PKA) and protein kinas G (PKG) contribute to smooth muscle relaxation (Makhlouf & Murthy, 1997). H-89 is a selective PKA inhibitor (Satake *et al.*, 1996). H-89 (10 µM) only relaxed longitudinal muscle tone by 42% without affecting circular muscle tone (Table 6). Pre-treatment of muscle strips with H-89 (10 µM) did not affect STW 5-induced relaxation in smooth muscle. STW 5 responses were non-significantly increased by 3 % and 17 % in longitudinal and circular muscle respectively (Figure 16 and Table 6).

On the other hand, application of methylene blue (50 µM) had no effect on basal muscle tone (Table 6). Methylene blue is a PKG and guanylyl cyclase inhibitor that consequently inhibits cGMP which is important for regulation of smooth muscle relaxation (Satake *et al.*, 1996). Application of 50 µM methylene blue prior to STW 5 did not affect its inhibitory action. The relaxing effect of STW 5 was reduced in longitudinal muscle by 10 % whereas increased in circular muscle by 18 % (Figure 16 and Table 6).

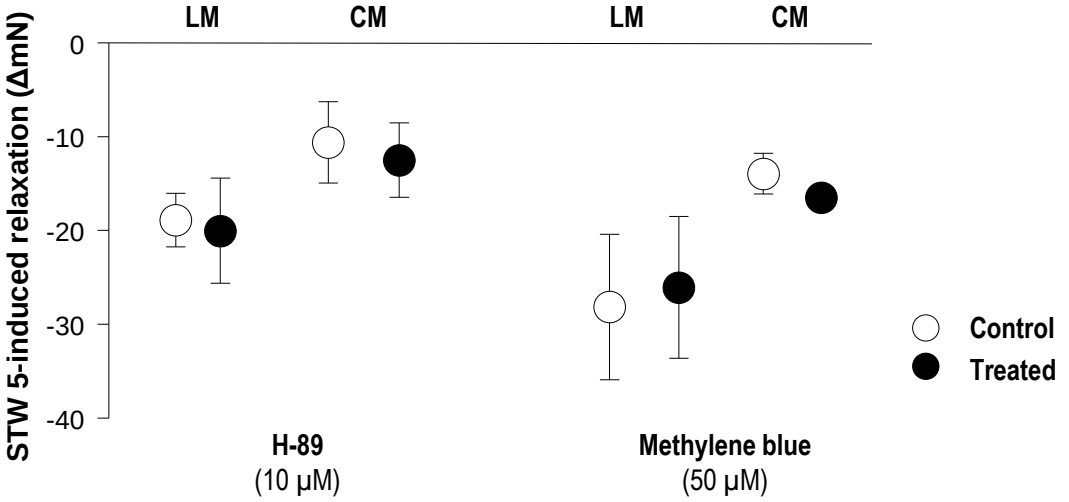

Figure 16: STW 5-induced relaxations are resistant to H-89 and methylene blue. Pre-treatment of longitudinal (LM) and circular muscle (CM) strips with PKA inhibitor H-89 or PKG inhibitor methylene blue did not affect STW 5-induced relaxation.

Calmodulin kinase II

KN-93 is a selective calmodulin kinase II inhibitor (Kim *et al.*, 2005b). Treatment of the muscle strips with KN-93 (10 µM) relaxed only longitudinal muscle tone (Table 6). Pre-incubation of KN-93 had no influence on STW 5-induced relaxation. The inhibitory effects were increased in both longitudinal and circular muscle by 6 % (Figure 17 and Table 6).

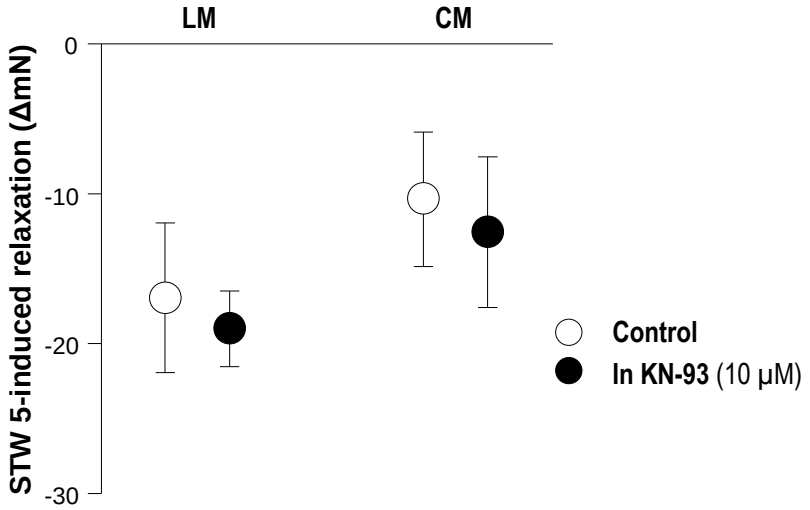

Figure 17: Inhibition of calmodulin kinase II had no effect on STW 5-induced relaxation. A CaM kinasa II inhibitor KN-93 did not influence STW 5-induced relaxations neither in longitudinal (LM) nor in circular muscle (CM).

Table 6: Effects of H-89, methylene blue and KN-93 on STW 5-induced relaxation

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	**STW 5-induced relaxation Control vs. treated (ΔmN)**	**Effect of the blocker on muscle tone (mN)**	**STW 5-induced relaxation Control vs. treated (ΔmN)**
H-89 (10 µM)	**54.2±4.7 vs. 32.2±5.4***	-18.9±2.8 vs. -20.0±5.6	37.7±2.1 vs. 35.8±2.4	-10.6±4.3 vs. -12.5±3.9
PKA inhibitor	***n* = 6; *P* ≤ 0.001***	*n* = 5; *P* = 0.8	*n* = 6; *P* = 0.3	*n* = 5; *P* = 0.1
Methylene blue (50 µM)	45.9±7.9 vs. 45.7±8.0	-28.2±7.8 vs. -26.1±7.6	47.6±6.6 vs. 48.2±6.7	-13.9±2.2 vs. -16.5±0.8
PKG, cGMP inhibitor	*n* = 6; *P* = 0.6	*n* = 6; *P* = 0.3	*n* = 7; *P* = 0.3	*n* = 7; *P* = 0.3
KN-93 (10 µM)	**62.3±3.8 vs. 45.8±5.0***	-17.0±5.0 vs. -19.0±2.5	50.9±5.9 vs. 49.7±5.3	-10.4±4.5 vs. -12.6±5.0
CaMK-II inhibitor	***n* = 4; *P* = 0.006***	*n* = 4; *P* = 0.6	*n* = 4; *P* = 0.8	*n* = 4; *P* = 0.6

(n) = number of muscle strips (equal to number of animals) studied. * mark significant differences.

Calcium channels

Because STW 5-induced relaxation was not neuronally- or NO-mediated as well as not influenced by any of the above discussed pathways, we hypothesised direct effects of STW 5 on intracellular Ca^{2+} handling. In smooth muscle, decreasing the intracellular calcium concentration $[Ca^{2+}]_i$ leads to relaxation. This decrease in $[Ca^{2+}]_i$ can be achieved through three different pathways:

a) Inhibition of extracellular Ca^{2+} influx.
b) Blockade of Ca^{2+} release from its intracellular stores.
c) Enhancement of Ca^{2+} reuptake into its stores.

A. Calcium reuptake

Calcium is stored into its intracellular stores (sarcoplasmic reticulum, SR). Cytoplasmic Ca^{2+} is pumped into SR via sarcoplasmic endoplasmic reticulum calcium ATPase pump (SERCA). Ca^{2+} reuptake leads to reduction of $[Ca^{2+}]_i$ level and consequently muscle relaxation. Thapsigargin is a SERCA inhibitor (Rosado, 2006). Application of 0.3 µM thapsigargin increased the basal muscle tone in both muscle layers (Table 7). Pre-treatment with thapsigargin (0.3 µM) non-significantly reduced the inhibitory effect of STW 5 in both longitudinal and circular muscles by 8 % and 13 % (Figure 18 and Table 7).

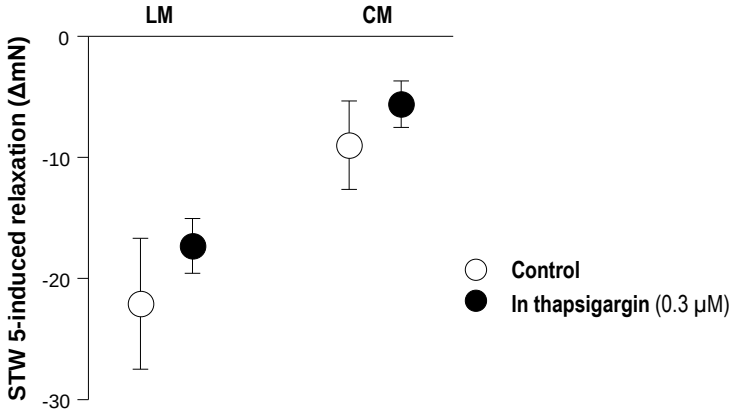

Figure 18: Inhibition of SERCA did not affect STW 5-induced relaxation. In both muscle layers longitudinal (LM) and circular (CM), application of SERCA-ATPase pump inhibitor, thapsigargin has no influence on STW 5 effects.

Table 7: Effects of thapsigargin on STW 5-induced relaxation in corpus gastric smooth muscle

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	STW 5-induced relaxation Control vs. treated (ΔmN)	Effect of the blocker on muscle tone (mN)	STW 5-induced relaxation Control vs. treated (ΔmN)
Thapsigargin (0.3 µM)	**43.1±5.8 vs. 51.6±5.5***	-22.1±5.4 vs. -17.3±2.3	**35.8±4.8 vs. 45.4±6.3***	-9.0±3.7 vs. -5.6±1.9
SERCA blocker	***n* = 5; *P* = 0.003***	*n* = 5; *P* = 0.5	***n* = 5; *P* = 0.04***	*n* = 5; *P* = 0.2

(n) = number of muscle strips (equal to number of animals) studied. * mark significant differences.

B. Calcium release

Intracellular stored calcium is released through ryanodine (RyRs) and inositol triphosphate (IP_3-R) receptors located in the membrane of the sarcoplasmic reticulum. Blockade of these receptors inhibits calcium release and consequently causes smooth muscle relaxation. Ryanodine, ruthenium red and caffeine were used as blockers for RyRs (Wray & Burdyga, 2010). These antagonists exhibited three different actions on basal muscle tone: ruthenium red did not affect basal muscle tone whereas ryanodine increased only longitudinal muscle tone (Table 8). Caffeine significantly relaxed both muscle layers, this relaxation may be due to its phosphodiesterase inhibition activities.

None of the blockers altered the relaxing effect of STW 5. Ryanodine (10 µM) decreased the relaxing effect of STW 5 by 3 % and 9 % in longitudinal and circular muscle respectively, whilst ruthenium red (10 µM) decreased them by 5 % and 22 % respectively. Caffeine (1 mM) non-significantly reduced the inhibitory effect of STW 5 by 10 % in longitudinal muscle and increased the response in circular muscle by 13 % (Figure 19 and Table 8).

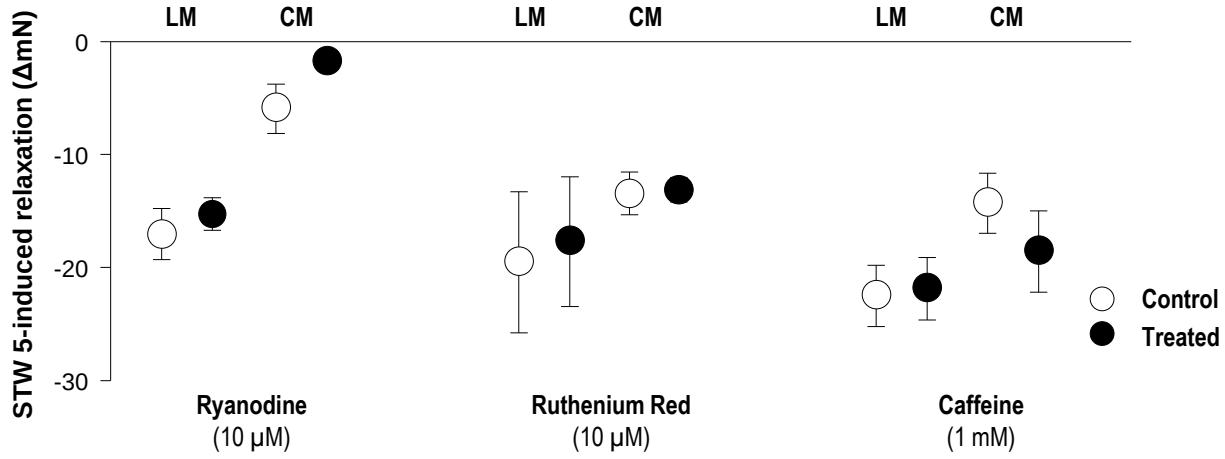

Figure 19: The intracellular calcium stores had no effect on STW 5-induced relaxation. pre-treatment of the muscle strips with ryanodine receptors blockers ryanodine, ruthenium red and Caffeine did not influence STW 5-induced relaxation.

Table 8: Effects of ryanodine receptor blockers on STW 5-induced relaxation

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	**STW 5-induced relaxation Control vs. treated (ΔmN)**	**Effect of the blocker on muscle tone (mN)**	**STW 5-induced relaxation Control vs. treated (ΔmN)**
Ryanodine (10 µM)	**23.2±2.2 vs. 29.3±2.8***	-17.1±2.3 vs. -15.3±1.5	25.7±2.2 vs. 25.9±2.2	-5.9±2.2 vs. -1.8±0.5
RyRs blocker	***n* = 6; *P* = 0.02***	*n* = 6; *P* = 0.4	*n* = 6; *P* = 0.4	*n* = 6; *P* = 0.1
Ruthenium red (10 µM)	21.9±2.9 vs. 23.6±3.7	-19.7±6.2 vs. -17.7±5.8	23.2±1.1 vs. 23.7±1.3	-13.5±1.9 vs. -13.2±1.1
RyRs blocker	*n* = 10; *P* = 0.3	*n* = 10; *P* = 0.8	*n* = 8; *P* = 0.4	*n* = 8; *P* = 0.9
Caffeine (1 mM)	**36.9±3.9 vs. 16.3±3.7***	-22.5±2.7 vs. -21.9±2.8	**40.5±2.1 vs. 9.4±1.7***	-14.3±2.7 vs. -18.6±3.6
RyRs, A1&A2A blocker	***n* = 9; *P* = 0.004***	*n* = 9; *P* = 0.7	***n* = 8; *P* ≤ 0.001***	*n* = 8; *P* = 0.2

(n) = number of muscle strips (equal to number of animals) studied. § mark non-normally distributed data. * mark significant differences.

C. Calcium influx

The third and major mechanism to control intracellular calcium level is to alter calcium influx from the extracellular pool. Extracellular Ca^{2+} entry is governed by different channels: store-operated (SOCs), voltage-dependent (VDCCs) and/or receptor operated calcium channels (ROCs). Blockade of these channels inhibits Ca^{2+} influx, decreases $[Ca^{2+}]_i$ level and consequently leads to muscle relaxation. In the following sections, the implications of these channels in STW 5-induced relaxation were studied.

- Non-selective cation channel blockers

Lanthanum chloride ($LaCl_3$) is a non-selective calcium channel blocker (Kwan *et al.*, 1990). Application of $LaCl_3$ (200 µM) did not influence the basal muscle tone by itself (Table 9). Pre-incubation of the gastric corpus muscle strips with $LaCl_3$ (200 µM) had no effect on STW 5-induced relaxation in both longitudinal and circular muscle, both responses increased by 7 % and 14 % respectively (Figure 20 and Table 9).

- Voltage sensitive calcium channels

The T-type calcium channel is a voltage dependent calcium channel (VDCC). This channel can be selectively blocked by mibefradil (Martin *et al.*, 2000). As shown in Table 9, 0.1 µM mibefradil relaxed only longitudinal muscle basal tone. Mibefradil (0.1 µM) did not influence the relaxation induced by STW 5. This relaxation was non-significantly increased by 7 % and 37 % in longitudinal and circular muscle respectively (Figure 20 and Table 9).

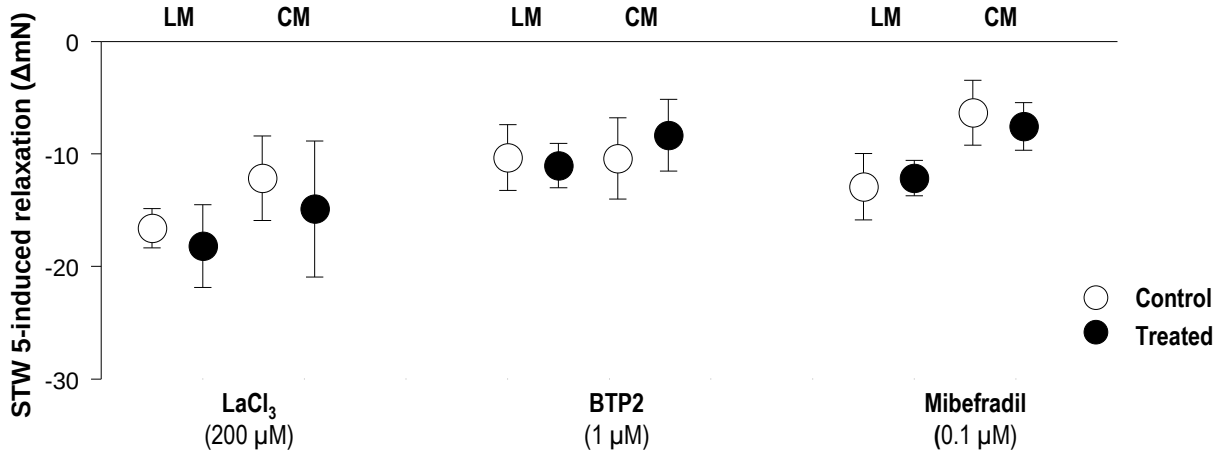

Figure 20: STW 5-induced relaxation is resistant to $LaCl_3$, BTP2 and mibefradil. Pre-treated muscle strips with non-selective calcium channel blocker: $LaCl_3$, selective CRAC antagonist: BTP2 or selective blocker of T-type calcium channel: mibefradil showed comparable responses to STW 5 as in control untreated strips.

Table 9: Effects of $LaCl_3$, BTP2 and mibefradil on STW 5-induced relaxation

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	**STW 5-induced relaxation Control vs. treated (ΔmN)**	**Effect of the blocker on muscle tone (mN)**	**STW 5-induced relaxation Control vs. treated (ΔmN)**
$LaCl_3$ (200 µM)	57.9±10.5 vs. 60.9±11.9	-16.6±1.8 vs. -18.2±3.7	42.9±8.6 vs. 44.7±8.8	-12.2±3.7 vs. -14.9±6.1
Cation channel blocker	*n* = 3; *P* = 0.4	*n* = 3; *P* = 0.3	*n* = 3; *P* = 0.3	*n* = 3; *P* = 0.4
Mibefradil (0.1 µM)	**32.4±1.7 vs. 24.8±3.0***	-12.9±2.9 vs. -12.2±1.6	37.0±2.6 vs. 35.9±2.8	-6.4±2.9 vs. -7.6±2.1
T-type Ca^{2+}channel blocker	***n* = 6; *P* = 0.006***	*n* = 6; *P* = 0.7	*n* = 6; *P* = 0.1	*n* = 6; *P* = 0.5
Nifedipine (1 µM)	**33.0±3.2 vs. 24.1±2.9***	**-14.3±2.1 vs. -9.6±2.4***	**40.0±2.1 vs. 28.7±1.9***	**-9.1±2.9 vs. -3.7±1.1**
L-type Ca^{2+} channel blocker	***n* = 10; *P* ≤ 0.001***	***n* = 10; *P* = 0.03***	***n* = 6; *P* = 0.004***	***n* = 6; *P* = 0.04**
S-(-)-Bay K 8644 (1 µM)	**36.9±3.8 vs. 59.3±5.3***	**-17.3±4.7 vs. -23.6±2.8***	**36.0±5.0 vs. 53.9±3.6***	**-8.5±2.0 vs. -19.8±3.5***
L-type Ca^{2+} channel agonist	***n* = 6; *P* = 0.01***	***n* = 6; *P* = 0.04***	***n* = 6; *P* = 0.009***	***n* = 6; *P* = 0.005***
BTP2 (1 µM)	**33.0±2.6 vs. 24.9±2.0***	-10.3±2.9 vs. -11.0±2.0	**42.9±7.3 vs. 29.5±10.2***	-10.4±3.6 vs. -8.3±3.2
CRAC blocker	***n* = 6; *P* ≤ 0.001***	*n* = 6; *P* = 0.8	***n* = 5; *P* = 0.02***	*n* = 5; *P* = 0.4

(n) = number of muscle strips (equal to number of animals) studied. * mark significant differences.

Another VDCC is the L-type calcium channel which is selectively antagonized by nifedipine and activated by S-(-)-Bay K 8644 (Franckowiak *et al.*, 1985;Bossert & Vater, 1989). Nifedipine (1 µM) significantly relaxed the resting muscle tone in both longitudinal and circular muscle (Table 9). Pretreatment of smooth muscle with nifedipine (1 µM) significantly reduced the relaxation induced by STW 5 in both longitudinal and circular muscle by 32 % and 39 % respectively (Figure 21 and Table 9).

In contrary, application of the selective agonist S-(-)-Bay K 8644 significantly increased the basal muscle tone of longitudinal and circular muscle (Table 9). Pre-incubation of S-(-)-Bay K 8644 significantly increased STW 5-induced relaxation in both longitudinal and circular muscle by 85 % and 169 % respectively (Figure 21 and Table 9).

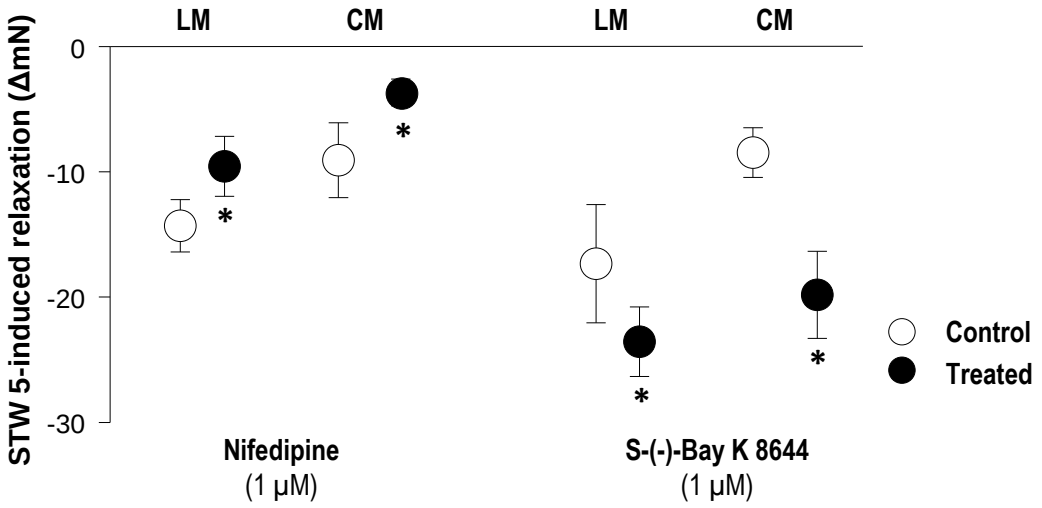

Figure 21: Effects of nifedipine and Bay K 8644. STW 5-induced relaxation in both longitudinal (LM) and circular (CM) is significantly reduced in the L-type calcium channel blocker nifedipine whilst increased in the channel agonist S-(-)-Bay K 8644. * mark significant differences compared to control.

- Store-operated calcium channels (SOCs)

BTP2 is a selective CRAC (calcium release activated channel) antagonist with higher affinity to block Orai1 receptors (He *et al.*, 2005;Schleifer *et al.*, 2012). BTP2 (1 µM) significantly relaxed both longitudinal and circular basal muscle tone (Table 9). However, BTP2-pretreated tissue showed comparable STW 5-induced relaxation to untreated tissues. The inhibitory effect of STW 5 was non-significantly increased in longitudinal muscle by 2 % and decreased in circular muscle by 23 % (Figure 20 and Table 9).

- Transient receptor potential (TRP channels)

SKF-96365 is an antagonist for store-operated calcium channel (SOCs), receptor-operated calcium channels (ROCs) and TRPC channels (Merritt *et al.*, 1990;Tang *et al.*, 2010;Song *et al.*, 2014). On its own, SKF-96365 (10 µM) significantly relaxed the resting tone of both longitudinal and circular muscles (Table 10). Pre-incubation of SKF-96365 (10 µM)

significantly reduced STW 5-induced relaxation by 64 % and 17 % in longitudinal and circular muscles respectively (Figure 22 and Table 10).

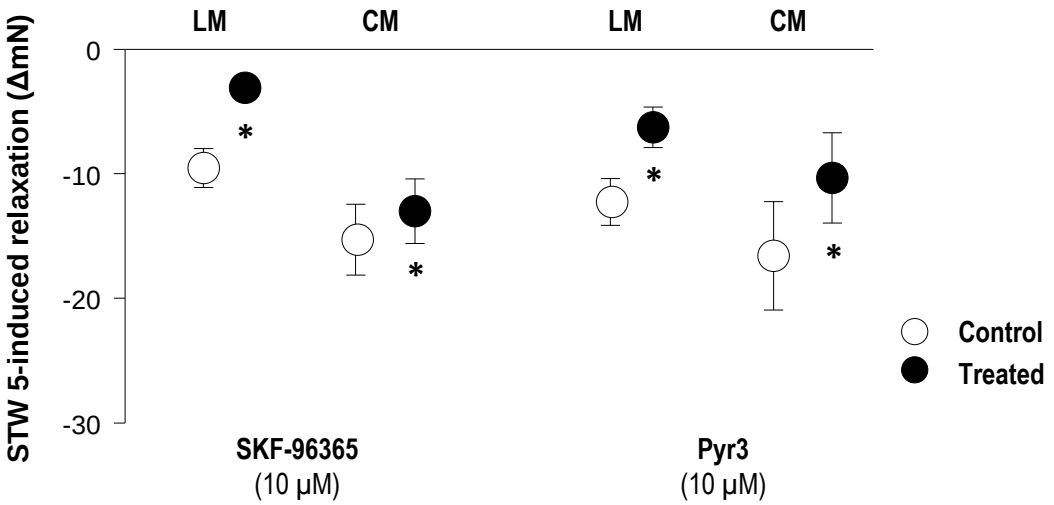

Figure 22: STW 5-induced relaxation is partly TRPC- and TRPC3-dependent. Application of SKF-96365 significantly reduced STW 5-induced relaxation in both longitudinal (LM) and circular muscle (CM). SKF-96365 is SOC, ROC and TRPC channel blocker. The results were similar with selective TRPC3 blocker Pyr3. * mark significant differences.

Since BTP2 had no effect on STW 5-induced relaxation, whereas SKF-96365 significantly decreased it, we hypothesised that this effect is due to inhibition of TRPC and not SOC channels.

To test this hypothesis, the TRPC blocker 2-APB was applied. In addition to its IP_3-R antagonising effect, 2-APB is also a non-selective TRP channel blocker and often used to predominantly block TRPC (Tang *et al.*, 2010). Application of 2-APB (30 µM) in the organ bath significantly relaxed longitudinal and circular muscle tone (Table 10). Pre-treatment of longitudinal muscle strips with 2-APB (30 µM) lead to significant reduction in STW 5-induced relaxation by 82% (Figure 23 and Table 10). Surprisingly, in circular muscle the inhibitory effect of STW 5 was fully reversed and now revealed a prokinetic effect of STW5 (Figure 23 and Table 10). As demonstrated in Figure 23 this contraction was abolished in nifedipine (1 µM).

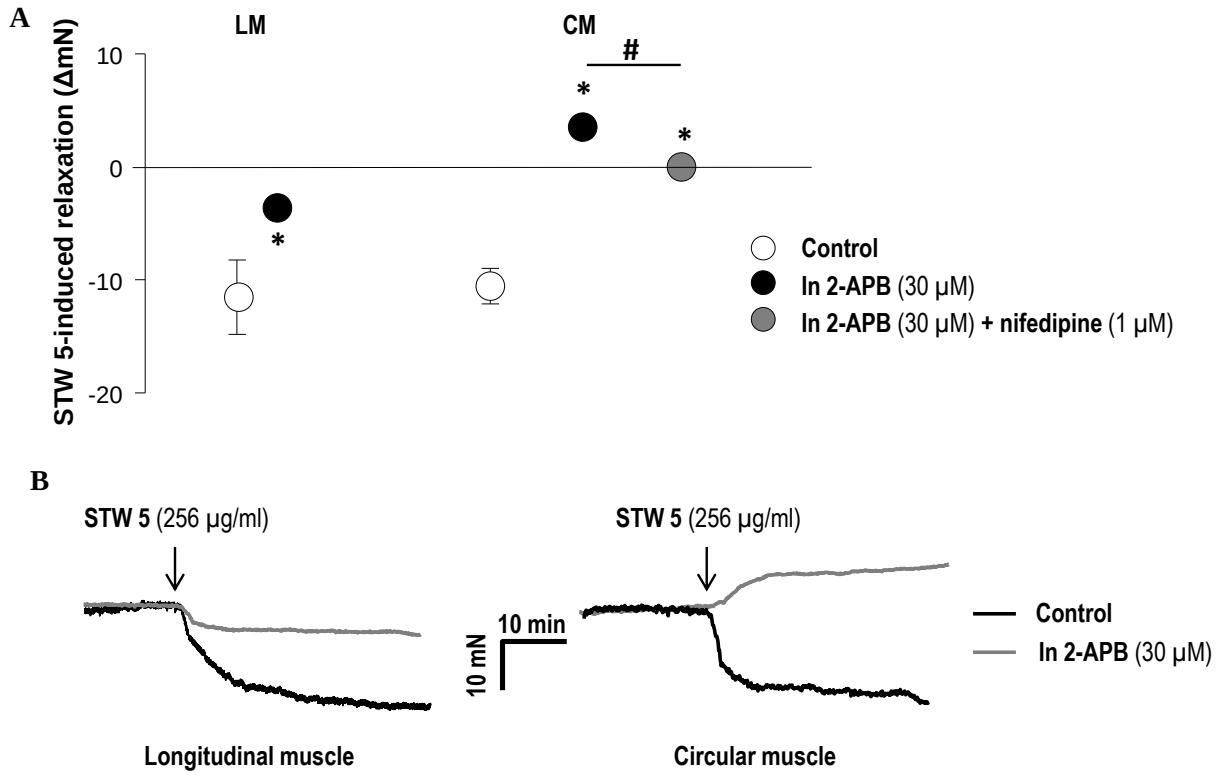

Figure 23: STW 5-induced relaxation is 2-APB-sensitive. Panel (A) shows that pre-application of 2-APB significantly reduced the STW 5-induced relaxation in longitudinal muscle. However in circular muscle the inhibitory effect of STW 5 was fully reversed into contractions revealing prokinetic effects of STW 5. This contraction was abolished in nifedipine. * mark significant differences from control. # marked significance between the treated strips. Panel (B) shows representative traces for the effects of 2-APB on STW 5 effects on longitudinal (to the left) and circular muscle (to the right).

For further confirmation as well as identification of the specific TRPC channel, the more selective antagonist, Pyr3 was used. Pyr3 is a selective antagonist for TRPC3 channels (Kiyonaka *et al.*, 2009). Application of Pyr3 (10 µM) relaxed longitudinal and circular muscle tone (Table 10). Pyr3-treated muscle strips showed significantly lower responses to STW 5 compared to controls. The inhibitory effects were reduced by 53 % and 43 % in longitudinal and circular muscle respectively (Figure 22 and Table 10).

The TRP channel blocker AP-18 was also tested. AP-18 is a transient receptor potential ankyrin1 (TRPA1) selective blocker (Cheah *et al.*, 2014). AP-18 (10 µM) relaxed longitudinal and circular muscle tone (Table 10). Selective blockade of TRPA1 channels by AP-18 (10 µM) substantially decreased STW 5-inhibitory action on muscle tone. STW 5 responses were significantly reduced in longitudinal and circular muscle by 85 % and 84 % respectively (Figure 24 and Table 10).

On the other hand, application of cinnamaldehyde (CNA) (100 µM) significantly increased the relaxation evoked by STW 5 in both longitudinal and circular muscle by 98 % and 76 % respectively (Figure 24 and Table 10). Cinnamaldehyde (CNA) is a TRPA1 agonist (Cheah *et al.*, 2014). Application of CNA relaxed both longitudinal and circular muscle tone however this relaxation was significant only in circular muscle (Table 10).

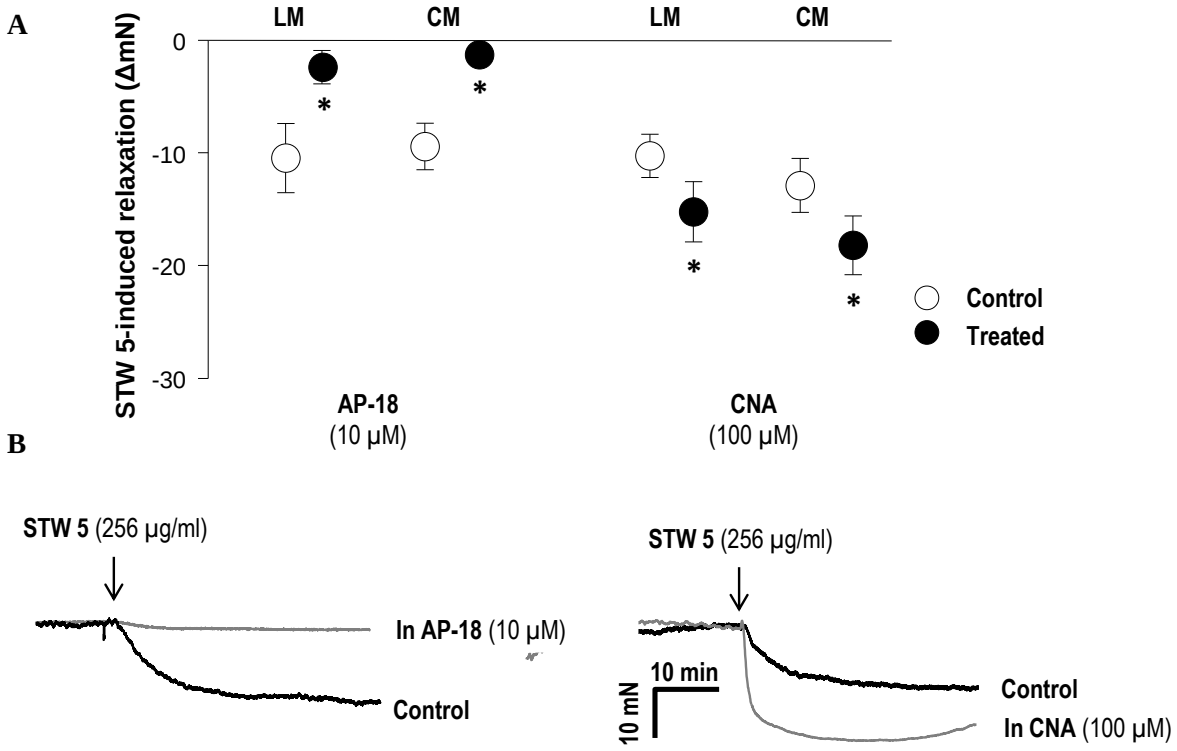

Figure 24: STW 5-induced relaxation is TRPA1-mediated. Panel (A) shows that STW 5-induced relaxation in both longitudinal (LM) and circular (CM) is significantly reduced in TRPA1 blocker, AP-18. In contrary, the effect was increased in TRPA1 agonist CNA. * mark significant differences. Panel (B) shows representative traces for the effects of AP-18 (to the left) and CNA (to the right) on STW 5 effects on longitudinal muscle.

Because CNA which is a TRPA1 agonist relaxed the basal muscle tone as AP-18, a TRPA1 antagonist, we aimed to ensure its agonistic specificity. CNA (100 µM) was applied in organ baths containing muscle strips pre-treated with AP-18 (10 µM). AP-18 did not completely abolish CNA effects however the relaxation was significantly decreased in longitudinal and circular muscles by 55 % and 32 % respectively (Figure 25). This partial inhibition revealed involvement of other pathways in CNA actions like TRPV1 activation.

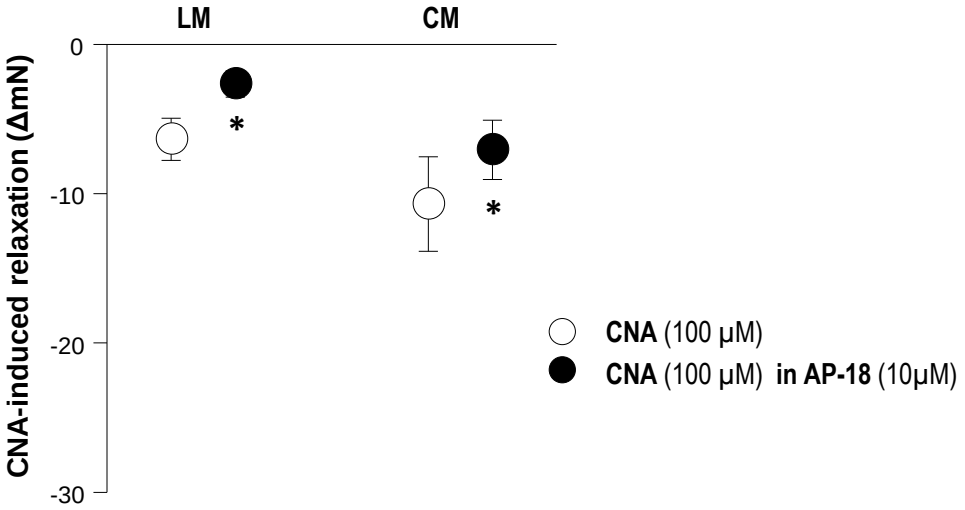

Figure 25: CNA-induced relaxation is significantly reduced in AP-18 in both longitudinal (LM) and circular (CM) muscle. * mark significant differences.

To confirm the involvement of TRPA1 in the mechanism of action, another TRPA1 antagonist HC-030031 was used. Although antagonizing TRPA1 by HC-030031 has been shown to improve guinea pig gastric accommodation (Koseki *et al.*, 2012), we did not observe any significant changes in the basal muscle tone after application of 10 µM HC-030031 (Table 10). As well, STW 5 evoked comparable relaxations in both HC-030031-treated and non-treated tissues. The inhibitory effect of STW 5 on treated muscle strips was increased by 23 % in longitudinal muscle and decreased by 11 % in circular muscle (Figure 26 and Table 10).

We also have evidences that HC-033031 did not block AITC- and CNA-induced secretion in guinea pig (personal communication, Dr. Dagmar Krueger). The most likely reason for the observed discrepancies is that Koseki *et al*. (2012) used conscious guinea pigs.

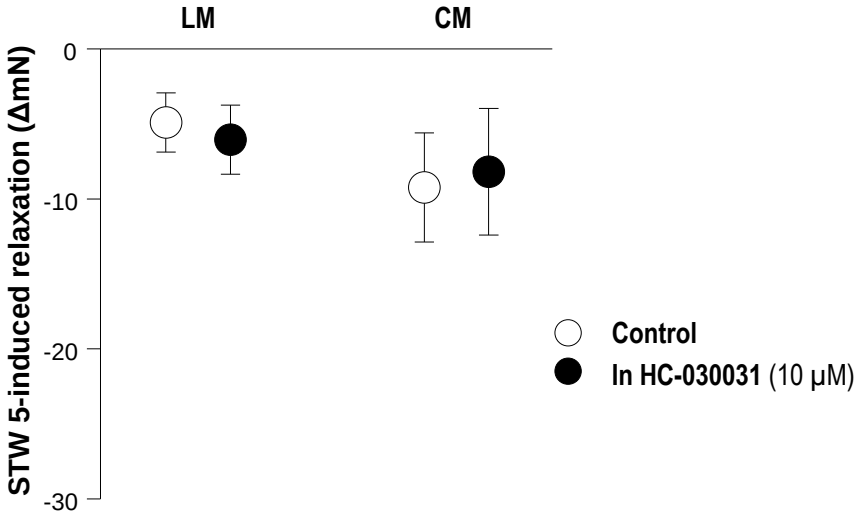

Figure 26: TRPA1 blocker HC-30031 did not affect STW 5-induced relaxation in both LM and CM.

- Protein kinase C

One of the intracellular signalling pathways that control smooth muscle activity is protein kinase C (PKC). PKC regulates SOCs/TRP channels and playing important roles in several signal transduction cascades (Andrea & Walsh, 1992;Venkatachalam *et al.*, 2003). PMA is a PKC activator whereas chelerythrine is an inhibitor (Herbert *et al.*, 1990;Meininger *et al.*, 1999). Application of PMA (10 µM) induced small, yet significant, increases in the basal muscle tone in both longitudinal and circular muscle strips (Table 10). Pre-treatment of the tissue with PMA (10 µM) significantly decreased STW 5-induced relaxation in longitudinal and circular muscle by 46 % and 51 % respectively (Figure 27 and Table 10). In contrary, application of chelerythrine (3 µM) relaxed longitudinal and circular muscle tone (Table 10). Additionally, pre-incubation of chelerythrine chloride (3 µM), significantly increased STW 5-induced relaxation in longitudinal and circular muscle by 181 % and 124 % respectively (Figure 27 and Table 10).

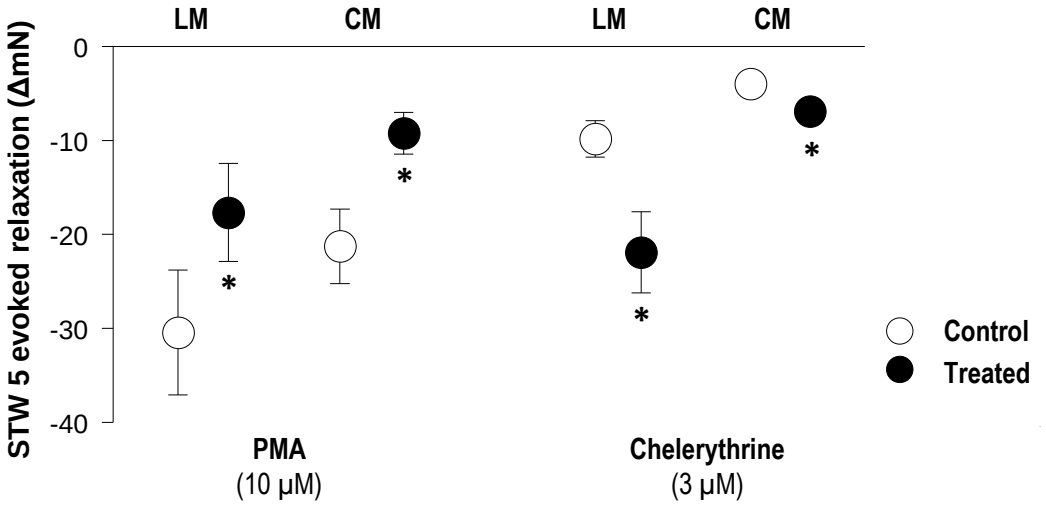

Figure 27: Effects of PMA and chelerythrine. STW 5-induced relaxation in both LM and CM is significantly reduced in PKC activator; PMA whilst increased in PKC inhibitor; chelerythrine chloride. * mark significant differences compared with control.

Table 10: Effects of TRP channels and PKC agonists/antagonists on STW 5-induced relaxation

	Corpus longitudinal muscle		Corpus circular muscle	
	Effect of the blocker on muscle tone (mN)	**STW 5-induced relaxation Control vs. treated (ΔmN)**	**Effect of the blocker on muscle tone (mN)**	**STW 5-induced relaxation Control vs. treated (ΔmN)**
SKF-96365 (10 μM)	**36.0±3.5 vs. 21.0±2.6***	**-9.6±1.6 vs. -3.0±0.6***	**45.6±2.5 vs. 27.1±2.9***	**-15.3±2.9 vs. -13.0±2.6***
SOC,ROC, TRPC antagonist	***n* = 8; *P* = 0.001***	***n* = 7; *P* = 0.01* §**	***n* = 8; *P* ≤ 0.001***	***n* = 6; *P* = 0.04***
Pyr3 (10 μM)	**29.3±4.7 vs. 19.7±2.4***	**-12.3±1.9 vs. -6.3±1.6***	**47.9±3.9 vs. 33.9±4.0***	**-16.6±4.4 vs. -10.3±3.6***
TRPC3 antagonist	***n* = 7; *P* = 0.02***	***n* = 7; *P* ≤ 0.001***	***n* = 5; *P* = 0.04***	***n* = 5; *P* = 0.01***
2-APB (30 μM)	**32.5±3.2 vs. 21.3±2.9***	**-13.7±3.4 vs. -2.0±0.5**	**47.8±6.9 vs. 20.0±3.7***	**-8.7±1.9 vs. 3.0±0.7***
IP_3-R, TRP, TRPC blocker	***n* = 6; *P* = 0.01* §**	***n* = 6; *P* = 0.004 §**	***n* = 5; *P* = 0.004***	***n* = 5; *P* = 0.004***
AP-18 (10 μM)	**26.3±2.1 vs. 20.5±1.3***	**-10.5±3.1 vs. -2.4±1.5***	**55.0±2.8 vs. 42.5±1.9***	**-9.4±2.1 vs. -1.3±0.5***
TRPA1 antagonist	***n* = 6; *P* = 0.01***	***n* = 6; *P* = 0.01***	***n* = 5; *P* = 0.006***	***n* = 5; *P* = 0.02***
CNA (100 μM)	38.6±5.5 vs. 33.2±6.6	**-10.3±1.9 vs. -15.2±2.7***	**44.1±2.4 vs. 35.5±2.1**	**-12.9±2.4 vs. -18.2±2.6***
TRPA1 agonist	*n* = 6; *P* = 0.3	***n* = 6; *P* = 0.04***	***n* = 7; *P* = 0.004**	***n* = 7; *P* = 0.02***
HC-030031 (10 μM)	29.5±3.6 vs. 27.9±3.3	-4.9±1.9 vs. -6.1±2.3	30.0±3.7 vs. 27.0±4.1	-9.2±3.6 vs. -8.2±4.2
TRPA1 antagonist	*n* = 6; *P* = 0.06	*n* = 6; *P* = 0.5	*n* = 6; *P* = 0.1	*n* = 6; *P* = 0.7
PMA (10 μM)	**43.7±8.0 vs. 48.5±8.9***	**-30.5±6.6 vs. -17.7±5.2***	**47.6±3.1 vs. 48.0±3.1***	**-21.3±3.9 vs. -9.2±2.2***
PKC activator	***n* = 6; *P* = 0.04***	***n* = 6; *P* = 0.02***	***n* = 7; *P* = 0.01***	***n* = 7; *P* = 0.04***
Chelerythrin (3 μM)	**31.2±3.1 vs. 27.1±2.0***	**-9.8±1.9 vs. -21.9±4.3***	**34.2±1.8 vs. 31.9±1.4***	**-4.0±1.1 vs. -6.9±1.2***
PKC inhibitor	***n* = 6; *P* = 0.02***	***n* = 6; *P* = 0.01***	***n* = 8; *P* = 0.008* §**	***n* = 7; *P* = 0.03***

(n) = number of muscle strips (equal to number of animals) studied. § mark non-normally distributed data. * mark significant differences.

3.2 Effects of STW 5 and its individual components on human intestinal motility

The rationale for this part of the thesis was to study the so far unknown effects of STW 5 and its individual components on human intestinal motility.

As discussed in section 3.1.1, because ethanol by itself has been shown to exert a profound dual effect on gastrointestinal motility in a dose-dependent manner (Wali *et al.*, 1987), the current study was conducted with ethanol-free STW 5 and its nine components.

Comparative study between fresh and overnight stored tissues

In some experiments due to late tissue supply, it was impossible to do the experiments on the same day of the surgical operations. Firstly, we aimed to study the differences between fresh and overnight stored human intestinal smooth muscle. Tissues viabilities were tested by applying electrical field stimulation (EFS). EFS evoke nerve-mediated contractions. The contractions were biphasic composed of an on-response during EFS followed by an off-response immediately after cessation of EFS pulses. Fresh human tissues were dissected and stored overnight in oxygenated Krebs buffer at 4 ºC in the fridge. In a paired design, comparative study was conducted between fresh versus overnight stored tissues from the same patients. The results showed that neither On- nor Off-responses were significantly changed in both circular and longitudinal muscles dissected from small and large intestine (Figure 28 and Table 17 in the appendix). Hence, in the following sections data from fresh and overnight stored tissues were pooled together. This comparative study was done by Dr. Dagmar Krueger.

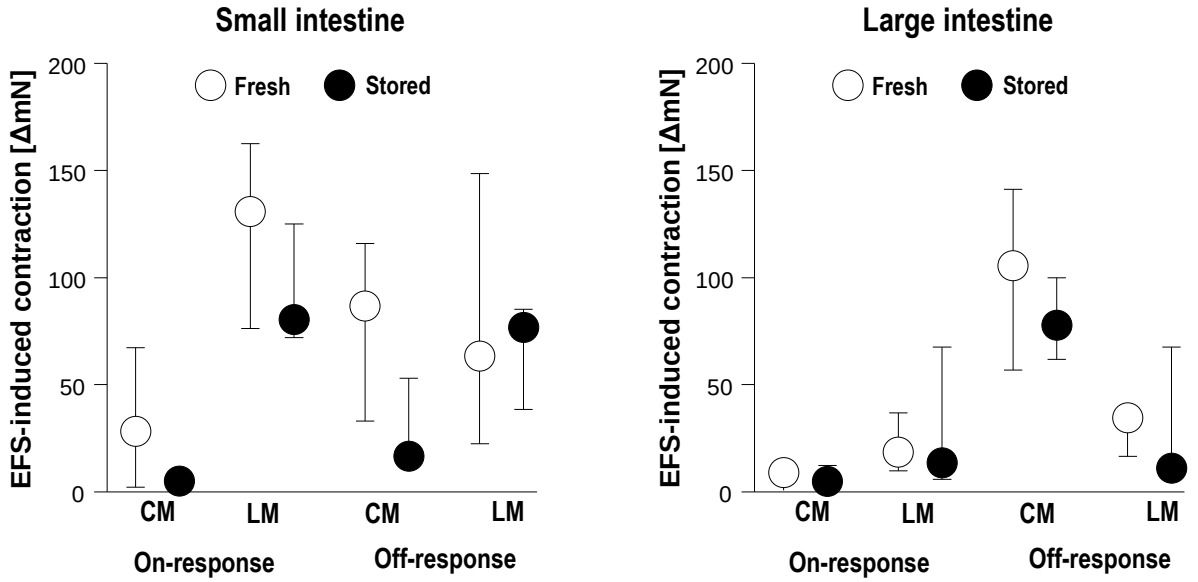

Figure 28: Fresh and overnight stored human intestinal smooth muscles respond similarly to EFS. Neither on- nor off-responses of EFS-induced nerve mediated contraction was altered due to overnight storage of the tissue. Both responses were comparable in fresh and overnight stored tissues dissected from the same patient. These comparable responses were applicable for circular (CM) and longitudinal (LM) muscles dissected from small and large intestine. Data are presented as median [75/25] percentile.

In preliminary experiments, muscle strips were mounted in the organ bath at pre-tension of 15 mN and allowed to equilibrate. Sixty to ninety minutes later, EFS was performed. After 20-25 min, carbachol was applied to increase the muscle tone to 40-45 mN prior to STW 5 application. However, these data were excluded from further analysis for the following reasons:

(1) Muscle strips from the same patient responded differently to the same dose of carbachol (Figure 29).

(2) Consequently, the applied carbachol doses to reach 40 mN varied ranging from 0.05 nM to 1 µM. This made direct comparison of effects impossible.

(3) In some experiments, carbachol evoked large transient increases in the muscle tone which shortly declined to the pre-application level (Figure 29).

(4) Finally and most importantly, there was a correlation between carbachol-treated muscle tension and the relaxing effect of STW 5. The higher the muscle tone was, the more relaxation STW 5 could exert (Figure 30 and Table 11). These correlations were comparable for all concentrations in circular and longitudinal muscles from small and large intestine. Therefore, the data were pooled together. Bethanechol at concentrations of 0.5 – 10 µM had similar effects.

As an alternative, it was tested if it is also possible to adjust the pre-tension manually to values of 40-45 mN. In these experiments, pre-tension and the relaxing effect of STW5 showed no correlation. This technique was therefore used for the motility experiments.

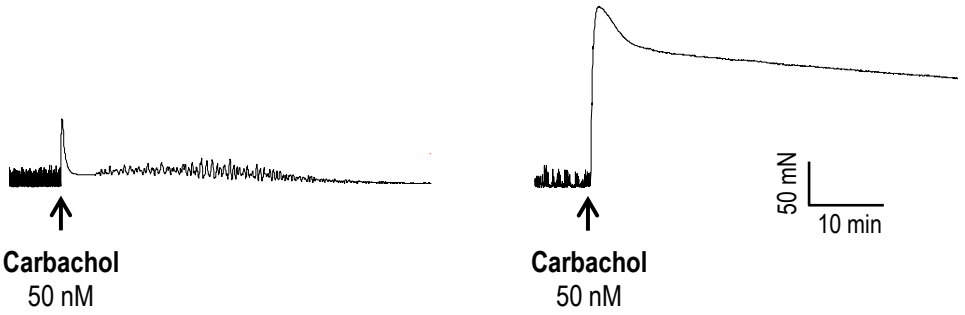

Figure 29: Representative traces for carbachol responses in human tissue. Application of the same dose of carbachol evoked different responses in muscle strips from the same patient. the left trace shows transient increase in muscle tone of jejunum whereas the right one from adjacent muscle strip from the same patient shows large slowly declining response for the same dose of carbachol under the same experimental procedures.

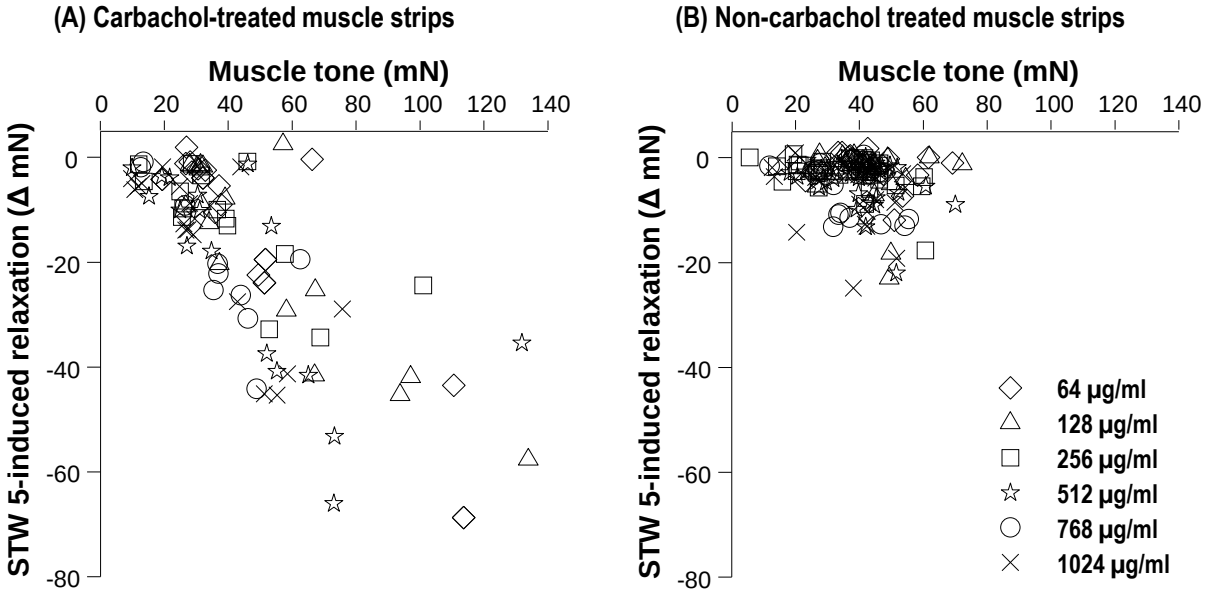

Figure 30: Carbachol influences STW 5 evoked relaxation in human intestine. Panel (A) Carbachol-treated muscle strips showed significant correlation between muscle tone and STW 5 induced relaxation. Panel (B) In non-carbachol treated muscle strips, STW 5 (64-1024 µg/ml) inhibitory effects were independent of the muscle tone.

Table 11: Correlation between muscle tone and STW 5 evoked relaxation in human intestine

	Carbachol-treated muscle			Non-carbachol treated muscle		
STW 5	**Correlation coefficient**	***P* value**	**No.[1]**	**Correlation coefficient**	***P* value**	**No.[1]**
64 µg/ml	-0.70	**0.0002***	22	-0.35	0.11	22
128 µg/ml	-0.74	**0.0004***	17	-0.17	0.42	25
256 µg/ml	-0.72	**0.001***	16	-0.33	0.07	33
512 µg/ml	-0.79	**0.000002***	17	-0.17	0.34	34
768 µg/ml	-0.69	**0.02***	10	-0.36	0.11	22
1024 µg/ml	-0.73	**0.0003***	18	-0.15	0.48	25

[1] No. indicates the number of tissues (equal to number of patients) studied. * mark significant correlations.

3.2.1 Effects of STW 5 on human intestinal motility

In order to investigate the effects of STW 5 on human intestinal motility, different concentrations of STW 5 ranging from 64-1024 µg/ml were added to the organ baths containing circular and longitudinal muscle strips dissected from human small and large intestine. Pre- and post-treatment changes in muscle activities were compared in each individual tissue. STW 5 was applied in a non-cumulative manner. Independent of the concentration, STW 5 evoked immediate relaxation on muscle tone within 5 ± 2 min whereas the inhibition of phasic contractility reached its maximum after 33 ± 6 min. Consequently (as described in materials and methods, section 2.4.3) three different time intervals related to STW 5 application time (t = 0 min) were analysed: 0, 30 and 50 min after STW 5 application. Each interval was 10 min long. These analysed intervals were statistically tested against the pre-STW 5 application (t = -10 min). Analysed parameters were: A) muscle tone, B) contractile amplitude, C) frequency of contractions and D) motility index. Throughout the following sections, only significant data at 50 min was described whilst other analysed periods were demonstrated in the tables and the figures.

The intestinal smooth muscle preparations exhibited spontaneous contractile activity. After addition of STW 5 the phasic contractions decreased in frequency and amplitude. Dependent on the concentration and the region, the phasic activity ceased in some preparations. In addition, STW5 decreased dose dependently the muscle tone as indicated by a decrease in the baseline. However, in some tissues STW 5 evoked an increase in the amplitude of phasic contractions which usually was accompanied with a decrease in muscle tone. It is not known whether this increase in contractile force is merely due to the altered muscle tone or a genuine effect of STW5 (Figure 31).

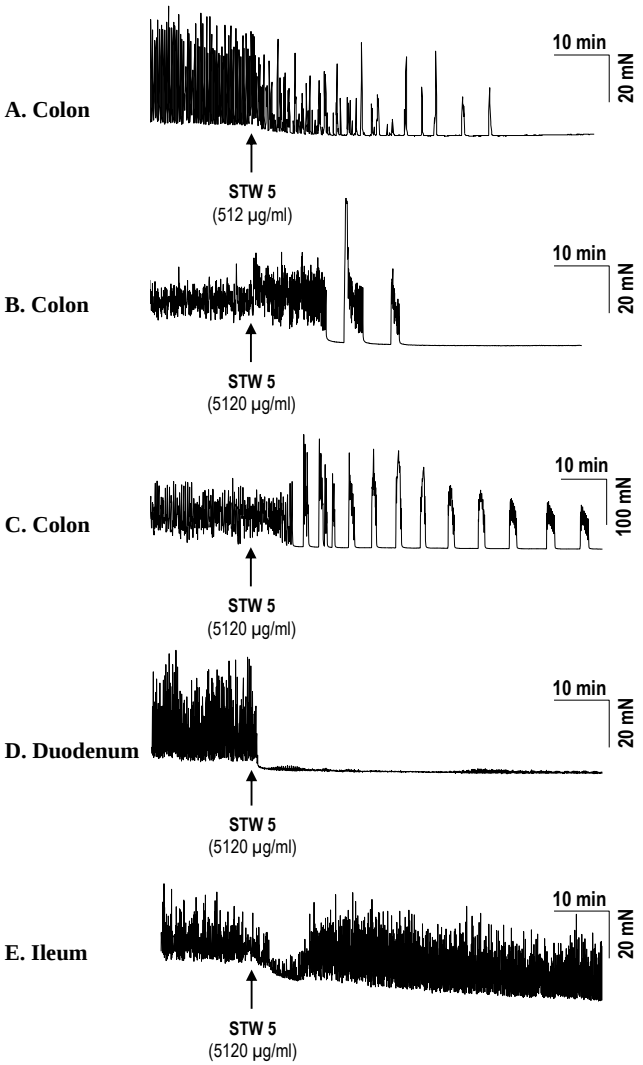

Figure 31: Representative traces for the effects of STW 5 on different regions of human intestine. Although STW 5 relaxes the circular muscle tone of all intestinal regions, it has region-specific effects on the phasic contractility. Panel (A) shows that STW 5 (512 µg/ml) decreases motility index in large intestine whilst tenfold higher concentration changes the contractile pattern into clusters with large transient increase in the phasic contractility followed by periods of quiescence (B), in three tissues out of eleven the cluster formation continued till the end of the recording time which was 60 min (C). In duodenum and jejunum, the phasic contractility was completely inhibited (D) whilst Ileum smooth muscle showed initial inhibition followed by non-significant increase (E).

A. Effects of STW 5 on muscle tone

STW 5 induced a dose-dependent relaxation on human intestinal muscle tone. This relaxation was region-specific and layer-dependent. Application of STW 5 (64-1024 µg/ml) on circular muscle strips of the small intestine induced significant and dose dependent relaxation of muscle tone. Muscle tone was reduced in 64, 128, 256, 512, 768 and1024 µg/ml STW 5 by 2 % (non-significant, n.s), 5 %, 5 %, 7 %, 6 % and 7 % respectively whereas in the longitudinal muscle, same concentrations inhibited the muscle tone by 0.6 % (*n.s*), 7 %, 5 % (*n.s*), 9 %, 13 % and 14 % respectively (Figure 32, Table 18 and Table 19 in the appendix).

In large intestinal circular muscle, application of 64, 128, 256, 512, 768 and1024 µg/ml STW 5 also significantly and dose dependently inhibited muscle tone by 9 %, 9 %, 23 %, 21 %, 26 % and 17 % respectively (Figure 32 and Table 20 in the appendix). Although, STW 5 (64, 128, 256, 512, 768 and 1024 µg/ml) showed tendencies to relax the longitudinal muscles, only the concentration of 512 µg/ml showed a significant reduction by 13 % (Figure 32 and Table 21 in the appendix).

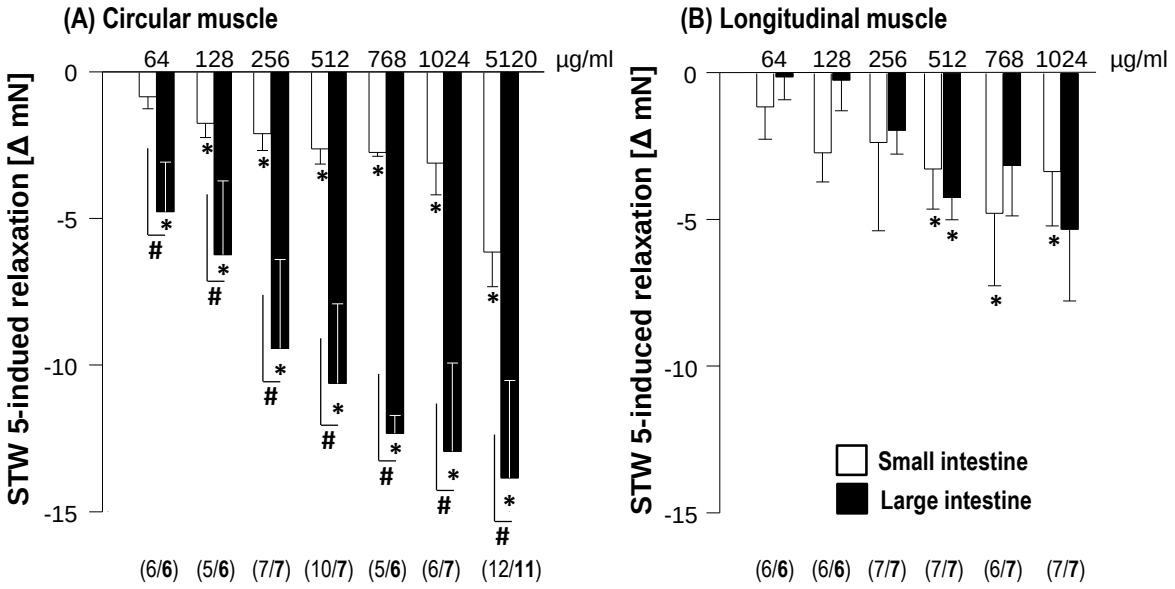

Figure 32: STW 5 effects on human intestinal muscle tone are concentration-dependent, region- and layer-specific. Panel (A) shows that STW 5 (64-5120 µg/ml) induced significant and dose dependent relaxation on circular muscle tone of small and large intestine. Panel (B) shows the tendencies of STW 5 to relax the muscle tone of small and large intestinal longitudinal muscle however only higher concentrations showed significance reduction. The effects are more prominent in large than in small intestine and in circular than in longitudinal muscle. Numbers in parentheses indicate the number of muscle strips (equal to number of patients) studied. (Normal/**bold**) numbers represent the tissues used to study small and large intestine respectively. * mark significant reduction in muscle tone. # mark significant differences between small and large intestine.

The effects were more prominent in the large than in the small intestine and more pronounced in the circular than in the longitudinal muscle. The inhibitory effects of STW 5 were significantly higher in large than in small intestinal circular muscles: (64 µg/ml, $P = 0.04$), (128 µg/ml, $P = 0.03$), (256 µg/ml, $P = 0.01$), (512 µg/ml, $P = 0.01$), (768 µg/ml, $P \leq 0.001$) and (1024 µg/ml, $P = 0.04$).

Only in large intestine, the same concentration induced a significantly higher relaxation in circular than in longitudinal muscle: (64 µg/ml, $P = 0.02$), (128 µg/ml, $P = 0.01$), (256 µg/ml, $P = 0.009$), (512 µg/ml, $P = 0.02$), (768 µg/ml, $P = 0.01$) and (1024 µg/ml, $P = 0.04$). In contrary, small intestine showed no significant difference between circular and longitudinal muscle in response to STW 5: (64 µg/ml, $P = 0.8$), (128 µg/ml, $P = 0.4$), (256 µg/ml, $P = 1.0$), (512 µg/ml, $P = 0.7$), (768 µg/ml, $P = 0.5$) and (1024 µg/ml, $P = 0.9$).

Because the responses were more prominent in the circular muscle, the higher concentration of STW 5 (5120 µg/ml) was applied only to circular muscle of both small and large intestine. At this concentration which is still below the recommended therapeutic dose of 51.3 mg/ml, STW 5 evoked an immediate long lasting relaxation on small and large intestinal circular muscle. The relaxation was significantly higher in large than in small intestine 32 % vs. 16 % respectively ($P = 0.04$) (Figure 32).

Measurement of muscle layers thickness

Since the effect of STW 5 was more prominent in circular than in longitudinal muscle and significant only in large intestine, we hypothesized that these differences in responses could be attributed to different amounts of muscle between both layers. Accordingly, the thickness of each layer was assessed by fluorescent microscopy. The amount of each muscle layers was measured as the area of the respective layer in transverse sections. In both small and large intestine, the same assessed region showed a significantly thicker circular muscle layer than longitudinal muscle. In small intestine, the amount of longitudinal muscle layer represents 80 % of the circular muscle layer, whereas in large intestine the ratio was 39 % of the circular muscle (Figure 33). From these findings we could conclude that the layer-specific effects of STW 5 are due to different muscle thickness of each layer. At concentration of 1024 µg/ml, STW 5 response in longitudinal muscle was 34 % compared to its response in circular muscle. On the other hand, the lower differences between circular and longitudinal small intestinal muscle layers could explain the comparable effects of STW 5 in both layers of the small intestine.

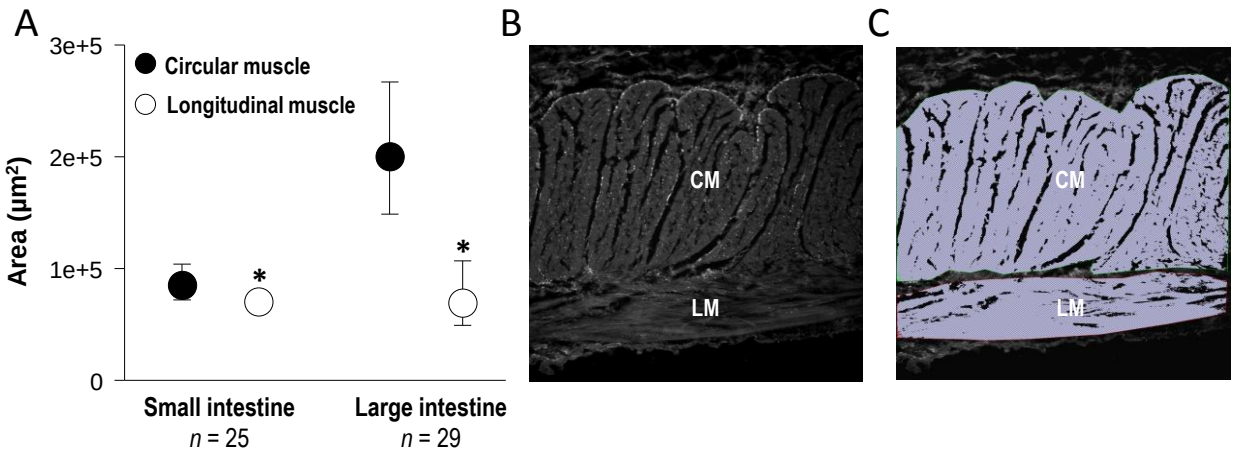

Figure 33: Circular muscle layer is thicker than longitudinal one. Panel (A) shows that in both small and large intestine, the amount of circular muscle is higher than longitudinal muscle. Panels (B) and (C) shows that same region in transverse section of descending colon has thicker circular muscle layer (CM) compared with longitudinal muscle layer (LM). Only muscle bundles marked as grey areas were assessed, black spots were excluded. *n* is the number of sections (equal to number of patients) studied. * mark significant difference ($P \leq 0.001$).

B. Effects of STW 5 on contractile amplitude

Application of different concentrations of STW 5 (64-1024 µg/ml) decreased the contractile amplitudes of human intestinal smooth muscle.

In small intestine, only higher concentration of 1024 µg/ml significantly reduced the contractile amplitudes of both circular and longitudinal muscles by 38 % and 52 % respectively. On the other hand, application of 64, 256, 768 and 1024 µg/ml STW 5 on large intestinal circular muscles significantly reduced the amplitudes of contractions by 37 %, 31 %, 70 % and 33 % respectively. None of the STW 5 concentrations affected the amplitude of the large intestinal longitudinal muscle.

At higher concentration of 5120 µg/ml, STW 5 exhibited a region dependent effect on the phasic contractility of different regions of the gut: duodenum, jejunum, ileum and large intestine. Because of the comparable effects on duodenum and jejunum, their data were pooled together. In duodenum and jejunum the contractile amplitude was significantly reduced by 68 %. In contrary, ileum showed an initial inhibition of 19 % followed by 27 % increase in the contractile amplitude however both were not significant (Figure 31). Application of STW 5 (5120 µg/ml) altered the phasic contractility pattern of the large intestinal circular muscle into clusters which was characterised by a transient large contractile

amplitude 52 % followed by inhibition of 72 %. For all concentrations, absolute values, percentage changes, number of tissues studied and *P* values are listed in Figure 35
Figure 35-Figure 41 and Tables 18-21 in the appendix.

C. Effects of STW 5 on contractile frequency

Application of different concentrations of STW 5 (64-1024 µg/ml) decreased the frequency of contractions of human intestinal smooth muscle.

In small intestinal circular muscle, application of 64, 256, 512 and 1024 µg/ml STW 5 significantly reduced the frequencies of contractions by 24 %, 27 %, 33 % and 38 % respectively, whereas in small intestinal longitudinal muscle only 1024 µg/ml STW 5 significantly decreased it by 41 %.

STW 5 (128, 512, 768 and 1024 µg/ml) significantly reduced the contractile rate of large intestinal circular muscle by 16 %, 17 %, 52 % and 22 % respectively. Only the higher concentration of 1024 µg/ml STW 5 significantly reduced the contractile frequency of large intestinal longitudinal muscle by 40 %. Despite STW 5 (5120 µg/ml) non-significantly changed the contractile frequencies of duodenum, jejunum and ileum, it induced an initial significant increase in the contractile frequencies of the large intestine by 80 % followed by 57 % inhibition.

For all concentrations, Absolute values, percentage changes, number of tissues studied and *P* values are listed in Figure 35
Figure 35-Figure 41 and Tables 18-21 in the appendix.

D. Effects of STW 5 on motility index

Application of different concentrations of STW 5 (64-1024 µg/ml) altered the phasic contractility of human intestinal smooth muscle. Phasic contractility was expressed as motility index which is the contractile amplitude multiplied by the frequency of contraction per minute.

At concentrations of 512 µg/ml and 1024 µg/ml, STW 5 decreased the phasic contractility in small intestinal circular muscles by 41 % and 63 % respectively, whereas in small intestinal longitudinal muscles only STW 5 (1024 µg/ml) reduced it by 61 %.

In human large intestinal circular muscle application of STW 5 (64, 128, 256, 512, 768 and 1024) significantly reduced the motility indexes by 27 %, 33 %, 43 %, 36 %, 69 % and 33% respectively. Again none of STW 5 concentrations affected the amplitude of the large

intestinal longitudinal muscle. At concentration of 5120 µg/ml, STW 5 significantly suppressed the phasic contractility of duodenum and jejunum by 66 % whilst remained unchanged in ileum. The contractility pattern of the large intestinal circular muscle was changed into clusters characterized by large transient increase in motility index by 159 % followed by inhibition of 58 % (three out of 11 tissues showed continuous cluster formations). For all concentrations, Absolute values, percentage changes, number of tissues studied and *P* values are listed in Figure 35-Figure 40 and Tables 18-21 in the appendix.

Effects of STW 5 on electrical field stimulation

Electrical field stimulation (EFS) was used to verify tissue viability and to study the effects of STW 5 on nerve-mediated contractions. With the chosen stimulation parameters (see Materials and Methods section), EFS induced nerve mediated, biphasic contractile responses. First an on-response during EFS followed by an off-response immediately after cessation of EFS pulses. Both on- and off-responses were reproducible before and after STW 5 application (Figure 34). For absolute values, number of the tissues studied and *P* values see Table 32, Table 33, Table 34and Table 35 in the appendix.

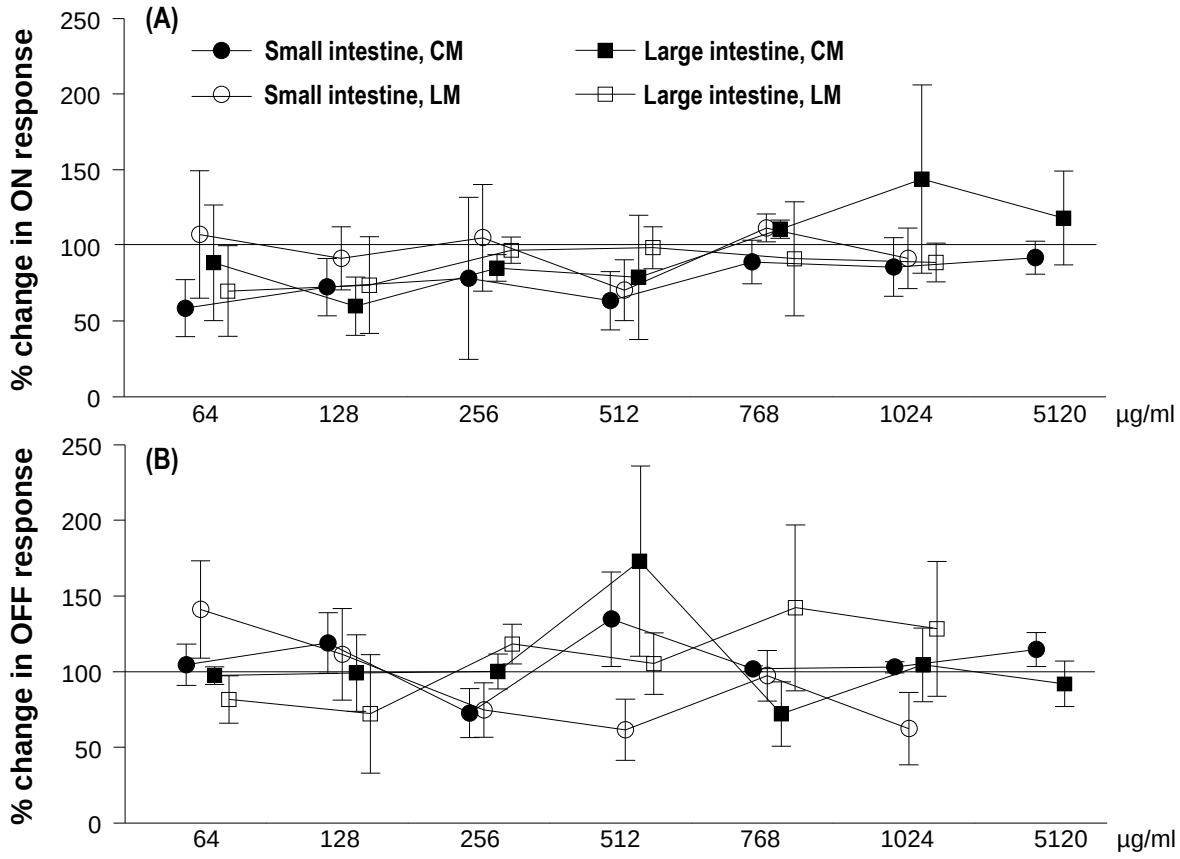

Figure 34: STW 5 had no influence on nerve mediated contractile on- and off-responses evoked by EFS in human intestinal smooth muscles. (A) The on-responses during the EFS remained unchanged in all applied concentrations of STW 5 (64-5120 µg/ml) at different kinds of muscle strips. (B) The off-responses which started after cessation of the EFS were reproducible before and after STW 5 (64-5120 µg/ml). STW 5 (5120 µg/ml) was performed only in circular muscles. CM indicates circular muscle whereas LM indicated longitudinal muscle. To avoid overlapping symbols, the values representing each concentration are slightly shifted.

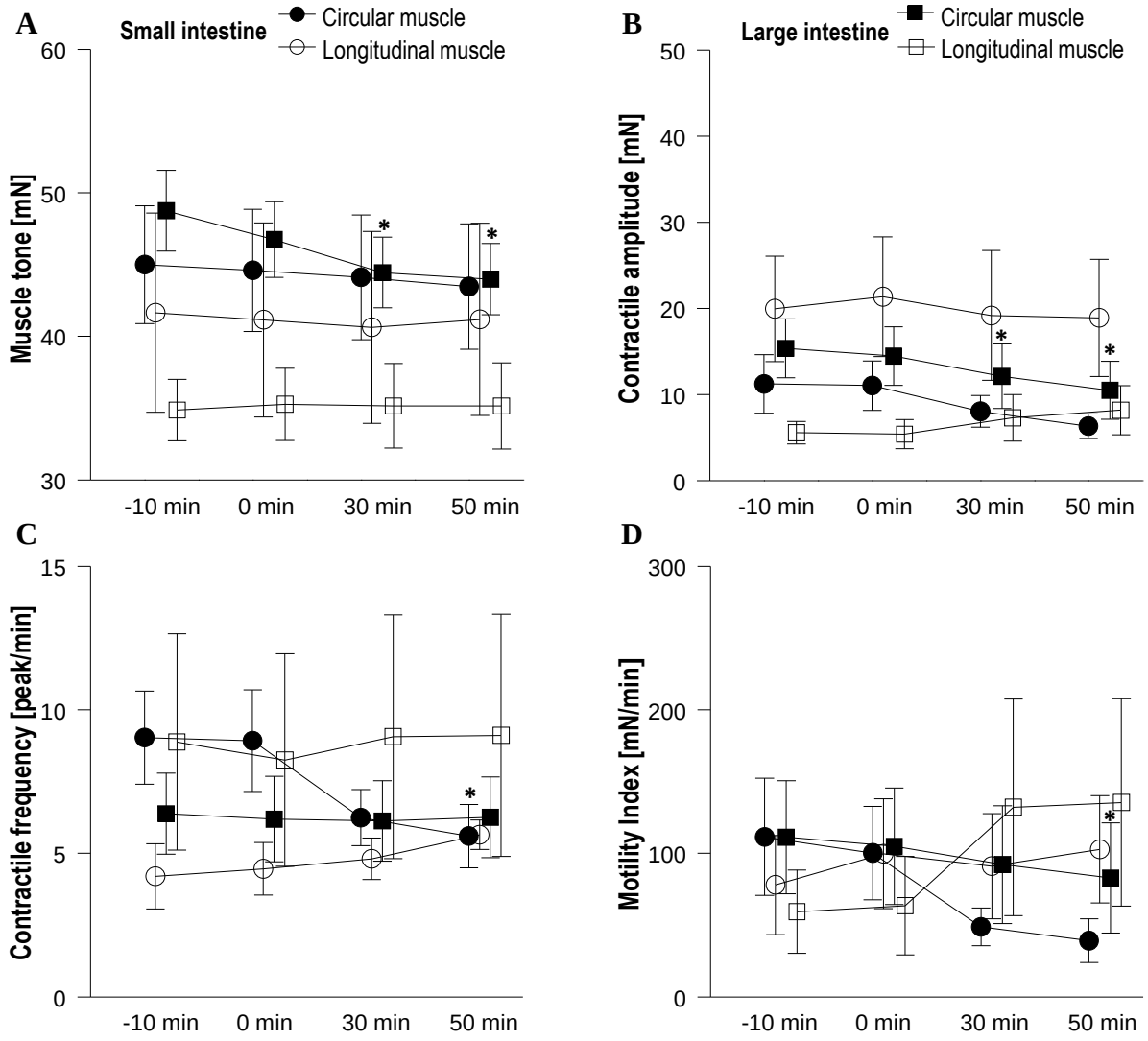

Figure 35: The effects of STW 5 (**64 µg/ml**) on human intestinal motility. Circular (CM) and longitudinal (LM) muscle strips from small and large intestine were used. STW 5 was applied at t=0 min. Post-treatment changes in muscle tone (A), contractile amplitude (B), frequency of contractions (C) and phasic contractility of the muscle (D) were recorded and compared with pre-application. * mark the significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

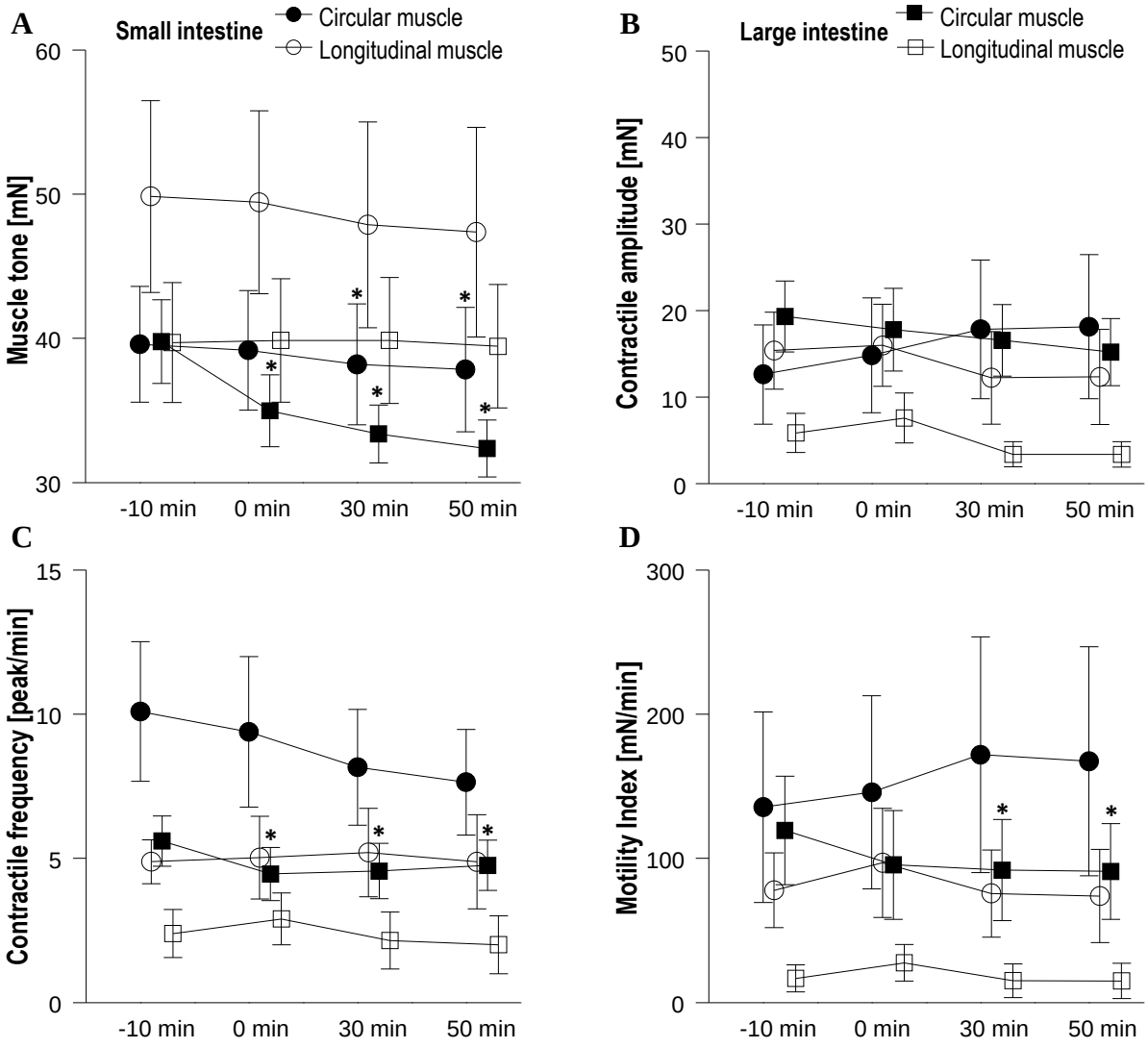

Figure 36: The effects of STW 5 (**128 µg/ml)** on human intestinal motility. Circular (CM) and longitudinal (LM) muscle strips from small and large intestine were used. STW 5 was applied at t=0 min. Post-treatment changes in muscle tone (A), contractile amplitude (B), frequency of contractions (C) and phasic contractility of the muscle (D) were recorded and compared with pre-application. * mark the significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

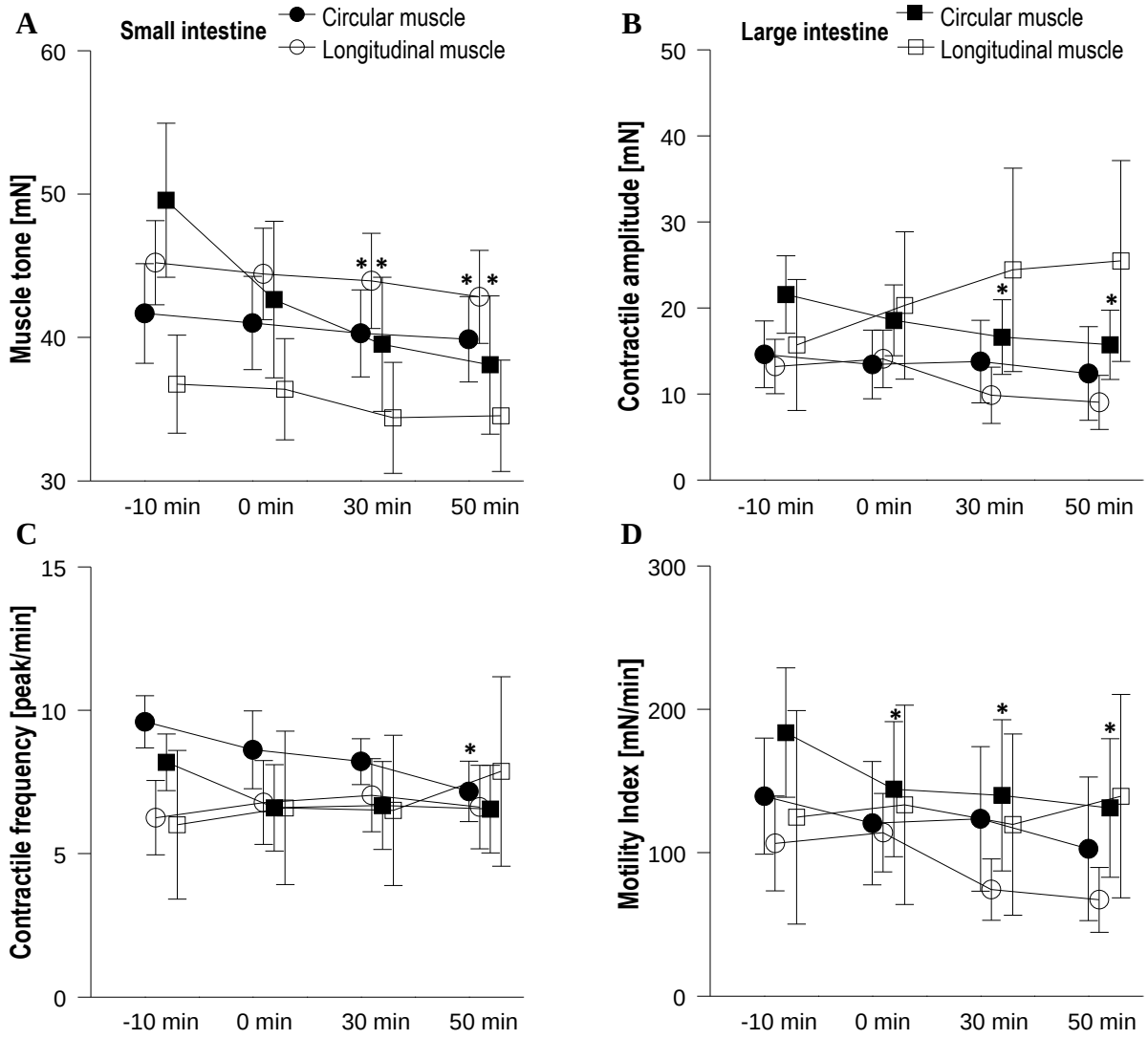

Figure 37: The effects of STW 5 (**256 µg/ml**) on human intestinal motility. Circular (CM) and longitudinal (LM) muscle strips from small and large intestine were used. STW 5 was applied at t=0 min. Post-treatment changes in muscle tension (A), amplitude of contractions (B), frequency of contractions (C) and phasic contractility of the muscle (D) were recorded and compared with pre-application. * mark the significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

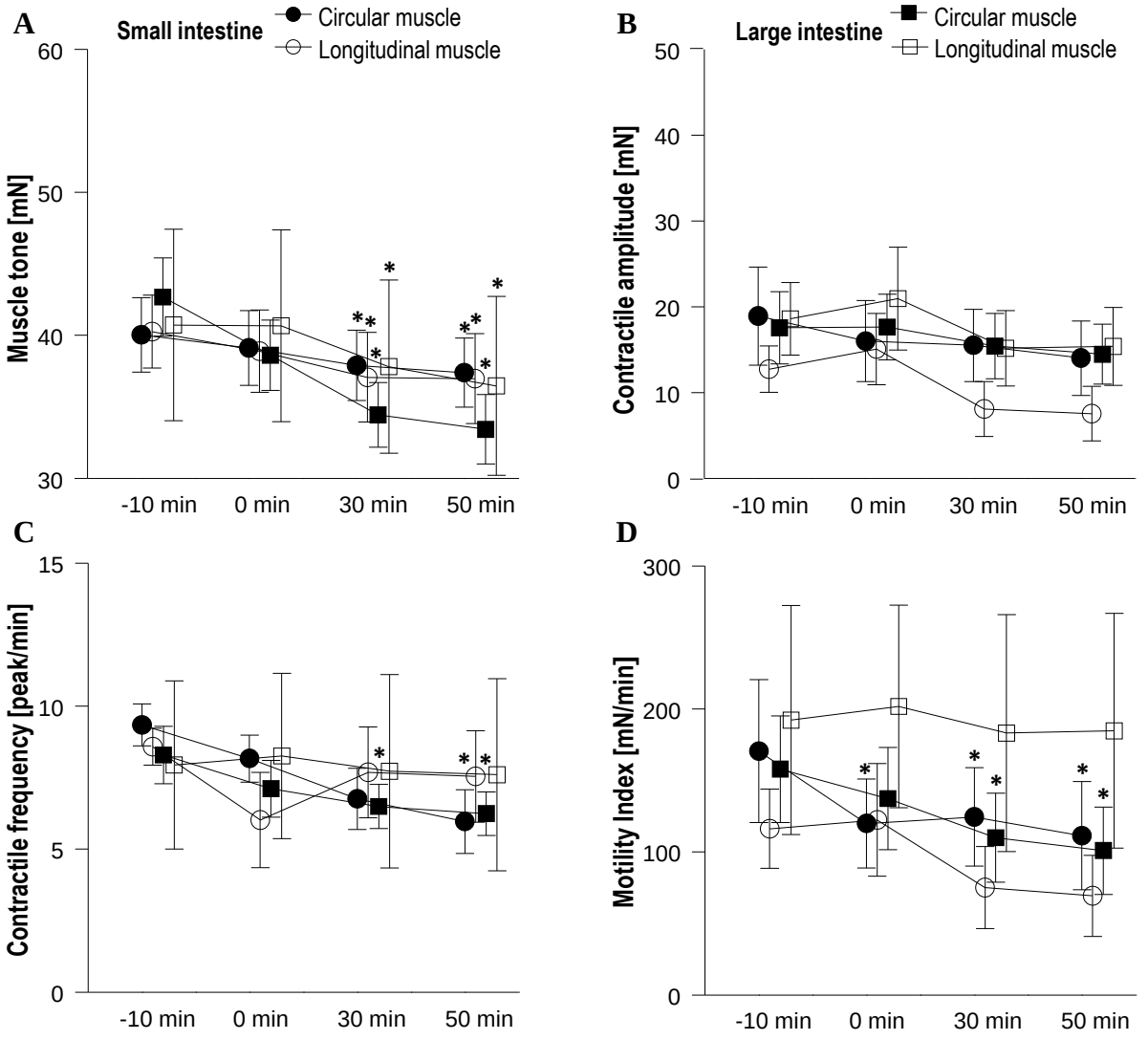

Figure 38: The effects of STW 5 (**512 µg/ml**) on human intestinal motility. Circular (CM) and longitudinal (LM) muscle strips from small and large intestine were used. STW 5 was applied at t=0 min. Post-treatment changes in muscle tension (A), amplitude of contractions (B), frequency of contractions (C) and phasic contractility of the muscle (D) were recorded and compared with pre-application. * mark the significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

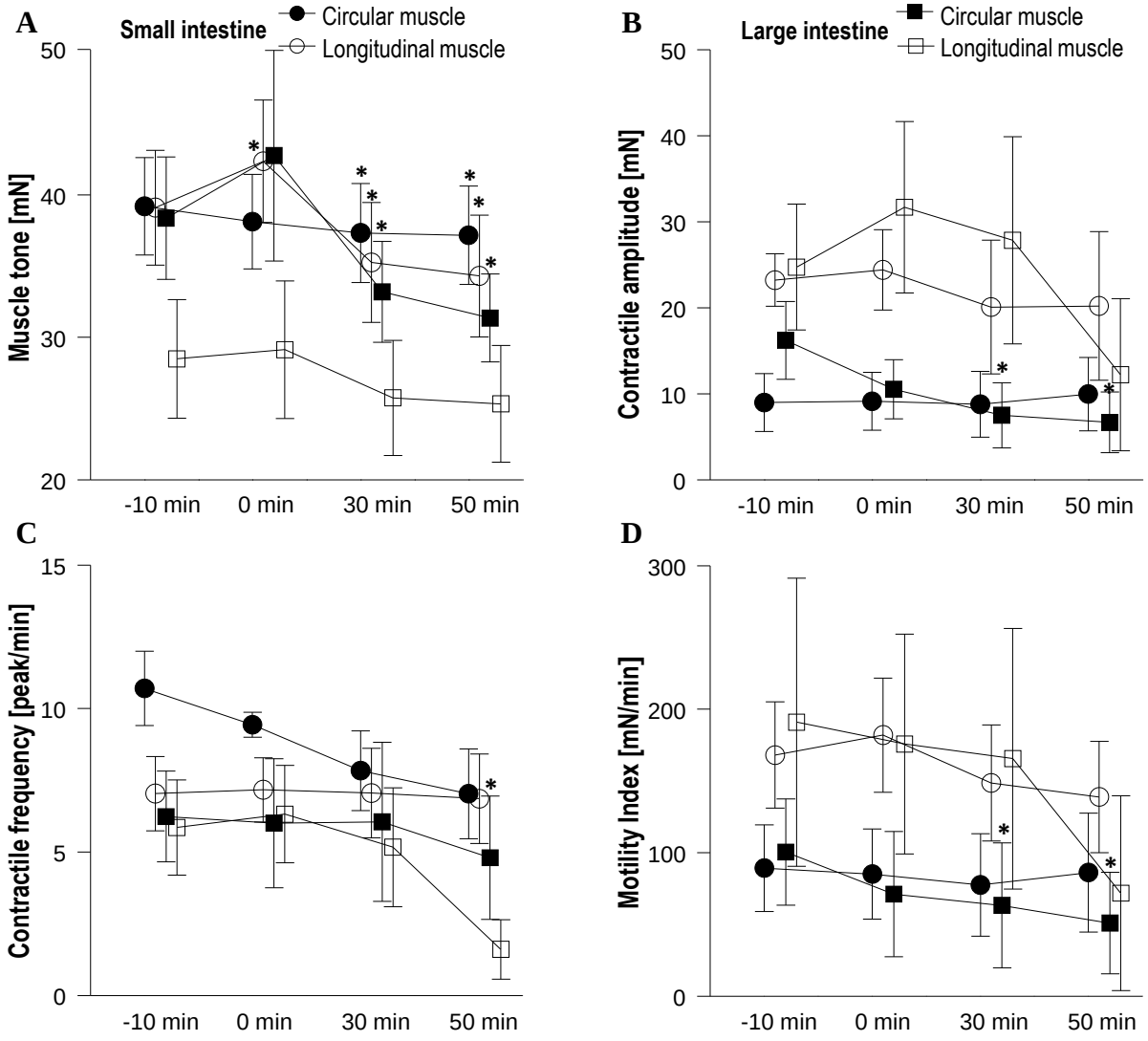

Figure 39: The effects of STW 5 (**768 µg/ml**) on human intestinal motility. Circular (CM) and longitudinal (LM) muscle strips from small and large intestine were used. STW 5 was applied at t=0 min. Post-treatment changes in muscle tension (A), amplitude of contractions (B), frequency of contractions (C) and phasic contractility of the muscle (D) were recorded and compared with pre-application. * mark the significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

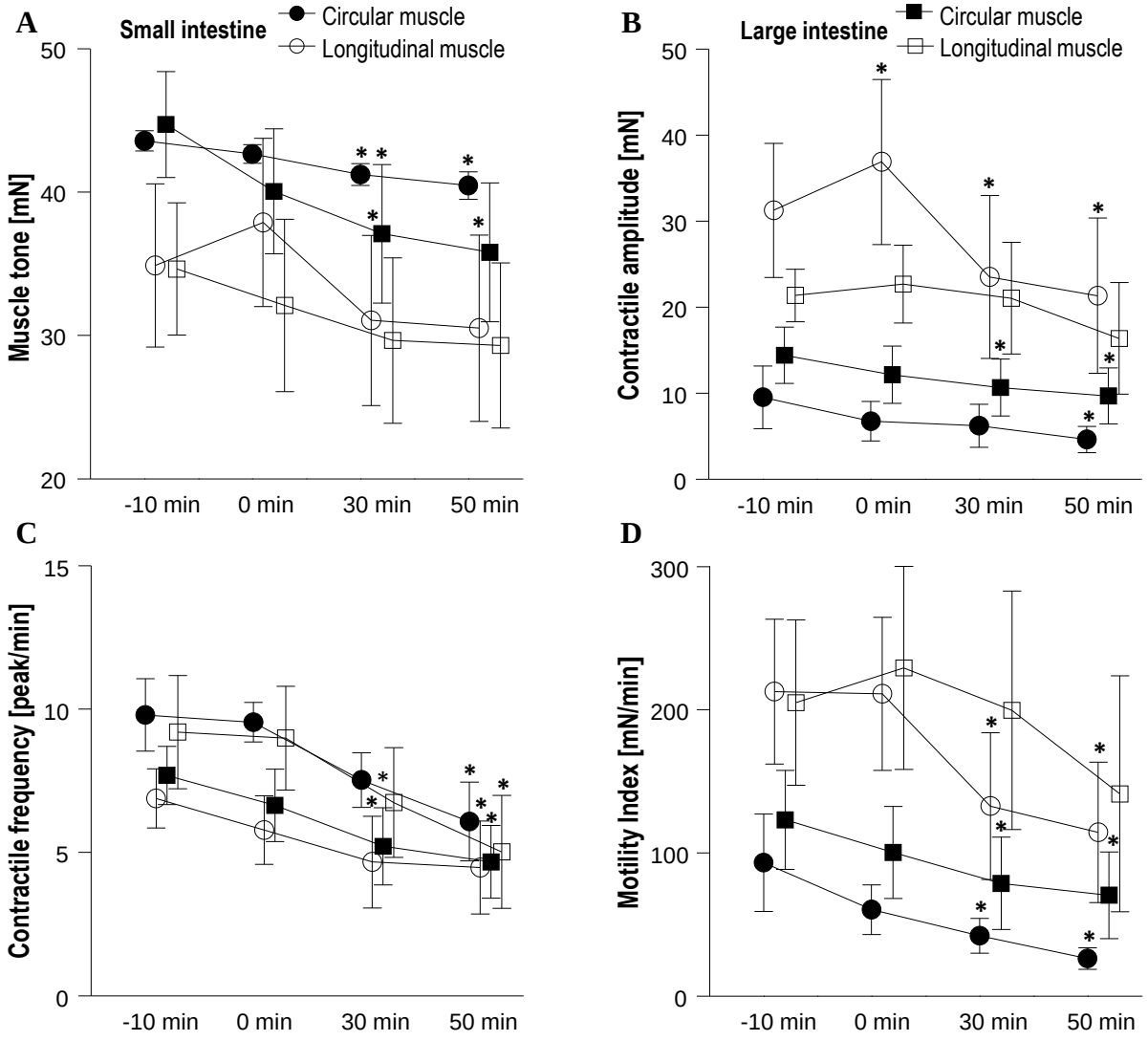

Figure 40: The effects of STW 5 (**1024 µg/ml**) on human intestinal motility. Circular (CM) and longitudinal (LM) muscle strips from small and large intestine were used. STW 5 was applied at t=0 min. Post-treatment changes in muscle tension (A), amplitude of contractions (B), frequency of contractions (C) and phasic contractility of the muscle (D) were recorded and compared with pre-application. * mark the significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

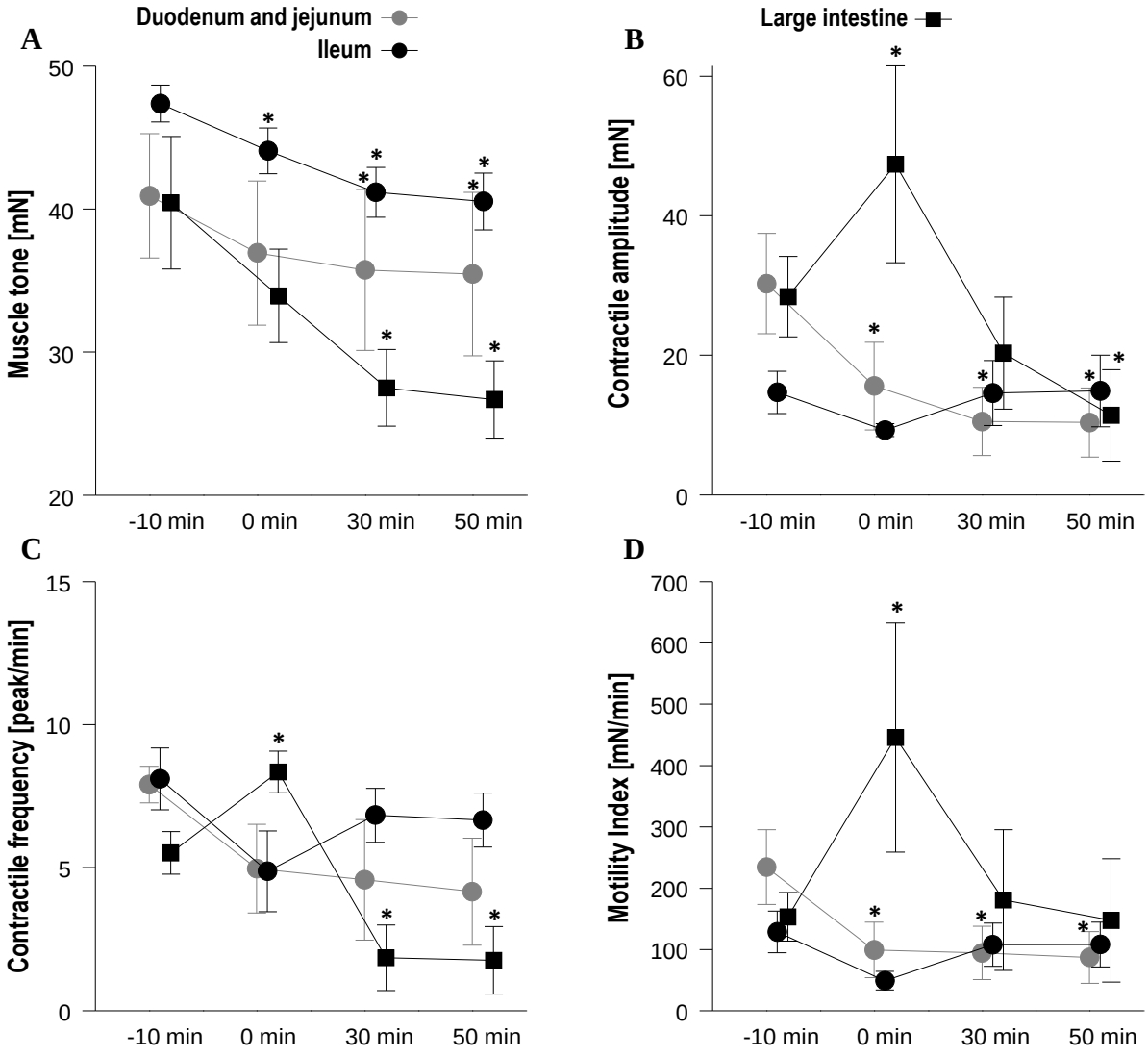

Figure 41: STW 5 (**5120 µg/ml**) has region-specific effects on human intestinal circular muscle. Panel (A) shows that STW 5 relaxed intestinal muscle tone. The relaxation was significantly higher in large than in small intestine. Panel (B) shows that STW 5 significantly decreased contractile amplitude in duodenum and jejunum whereas induced large transient increase in large intestinal amplitude. Panel (C) shows that the contractile frequency was initially increased in large intestine and remains unchanged in other regions. Panel (D) shows that the phasic contractility of large intestine was transiently increased whereas significantly inhibited in duodenum and jejunum and remain unchanged in ileum. * mark the significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

3.2.2 Effects of STW 5 components on human intestinal motility

The rationale for this part of the study is that after exploring the effects of STW 5 on human intestinal motility, we would like to investigate which extract(s) may be responsible for these actions. In this study, all nine individual components were applied on human intestinal circular muscle dissected from both small and large intestine. Extracts were added non-cumulatively at concentrations that corresponded to their concentrations in STW 5 (512 and 5120 µg/ml).

Briefly, at concentrations corresponding to their concentrations in 5120 µg/ml STW 5, apart from bitter candytuft and milk thistle, all other extracts contributed to STW 5 activities.
In small intestine: angelica, peppermint, liquorice, chamomile and caraway significantly relaxed muscle tone and inhibited phasic contractility of smooth muscle. Greater celandine significantly increased the motility index in duodenum and jejunum whereas it decreased the motility index in the ileum. Lemon balm did not affect the motility index (Figure 42).
In large intestine: angelica, peppermint and liquorice nearly mimicked the inhibitory effects of STW 5 on both tonic and phasic contractions whereas chamomile and lemon balm inhibited only muscle tone. Greater celandine increased muscle tone significantly without affecting phasic contractility of the large intestinal circular muscle (Figure 42).
The relaxation of the muscle tone started immediately after drug application within 5 ± 2 min. The onset of the reduction in phasic contractility was variable. In some extracts like peppermint it started immediately after application whereas in extracts it decreased gradually and peaked at different time intervals. Similar to STW 5 experiments, four different periods were analysed: immediately before application (-10 min), immediately after (0 min) and 30 and 50 min after extract application. Each analysed interval was 10 min long. These analysed intervals were statistically tested against the pre-STW 5 application, -10 min. Analysed parameters were: A) muscle tone, B) contractile amplitude, C) frequency of contractions and D) motility index. Throughout the following sections, only significant data at 50 min will be described whilst other analysed periods are demonstrated in the corresponding tables and the figures.

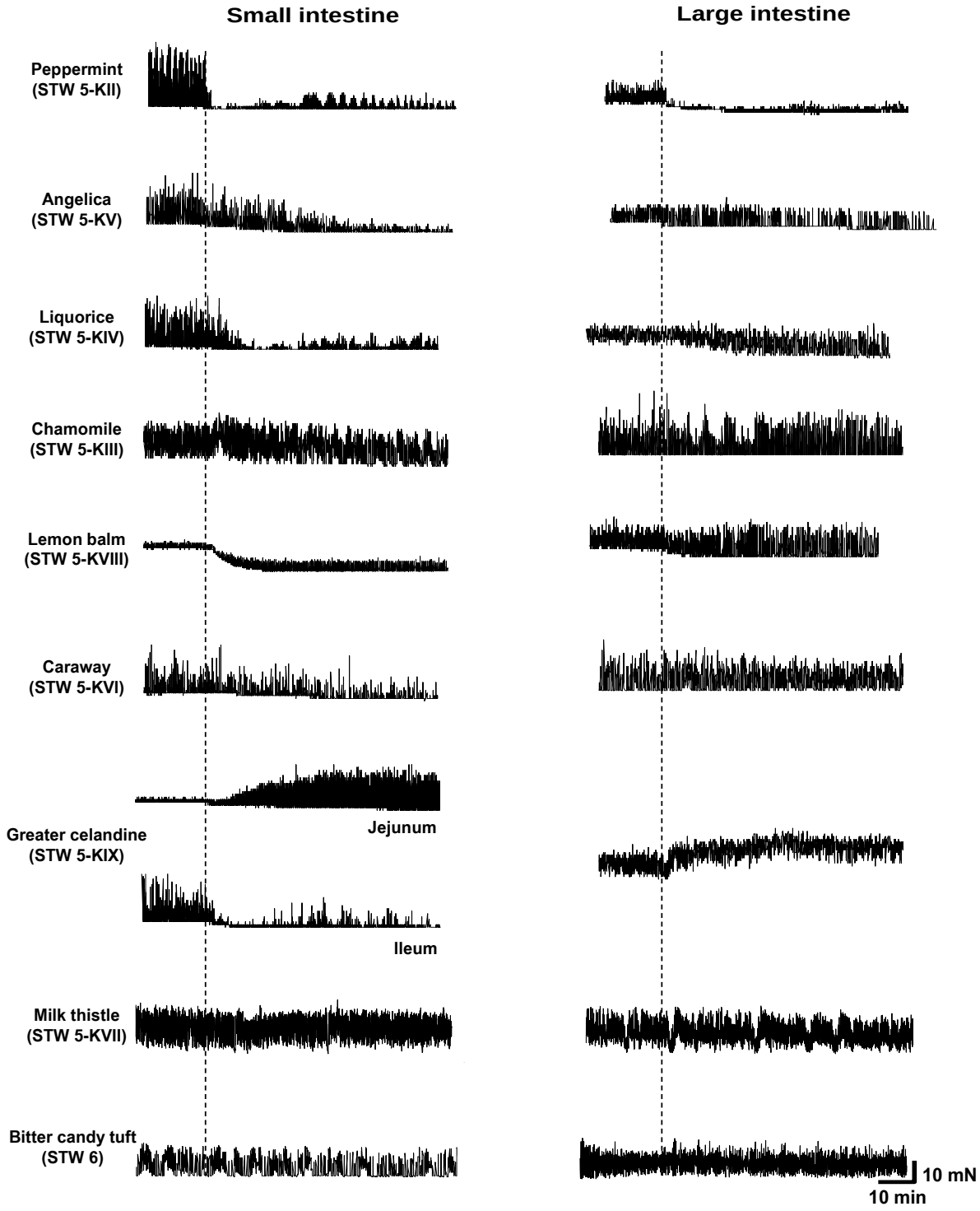

Figure 42: Representative traces for the effects of STW 5 individual components on human intestinal circular muscles from small intestine (left panel) and large intestine (right panel). The extracts were applied at final concentrations corresponding to their concentrations in STW 5 (5120 µg/ml) in the organ baths containing the muscle strips. The traces were taken from different experiments. The scale bar in the lower right corner applies to all traces. The dashed lines indicate the time of drug application.

Angelica

Application of 89.5 µg/ml angelica corresponding to STW 5 (512 µg/ml) did not change neither the muscle tone nor the motility index of small intestine whereas the tenfold higher concentration significantly inhibited both parameters by 9 % and 62 % respectively.

However, in large intestinal circular muscles angelica (89.5 µg/ml) reduced muscle tone and motility index by 6 % and 44 % respectively whilst the higher concentration of 895 µg/ml inhibited both by 39 % and 33 % respectively (Figure 42, Figure 43 and Table 22 in the appendix).

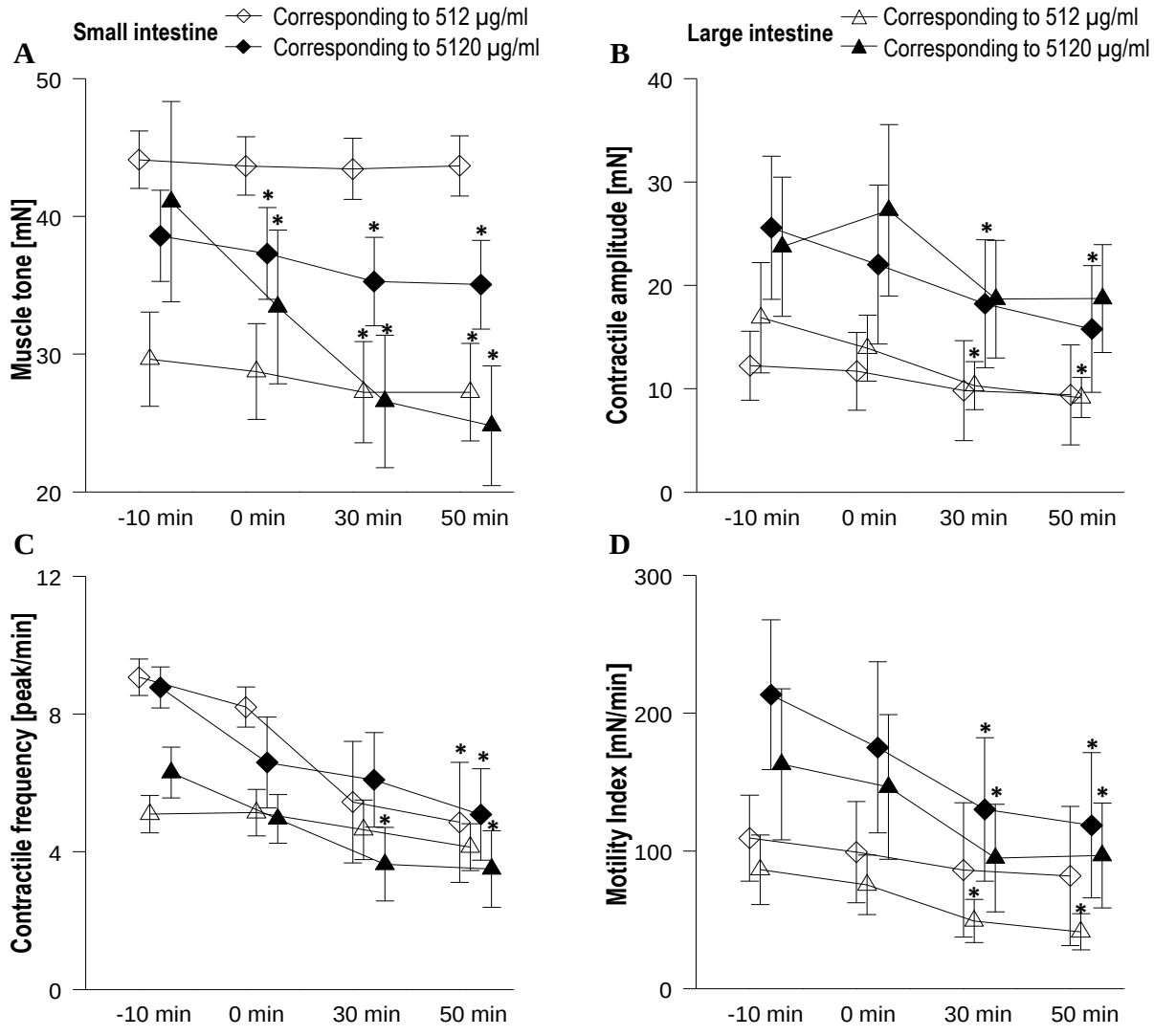

Figure 43: The effects of **angelica** on human intestinal motility. Angelica was applied at concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. * mark significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Peppermint

The peppermint concentration (37.2 µg/ml) that corresponded to 512 µg/ml STW 5 had no influences on neither the muscle tone nor the motility index of small intestinal circular muscle. Both parameters were reduced in tenfold higher concentration by 7 % and 52 % respectively. Both peppermint concentrations corresponding to 512 and 5120 µg/ml STW 5 significantly reduced the muscle tone of the large intestine by 19 % and 35 % respectively and inhibited the phasic contractility by 40 % and 62 % respectively (Figure 42, Figure 44 and Table 23 in the appendix).

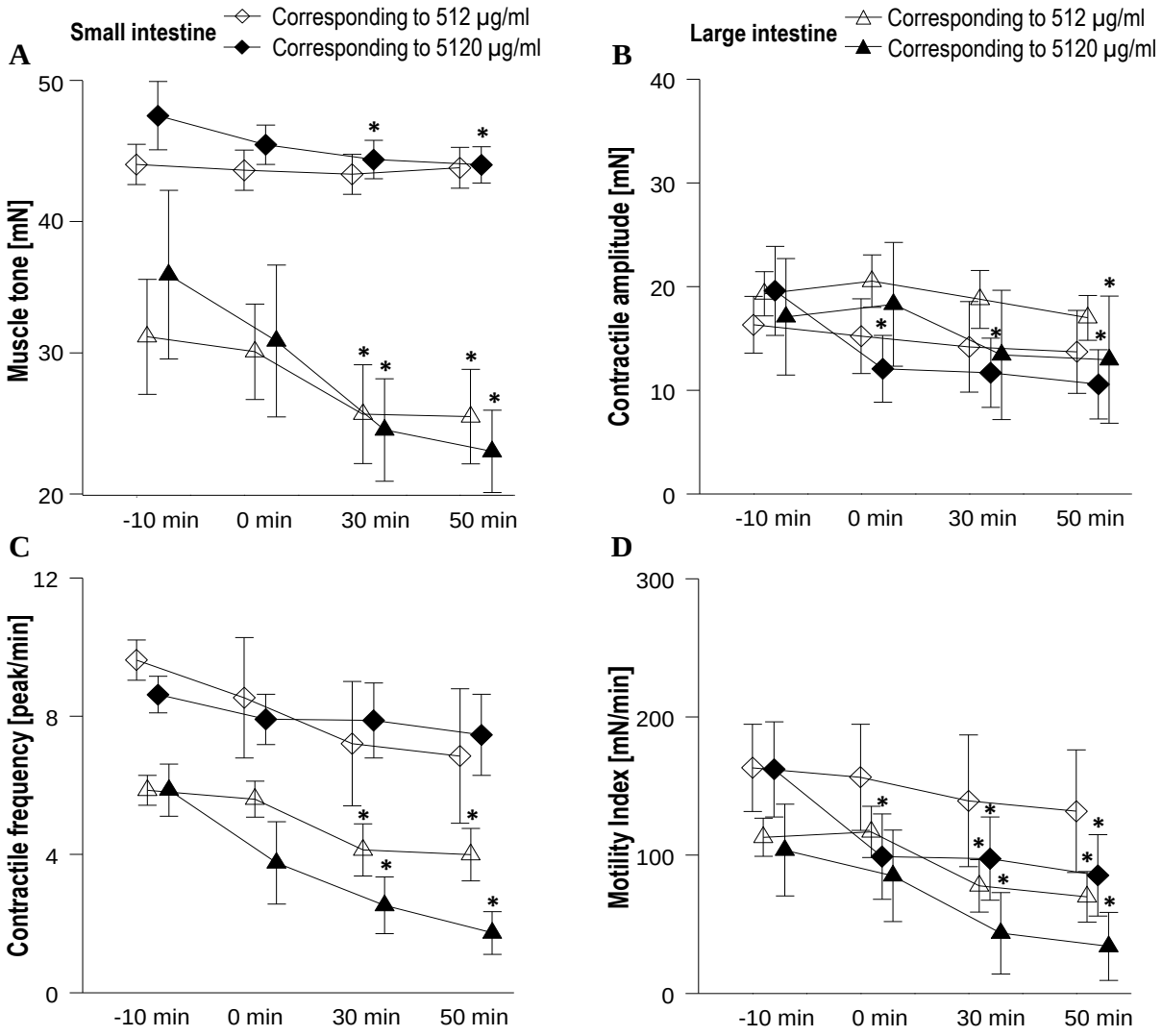

Figure 44: The effects of **peppermint** on human intestinal motility. Peppermint was applied at concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. * mark significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Liquorice

At a concentration of 80.1 µg/ml that corresponded to 512 µg/ml STW 5, liquorice reduced only the phasic contractions of small intestine by 41 % without affecting the muscle tone. The tenfold higher concentration reduced both muscle tone and motility index by 7 % and 67 % respectively.

In large intestine, liquorice (80.1 µg/ml) had no influence on both muscle tone and motility index. A concentration of 801 µg/ml evoked significant inhibition in muscle tone and motility index by 15 % and 48 % respectively (Figure 42, Figure 45 and Table 24 in the appendix).

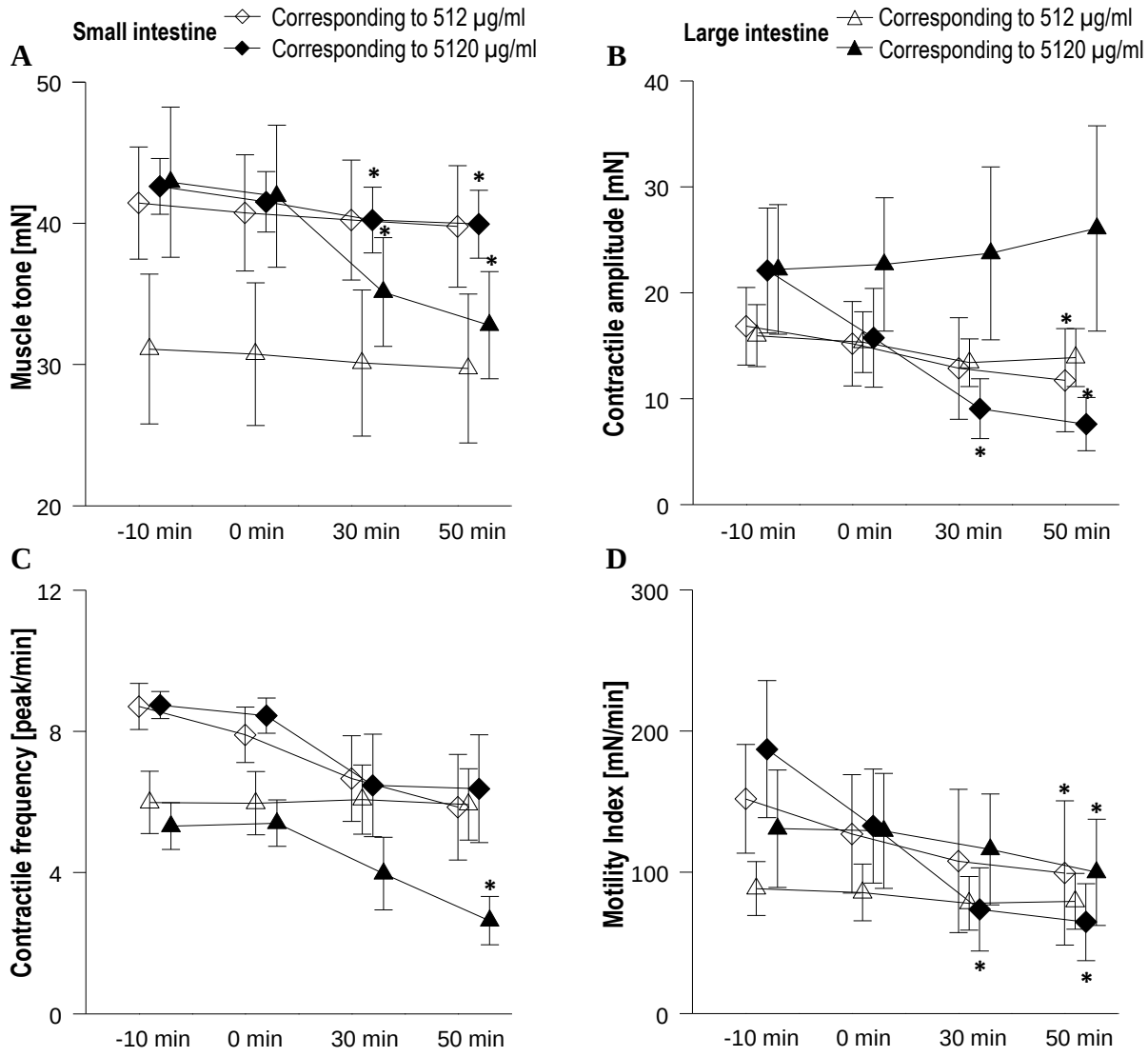

Figure 45: The effects of **liquorice** on human intestinal motility. Liquorice was applied at concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. * mark significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Chamomile

Only the muscle tone of small intestine was significantly reduced by 3 % after application of chamomile (114.3 µg/ml) which corresponded to its concentration in STW 5 (512 µg/ml), without affecting the motility index. At a concentration of 1143 µg/ml, both muscle tone and motility index were reduced by 9 % and 45 % respectively. Neither muscle tone nor motility index of large intestine was affected by lower concentration of chamomile where higher concentration significantly inhibited only the muscle tone by 13 % (Figure 42, Figure 46 and Table 25 in the appendix).

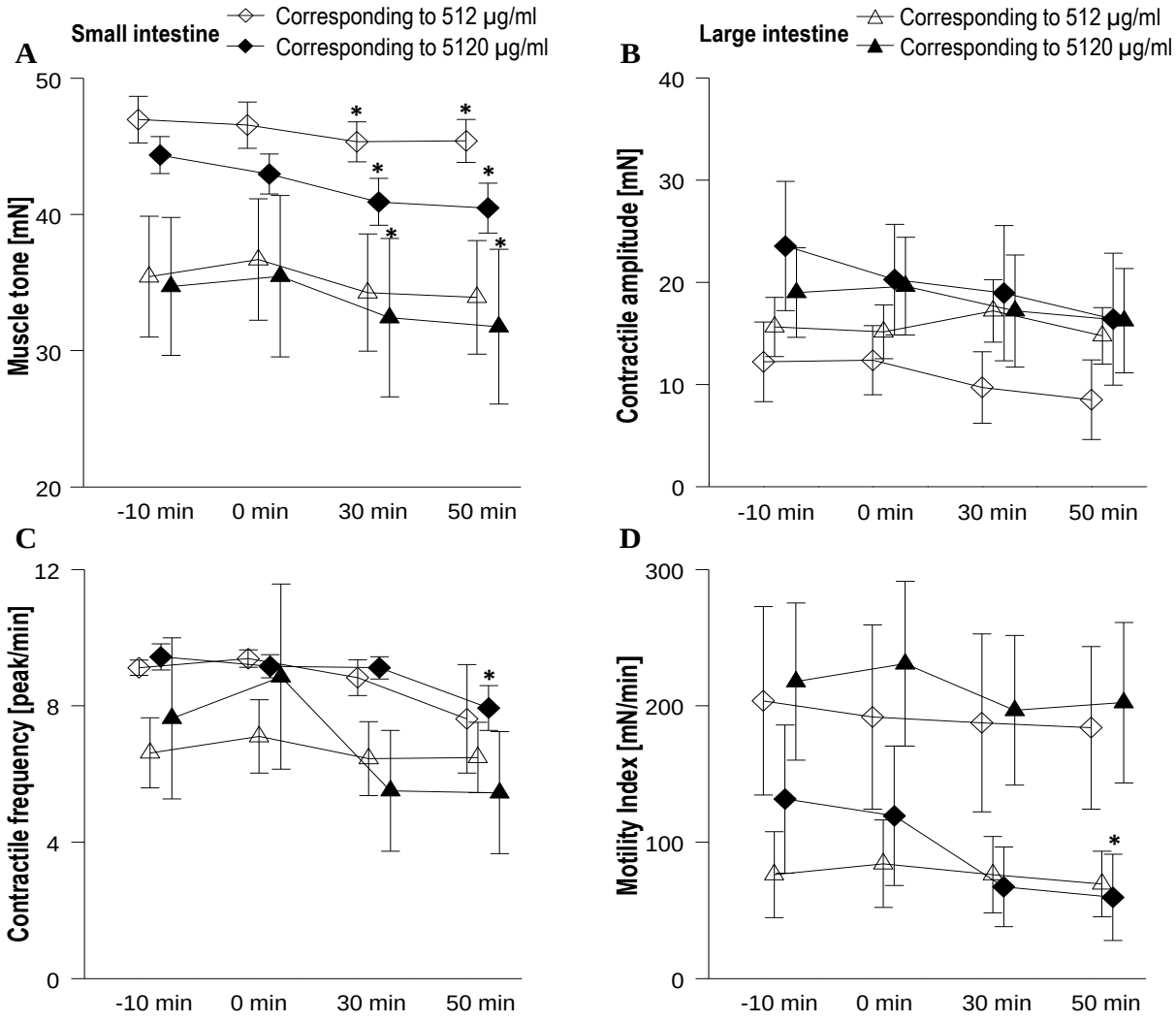

Figure 46: The effects of **chamomile** on human intestinal motility. Chamomile was applied in concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. * mark significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Lemon balm

Lemon balm at a concentration of 57.9 µg/ml that corresponded to STW 5 (512 µg/ml) had no influences on the muscle tone and motility index of either small or large intestinal muscle. Tenfold higher concentrations of 579 µg/ml reduced only muscle tone of both small and large intestinal circular muscle by 4 % and 20 % respectively. Phasic contractions were not affected in both concentrations (Figure 42, Figure 47 and Table 26 in the appendix).

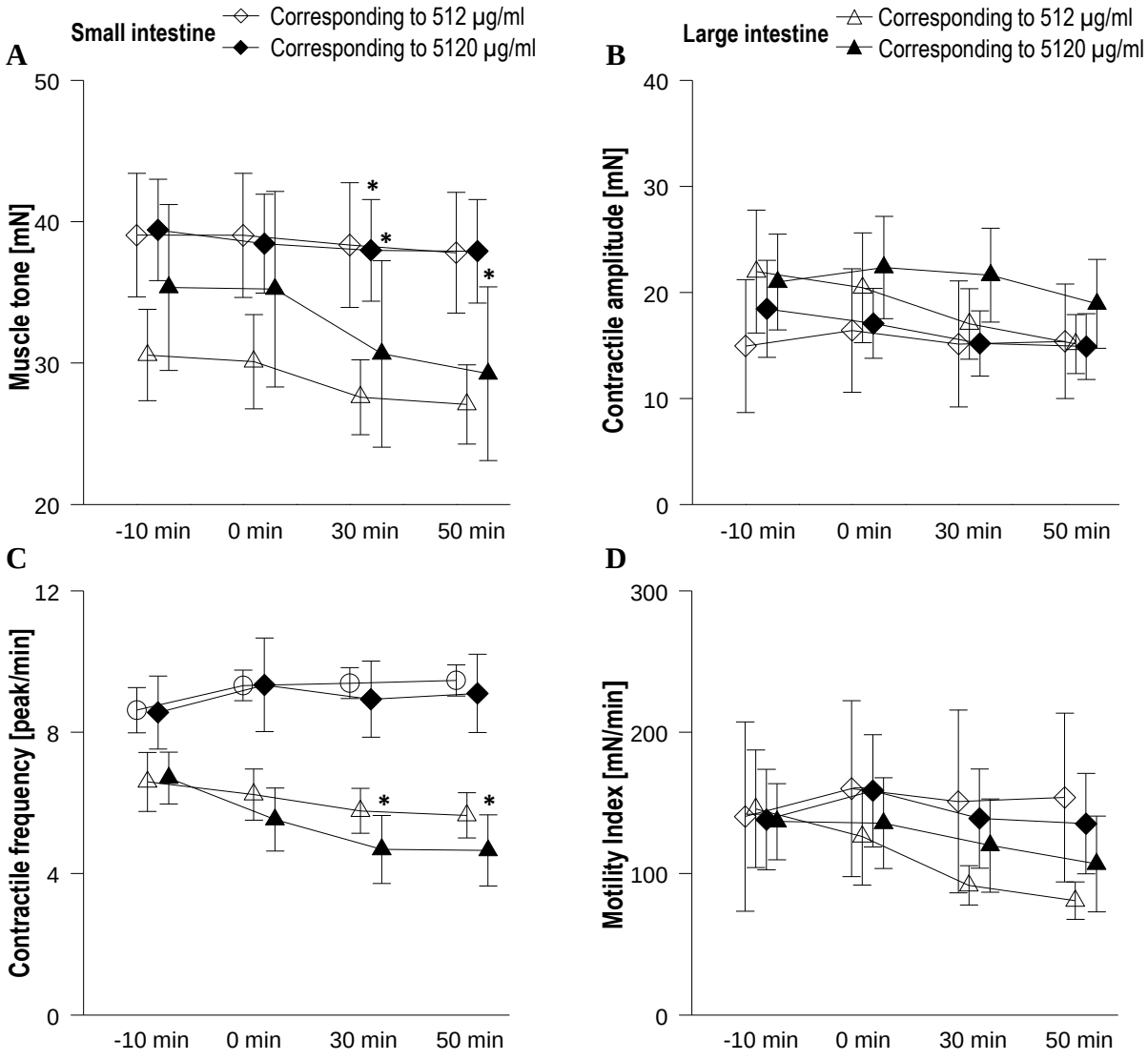

Figure 47: The effects of **lemon balm** on human intestinal motility; Lemon balm was applied at concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. * mark significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Caraway

Caraway (28.4 µg/ml) corresponding to 512 µg/ml STW 5 significantly inhibited phasic contractions of small intestine by 38 %. However, the tenfold higher concentration inhibited both muscle tone and motility index by 4 % and 33 % respectively. Large intestinal smooth muscle activities were not affected by either concentration (Figure 42, Figure 48 and Table 27 in the appendix).

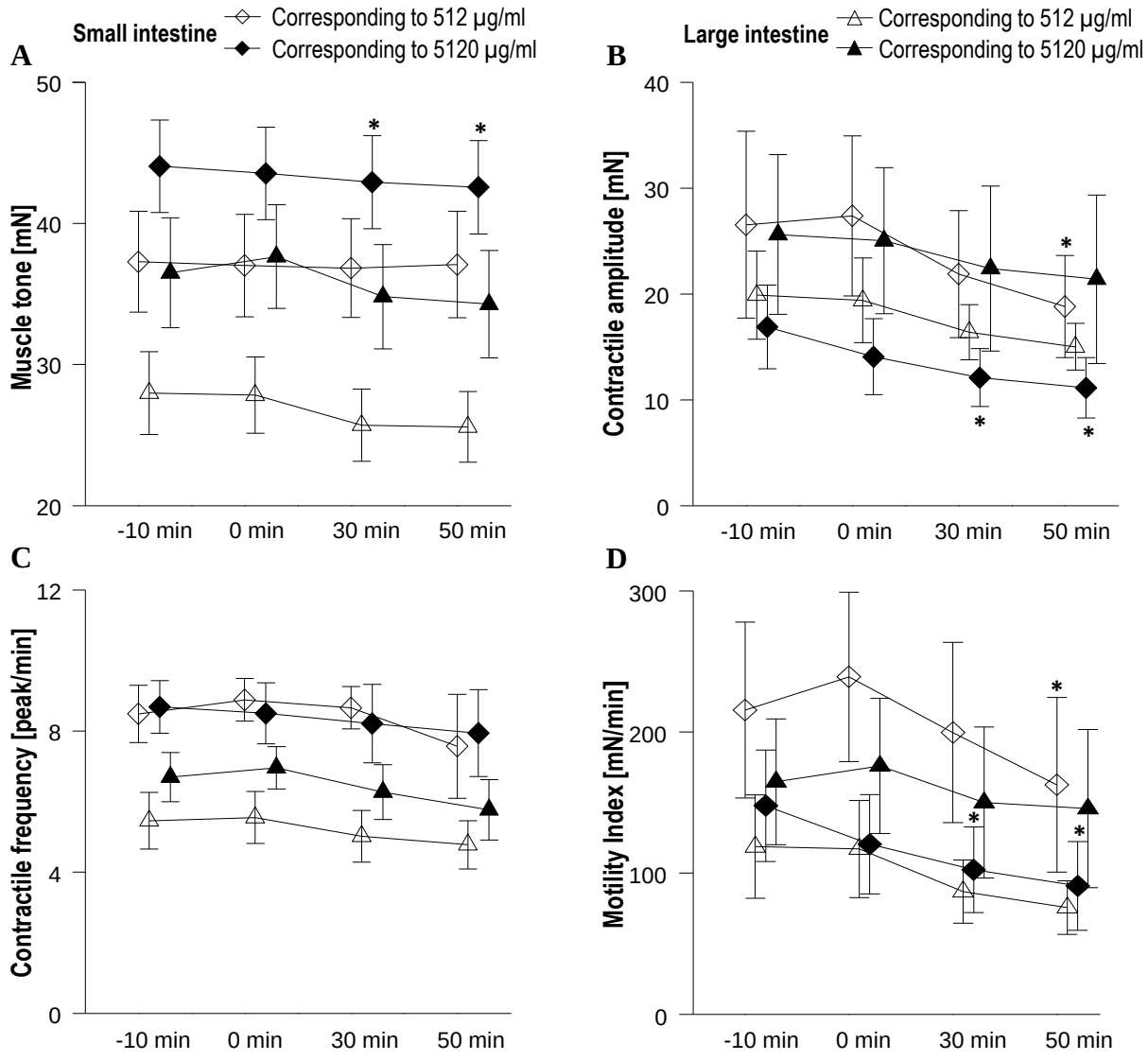

Figure 48: The effects of **caraway** on human intestinal motility; Caraway was applied at concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. * mark significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Greater celandine

Only at tenfold higher concentration corresponding to 5120 µg/ml STW 5, greater celandine (629 µg/ml) exhibited region-specific effects on human intestine. Because duodenum (D) and jejunum (J) behave similarly, their data were pooled together. Despite G. celandine relaxed the muscle tone of D+J and ileum by 9 % and 5 % respectively, it significantly increased the phasic contractility in D+J by 119 % whilst inhibited it in ileum by 36 %. The inhibition of ileum contractions was immediate and long lasting whereas the activation of D+J started slowly, peaked after 30 min and declined slowly. In large intestine, G. celandine induced immediate long lasting increase in muscle tone by 18 % without altering the phasic contractility Figure 42, Figure 49 and Table 28 in the appendix). At the lower concentration of 62.9 µg/ml, G. celandine did not affect human intestinal motility.

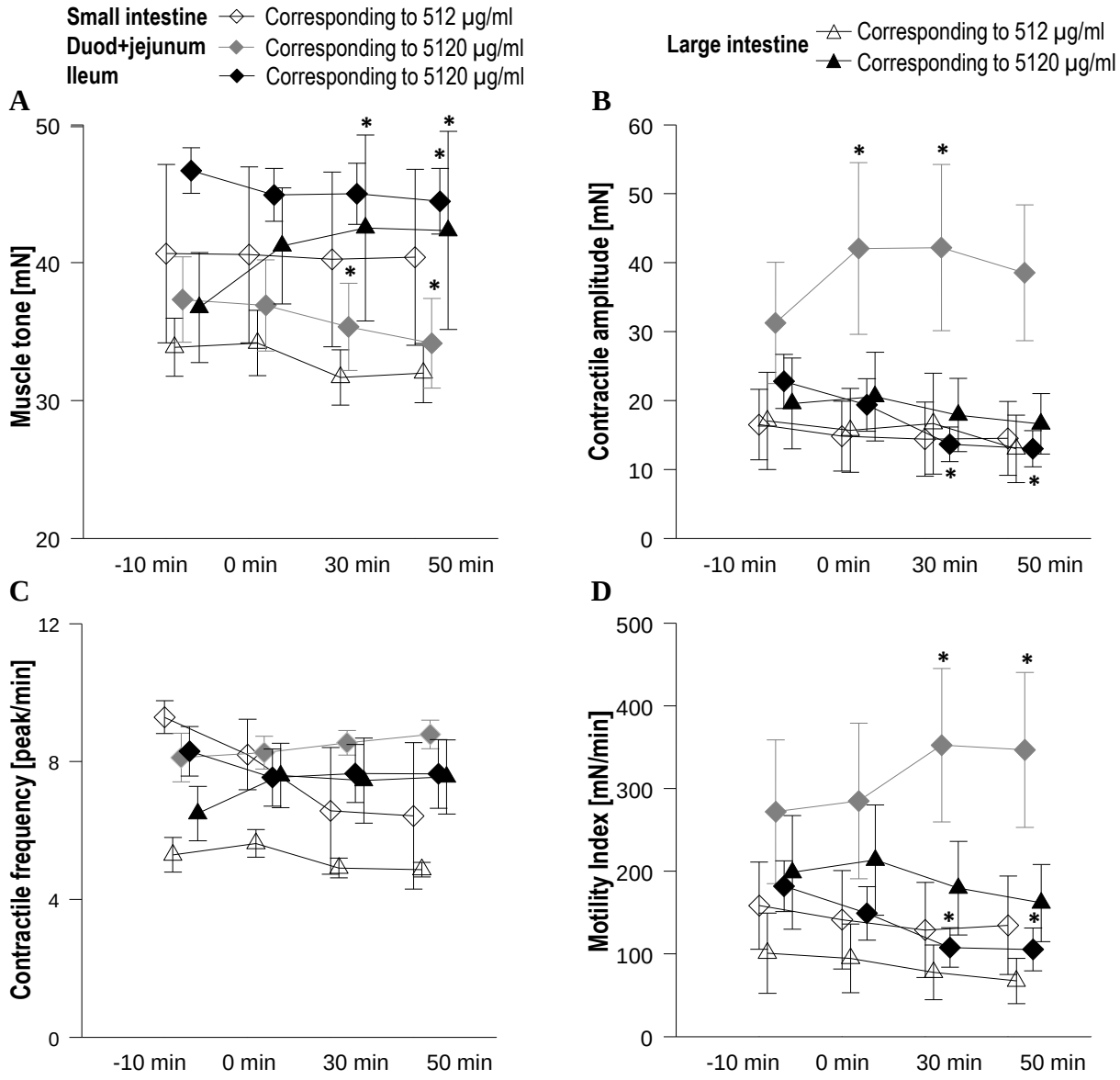

Figure 49: The effects of **greater celandine** on human intestinal motility; Greater celandine was applied in concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. * mark significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Milk thistle

Both concentrations of milk thistle (14.4 and 144 µg/ml) that corresponded to its concentration in STW 5 (512 and 5120 µg/ml) respectively had no effect on human intestinal motility (Figure 42, Figure 50and Table 29 in the appendix).

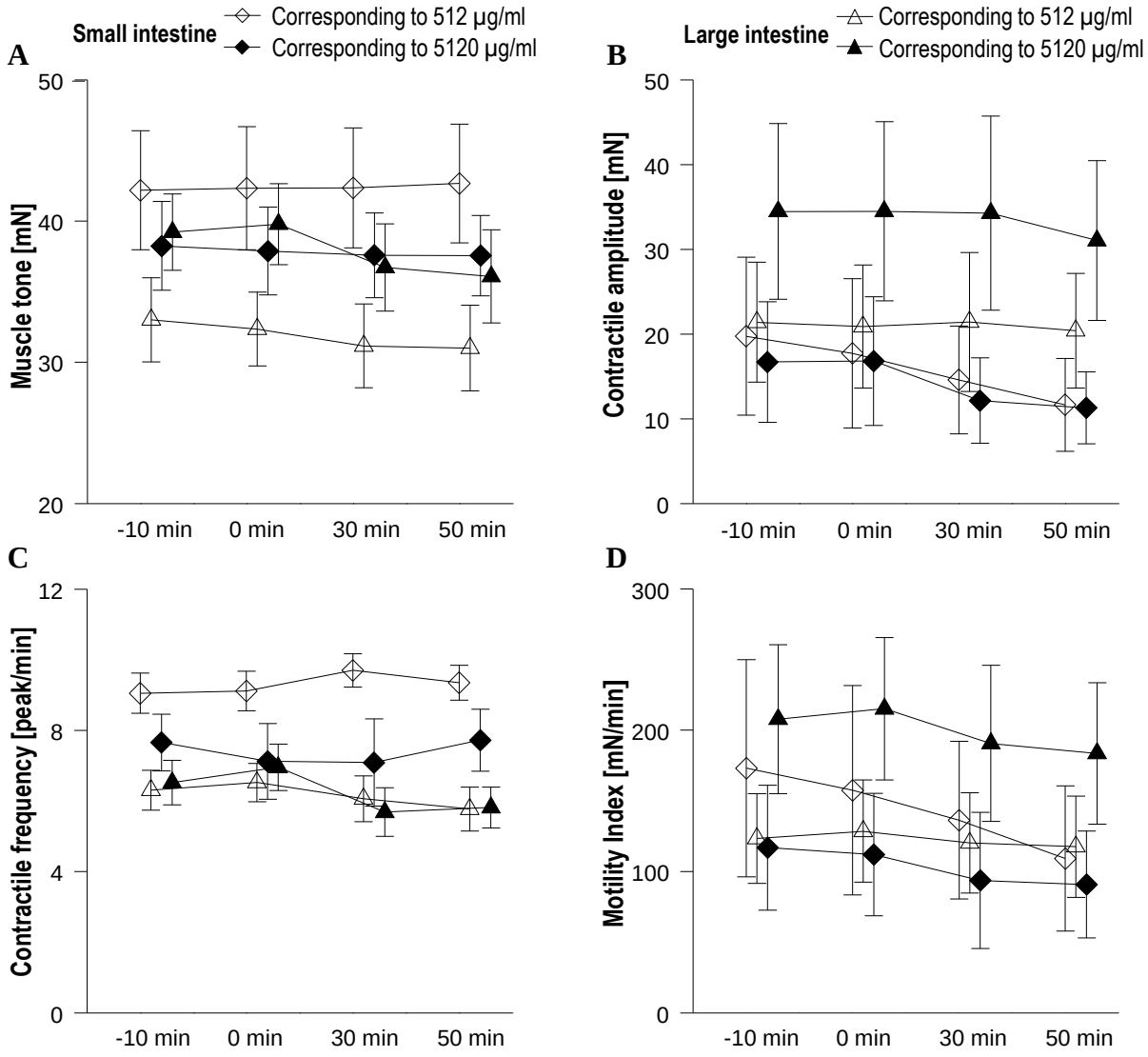

Figure 50: The effects of **milk thistle** on human intestinal motility; Milk thistle was applied in concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. There are no significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Bitter candytuft

Bitter candytuft had no influence on human intestinal smooth muscle. Both concentrations of 27.3 and 273 µg/ml that corresponded to STW 5 (512 and 5120 µg/ml) respectively, showed negligible changes in muscle tone and motility index (Figure 42, Figure 51 and Table 30 in the appendix).

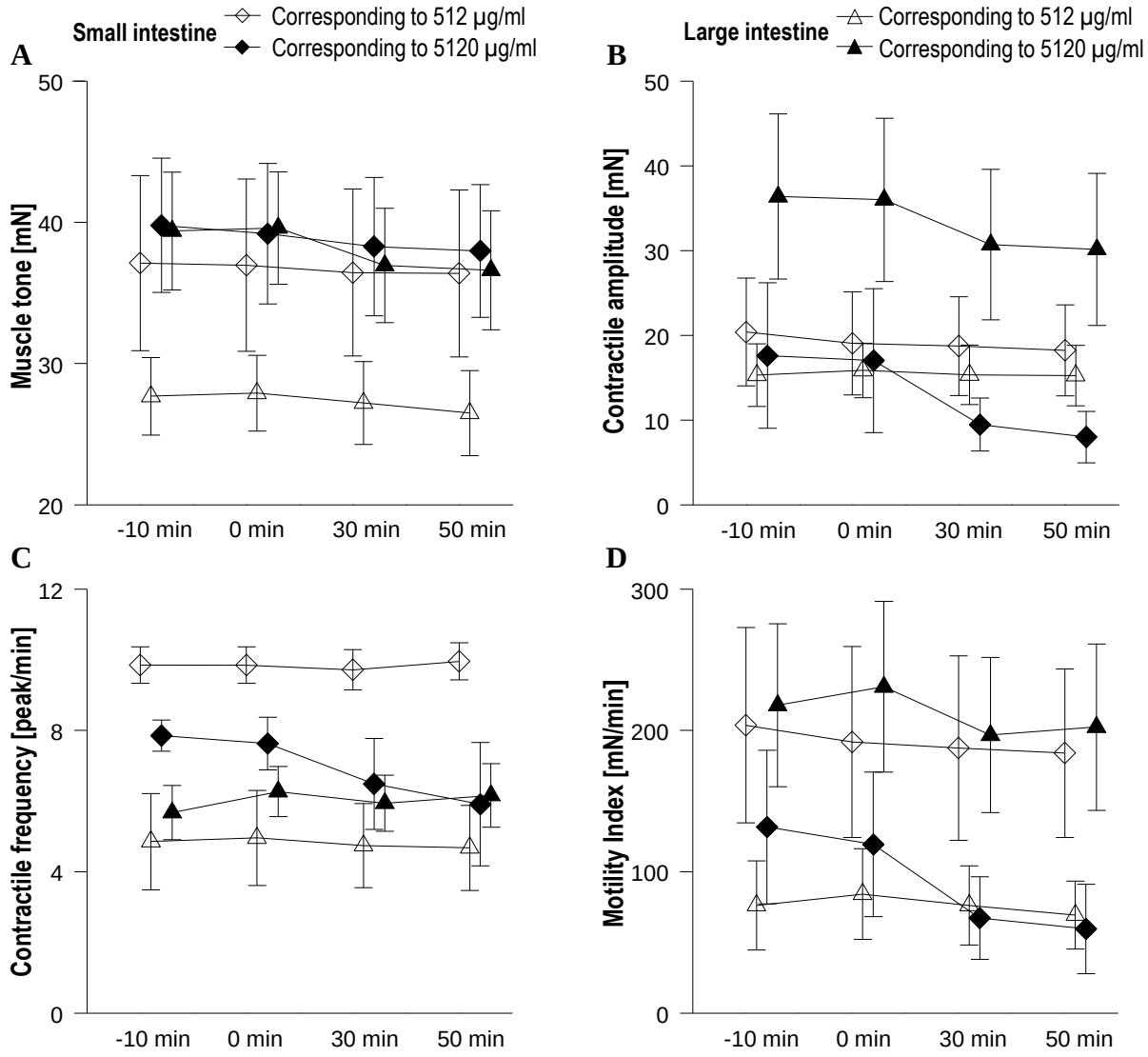

Figure 51: The effects of **bitter candytuft** on human intestinal motility. Bitter candytuft was applied in concentrations corresponding to 512 and 5120 µg/ml STW 5 on circular muscle strips from either small or large intestine. There are no significant differences between pre- (-10 min) and post-treatment (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Control tissues

In parallel, changes in the muscle tone and phasic contractility were recorded from control tissues. Control tissues were dissected from the same region, the same layer of the same patient and mounted in the organ baths without any treatment. Only the solvents were applied at the same time of drug application and similar four time intervals were analysed. The results revealed that the muscle tone and spontaneous contractility of the control human intestinal smooth muscle remained unchanged throughout the experimental period (Figure 52 and Table 31 in the appendix). These findings give evidences that the observed changes in muscle tone, contractile amplitude, contractile frequency and motility index are real effects of the applied drugs and not due to solvent application, time lapse, experimental artefacts or any other factors.

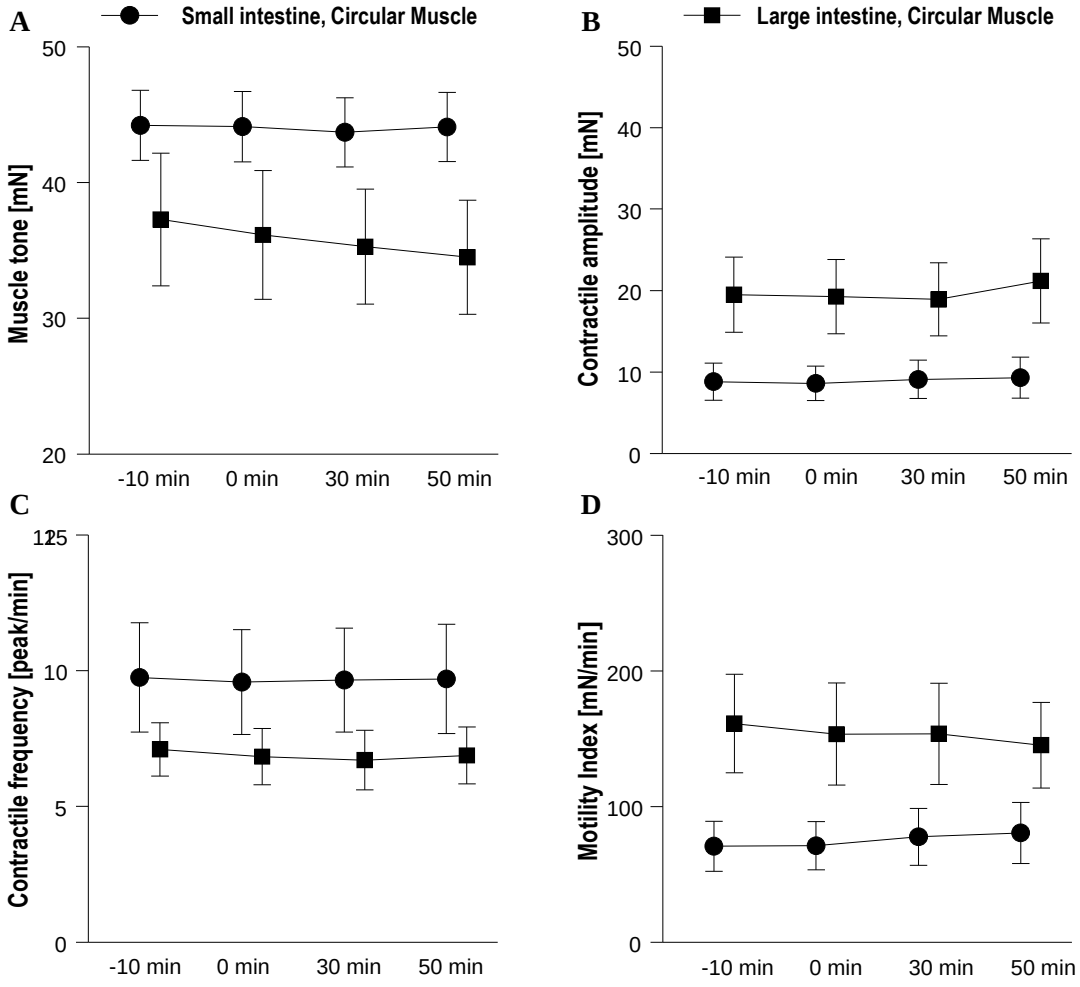

Figure 52: Control muscle strips showed stable muscle tone and contractility pattern throughout the recording time. There are no significant differences between pre- (-10 min) and post-solvent (0, 30 and 50 min). To avoid overlapping symbols, the values representing each time period are slightly shifted.

Spasmolytic effects of STW 5 components are neither synergistic nor additive

The rationale for this part was to study synergistic and/or additive effects of the individual components of STW 5 on human intestinal motility.

In small intestine at concentrations corresponded to their concentrations in STW 5 (5120 µg/ml) apart from milk thistle and bitter candytuft, the other seven extracts induced significant relaxation in muscle tone. The spasmolytic effects evoked by caraway and lemon balm were significantly lower than STW 5 effect ($P = 0.007$) whereas the effects of the other extracts were comparable to the effect of STW 5 ($P = 1.0$) (Figure 53).

In large intestine, only milk thistle, bitter candytuft and caraway had no significant spasmolytic effect whereas greater celandine increased the muscle tone. The decrease in muscle tone evoked by chamomile was significantly lower than STW 5 ($P = 0.002$) whilst the spasmolytic effects induced by the other extracts were comparable to STW 5 ($P = 0.7$) (Figure 53).

Similar to STW 5, the inhibitory effects of some extracts were significantly higher in large than in small intestine: STW 5 ($P = 0.04$), angelica ($P = 0.001$), peppermint ($P = 0.02$), lemon balm ($P = 0.002$) and greater celandine ($P = 0.002$).

In small intestine, the relaxation induced by STW 5 (-6.2 ± 1.2 mN) was significantly lower than the expected value of 21.6 mN which was the sum of the responses induced by individual application of the components ($P \leq 0.0001$). The results were similar in large intestine, the STW 5 response of (-13.8 ± 2.5 mN) was significantly lower than the calculated value of 47.8 mN ($P \leq 0.0001$). From these observations, we could conclude that the effects of STW 5 components on human motility are not synergistic or even additive otherwise one would expect a much larger effect of STW 5.

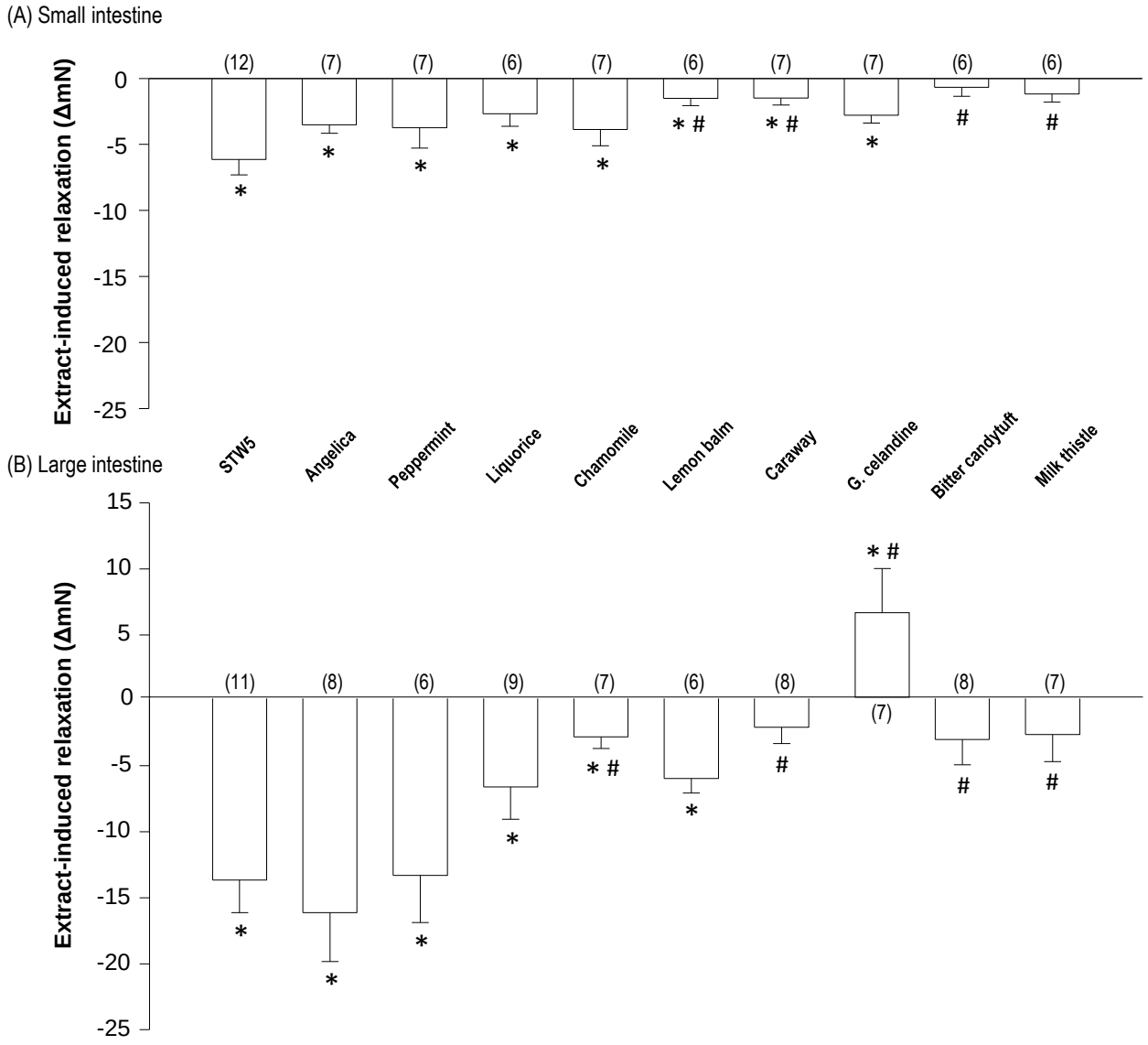

Figure 53: The effects of STW 5 components on human intestinal motility are not synergistic or additive. Application of STW 5 and its extracts at concentrations corresponding to 5120 µg/ml induced relaxation on the muscle tone. Panels (A) and (B) indicate the effects in small and large intestine respectively. The spasmolytic effects of some extracts were comparable to STW 5 effect. * mark significant changes compared to baseline tone. # mark significant differences from STW 5 effect. Numbers in parentheses indicate number of tissue (equal to number of patients) studied.

3.2.3 Pharmacology of STW 5-induced relaxation on human intestine

The rationale for this part of the thesis was to study the mechanism(s) of action by which STW 5 relaxes human intestinal smooth muscle. As concluded in the previous section, the relaxing effect of STW 5 on guinea pig proximal stomach is due to closure of TRPA1 and TRPC, in particular TRPC3 channels. These channels seem to be open in the resting state. STW 5 decreases the intracellular calcium level by closing them. Accordingly the present pharmacological study focused on these channels only. These experiments were conducted on human large intestinal circular muscles using STW 5 (5120 µg/ml) where the effects were relatively large.

In a paired design, blockers were applied in different organ baths containing muscle strips from the same patient.

Selective blockade of TRPA1 channels by HC-030031 (10 µM) significantly reduced muscle tone and phasic contraction by 12 % and 21 % respectively (Figure 54). Pre-treatment of human intestinal smooth muscle with HC-030031 (10 µM) significantly reduced STW 5-induced relaxation by 50 % (n = 7, P = 0.003) (Figure 55).

The results were similar in SKF-96365 (10 µM) which decreased muscle tone and motility index by 11 % and 12 % respectively (Figure 54). Pre-incubation of SKF-96365 (10 µM) significantly decreased STW 5-evoked relaxation by 35 % (n = 7; P = 0.03) (Figure 55).

Similarly, the selective TRPC3 antagonist Pyr3 (10 µM) decreased both tonic and phasic contractility by 9 % and 26 % respectively. Additionally, Pyr3-treated muscle strips showed significant lower response to STW 5 by 39 % (n = 7; P = 0.03) (Figure 55).

The phasic contractility were only non-significantly changed in all blockers (n = 8; P = 0.7).

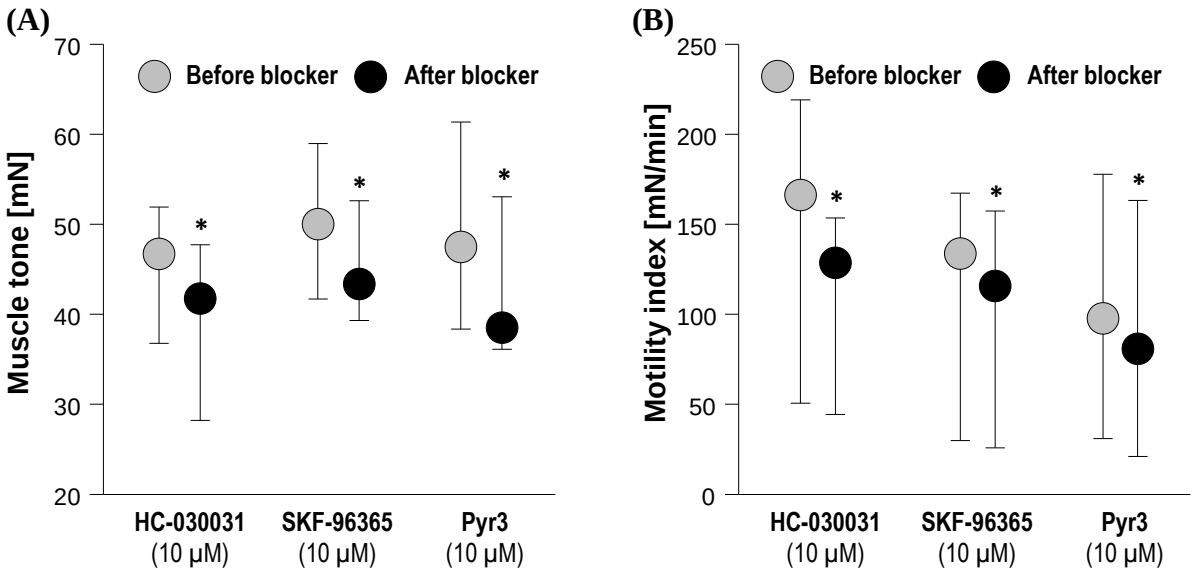

Figure 54: Both tonic and phasic contractions are significantly reduced by calcium channel blockers. Application of TRPA1 blocker HC-030031, TRPC antagonist SKF-96365 and TRPC3 antagonist Pyr3 significantly decreased muscle tone and motility index of human large intestinal circular muscle. Data are presented as median [75/25] percentile.

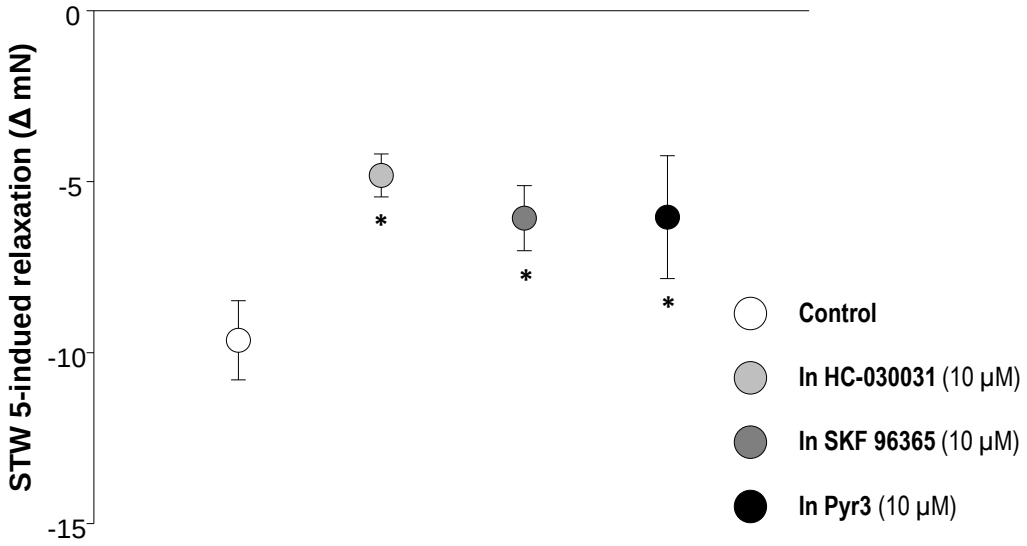

Figure 55: STW 5-induced relaxations in human intestinal circular muscle are significantly reduced in TRPA1 and TRPC in particular TRPC3 antagonists.

3.2.4 Summary of the effects of STW 5 and its components on human intestinal motility

Table 12 summarizes the effects of STW 5 and its nine individual components on human intestinal motility.

Table 12: Effects of STW 5 and its components on human intestinal motility

	Human intestinal motility			
	Small intestine		Large intestine	
	Muscle tone	Motility index	Muscle tone	Motility index
STW 5	↓	↓ D+J – Ileum	↓	↑↓ **
Angelica	↓	↓	↓	↓
Peppermint	↓	↓	↓	↓
Liquorice	↓	↓	↓	↓
Lemon balm	↓	–	↓	–
Chamomile	↓	↓	↓	–
Caraway	↓	↓	–	–
Gretear celandine	↓	↑ D+J ↓ Ileum	↑	–
Milk thistle	–	–	–	–
Bitter candytuft	–	–	–	–

Black downward arrows reveal decreased activities. Grey upward arrows reveal increased activities. Grey dashes indicate no effects. D+J is duodenum and jejunum. ** Significant increase followed by inhibition.

3.3 Secretory effects of STW 5 and its individual components on human intestinal epithelial cells

The rationale for this part of the study is that our previous study revealed the secretagogue effect of STW 5 in human intestinal epithelium and T84 cells (Krueger *et al.*, 2009). However, the possibility that only few components or even one single extract is responsible for this action had not been studied. In the following sections, the secretory effects of the individual component will be discussed.

Based on the previously published dose response study (Krueger *et al.*, 2009), basolatertal application of STW 5 at a concentration of 512 µg/ml evoked significant and reliable secretion in isolated human intestinal mucosal/submucosal preparations and T84 cells. In accordance, the nine individual extracts present in STW 5 were prepared at concentrations corresponding to their concentrations in 512 µg/ml STW 5. Since some of the effects were rather marginal, tenfold higher concentrations of STW 5 and its individual components (corresponding to 5120 µg/ml STW 5) were also used.

In preliminary experiments, the individual components were applied cumulatively (512 µg/ml, 5-fold and 10-fold). However, the responses were not reproducible (data not shown) and consequently each concentration was applied in a separate preparation.

STW 5, its nine extracts and particular combinations were dissolved in Krebs solution and applied as ethanol-free lyophilisates to avoid ethanol-induced secretion and decreased epithelium resistance (Sommansson *et al.*, 2014).

Because there are no significant differences between the responses of small and large intestine to the STW 5 components (except for liquorice), data from both regions were pooled (Table 13).

Table 13: Prosecretory actions of STW 5, its components and their combinations in human small and large intestinal preparations

	Small intestine ΔIsc [µA/cm²]		Large intestine ΔIsc [µA/cm²]		*P* value
STW 5 (512 µg/ml)	20.3 ± 8.0	*n* = 6	30.1 ± 7.6	*n* = 6	*P* = 0.4
sSTW 5 (512 µg/ml)	30 [9.5/44.8]	*n* = 9	16 [13.0/20.2]	*n* = 7	*P* = 0.3
Angelica (A) as in 512 µg/ml	15.4 ± 2.8	*n* = 8	14.1 ± 5.4	*n* = 6	*P* = 0.8
Angelica (A) as in 5120 µg/ml	19.4 ± 5.1	*n* = 7	13.9 ± 2.8	*n* = 6	*P* = 0.4
Peppermint (P) as in 5120 µg/ml	15.3 ± 5.8	*n* = 6	8.2 ± 2.7	*n* = 6	*P* = 0.3
Lemon balm (L) as in 5120 µg/ml	10.1 [6.8/38.0]	*n* = 6	6.0 [5.0/10.0]	*n* = 7	*P* = 0.1
Liquorice as in 5120 µg/ml	**-9.7 ± 1.7**	*n* = 6	**-2.4 ± 1.3**	*n* = 10	***P* = 0.005***
APL [1] as in 512 µg/ml	17.4 ± 3.3	*n* = 5	14.7 ± 2.6	*n* = 7	*P* = 0.52
APL [1] as in 5120 µg/ml	26.6 [22.0/31.8]	*n* = 5	23.2 [18.2/24.4]	*n* = 11	*P* = 0.5

[1] APL is a mixture of angelica, peppermint and lemon balm. (*n*) is the number of tissues. * marks significant differences between small and large intestine.

3.3.1 Secretory effects of STW 5 and sSTW 5

To ensure that neither isolation, extraction nor lyophilisation processes influence STW 5 action, all nine lyophilisates were combined at concentrations corresponding to their concentrations in 512 µg/ml STW 5, this mixture is referred to as sSTW 5. Basolateral application of STW 5 or sSTW 5 (both 512 µg/ml) induced comparable (P=1.0) significant increases in Isc in human intestinal preparations of 25.8 [6.6/38.6] µA/cm² or 17.7 [11.1/36.0] µA/cm² respectively (Figure 56). Similarly, STW 5 or sSTW 5 induced increased Isc in T84 cells of 5.2 [3.3/8.1] or 4.2 [2.4/5.4] µA/cm², respectively Figure 57. Again there was no difference between the two compounds (*P* = 0.3).

In human tissue, 5120 µg/ml and 512 µg/ml STW 5 evoked statistically comparable prosecretory responses although the response to the higher concentration was 26 % higher (*n* = 6; *P* = 0.1). However in T84 cells, the response of STW 5 (5120 µg/ml) was 568 % above that after 512 µg/ml and this reached significance (*n* = 6; *P* ≤ 0.001) (Figure 58).

Independent of the concentrations, the STW 5 and sSTW 5 responses peaked in human intestinal preparations after 17.5 ± 2.6 min (*n* = 18) and 19.1 ± 2.1 min; (*n* = 19), respectively (*P* = 0.6) and slowly declined to pre-drug baseline within 48.0 ± 9.9 min and 45.9 ± 4.2 min (*P* = 0.8), respectively. In T84 cells, STW 5 and sSTW 5 responses reached their maxima

after 9.7 ± 1.3 and 10.7 ± 1.8 min, (n = 6; P = 0.7) and gradually declined to pre-drug levels in 30.0 ± 1.5 and 33.0 ± 2.0 min (n = 6, P = 0.3), respectively.

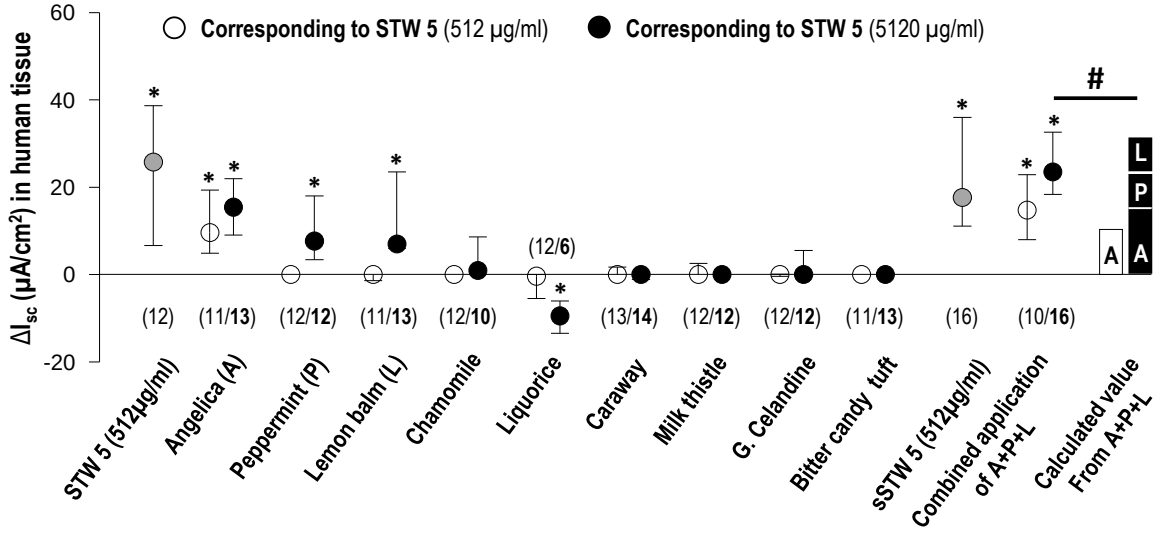

Figure 56: Effects of STW 5, its individual components and the combination of angelica, peppermint and lemon balm (APL) on human intestine secretion. STW 5 (512 µg/ml) increased Isc. At concentration corresponding to STW 5 (512 µg/ml), only angelica (A) evoked a comparable response to STW 5. At 10x higher concentrations, additionally, peppermint (P) and lemon balm (L) increased ion secretion while liquorice decreased it, only in small intestine. sSTW 5 induced a comparable effect to STW 5. Combination of extracts A+P+L (at the two given concentrations) evoked also comparable increases in Isc with STW 5; this increase was significantly lower than the value calculated from the effects of the individual extracts (#). Numbers in parentheses indicate the number of tissues (equal to number of patients) studied. Normal (to left) and bold (to right) numbers represent the preparations used to study 512 and 5120 µg/ml STW 5 respectively. * mark significant changes compared to baseline Isc. Data are presented as medians [75/25] percentile.

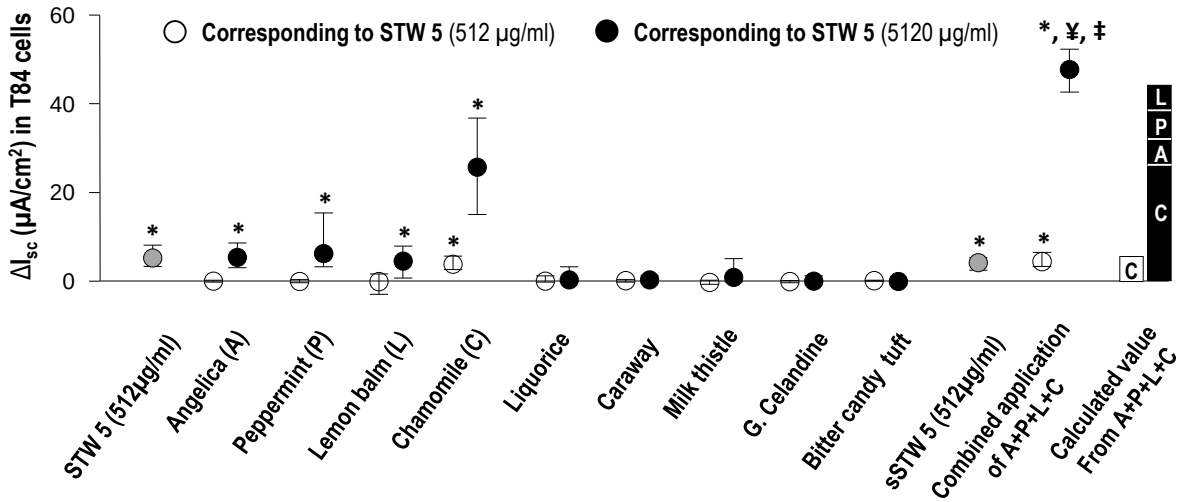

Figure 57: Effects of STW 5, its individual components and their combinations on secretory activity of T84 cells. At concentrations corresponding to 512 mg/ml, chamomile (C) evoked a comparable secretagogue action to STW 5 and sSTW 5. At 10x higher concentrations additionally angelica (A), peppermint (P) and lemon balm (L) evoked increases in Isc. Co-application of A+P+L+C as their concentration in 512 µg/ml evoked comparable response to STW 5, whereas tenfold higher concentrations evoked a huge increase in Isc which is comparable with calculated value. * Statistically significant from zero ($P \leq 0.001$), ¥ significantly different from STW 5 ($P \leq 0.001$), ‡ significantly different from combination corresponding to 512 µg/ml ($P \leq 0.001$). n=6 filters in each experiment. Data are presented as median [75/25] percentile.

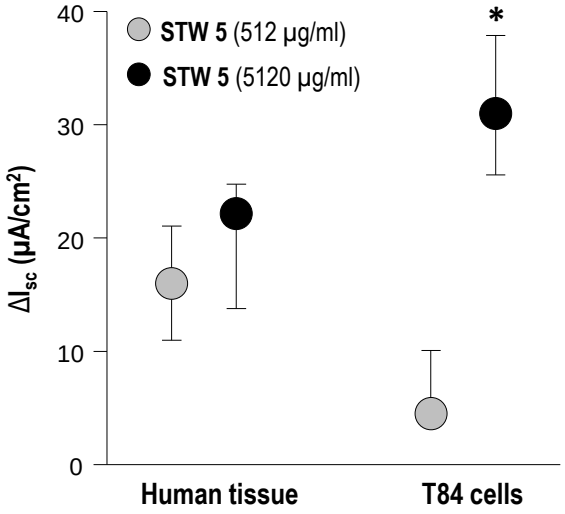

Figure 58: STW 5 (512 and 5120 µg/ml) evoked comparable increases in ion secretion only in human tissue. In T84 cells, STW 5 (5120 µg/ml) induced significantly higher response than STW 5 (512 µg/ml). n=6 patients or filters. * marks significant difference between both concentrations. Data are presented as median [75/25] percentile.

3.3.2 Effects of the individual extracts on the basal epithelial secretion

Of the nine extracts, only angelica evoked a prosecretory action in human intestine at concentrations corresponding to its concentrations in STW 5 (512 µg/ml), whereas in T84 cells, only chamomile showed an increase in Isc.

At tenfold higher concentrations, which corresponded to their concentrations in 5120 µg/ml STW 5, peppermint and lemon balm also evoked prosecretory actions in human intestine and T84 cells. At this concentration, liquorice showed anti-secretory action only in small intestine (Figure 56 and Figure 57).

Caraway, milk thistle, greater celandine and bitter candytuft had no statistically significant influence on Isc in human intestine or in T84 cells.

Representative traces of the effects of STW 5 and its components are shown in Figure 59 where its application as well as its prosecretory components and their combinations evoked reliable increases in short circuit current in both human tissue and T84 cells. Only liquorice showed a significant decrease in Isc in small intestine.

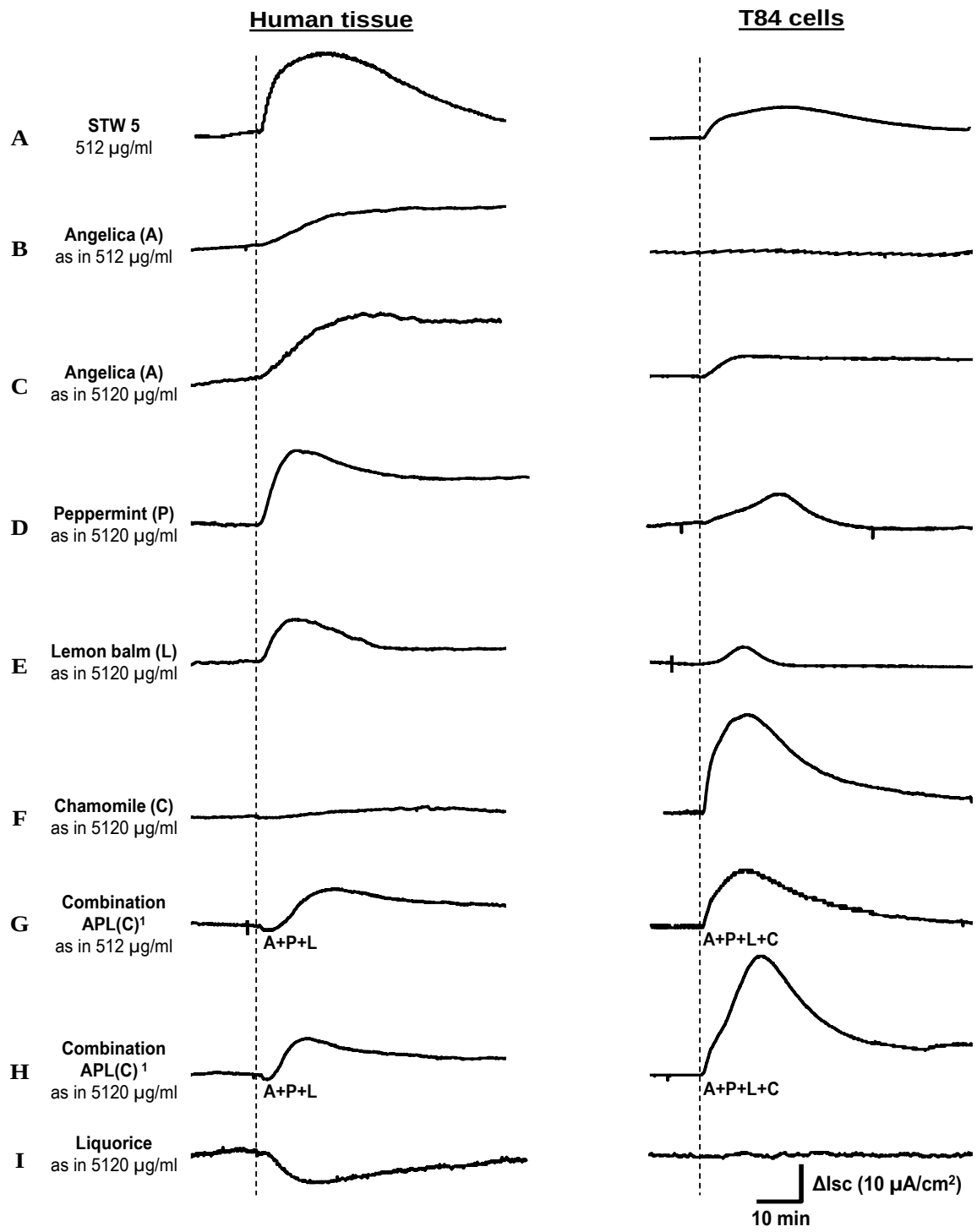

Figure 59: Representative traces for the effects of STW 5, its components and their combination on secretion in human intestine (left panel) and T84 cells (right panel). The traces were taken from different experiments. The scale bar in the lower right corner applies to all traces. The dashed lines indicate the time of basolateral drug application. [1] combination in human tissues is a mixture of angelica (A), peppermint (P) and lemon balm (L) while in experiments with T84 cells, chamomile (C) was added to the mixture.

Angelica

Basolateral application of 89.5 µg/ml angelica -corresponding to STW 5 (512 µg/ml)- significantly increased the Isc in human intestine by 14.9 ± 2.7 µA/cm^2, ($P \leq 0.001$). This increase did not statistically differ from STW 5 (512 µg/ml)-evoked secretion ($P = 0.09$). At this concentration, angelica evoked a negligible change in Isc in T84 cells (0.02 ± 0.1 µA/cm^2, $P = 0.9$).

At a concentration of 895 µg/ml -corresponding to STW 5 (5120 µg/ml) - angelica showed a prosecretory effect in human intestine (16.9 ± 3.0 µA/cm^2, $P \leq 0.001$). This increase in Isc was comparable with that evoked by a tenfold lower concentration and by STW 5 (512µg/ml) ($P = 0.6$). Angelica (895 µg/ml) also increased the Isc significantly by 5.5 ± 1.2 µA/cm^2 in T84 cells ($P = 0.007$) (Figure 56 and Figure 57).

The maximum responses were reached after 17.1 ± 1.9 and 11.3 ± 2.9 min in human tissue and T84 cells, respectively. The responses were sustained and long lasting till the end of the recording period which was 45 min (Figure 59).

Peppermint

Application of peppermint at a concentration corresponding to its concentration in STW 5 (512 µg/ml), 37.2 µg/ml had no secretory influences on human intestine or T84 cells (0.0 [0.0/0.0] µA/cm^2, $P = 0.5$ and 0.006 ± 0.2 µA/cm^2, $P = 1.0$), respectively. Higher concentration of 372 µg/ml showed prosecretory effects on human intestine epithelium and T84 cells with an Isc increase of 11.8 ± 3.2 µA/cm^2, $P \leq 0.001$ and 9.3 ± 3.5 µA/cm^2, $P = 0.04$), respectively (Figure 56 and Figure 57).

The peak of the response was reached after 11.1 ± 1.6 min and 13.2 ± 0.7 min in human tissue and T84 cells, respectively whereas declined to pre-application levels within 30.7 ± 2.3 and 41.0 ± 3.0 min, respectively (Figure 59).

Lemon balm

At a concentration corresponding to its concentration in STW 5 (512 µg/ml), lemon balm did not influence the secretion of human intestine (0.0 [-1.4/0.0] µA/cm^2, $P = 0.8$) or T84 cells (-0.006 ± 1.9 µA/cm^2, $P = 0.8$). Tenfold higher concentration of lemon balm (579 µg/ml) induced significant increases in Isc in human intestine and T84 cells by (7 [6.0/23.5] µA/cm^2, $P \leq 0.001$ and 4.3 ± 1.6 µA/cm^2, $P = 0.04$), respectively Figure 56 and Figure 57.

These effects were relatively short and transient. They peaked after 13.4 ± 2.4 min and 4.4 ± 0.4 min in human intestine and T84 cells, respectively and declined shortly within 34.2 ± 6.1 and 9.2 ± 0.4 min, respectively (Figure 59).

Chamomile

Basolateral application of chamomile had no effect on human intestinal secretion at both concentrations corresponding to STW 5 (512 and 5120 µg/ml) (0.0 [0.0/0.0] µA/cm^2, *P* = 0.9 and 3.4 ± 2.5 µA/cm^2, *P* = 0.2, respectively) Figure 56.

In T84 cells, application of chamomile at 114.3 and 1143 µg/ml evoked increases in Isc by (3.8 [2.7/5.7] µA/cm^2, *P* = 0.03 and 26.9 ± 5.7 µA/cm^2, *P* = 0.005), respectively (Figure 57).

Independent of the concentration, the responses peaked at 5.6 ± 0.7 min. While the response to the lower concentration was sustained, it lasted 41.2 ± 5.2 min at the higher concentration (Figure 59).

Liquorice

Only in small intestine, 801 µg/ml liquorice, a concentration corresponding to 5120 µg/ml STW 5, evoked a decreased Isc by (-9.7 ± 1.7 µA/cm^2, *P* = 0.002) (Figure 56). The maximum effect was reached after 11.7 ± 6.9 min and returned to pre-drug levels within 38.2 ± 6.2 min (Figure 59). Liquorice had no influence on secretory activity in T84 cells (0.3 [-0.2/3.3] µA/cm^2, *P* = 0.3) (Figure 57).

At lower concentration of 80.1 µg/ml, liquorice had no effect in both human intestine and T84 cells (-0.4 [-5.5/0.0] µA/cm^2, *P* = 0.1 and -0.04 [-0.2/1.2] µA/cm^2, *P* = 1.0, respectively) (Figure 56 and Figure 57).

Caraway

Basolateral application of caraway at 28.4 µg/ml which corresponded to its concentration in 512 µg/ml STW 5 had no effect on Isc in both human intestine (0.0 [0.0/1.7] µA/cm^2, *P* = 0.1) or T84 cells (0.1 ± 0.2 µA/cm^2, *P* = 0.6). The results were similar with the tenfold higher concentration of 284 µg/ml caraway (human intestine: 0.0 [-1.1/0.7] µA/cm^2, *P* = 1.0; T84 cells: 0.3 ± 0.4 µA/cm^2, *P* = 0.5) (Figure 56 and Figure 57).

Milk thistle

Basolateral application of milk thistle at 14.4 µg/ml which corresponded to its concentration in STW 5 (512 µg/ml) had no effect on Isc in both human intestine and T84 cells

(0.0 [0.0/2.5] µA/cm^2, P = 0.1 and -0.2 ± 0.3 µA/cm^2, P = 0.4) respectively. The results were similar with the tenfold higher concentration of 144 µg/ml milk thistle (human intestine: 0.0 [0.0/0.0] µA/cm^2, P = 1.0; T84 cells: 1.9 ± 1.1 µA/cm^2, P = 0.1) (Figure 56 and Figure 57).

Greater celandine

Basolateral application of greater celandine at 62.9 µg/ml which corresponded to its concentration in STW 5 (512 µg/ml) 5 had no effect on Isc in both human intestine (0.0 [-0.4/0.1] µA/cm^2, P = 0.9) or T84 cells (-0.1 ± 0.1 µA/cm^2, P = 0.4). The results were similar with the tenfold higher concentration of 629 µg/ml greater celandine (human intestine: 4.9 ± 2.7 µA/cm^2, P = 0.1; T84 cells: -0.3 [-0.8/1.2] µA/cm^2, P = 1.0) (Figure 56 and Figure 57).

Bitter candytuft

Basolateral application of bitter candytuft at 27.3 µg/ml which corresponded to its concentration in 512 µg/ml STW 5 had no effect on Isc in both human intestine (0.0 [0.0/0.0] µA/cm^2, P = 0.5) or T84 cells (0.1 ± 0.1 µA/cm^2, P = 0.1). The results were similar with the tenfold higher concentration of 273 µg/ml bitter candytuft (human intestine: 0.0 [0.0/0.2] µA/cm^2, P = 0.4; T84 cells: -0.2 ± 0.2 µA/cm^2, P = 0.3) (Figure 56 and Figure 57).

3.3.3 Combined application of the prosecretory compounds angelica, peppermint and lemon balm.

Only angelica, peppermint and lemon balm exerted prosecretory actions in the human intestine. Combined application of these extracts at concentrations corresponding to their concentrations in 512 or 5120 µg/ml STW 5 evoked comparable increases in Isc of 16.2 [9.0/21.1] µA/cm^2 ($P \leq 0.001$) or 23.5 [18.3/32.6] µA/cm^2 ($P \leq 0.001$), respectively. These responses were not different to those evoked by 512 µg/ml STW 5 or sSTW 5 (P = 0.06) (Figure 56).

In T84 cells, chamomile, besides angelica, peppermint and lemon balm also evoked an increase in I_{SC}. Co-application of the four extracts at concentrations corresponding to their concentrations in STW 5 (512 µg/ml) evoked an I_{SC} increase of 4.4 [3.3/6.5] µA/cm^2 (P = 0.004). This secretagogue action was comparable with STW 5 and sSTW 5 (both 512

µg/ml) ($P = 0.7$). However, at higher concentrations corresponding to those present in 5120 µg/ml STW 5, the increase in Isc was much larger (47.5 ± 3.4 µA/cm^2, $P \leq 0.001$) and significantly higher than STW 5 and sSTW 5 (512 µg/ml) ($P \leq 0.001$) (Figure 57). This increase in Isc was also significantly higher than STW 5 (5120 µg/ml) ($P = 0.007$).

The I_{SC} increase which was evoked by the prosecretory components in human intestine (23.5 [18.3/32.6] µA/cm^2) was significantly lower than the value of 31.1 µA/cm^2 which was the sum of the responses induced by individual application of the compounds ($P = 0.03$) (Figure 56). Conversely, in T84 cells the actual response of the combined application of the compounds was not different from the calculated sum of individual responses (47.5 ± 3.4 vs. 41.6 µA/cm^2, $P = 0.1$) (Figure 57).

3.3.4 Combined application of the non-secretory components

The results revealed that caraway, milk thistle, greater celandine, bitter candytuft and liquorice had no statistically significant influence on Isc in T84 cells and (with the exception of liquorice) in human intestine. To confirm this finding, a combination of these five extracts was applied at concentrations corresponding to their concentrations in STW 5 (5120 µg/ml). This combined application did not change the Isc (0.3 ± 0.1 µA/cm^2, $n = 5$; $P = 0.1$). Moreover, the resistance remained unchanged (230.8 [162.5/292.8] Ω / cm^2 vs. 250.0 [145.0/314.1] Ω / cm^2) before and after application, respectively, ($n = 5$; $P = 0.6$). These experiments were carried out in T84 cells.

3.3.5 Effects of STW 5 and its components on electrical field stimulation

Electrical field stimulation (EFS) evoked nerve-mediated secretion in human intestine. Although STW 5 had been shown to activate enteric neurons (Krueger *et al.*, 2009), application of STW 5 or sSTW 5 (both 512µg/ml) did not significantly potentiate the response of the human intestine to EFS (Figure 60). The results were similar with application of the nine individual extracts at concentrations corresponding to their concentrations in 5120 µg/ml STW 5 (Figure 60). Co-application of the prosecretory components angelica, peppermint and lemon balm at both concentrations corresponding to 512 and 5120 µg/ml STW 5 had also no influence on EFS-evoked increase in Isc (Figure 60). The most likely reason for these findings is that we used stimulus parameters which already induced supramaximal stimulation. Only at 5120µg/ml, STW 5 significantly reduced the response to EFS by (71%, $P = 0.002$) which could be due to local anaesthetic effect. However same concentration did not alter EFS-

evoked contractions in human intestinal smooth muscle (as discussed in section 3.2.1). See Table 36 in appendix for *P* values.

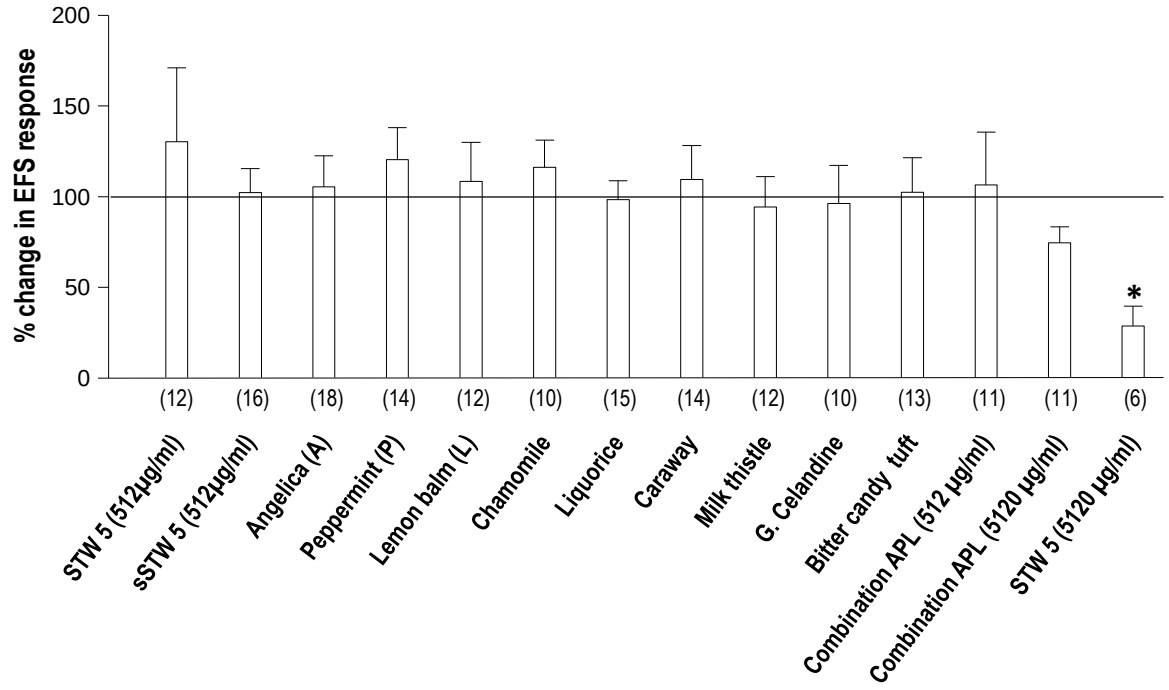

Figure 60: STW 5, its components and their combinations had no effect on EFS-evoked secretion in human intestine. Only STW 5 (5120 µg/ml) significantly reduced the response to EFS. Individual extracts were applied in concentrations corresponding to their concentrations in 5120 µg/ml STW 5. Numbers in parentheses indicate the number of tissues (equal to number of patients) studied. * marks significant reduction in response.

3.3.6 Effects of STW 5 and its components on resistance of human intestine and T84 cells

In order to study the effects of STW 5, its individual components and their combinations on the permeability and integrity of the human intestine, resistance of human intestine as well as T84 cells we measured before and after drug application. The results showed that application of STW 5, its individual components and their combinations at both concentrations of 512 and 5120 µg/ml did not change the resistance of both human intestinal and T84 cells (Figure 61). For absolute values, number of the tissues/filters and *P* values see Table 36 and Table 37 in the appendix.

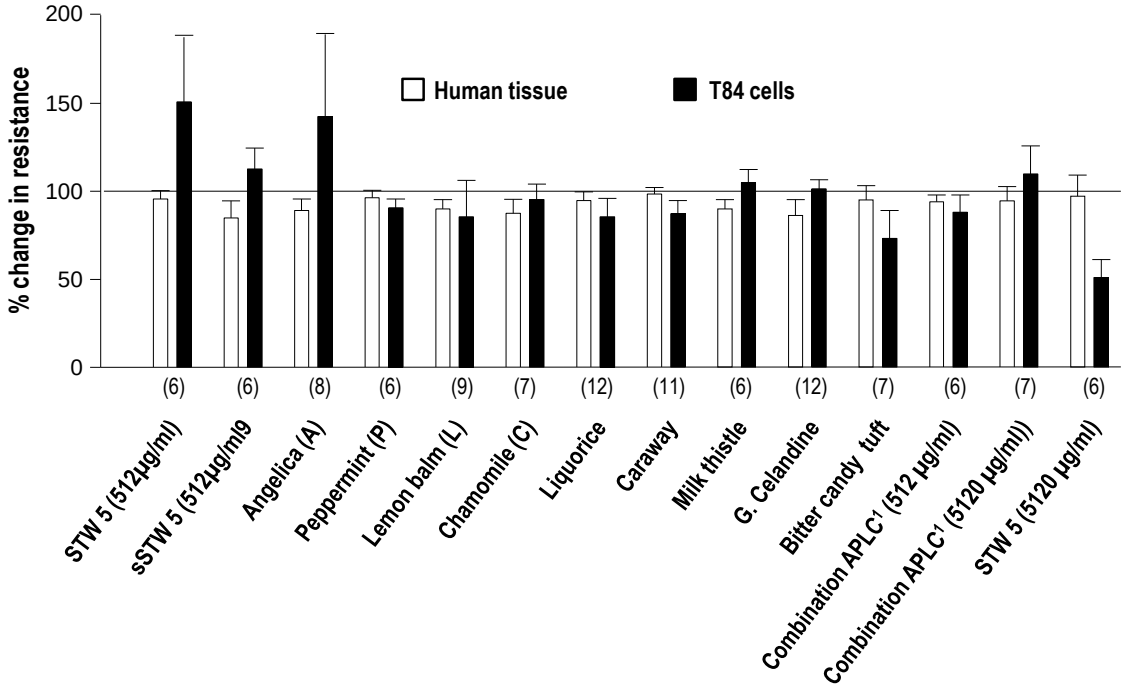

Figure 61: STW 5, its individual components and their combinations had no effect on the resistance of human intestine and T84 cells. The resistances of human intestine and T84 cells remain unchanged after application of STW 5 and its extracts. [1] APLC is mixture of angelica, peppermint and lemon balm in human intestine experiments. In T84 cells, chamomile was added to the mixture. Individual extracts were applied in concentrations corresponding to their concentrations in 5120 µg/ml STW 5. Numbers in parentheses indicate the number of tissues (equal to number of patients) studied. In T84 cells, n=6 filters in each experiments.

3.3.7 Pharmacology of changes in Isc induced by STW 5, its components and their combination in human intestine and T84 cells

The rationale for this part of the study is to identify the mechanism of action by which STW 5, its prosecretory components (angelica, peppermint, lemon balm and chamomile) and their combinations evoked increases in Isc in both human intestine and T84 cells. As well, to study the mechanism of liquorice induced decrease in Isc in human small intestine.

Briefly, the increase in Isc results from either increased luminal anion secretion (mainly Cl^- and/or HCO^{-3}) or enhanced cation absorption (mainly Na^+ and/or K^+). In a previous study, we concluded that the STW 5-induced increases in Isc in human intestine and T84 cells were partly due to enhanced chloride secretion via activation of epithelial chloride channels (Krueger *et al.*, 2009). There are two most prominent candidates responsible for chloride secretions: the cAMP-dependent cystic fibrosis transmembrane conductance regulator channels (CFTR) and calcium-activated chloride channels (CaCl).

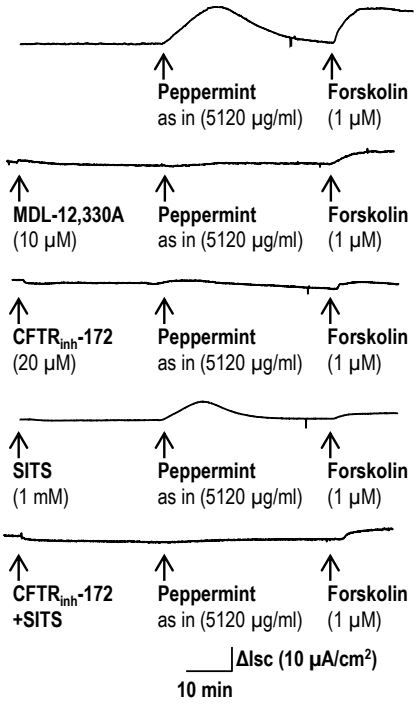

Figure 62: Representative traces for the effects of different blockers on peppermint-induced secretion in T84 cells. Basolateral application of peppermint at concentration corresponding to 5120 µg/ml STW 5 evoked an increase in Isc due to chloride secretion involving cAMP-dependent and Ca^{2+}-activated chloride channels. Blockade of both channel types by the selective cAMP antagonist MDL-12,330A, the CFTR chloride channel blocker $CFTR_{inh}$-172 or the CaCl channel antagonist SITS significantly decrease the induced Isc. However none of them was able to inhibit the response. Co-application of both channels blockers did not abolish the response. The traces were taken from the same experiment. The scale bar in the lower right corner is valid for all traces.

To further characterize the mode of action of basolateral applications of STW 5, its components and their combinations, blockers of ion channels were applied in human intestine and T84 cells.

MDL-12,330A is an adenylate cyclase inhibitor which was applied basolaterally at a concentration of 10 µM. Pre-treatment of human intestine with MDL-12,330A significantly reduced the increase in Isc evoked by STW 5, sSTW 5 (both 512 µg/ml), angelica (A) (corresponding to 512 and 5120 µg/ml STW 5), peppermint (P), lemon balm (L) (both at concentrations which corresponded to 5120 µg/ml STW 5) and the mixture of A+P+L (at both concentrations) (Figure 63 and Table 38 in the appendix). The results were similar in T84 cells besides decreasing the increase in Isc evoked by chamomile (at concentrations corresponding to 512 and 5120 µg/ml STW 5) (Figure 62, Figure 64 A and Table 38 in the appendix). This reduction in responses supported the involvement of cAMP-dependent CFTR chloride channels in chloride secretion.

The involvement of CFTR chloride channels was further confirmed by apical application of $CFTR_{inh}$-172 (20 µM) in human intestine and T84 cells. $CFTR_{inh}$-172 is a highly selective blocker for these channels. Similar to MDL-12,330A results, pre-incubation of $CFTR_{inh}$-172 significantly reduced the increases in Isc evoked by STW 5 and its components in human intestine (Figure 63) and T84 cells (Figure 62 and Figure 64A). The percentage reduction in responses and *P* values were presented in Table 38 in the appendix.

Both in human intestine and in T84 cells none of these two inhibitors fully blocked the response. This observation suggests the involvement of calcium activated chloride channels (CaCl). To test the involvement of this channel, the CaCl antagonist SITS (1mM) was apically applied. The results showed that both in human tissue and in T84 cells, the increases in Isc were significantly reduced in SITS (Figure 62, Figure 63 and Figure 64A). The percentage reduction in responses and *P* values were presented in Table 38 in the appendix.

To test if these channels are completely responsible for STW 5 secretions, $CFTR_{inh}$-172 and SITS were apically co-applied. Pre-treatment of the T84 cells with the combined application of the two blockers, significantly reduced the increases in Isc evoked by STW 5, its components and their combination (Figure 64 B and Table 38 in the appendix).

Only in small intestine, liquorice induced decrease in Isc. Apical application of amiloride (10 µM) significantly reduced the effect by 72 %, ($P = 0.01$). amiloride is a blocker for epithelial sodium channel (ENaC) and sodium hydrogen exchanger (Figure 63).

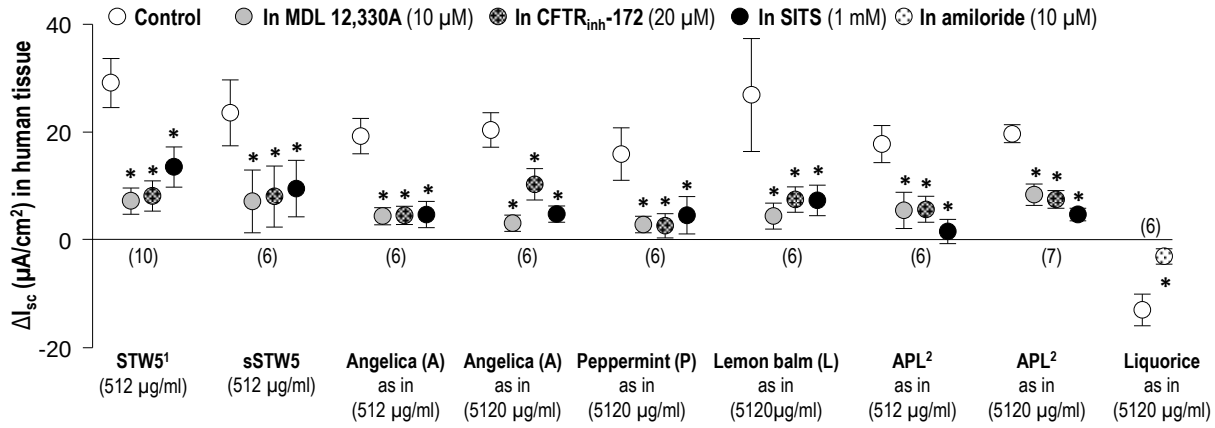

Figure 63: In human intestine, STW 5, its components and particular combinations evoked increases in Isc. These increases are due to increased chloride secretion via CFTR and CaCl chloride channels. The effects were significantly reduced in selective adenylate cyclase blocker MDL 12,330A, the CFTR channel antagonist $CFTR_{inh}$-172 and the CaCl channel inhibitor SITS. Only in small intestine, liquorice-induced decrease in Isc is significantly reduced in amiloride. [1] STW 5 data are from our previous study (Krueger *et al*., 2009). [2] APL is a combined application of a mixture from angelica (A), peppermint (P) and lemon balm (L). Numbers in parentheses indicate the number of tissues (equal to number of patients) studied. Extracts and blockers were applied in separate tissues from the same patient. * mark significant differences from controls.

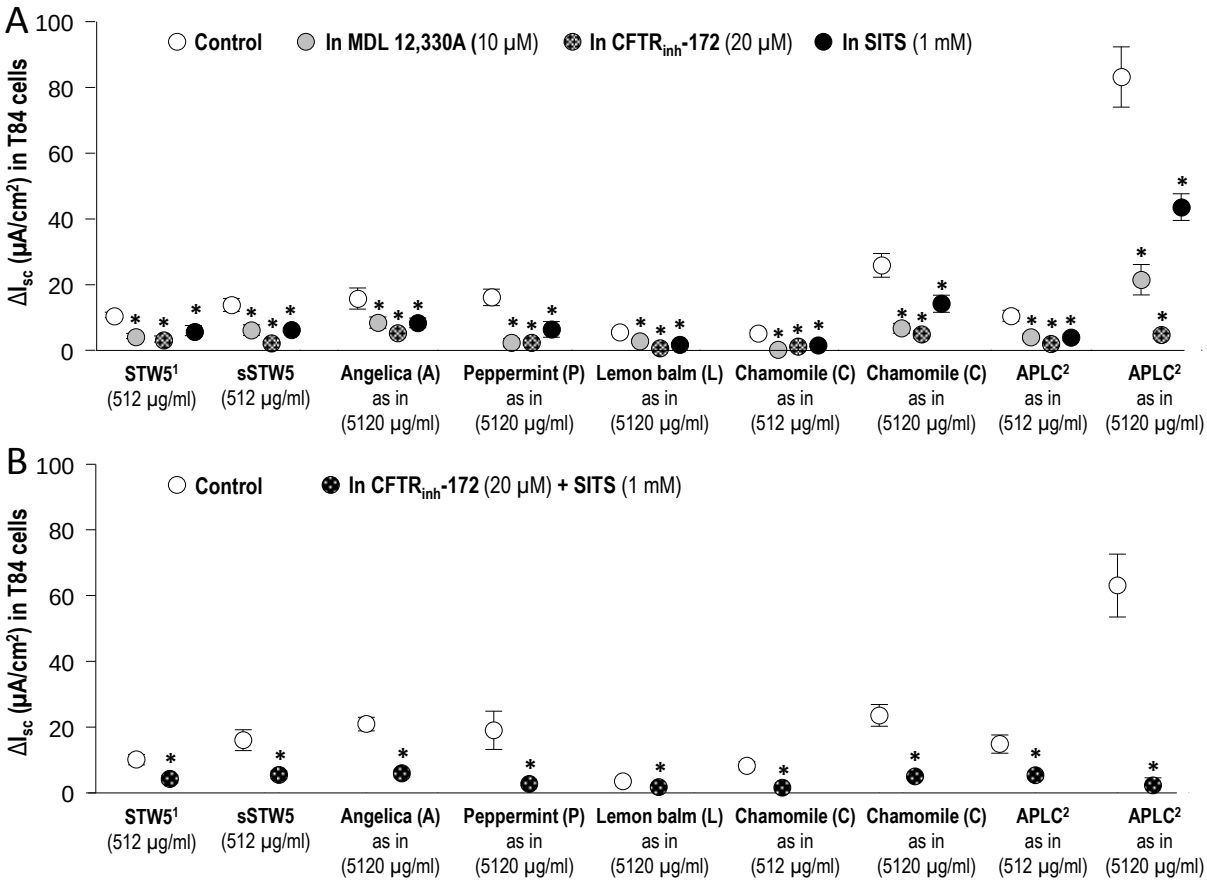

Figure 64: In T84 cells, STW 5, its components and particular combinations evoked increases in Isc. These increases are due to increased chloride secretion via CFTR and CaCl chloride channels. Panel (A) shows that the effects were significantly reduced in selective adenylate cyclase blocker MDL 12,330A, the CFTR channel antagonist $CFTR_{inh}$-172 and the CaCl channel inhibitor SITS. Panel (B) shows that co-application of $CFTR_{inh}$-172 and SITS did not abolish the response. [1] STW 5 data are from our previous study (Krueger *et al*., 2009). [2] APLC is a combined application of a mixture from angelica (A), peppermint (P), lemon balm (L) and chamomile (C). n=6 filters for each experiment. Extracts and blockers were applied in separate filters from the same batch. * mark significant differences from controls.

Prosecretory effects of STW 5 in bicarbonate depleted buffer.

In a paired design, the prosecretory response of STW 5 (512 µg/ml) in human epithelium was significantly reduced in air-bubbled bicarbonate-free HEPES-Krebs solution by 74 % when compared with the control response in bicarbonate buffered Krebs solution. The response to EFS was significantly reduced by 79 % in HEPES solution (Figure 65). Surprisingly, in T84 cells the reduction of the STW5 response in bicarbonate-free HEPES-Krebs was only non-significantly reduced by 16 % (Figure 65).

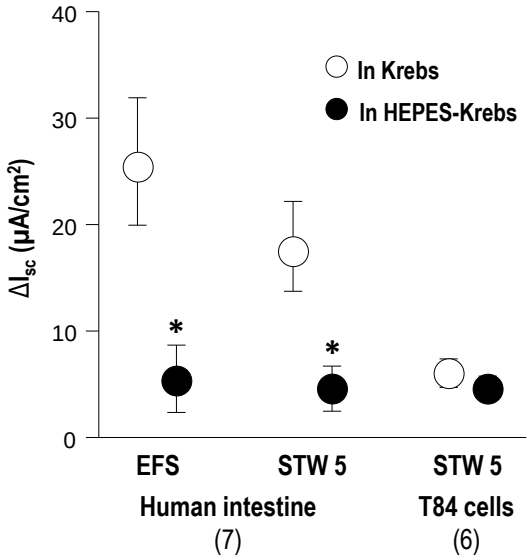

Figure 65: STW 5- and EFS-evoked increases in Isc were significantly reduced in HEPES buffer. STW 5 (512 µg/ml) evoked an increase in Isc when basolateral applied in bicarbonate-free solution. In human intestine, the response was significantly lower compared to the control response in Krebs solution. However, in T84 cells, the responses were comparable. Numbers in parentheses indicate the number of tissues/filters studied. * mark significant differences ($P < 0.05$).

Nerve-mediated action of angelica and chamomile

The reason for this experiment is that at concentrations corresponding to their concentrations in STW 5 (512 µg/ml) angelica increased the Isc only in human intestine whereas chamomile had effects only in T84 cells. We provided evidences that STW 5 activates enteric neurons (Krueger *et al.*, 2009). We hypothesized that chamomile may inhibit the nerve-driven secretion in human intestine and directly activate epithelial cells. In human intestine these effects could offset each other.

Pre-treatment of the human intestine with the fast sodium channel blocker tetrodotoxin (TTX, 1 µM) significantly inhibited the secretory effect of angelica by 79 % ($P = 0.03$). Chamomile showed no increase in Isc after TTX ($P = 0.7$) (Figure 66).

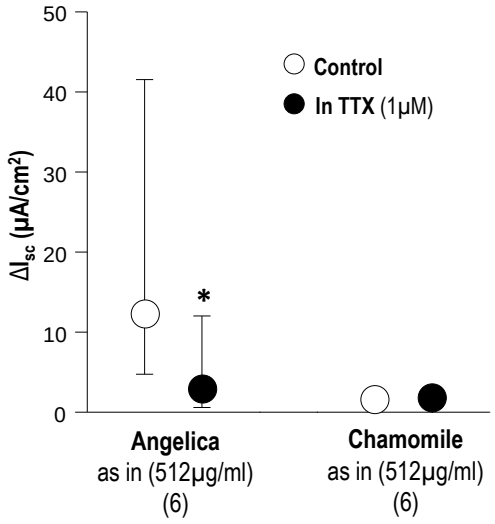

Figure 66: Angelica-evoked increase in Isc in human intestine is partly nerve mediated whilst chamomile is not. Pre-incubation of TTX (1 µM) significantly reduced angelica-evoked ion secretion. Chamomile did not evoke any secretory response in TTX. Numbers in parentheses indicate the number of tissues (equal to number of patients) studied. * marks significant difference ($P < 0.05$). Data are presented as median [75/25] percentile.

3.3.8 Effect of STW 5 and its components on forskolin induced secretion in T84 cells

In T84 cells, the cAMP activator forskolin (1 µM) was applied in all experiments as a second stimulus. Basolateral application of forskolin was used to trigger a cAMP-dependent chloride secretion and thus to check the viability of the T84 cells and the effectiveness of the blockers.

Forskolin responses after individual components

Basolateral application of the cAMP activator forskolin (1 µM) induced a significant increase in Isc in non-treated T84 cells. Pretreatment with the individual components of STW5 at concentrations corresponding to those in 512 or 5120 µg/ml STW 5 did not change the response to forskolin (Figure 67 and Table 14).

Table 14: The effect of STW 5 and its components on forskolin induced secretion in T84 cells

	Corresponding to 512 µg/ml			**Corresponding to 5120 µg/ml**		
	% change		***P* value**	**% change**		***P* value**
Angelica	13 %	*n* = 6	*P* = 1.0	-21 %	*n* = 6	*P* = 0.2
Peppermint	27 %	*n* = 5	*P* = 1.0	5 %	*n* = 6	*P* = 0.2
Lemon balm	2 %	*n* = 7	*P* = 1.0	-37 %	*n* = 6	*P* = 0.2
Chamomile	0.4 %	*n* = 6	*P* = 1.0	-23 %	*n* = 6	*P* = 0.2
Liquorice	23 %	*n* = 6	*P* = 1.0	-46 %	*n* = 6	*P* = 0.2
Caraway	18 %	*n* = 5	*P* = 1.0	3 %	*n* = 5	*P* = 0.2
Milk thistle	14 %	*n* = 5	*P* = 1.0	-6 %	*n* = 5	*P* = 0.2
Greater celandine	48 %	*n* = 5	*P* = 1.0	13 %	*n* = 5	*P* = 0.2
Iberis amara	16 %	*n* = 5	*P* = 1.0	9 %	*n* = 5	*P* = 0.2

Positive and negative values indicate percentage increase and decrease in the responses, respectively. (*n)* is the number of T84 filters.

However, T84 cells pretreated with 512 µg/ml STW 5, 512 µg/ml sSTW 5 or 5120 µg/ml STW 5 showed a significant reduction in the forskolin response by 61 %, 65 % and 100 %, respectively (Figure 68). Similarly, the forskolin responses were significantly inhibited by 35 % and 100 % after the combination of the four prosecretory components which applied at both concentrations corresponding to 512 and 5120 µg/ml, respectively (Figure 68).

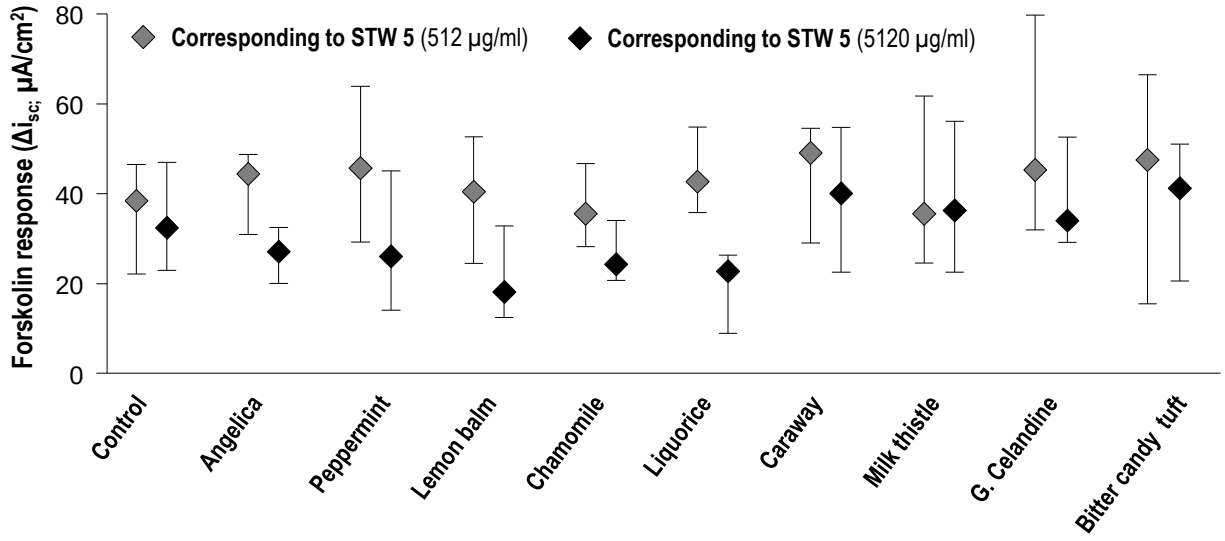

Figure 67: The nine components of STW 5 had no effect on forskolin induced secretion in T84 cells. Application of forskolin (1µM) as a second stimulus after each extract at concentrations corresponding to 512 and 5120 µg/ml STW 5 increased Isc. These increases were comparable to forskolin responses in non-treated T84 cells. Data are presented as median [75/25] percentile.

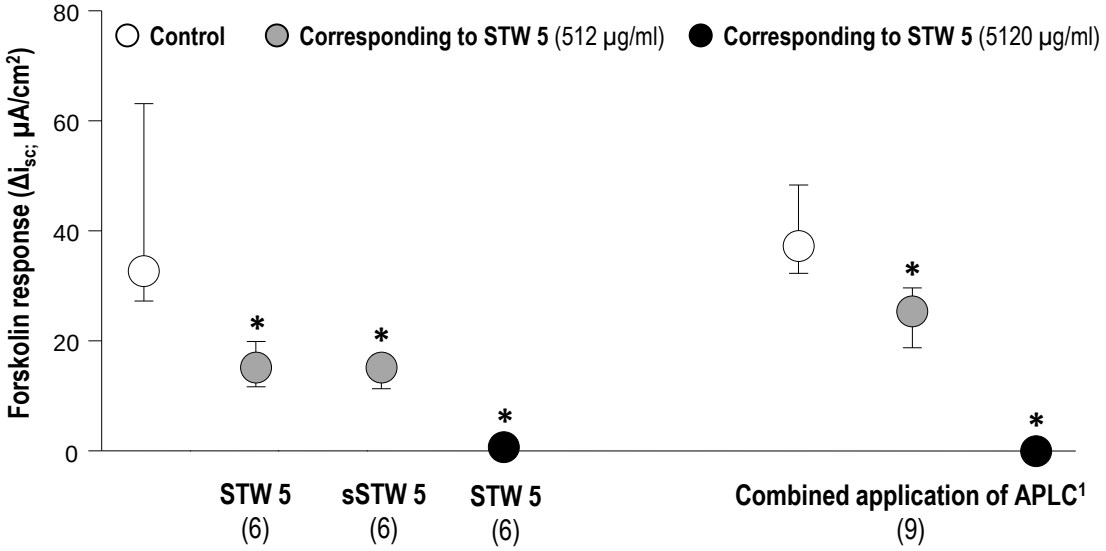

Figure 68: STW 5 and combined application of its prosecretory components significantly reduced the forskolin-induced secretion in T84 cells. [1]APLC is combined application of angelica, peppermint, lemon balm and chamomile. At concentrations corresponding to 512 µg/ml, STW 5, sSTW 5 and APLC significantly reduced the forskolin-induced increases in Isc. At tenfold higher concentration, STW 5 and APLC abolished the forskolin response. * mark significant differences ($P < 0.05$). The numbers in parentheses indicate number of filters. Data are presented as median [75/25] percentile.

Forskolin and Bethanechol induced increases in Isc through different pathways

As shown in the previous section, pretreatment of T84 cells with 5120 µg/ml STW 5 or APLC abolished the forskolin evoked secretion. We hypothesized that both STW 5/APLC and forskolin induced ion secretin via the same pathway: cAMP-dependent chloride channels. Higher concentrations of STW 5 and APLC activate these channels and drives them to their maximum capacity of secretion so that application of any other stimulus which acts through the same channels cannot induce further ion secretion. To test this hypothesis, forskolin (1 µM) was applied in T84 cells pretreated with forskolin (1 µM) and as shown in Figure 69 second forskolin application did not evoke any increase in Isc. On the other hand, application of another stimulus bethanechol (100 µM) evoked secretion via CaCl channels (Figure 69).

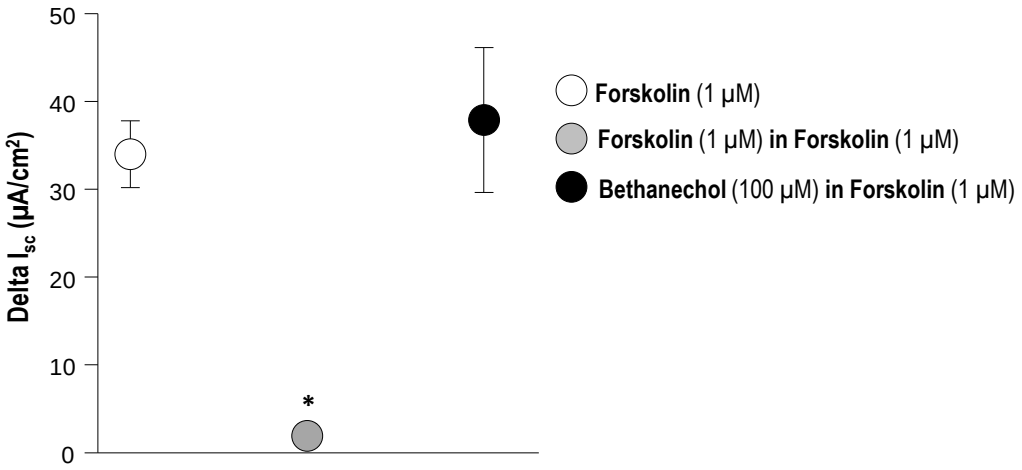

Figure 69: A second forskolin application has no influence on ion secretion in T84 cells. Application of bethanechol as a second stimulus after forskolin evoked significant increase in Isc. *n* = 6 filters. * marks significant differences ($P < 0.05$).

Because there were no forskolin response after STW 5 and APLC (both at 5120 µg/ml), T84 cells viabilities were checked by application of Bethanechol (100 µM). The bethanechol responses were reduced after STW 5 (5120 µg/ml) and combined application of APLC (at concentrations corresponding to their concentrations in 5120 µg/ml) by 68% and 70% respectively (Figure 70).

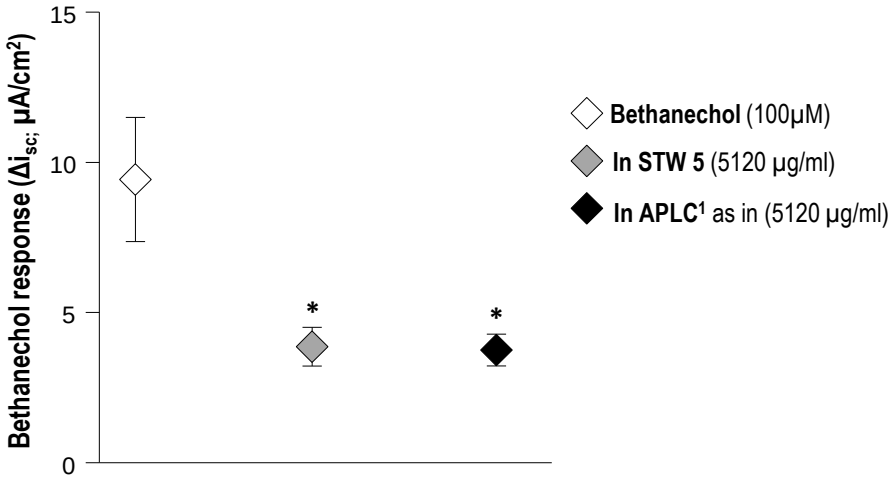

Figure 70: STW 5 (5120 µg/ml) and combined application of APLC at concentration corresponding to 5120 µg/ml STW 5 significantly reduced the bethanechol (100 µM) induced increases in Isc in T84 cells. [1]APLC is combined application of angelica, peppermint, lemon balm and chamomile. (*n*) = 6 filters. * mark significant differences ($P < 0.05$).

3.3.9 Summary of the effects of STW 5 and its components on human intestinal motility and secretion

Table 15 summarizes the effects of STW 5 and its nine individual components on human intestinal motility and secretion.

Table 15: Effects of STW 5 and its components on gastric and intestinal motility as well as intestinal secretion

	Human intestinal motility				Human intestinal secretion	
	Small intestine		Large intestine		Human	T84 cells
	Muscle tone	Motility index	Muscle tone	Motility index		
STW 5	↓	↓ D+J — Ileum	↓	↑↓ **	↑	↑
Angelica	↓	↓	↓	↓	↑	↑
Peppermint	↓	↓	↓	↓	↑	↑
Liquorice	↓	↓	↓	↓	↓ Small only	—
Lemon balm	↓	—	↓	—	↑	↑
Chamomile	↓	↓	↓	—	—	↑
Caraway	↓	↓	—	—	—	—
Gretear celandine	↓	↑ D+J ↓ Ileum	↑	—	—	—
Milk thistle	—	—	—	—	—	—
Bitter candytuft	—	—	—	—	—	—

Black downward arrows reveal decreased activities. Grey upward arrows reveal increased activities. Grey dashes indicate no effects. D+J is duodenum and jejunum. ** Significant increase followed by inhibition.

4. Discussion

The herbal medicine Iberogast® (STW 5) is a hydroethanolic multi-herbal drug combination from nine plant extracts: angelica roots, peppermint leaves, lemon balm leaves, chamomile flowers, liquorice roots, caraway fruits, milk thistle fruits, greater celandine herb and fresh bitter candytuft plant (Wegener & Wagner, 2006). STW 5 is successfully used to treat functional dyspepsia and irritable bowel syndrome (Rosch *et al.*, 2002a;Madisch *et al.*, 2004b). However, there is still much to learn about its mode of action at a molecular level.

This is the first study to investigate the effects of STW 5 and its individual components on different regions of the human gut, providing mechanistic insights into its mode of action as the basis for its clinical use. Several experimental protocols were conducted in order to achieve this goal. Firstly, the mechanism of action by which STW 5 induced relaxation in guinea pig proximal stomach was studied. Secondly, the effects of STW 5 and its individual components on circular and longitudinal muscle of human small and large intestine were screened, as well their mechanism of action. Thirdly, the secretory actions of STW 5 and its individual components were investigated in human small and large intestine as well as in human colonic epithelial T84 cell line.

Briefly, this study provided evidences that STW 5 has inhibitory actions on both tonic and phasic contractions of human intestinal smooth muscle. These effects are concentration-dependent, region- and layer-specific. Furthermore, we identified the individual extracts responsible for these actions. The study also demonstrated that this inhibitory effect as well as STW 5-induced relaxation in guinea pig proximal stomach are myogenic and due to the inhibition of calcium influx via direct blockade of cation channels namely TRPA1 and TRPC (specifically TRPC3) and regulated by intracellular protein kinase C. Finally this study also reported that the prosecretory effect of STW 5 is mainly due to angelica, peppermint, lemon balm with an additional contribution of chamomile in the T84 epithelial cell line. Their action involves activation of cAMP-dependent (cystic fibrosis transmembrane conductance;) CFTR-chloride channels and calcium-activated chloride channels (CaCl).

4.1 Mechanism of STW 5-induced relaxation in proximal stomach

In a previous *in vitro* study, it was shown that STW 5 has a relaxing effect on guinea pig proximal stomach (Hohenester *et al.*, 2004). Similar observations were also reported *in vitro* and *in vivo* in human proximal stomach (Schemann *et al.*, 2006;Pilichiewicz *et al.*, 2007). It was also shown that this effect is neither nerve mediated nor nitric oxide dependent. This action was proposed to be involved in its clinical efficacy. However, to date the mechanism(s) of action by which STW 5 relaxes the smooth muscle remains unclear.

This is the first pharmacological study aimed to determine the mechanism of action by which STW 5 induces relaxation in gastric smooth muscle. The most striking results of the present study are that STW 5 evoked robust and sustained relaxation of gastric corpus muscle strips. This effect is significantly higher in longitudinal than in circular muscle. The pharmacological studies showed that STW 5 induced muscle relaxation was due to inhibition of extracellular calcium influx by functional antagonism of transient receptor potential ankyrin 1 (TRPA1) and transient receptor potential canonical (TRPC), in particular TRPC3, channels. These channels seemed to be open in the resting state of the gastric smooth muscle. Application of STW 5 closed these channels resulting in decrease of $[Ca^{2+}]_i$ and consequently smooth muscle relaxation. Furthermore, protein kinase C might be involved as an additional target of STW 5.
STW 5 was dissolved in Krebs solution to avoid the profound effects of ethanol on ileum and gastric smooth muscle (Wali *et al.*, 1987;Zheng *et al.*, 1997). The aqueous solution was applied at a final concentration of 256 µg/ml. This concentration evoked a reliable relaxation on gastric proximal smooth muscle (Hohenester *et al.*, 2004). Throughout all experiments, it was crucial to keep the pH close to 7.4 to avoid tonic and phasic inhibitory effects of excess CO_2 bubbling (Fujimoto *et al.*, 2011).

In 2004, Hohenester *et al*. showed that STW 5 provoked a relaxation in guinea pig proximal stomach and provided evidences that this inhibition was neither nerve mediated nor nitric oxide dependent suggesting a direct myogenic effect of STW 5 on smooth muscle cells (Hohenester *et al.*, 2004). There are several pathways and intracellular signalling cascades that contribute to the contractile machinery of the smooth muscle. In order to clarify which pathways or intracellular signalling cascades are responsible for or involved in STW 5-induced relaxation, several agonists and antagonists were applied prior to STW 5 and the

changes in STW 5 responses were recorded, analysed and statistically tested against corresponding controls.

In various cell, tissue and animal models others have demonstrated effects of STW5 on a plethora of cell functions. This seemingly involved a number of different receptors and intracellular pathways. Some of these mechanisms also regulate smooth muscle activity and we tested whether these pathways may also be involved in the relaxation of gastrointestinal smooth muscle.

It has been reported that adenosine receptors mediate the anti-inflammatory activity of STW 5 in isolated rat small intestine (Michael *et al.*, 2012). It is not clear how in this model STW 5 reversed the tissue damage, in particular as there appear to be massive necrosis. However, the present study showed that selective blockade of adenosine A1, A2A and A2B receptors had no influence on gastric smooth muscle relaxation, although adenosine itself strongly relaxes the stomach.

Since serotonin receptors are known to play a crucial role in the etiology of FD and IBS, one clinical study showed equivalent efficacy of STW 5 to cisapride, 5-HT_4 agonist, in treatment of patients with functional dyspepsia (Rosch *et al.*, 2002b). Moreover, in 2006 Simmen *at al*, showed higher binding affinity of STW 5 to 5-HT_4 receptors in homogenized rat intestine (Simmen *et al.*, 2006). Nevertheless, the results of the present study showed no influence of 5-HT_4 receptors on STW 5-induced gastric relaxation when selectively antagonised by piboserod. The 5-HT_4 agonist prucalopride had no effect on the muscle tone. The discrepancy may be due to the high STW 5 dose used in the binding studies.

Prostaglandins and the cyclooxygenase system have been shown to play an important role in the protective effects of STW 5 against peptic ulcer in rats (Khayyal *et al.*, 2006). Blockade of these pathways by pre-incubation with the COX-II inhibitor piroxicam had no influence on the inhibitory effect of STW 5. Again, the STW 5 dose (2.5 - 10 ml/kg) used in the *in vivo* studies which demonstrated anti-ulcer effects were higher than those used in my *in vitro* experiments. Although unlikely, species differences could account for the different pharmacology. In any case, the guinea pig seems to be a good model to study mechanisms of gastric relaxation because STW5 evokes gastric accommodation in human and guinea pig stomach alike (Schemann *et al.*, 2006).

It has been shown that different calcium signals in the vascular and gastric smooth muscle target different types of Ca^{2+}-sensitive K^+ channels mediating smooth muscle relaxation (Ledoux *et al.*, 2006;Kim *et al.*, 2009b). The results of the present study showed that blockade

of small-, intermediate- and big-conductance calcium-activated potassium channels had no influence on STW 5-induced relaxation.
In 1997, Makhlouf and Murthy concluded that cAMP-dependent protein kinase A (PKA), cGMP-dependent protein kinase G (PKG) and calmodulin kinase II (CaMK-II) contribute to smooth muscle contractile activity. They also reported a cross talk between PKA and PKG (Makhlouf & Murthy, 1997). The present study demonstrated that blockade of PKA,PKG and CaMK-II played no role in the mechanism mediating the inhibitory effect of STW 5.

The lack of effects of the above mentioned pathways ruled out receptor operated mechanisms hitherto described for STW 5 in other cell systems. We therefore hypothesised direct effects of STW 5 on intracellular Ca^{2+} handling or indirectly via ligand gated ion channels that have some selectivity for Ca^{2+}. This notion was supported by the previously described calcium antagonistic features of coumarins and herbal alkaloids (Kong *et al.*, 1986;Harmala *et al.*, 1992).

In smooth muscle, increasing the intra cellular calcium level $[Ca^{2+}]_i$ provides the main trigger for contraction. To date, two main sources of $[Ca^{2+}]_i$ are recognized; extracellular fluid and intracellular stores (sarcoplasmic reticulum, SR). Ca^{2+} entry provides the major access route to elevate $[Ca^{2+}]_i$ and can be achieved via store-operated (SOCs), voltage-dependent (VDCCs) and/or receptor operated calcium channels (ROCs). Ca^{2+} release from the SR store is governed mainly by inositol 1,4,5-trisphosphate receptors (IP_3-R) and ryanodine receptors (RyR). Increased $[Ca^{2+}]_i$ activates the calcium uptake into SR by SERCA (sarcoplasmic endoplasmic reticulum calcium ATPase pump). The two calcium sources are dependent on each other. Calcium influx regulates calcium release and calcium release regulates calcium influx (Chalmers *et al.*, 2007).

The results demonstrated that inactivation of RyRs by pre-incubation with ryanodine, ruthenium red and caffeine had no influence on STW 5-induced relaxation. As well, blockade of SERCA by thapsigargin did also not affect the tone changes generated by STW 5. It is therefore concluded, that intracellular Ca^{2+} stores are not involved in STW 5 actions on smooth muscle.

Store-operated calcium channels (SOCs) are regulated by the Ca^{2+} release from its stores and hence SR calcium concentration. The transient receptor potential (TRP) superfamily of

channels contains several members that may serve the function of store-operated channels (SOCs). Of these channels, transient receptor potential ankyrin 1 (TRPA1), which is a non-selective cation channel with Ca^{2+} conductance has been reported to regulate various gut functions including intestinal contraction, pain-induced gastric distension and delayed gastric emptying (Penuelas *et al.*, 2007;Doihara *et al.*, 2009;Nozawa *et al.*, 2009;Kondo *et al.*, 2010). Recently, TRPA1 activation was shown to suppress gastric accommodation in guinea pig stomach (Koseki *et al.*, 2012). Tracheal smooth muscle TRPA1 channels can be activated by cinnamaldehyde (CNA) and selectively antagonised by AP-18 (Cheah *et al.*, 2014).

Another member of TRP channels that function as SOCs is the canonical type channel (TRPC). TRPC channels, particularly TRPC3, showed a strong functional link to SOCs activity and are described as a component of SOC channels (He *et al.*, 2005). Pyr3 is a selective blocker for TRPC3 channels (Kiyonaka *et al.*, 2009). SKF-96365 was originally described as a non-selective SOC and ROC blocker (Merritt *et al.*, 1990). More and more evidence suggest that SKF-96365 is also a TRPC channel antagonist based on findings that SKF-96365 inhibited the extracellular calcium influx via TRPC channels in human malignant gliomas and pulmonary artery smooth muscles (Bomben & Sontheimer, 2008;Tang *et al.*, 2010;Song *et al.*, 2014). BTP2 is a selective Ca^{2+} release activated Ca^{2+} (CRAC) antagonist (He *et al.*, 2005). CRAC is a SOC channel with remarkable selectivity for Ca^{2+} and virtually impermeable to other cations (Venkatachalam *et al.*, 2001).

The results showed that pre-treatment of the gastric corpus muscle strips with AP-18 nearly abolished the relaxation evoked by STW 5. Incubation of the TRPA1 agonist CNA significantly increased the inhibitory effect of STW 5 (Figure 71). One of the possible explanations is that CNA potently activates TRPV1 channels which may affect the relaxation by other TRP channels. The activation of TRPV1 may also explain the observed relaxation after CNA application. While AP-18 almost reversed STW 5 responses, another TRPA1 antagonist HC-030031 had no effect. This was surprising because HC-030031 decreased gastric tone in conscious guinea pigs (Koseki *et al.*, 2012). I have no explanation for this finding except that HC-030031 did also not block TRPA1-mediated secretion induced by AITC in guinea pig intestine (personal communication Dr. Dagmar Krueger). It may be a species related issue with TRPA1 affinity or accessibility but there is no evidence in the literature to support this speculation.

Similarly, however to a lesser degree, application of the selective TRPC3 antagonist Pyr3, or the broad range TRPC blocker SKF-96365 reduced the STW 5 induced relaxation (Figure 71). BTP2 (Pyr6) was also described as a broad range TRPC blocker but had no effect on STW 5-induced relaxation. The apparent discrepancy between Pyr3 and BTP2 effects is best explained by additional effect of BTP2 on Orai, one of the store operated calcium channels (Schleifer *et al.*, 2012). These channels are inhibited by concentrations of BTP2 in the low µM range. At this concentration the effect on TRPC3 is rather low and may explain the lack of effect of BTP2 on STW 5 responses. The spasmolytic effect of BTP2 itself is very likely due to its inhibition of Orai.

Moreover, pre-incubation with 2-APB reduced STW 5-induced relaxation in the longitudinal muscle. In the circular muscle, 2-APB fully reversed the action and revealed a nifedipine-sensitive contractile action of STW 5. This agrees with the interpretation of the region specific effects of STW 5. STW 5 has a prokinetic action in the antrum and lower esophageal motility, an effect blocked by nifedipine (personal communication Prof. Michael Schemann). The region-specific functional expression of channels relaxing or contracting smooth muscle is therefore responsible for the different actions of STW 5. 2-APB is an IP_3-R antagonist but at the same time it is a broad range TRPC channel blocker (Tang *et al.*, 2010). I believe that its effect is mainly due to TRPC antagonism as the data from ryanodine, ruthenium red, caffeine, thapsigargin and BTP2 showed that the intracellular calcium store had no role in the mechanism of STW 5 induced relaxation.

These results lead to a hypothesis that TRPA1 and to a lesser degree also TRPC, in particular TRPC3, are the primary targets for STW 5. According to this hypothesis, STW 5 acts as a TRPA1 and TRPC antagonist which resulted in decreased $[Ca^{2+}]_i$ consequently leading to smooth muscle relaxation (Figure 71). This was additionally confirmed by recording changes in intracellular calcium in TRPA1-expressing HEK293 cells. The results showed that STW 5 blocks calcium transients induced by the TRPA1 agonist AITC with similar potency as the selective TRPA1 antagonist HC-030031 (personal communication; Eva Kugler). The TRPA1 antagonistic action of STW 5 may be one explanation for its clinical use in functional dyspepsia. Impaired gastric accommodation is one possible pathology in functional dyspepsia (Tack *et al.*, 1998) and it has been recently shown that TRPA1 antagonism improved gastric accommodation in conscious guinea pigs (Koseki *et al.*, 2012). This fully agrees with the mode of action of STW 5 to relax gastric smooth muscle by TRPA1 blockade.

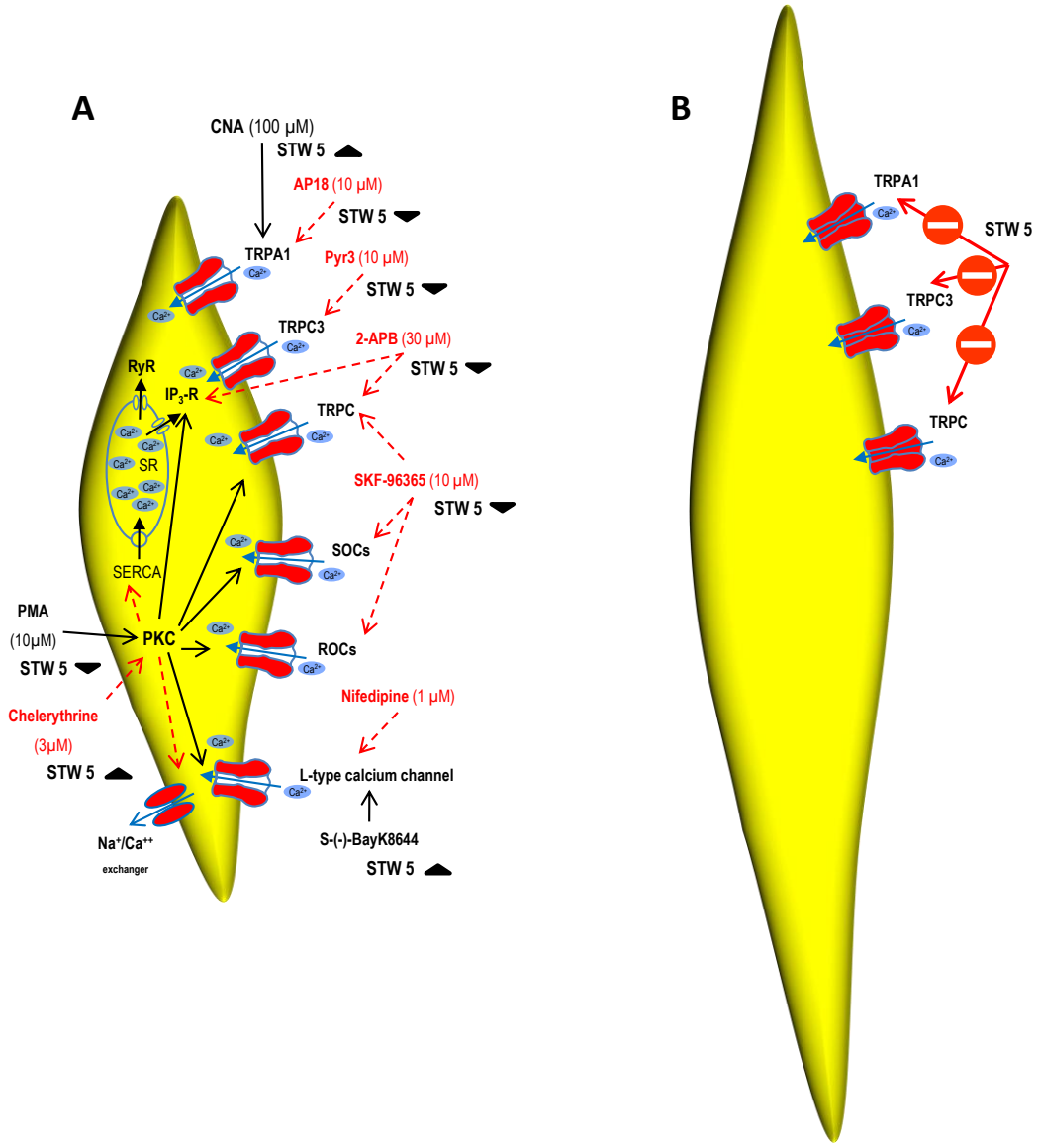

Figure 71: STW 5-induced relaxation in gastric proximal smooth muscle is mainly due to closure of TRPA1 and TRPC, in particular TRPC3 channels. Panel (A) shows a smooth muscle in resting state. During the resting state, Ca^{2+} channels seem to be open as application of channel blockers (in red) relaxes the muscle. On the other hand channel activators (in black) contract the muscle. Both antagonists and agonists affected the relaxing effects of STW 5. Upward and downward triangles indicate increase and decrease in the response, respectively. Panel (B) shows that application of STW 5 blocks TRPA1 and TRPC channels, in particular TRPC3. This blockade inhibits calcium influx and hence decrease $[Ca^{2+}]_i$ leads to muscle relaxation. PKC might be involved as an additional target.

Amongst the intracellular signalling pathways is protein kinase C (PKC), which is an ubiquitous family of protein kinase enzymes possessing feedback regulation of SOCs/TRP channels and playing important roles in several signal transduction cascades (Venkatachalam *et al.*, 2003). PKC can be selectively activated by phorbol derivatives such as phorbol-12 myristate, 13-acetate (PMA) and phorbol-12,13-dibutyrate (PDBu) and potently and selectively inhibited by chelerythrine, an alkaloid extracted from greater celandine (Herbert *et al.*, 1990;Colombo & Bosisio, 1996).

Data concerning the interaction between PKC and calcium regulations are contradictory. Some research groups showed that activation of PKC increased $[Ca^{2+}]_i$ via many pathways involved in calcium ion movement across plasma- and/or SR-membranes, such as activation of L-type calcium channel, ROCs and IP_3-R and inhibition of SERCA and Na^+/Ca^{2+} exchanger in rabbit thoracic aorta and porcine carotid artery (Andrea & Walsh, 1992). In contrast, others concluded that activation of PKC decrease $[Ca^{2+}]_i$ by inhibiting TRPC3, SOCs (Venkatachalam *et al.*, 2003) or L-type calcium channel in A7r5 cells (Nastainczyk *et al.*, 1987) as well as activating SERCA increasing the calcium uptake in bovine aortic microsomes (Fukuda *et al.*, 1990) and Na^+/Ca^{2+} exchanger on plasma membrane of A7r5 cells (Vigne *et al.*, 1988). The diverse often conflicting findings may be due to different models like the use of intact tissue versus vascular smooth muscle cell line (A7r5 cells). There is no evidence for PKC/TRPA1 interaction (Mandadi *et al.*, 2011;Petho & Reeh, 2012). There is also no data available for gastric smooth muscle. The observations of the present study showed that activation of PKC by PMA induced a small, yet significant muscle contraction reflecting an increase in $[Ca^{2+}]_i$ whilst PKC inhibition by chelerythrine significantly relaxed the muscle. Additionally, preliminary experiments showed that PMA-induced contraction is significantly reduced in the TRPA1 blocker AP-18.

Moreover, the results of the present study showed that activation of PKC by PMA significantly reduced the STW 5-induced relaxation whereas its blockade by chelerythrine significantly increased the STW 5 inhibitory action. It has been established that 50 µM decursin derived from angelica is a PKC activator and used in treatment of malignancies (Ahn *et al.*, 1996;Kim *et al.*, 2005a;Harn *et al.*, 2011). Angelica was shown to mimic the inhibitory effect of STW 5 (Schemann *et al.*, 2006). Menthol responsiveness was significantly inhibited in DRG neuron pre-treated with PKC activator PDBu (Sarria & Gu, 2010).

Interference with other pathways also resulted in an altered STW 5 response. However, for several reasons these are not considered as main targets. In principle, the effect of every drug which alters muscle tone depends on the resting muscle tone just prior to drug application. The potency of a drug reducing muscle tone will decrease if the baseline muscle tone is low; at a muscle tone of zero the effect will not be demonstrable anymore. This methodological limitation has to be considered. Thus, it is unlikely that PKC is the primary target for STW 5 because PKC inhibition potentiates but did not abolish STW 5 induced relaxation. Additionally, concentrations of the angelica ingredient decursin, which seems responsible for the PKC activating properties (Ahn *et al.*, 1996;Kim *et al.*, 2005a), was much lower in the muscle strip experiments. It is also unlikely that STW 5 acts as a blocker of voltage sensitive calcium channels (VDCC). Firstly, T-type Ca^{2+} channels may be ruled out as its blockade did not affect STW 5 induced relaxation. Secondly, blockade of L-type Ca^{2+} channels is not likely although peppermint and menthol, both present in STW 5 relax smooth muscle by blocking VDCC (Hawthorn *et al.*, 1988;Amato *et al.*, 2014). The L-type calcium channel is a VDCC that can be antagonised by nifedipine (Bossert & Vater, 1989) and selectively activated by S-(-)-Bay k 8644 (Franckowiak *et al.*, 1985). Pre-treatment of the muscle strips with nifedipine significantly reduced STW 5-induced relaxation. This would at first sight suggest an effect of STW 5 on L-type channels. However, in these experiments carbachol was applied to the preparations to increase muscle tone that had been dramatically reduced by nifedipine. Carbachol has been shown to induce its contraction via sustained increase $[Ca^{2+}]_i$ through VDCC influx mechanisms in human and guinea pig corporal gastric smooth muscle and porcine airways smooth muscles (Kumasaka *et al.*, 1996;Kim *et al.*, 2008;Kim *et al.*, 2009a). This interference of carbachol with VDCC may affect STW 5 responses. On the other hand, incubation of the tissues with S-(-)-Bay k 8644 prior to STW 5 resulted in a significantly higher inhibitory effects on muscle tone. Because of the high affinity of S-(-)-Bay k 8644 (Bellemann & Franckowiak, 1985), this effect can only be explained when the main effect of STW 5 is not mediated through L-type channels. Moreover, if STW 5 would act as a VDCC antagonist one would have suggested that its effect is reduced in S-(-)-Bay k 8644. To verify this, preliminary experiments were carried out in which tissues were incubated with STW 5 before addition of S-(-)-Bay k 8644. In these experiments, S-(-)-Bay k 8644 still evoked a contractile response that was not affected by STW 5. This further supports the hypothesis that the main effect of STW 5 is not mediated through L-type calcium channels.

In conclusion, STW 5 has a relaxing effect on gastric proximal smooth muscle. The relaxation is more prominent in longitudinal than in circular muscle. The inhibitory effect is mainly due to closure of TRPA1 and TRPC, in particular TRPC3 channels. These channels seem to be open in the resting state as the specific blockers induced relaxation. STW 5 decreases the intracellular calcium level by closing them. Activation of PKC might be involved as additional target.

4.2 STW 5 Effects on human intestinal motility

This is the first report describing the effects of STW 5 and its individual components on human intestinal motility. To perform this study, circular and longitudinal muscle strips dissected from human small and large intestine were used. The muscle tone and the motility index were analysed. The latter is the product of contractile force and frequency and represents phasic muscle activity.

STW 5 decreased both tonic and phasic contractile activity in isolated human intestinal smooth muscle strips. The effects were concentration dependent as well as region- and layer-specific. The relaxation of the muscle tone is more prominent and occurs already at low doses in circular than in longitudinal muscle and more pronounced in large than in small intestine. In the duodenum and jejunum, reduction of phasic contractions occurred only at the highest dose of 5120 µg/ml. In the large intestine, reduced phasic motility was already observed at lower doses. In the guinea pig stomach, STW 5 increased phasic activity in the antrum but decreased tone in the corpus. An increased contractile force was sometimes also seen in intestinal muscle strips indicated by large amplitude clustered contractions. However, it is not known whether this increase in muscle activity is mediated by increased Ca^{2+} influx through L-type Ca^{2+} channels as is the case in the gastric antrum. The decrease in muscle tone is prevailing in small and large intestine and the results from pharmacological intervention revealed antagonism of TRPA1 and TRPC3 as most relevant mechanism. In this way the mode of spasmolytic action of STW 5 is comparable in the gastric corpus and intestine.
The results further revealed that apart from bitter candytuft and milk thistle all other extracts contribute to STW 5 actions in that they evoked a relaxation. Similar to STW 5, the reduction in muscle tone was more pronounced in large than in small intestine. In the small intestine, peppermint, angelica, chamomile, liquorice and caraway had additionally inhibitory effects on the phasic contractions. Greater celandine significantly increased the motility index in duodenum and jejunum and decreased it in ileum. Lemon balm had no effect on motility index. In the large intestine, peppermint, liquorice, angelica, chamomile and lemon balm decreased muscle tone. Peppermint, liquorice and angelica also inhibited the phasic contractility while greater celandine increased the muscle tone without affecting the motility index.

The results revealed that STW 5 has inhibitory effects on human intestinal smooth muscle. Similar effects were shown for histamine/acetylcholine pre-contracted muscle of guinea pig

and rat intestine (Ammon *et al.*, 2006). The spasmolytic effects were concentration-dependent, similar dose-related actions had been reported for guinea pig stomach and ileum (Hohenester *et al.*, 2004;Schemann *et al.*, 2006;Ammon *et al.*, 2006;Heinle *et al.*, 2006). The stronger effects of STW 5 in large compared to small intestine was previously also reported in guinea pig and rat (Ammon *et al.*, 2006). The observed differences in responses between longitudinal and circular muscle had been described for many stimuli in different regions of the gut from various species (Brownlee & Harry, 1963;Kuriyama *et al.*, 1975;Miyazaki *et al.*, 1991;Nieto *et al.*, 2000;Undi *et al.*, 2009). These differences in responses between circular and longitudinal muscle could be related to the higher thickness of former compared with the later.

STW 5 and its nine extracts had been shown to decrease mice intestinal smooth muscle resting membrane potential and slow wave rhythmicity. The frequency and amplitude of the slow waves were significantly decreased by STW 5. This effect was a summation of effects of the nine components at which angelica and chamomile completely blocked the slow waves, bitter candytuft increased the frequency and amplitude, greater celandine herb reduced frequency and amplitude of the slow wave, peppermint leaf reduced frequency and left amplitude unchanged and liquorice root whereas caraway fruit and lemon balm leaf had no effects (Storr *et al.*, 2004;Sibaev *et al.*, 2006).

The highest concentration of STW 5 (5120 µg/ml) evoked region-dependent changes in phasic contractions. In the small intestine, phasic contractions were significantly reduced in duodenum and jejunum, whereas in ileum, the phasic contractions were initially decreased and then increased however none of them was significant. In the large intestine, STW 5 evoked a transient increase followed by complete inhibition of the phasic contractions (only 3 out of 11 tissues showed ongoing clustered contractions in the presence of STW 5).Similar bi-phasic responses were also observed in guinea pig proximal stomach (Hohenester *et al.*, 2004). Based on the results from the present study, these biphasic responses may reflect the dual mode of action of STW 5 in gut smooth muscle; STW 5 potentiates L-type Ca^{2+} channel activity and at the same time inhibits TRPA1 and TRPC3 mediated Ca^{2+} influx. The tonic effect at the higher concentration was previously recorded (Ammon *et al.*, 2006). Similar region-specific effects of STW 5 were also reported for human and guinea pig stomach; relaxation of proximal stomach vs. enhancement of the antrum contractions (Hohenester *et al.*, 2004;Schemann *et al.*, 2006;Pilichiewicz *et al.*, 2007). These mixed effects in different regions of the human gut might explain the efficacy of STW 5 in treatment of all IBS subtypes (IBS-C, IBS-D or IBS-M) (Madisch *et al.*, 2004b).

Regarding the identification of the single extracts, the results showed that apart from bitter candy tuft and milk thistle, the other extracts exhibit different inhibitory potencies on human intestinal smooth muscle. Peppermint > liquorice > angelica nearly mimicked the inhibitory effect of STW 5 on the phasic contractions.

It is unlikely that the clinical efficacy of STW 5 to treat irritable bowel syndrome can be fully explained by the action of peppermint. Nevertheless, the conducted experiments for peppermint showed significant potent concentration-dependent inhibition of muscle tone as well as phasic contractility of both small and large intestine. Similar changes occurred in circular muscle of human colon with the difference that in this study contractile amplitude but not frequency decreased (Amato *et al.*, 2014). This difference may be attributed to cumulative application of different concentrations of peppermint oil. Clinical trials showed a relaxing effect and a significant delay in human orocaecal transit time for peppermint (Micklefield *et al.*, 2000;Goerg & Spilker, 2003;Micklefield *et al.*, 2003). Peppermint is successfully used in treatment of IBS (Koch, 1998;Cappello *et al.*, 2007). It also relaxes human oesophageal and gastric smooth muscle (Pimentel *et al.*, 2001;Hiki *et al.*, 2003;Hiki *et al.*, 2011). Similar actions were reported for other species (Forster *et al.*, 1980;Heinle *et al.*, 2006;de Sousa *et al.*, 2010;Guo *et al.*, 2014). The superiority of peppermint over the other extracts present in STW 5 was previously reported (Heinle *et al.*, 2006).

The results also showed potent inhibitory effects of liquorice on tonic and phasic contractions of circular smooth muscle in small and large intestine that may support its use in Japanese herbal medicine Hangeshasin-to in the treatment of diarrhoea (Suzuki *et al.*, 2009). An *in vivo* study illustrated the inhibitory effect of liquorice on rat small intestinal motility (Lee *et al.*, 2013). The antispasmodic effects of liquorice were observed in guinea pig stomach and ileum (Schemann *et al.*, 2006;Heinle *et al.*, 2006) as well as in mouse jejunum (Sato *et al.*, 2006;Sato *et al.*, 2007;Nagai *et al.*, 2007).

The present study provided evidence that angelica is a potent inhibitor for both tonic and phasic contractions. In accordance, similar inhibitory effects were demonstrated in guinea pig stomach and ileum (Reiter & Brandt, 1985;Schemann *et al.*, 2006;Heinle *et al.*, 2006). The spasm-relieving potential of angelica was also shown in rat blood vessels (Du *et al.*, 2007).

As most of the components of STW 5 have effects on smooth muscle, on would expect also an effect of STW 5 on blood pressure which has not been reported so far. This can be explained either by a slow absorbtion or a degradation of STW 5 components. Additionally,

all active components entering the blood stream would be strongly diluted in the blood and other body fluids.

In accordance with the present study, chamomile inhibited tonic and phasic contractions of the guinea pig ileum (Ammon *et al.*, 2006;Heinle *et al.*, 2006). The antispasmodic properties of chamomile have previously been demonstrated in guinea pig ileum and porcine vascular smooth muscle (Forster *et al.*, 1980;Achterrath-Tuckermann *et al.*, 1980;Roberts *et al.*, 2013).

The results of the present study showed significant inhibitory effect of caraway only on the small intestinal motility and agrees with the spasmolytic action in guinea pig ileum (Forster *et al.*, 1980;Heinle *et al.*, 2006). Region-specific effects of caraway were demonstrated (Reiter & Brandt, 1985).

The contribution of peppermint, chamomile, liquorice and caraway in STW 5 activity is supported by the clinical efficacy of STW 5 II which lacks angelica, milk thistle and greater celandine, in treatment of IBS patients (Madisch *et al.*, 2004b). However, the in vitro data of the present study favour a significant contribution of angelica to STW 5 effect, in particular at the lower concentrations.

The data revealed antispasmodic effect of lemon balm extract which is in accordance with a previous study (Heinle *et al.*, 2006) and supported by an *in vivo* study demonstrating the inhibitory effects of lemon balm and chamomile on mice and guinea pig intestinal motility (Forster *et al.*, 1980;Savino *et al.*, 2008).

The effects of greater celandine were variable. In small intestine, greater celandine relaxed the small intestinal circular muscle but it increased in the duodenum and jejunum contractile amplitude and frequency while it decreased phasic motility in the ileum. In the large intestine, greater celandine increased the muscle tone without affecting the motility index. Differential effects in different gastrointestinal regions had been demonstrated before in animal studies (Hiller *et al.*, 1998;Schemann *et al.*, 2006). While greater celandine had antispasmodic action in guinea pig ileum it increased tonic and phasic contractions in proximal and distal stomach.

In contrary to previous findings where Ammon et al., (2006) showed that bitter candytuft potentiated tonic and phasic contractility in guinea pig ileum, the present study reported no changes in both tonic and phasic contractions either in small or large intestine. Interestingly, STW 5 and STW 7 (STW 5 without bitter candytuft) exerted similar spasmolytic effects on pre-contracted muscle (Ammon *et al.*, 2006). Bitter candy tuft had also no effect in relieving IBS symptoms (Madisch *et al.*, 2004b).

STW 5-induced relaxation in the stomach was neither nerve mediated nor nitric oxide dependent suggesting a direct myogenic action (Hohenester *et al.*, 2004). In the present study a direct influence on calcium influx is strongly suggested as the primary mode of inhibitory action. The results provide evidence that the relaxing effect of STW 5 is due to reduction of the intracellular calcium level via closure of TRPA1 and TRPC in particular TRPC3 channels. In accordance, the spasmolytic effects of furanocoumarins on smooth muscle preparations and angelica involved reduced calcium influx (Kong *et al.*, 1986;Harmala *et al.*, 1992). Similar findings were reported for peppermint and its main active constituent menthol (Hills & Aaronson, 1991;Amato *et al.*, 2014). It is noteworthy that coumarins and menthol are the main active constituent of angelica and peppermint, respectively (Kroll & Cordes, 2006) and strikingly, peppermint and angelica were the two main components mimicking the inhibitory effect of STW 5 on intestinal motility.

It is concluded that STW 5 has a dose-dependent inhibitory effect on human intestinal smooth muscle. The effect is region-dependent and layer-specific and mainly caused by peppermint, angelica and liquorice. Its inhibitory effect involves decreased intracellular calcium level via the closure of TRPA1/TRPC3 family. These channels seem to be open in the resting state. STW 5 decreases the intracellular calcium level by closing them.

4.3 STW 5 effect on intestinal ion secretion

In 2009, Krueger *et al*. reported the prosecretory effect of STW 5 in an intestinal epithelial cell line and in human small and large intestinal mucosal / submucosal preparations and proposed that this action may be involved in its clinical efficacy (Krueger *et al.,* 2009). Results from the present study extend this observation by identifying the individual components responsible for the prosecretory action.

The results showed that out of the nine herbal extracts composing STW 5 it was mainly angelica which mimicked the prosecretory action of STW 5 in human intestine. At higher concentrations, peppermint and lemon balm contributed. The prosecretory potentials were comparable in small and large intestine. Only in T84 cells, chamomile showed additional secretagogue activity. The prosecretory effects involved activation of epithelial cAMP- and calcium-activated chloride channels. Only in small intestine, liquorice exerted anti-secretory effects by increasing Na^+ absorption because the effect was reversed by amiloride.

The extracts seem to contain all relevant components. This conclusion is based on the finding that remixing the nine extracts (sSTW 5) produced effects very much comparable to those of STW 5. This suggests that there is no loss of biological activity during the extraction process.

STW 5, sSTW 5, individual components and their combinations were basolaterally applied in a concentration corresponding to 512 µg/ml, a concentration that evoked reliable increases in ion secretion in both human tissue and T84 cells (Krueger *et al.,* 2009). In parallel, tenfold higher concentrations corresponding to 5120 µg/ml were studied. This concentration is still below the clinical therapeutic dose of 51.3 mg/ml present in 20 drops (1 ml) Iberogast®.

The main biologically active ingredients have been identified in the present study. The further identification of the molecules was not feasible as the nine extracts contain more than 300 defined phytochemicals (Wegener & Wagner, 2006).

Osthole and furocoumarins are coumarins' derivatives which are considered to be the main active constituents in angelica archangelica (Kroll & Cordes, 2006). The present study identified angelica as the main active extract mimicking STW 5 secretagogue effects via activation of CFTR and CaCl channels. These findings are in agreement with the recently reported data describing osthole as a very potent natural activator for chloride secretion in rat colonic mucosa via activation of CFTR channels (Yang *et al.*, 2011). Furocoumarin also has inhibitory effects on acetylcholine esterase which could lead to the activation of $CaCl^-$ channels through neural pathways (Sigurdsson & Gudbjarnason, 2007). This seems unlikely as STW 5 did not increase nerve mediated ion secretion which is partly mediated by acetylcholine (Krueger *et al.*, 2009).

At a concentration corresponding to 512 µg/ml STW 5 the angelica-evoked response was significantly reduced in TTX which is in accordance with the nerve-mediated action of STW 5 shown previously (Krueger *et al.,* 2009). This finding could explain that, at least at low concentrations, angelica increased ion secretion only in human tissue and not in T84 cells. Angelica-containing preparations have long been used in ancient traditional medicine in the far-east and western countries to treat many diseases including gastrointestinal disorders (Sarker & Nahar, 2004). It's essential oils exhibit antimicrobial activity against many gastrointestinal infectious agents (Fraternale *et al.*, 2014).

Menthol is the main active constituent in peppermint (30-55%) (Grigoleit & Grigoleit, 2005).This study revealed that peppermint activates apical CFTR and CaCl channels in small and large intestinal epithelium and T84 cells. Similar activation of the apical CFTR channels by menthol was reported in human epithelial airways Calu-3 cells (Morise *et al.*, 2010).

The present results showed secretagogue action of lemon balm via activation of chloride channels. As outlined above it is questionable whether its cholinesterase inhibitor properties contribute to this action (Howes & Perry, 2011). Involvement of peppermint and lemon balm in STW 5-induced secretion is supported by the efficacy of STW 5 II (STW 5 without angelica, milk thistle and greater celandine) in IBS treatment (Madisch *et al.*, 2004b).

It has been shown in this study that co-application of the prosecretory components, at concentration corresponding to 512 µg/ml STW 5 evoked comparable responses to STW 5 (512 µg/ml) in both human tissue and T84 cells, which supported the notion that these extracts play a central role for the prosecretory action of STW 5. There is no evidence for synergistic actions because combined application of those extracts that had no prosecretory effect evoked no responses (see section 3.3.3).

The results demonstrated that in human tissue at concentration of 5120 µg/ml, STW 5 and the combination of its prosecretory components (angelica, peppermint and lemon balm) evoked comparable responses to STW 5 (512 µg/ml). These responses are significantly lower than the expected value calculated from their individual responses. In T84 cells the responses were significantly higher than STW 5 and comparable to the calculated value. This observation could be explained, at least in part, by the nerve-mediated activity of STW 5 (Krueger *et al.*, 2009) and the naturally-occurring extracellular factors regulating chloride secretion in intact human mucosal/submucosal preparations which are not existing in cultured T84 cells such as mast cells, neutrophils and fibroblasts (Barrett, 1993). These factors may play a role in controlling secretion based on the fact that the regulation of chloride secretion is a complex

interplay between excitatory and inhibitory influences from neurotransmitters and chemical messengers released from epithelial endocrine and immune cells (Cooke, 1998).

It is well-established that mast cell activation leads to chloride secretion by releasing mediators such as histamine and prostaglandins, and activation of the enteric nervous system, reviewed by (Barrett, 1993). Recently chamomile was reported to have a potent anti-allergic activity by inhibition of histamine release from mast cells (Chandrashekhar *et al.*, 2011). We hypothesize that chamomile may inhibit degranulation of mast cells in intact human specimens which has a negative impact on chloride secretion. This may then offset the direct epithelial prosecretory action. Absence of mast cells as well as other extracellular factors in cultured T84 cells could explain its prosecretory response only in T84 cells and not in human tissues. These data may be supported by the spasmolytic activity of chamomile in histamine-induced contraction in guinea pig ileum smooth muscle (Ammon *et al.*, 2006;Heinle *et al.*, 2006).

The results showed anti-secretory effect of liquorice in small intestine which was significantly reduced in amiloride. In addition to its epithelial sodium channel (ENaC) blockade activity, amiloride is also a potent antagonist for Na^+/H^+ exchanger (Garty & Palmer, 1997;Harris & Fliegel, 1999). Since ENaC are mainly expressed in large intestine (Kato & Romero, 2011), this effect could also be due to inhibition of Na^+ absorption via apical Na^+/H^+ exchanger which is highly expressed in small intestine (Counillon *et al.*, 1993;Harris & Fliegel, 1999;Kato & Romero, 2011). Liquorice seems to have a region-specific activity as it has no influence on secretion in large intestine nor in T84 cells which are derived from human colon adenocarcinoma cell line. This decrease in Isc as well as its inhibitory effect on intestinal motility could be supported by the use of the Japanese herbal medicine Hangeshasin-to containing liquorice in treatment of diarrhoea (Suzuki *et al.*, 2009). Bitter candytuft had no influence on ion secretion neither in human intestine nor in T84 cells.

Cystic fibrosis trans-membrane conductance regulator (CFTR) is a cAMP-dependent chloride channel highly expressed in the apical membrane of gut epithelia and considered to be the main gate responsible for chloride secretion (Nilius & Droogmans, 2003). CFTR channels can be directly and selectively blocked by $CFTR_{inh}$-172 or indirectly through inhibition of the adenylate cyclase by MDL-12,330A (Thiagarajah *et al.*, 2004;Murek *et al.*, 2010). The responses of the extracts and their combinations were significantly reduced in both blockers providing strong evidence for enhanced chloride secretion. Similar reduction in STW 5-induced secretions by these blockers has been observed before (Krueger *et al.*, 2009).

However, neither the blockade of the CFTR chloride channel, nor the inhibition of adenylate cyclase completely prevented the secretion, suggesting involvement of other chloride secretory pathway via calcium-activated chloride channels (CaCl). Recently, the transmembrane protein TMEM 16A (Anoctamin 1) was identified as a candidate for such a CaCl (Yang *et al.*, 2008). SITS is a potential inhibitor of TMEM 16A (De La *et al.*, 2008) and described as CaCl blocker (Gawenis et al., 2010). The secretory responses of sSTW 5, all extracts and their combinations were significantly reduced in SITS which has been also reported for STW 5 (Krueger *et al.*, 2009). The dual mechanism is believed to be of clinical relevance as these extracts can evoke secretion even if, under any pathological condition, one of these channels is down regulated (Krueger *et al.*, 2009).

The reduced responses in a mixture of $CFTR_{inh}$-172/SITS were comparable to the reduction in $CFTR_{inh}$-172 alone providing evidence that the ion secretion is mainly through CFTR channels. In harmony, similar non-synergistic or non-additive effects of $CFTR_{inh}$-172/SITS mixture have been reported in preliminary study with T84 cells (own unpublished data; Bachelor thesis, Lisa Gruber, 2007). These incomplete inhibitions suggest additional contribution of other ion channels. One candidate is bicarbonate. The results showed that in human tissue, EFS- and STW 5-evoked secretion were significantly reduced in HEPES Krebs which suggested bicarbonate secretion. Under physiological conditions, the anion secretion in the proximal colon seems to be primarily mediated by the $Na^+/K^+/2Cl^-$ co-transporter and to a small extent by Cl^-/HCO_3^- exchanger which is coupled to Na^+/ H^+ exchange proteins (Gawenis *et al.*, 2010). In addition to chloride secretion, Cl^-/HCO_3^- exchanger and Na^+/HCO_3^- co-transporter contribute to bicarbonate secretion via the CFTR channel (Verkman & Galietta, 2009). In addition, cation absorption may be changed by STW 5. Although, the role of sodium was ruled out (Krueger *et al.*, 2009), the involvement of K^+ uptake is still conceivable. Potassium ions play an important role in the maintenance of epithelial anion secretion and are absorbed through basolateral K^+ channels, intermediate-conductance (IK1) channels, calcium-dependent K^+ channels and KvLQT1 channels which are regulated by cAMP-dependent and CaCl channels (Moser *et al.*, 2008).

We lack data on TRP channel involvement in the prosecretory action of STW 5. In preliminary experiments I found that HC-030031 indeed reduced the STW 5 induced secretion. This effect is puzzling and is difficult to conceive because TRPA1 activation, but not TRPA1 inhibition, has been shown to enhance anion secretion in human intestine (Kaji *et al.*, 2012).

The findings that the resistance of human tissue and T84 cells were not changed after the extract(s) application are consistent with the lack of effect of STW 5 (personal communication Dr. Dagmar Krueger). Interestingly, STW 5 appear to rescue mucosal integrity after inflammatory challenge by 5-fluorouracil (Wright *et al.*, 2009), which may be related to its anti-inflammatory effects (Wadie *et al.*, 2012) rather than direct improvement of mucosal barrier.

In T84 cells, viabilities were checked by forskolin which was applied as a second stimulus after the drugs. Forskolin is an adenylate cyclase activator (Seamon & Daly, 1981). Compared to respective controls, the forskolin responses were not touched after any of the single extracts (at both concentrations), significantly reduced after STW 5 and the combination at 512 µg/ml and completely abolished after STW 5 and the combination at 5120 µg/ml. These significant reductions and inhibitions led to the conclusion that forskolin and the extracts activate the same pathway. This is in agreement with the finding that repeated forskolin application always resulted in smaller responses.

There are many drug categories with different targets developed to treat IBS, among them are drugs regulating intestinal secretion (Manabe *et al.*, 2010). Generally, secretogouges such as Lubiprostone and Linaclotide -regardless of their mechanism of action- are successfully used in the treatment of constipation, chronic idiopathic constipation and IBS-C (Andresen *et al.*, 2007;Johanson *et al.*, 2008;Thomas & Allmond, 2013) however, some adverse effects were reported (Chamberlain & Rao, 2012). On the other hand, the CFTR antagonist Crofelemer is a novel therapy option for treatment of secretory diarrhoea (Crutchley *et al.*, 2010;Cottreau *et al.*, 2012) and also showed an increase in pain- and discomfort-free days in female patients with IBS-D (Mangel & Chaturvedi, 2008).

In conclusion, identification of region and target specific action of the extracts suggests the potential for disease-targeted combinations or monotherapy. An already existing example is the efficacy of enteric coated peppermint oil in relieving IBS symptoms (Rees *et al.*, 1979;Cappello *et al.*, 2007;Merat *et al.*, 2010). Similar efficacy was observed in a study using a dietary integrator which contained angelica to improve IBS symptoms (Astegiano *et al.*, 2006). However, the contribution of angelica is unclear. Strikingly, STW 5 improved symptoms in constipation as well as in diarrhoea predominant IBS which is unexpected given the spasmolytic and prosecretory action of STW 5. Since there is no symptom-specific

evaluation available it is not known whether the symptom improvement is based on pain-relief rather than normalisation of stool behaviour.

Altered motility, discomfort and pain are common symptoms in patients with functional gastrointestinal disorders (FGIDs) (Brierley & Kelber, 2011). Most frequent and symptomatically overlapping are functional dyspepsia (FD) and irritable bowel syndrome (IBS) (Suzuki & Hibi, 2011;Ottillinger *et al.*, 2013).

Iberogast® as well as its individual components have prosecretory actions on human intestinal mucosa (Krueger *et al.*, 2009). Additionally, it possesses region-specific effects on gastric fundus, corpus and antrum (Hohenester *et al.*, 2004;Schemann *et al.*, 2006). These actions were further confirmed in a clinical pharmacological study (Pilichiewicz *et al.*, 2007). Moreover, implication of TRPA1 in the mode of action of STW 5 may play an additional important role in the reduction of inflammation and nociception usually associated with gastrointestinal disorders (Kondo *et al.*, 2010;Koseki *et al.*, 2012;Koivisto *et al.*, 2013). As well, peppermint influenced this action through TRPM8 (Liu *et al.*, 2013). Furanocoumarins, the major active constituent of angelica was recently described as TRPV1 modulator which may help in its pain relieving action (Chen *et al.*, 2014). Additionally, chamomile was recently reported to have mast cell stabilizing activity (Chandrashekhar *et al.*, 2011). This may also have anti-nociceptive effects as mast cell mediators provoke visceral pain (Barbara *et al.*, 2007). In animal model STW 5 reduced sensitivity of visceral afferents to mechanical and chemical stimulation (Mueller et al., 2006). This study did not reveal this anti-nociceptive mode of action of STW 5. It may very well be that the TRPA1 antagonism found in the present study play a major role because TRPA1 is expressed on visceral nociceptors (Brierley *et al.*, 2009;Blackshaw *et al.*, 2010).

Summary

Table 16 summarizes the effect of STW 5 and its individual components on gastric and intestinal motility as well as their effects on intestinal secretion.

Table 16: Effects of STW 5 and its components on gastric and intestinal motility and intestinal secretion

	Human intestinal motility				Human intestinal secretion		Guinea pig gastric motility	
	Small intestine		Large intestine		Human Small & large	T84 cells	Proximal	Antrum
	Muscle tone	Motility index	Muscle tone	Motility index	ΔI_{sc}	ΔI_{sc}	Muscle tone	Muscle tone
STW 5	↓	↓ D+J – Ileum	↓	↑↓ **	↑	↑	↓	↑
Angelica	↓	↓	↓	↓	↑	↑	↓	↑
Peppermint	↓	↓	↓	↓	↑	↑	↑↓ *	↑↓ *
Liquorice	↓	↓	↓	↓	↓ Small only	–	↓	↑
Lemon balm	↓	–	↓	–	↑	↑	↑	↑
Chamomile	↓	↓	↓	–	–	↑	↓	↑
Caraway	↓	↓	–	–	–	–	↑	↑
Gretear celandine	↓	↑ D+J ↓ Ileum	↑	–	–	–	↑	↑
Milk thistle	–	–	–	–	–	–	↑↓ *	↑↓ *
Bitter candytuft	–	–	–	–	–	–	↑	↑

Black downward arrows reveal decreased activities. Grey upward arrows reveal increased activities. Grey dashes indicate no effects. D+J is duodenum and jejunum. * Inconsistent effects some tissues showed an increase whilst others showed a decrease. ** Significant increase followed by inhibition. Guinea pig data were previously published (Schemann *et al*., 2006).

Appendix

Table 17: Effects EFS-evoked contraction on fresh versus overnight stored human intestinal muscle

	On-response			**Off-response**		
	Fresh vs. overnight stored	**n**	***P* value**	**Fresh vs. overnight stored**	**n**	***P* value**
Small intestine						
Circular muscle	33.1 ± 16.9 vs. 2.4 ± 1.0	$n = 5$	$P = 0.2$	76.6 ± 21.4 vs. 17.8 ± 9.9	$n = 5$	$P = 0.1$
Longitudinal muscle	121.3 ± 23.3 vs. 94.5 ± 12.7	$n = 5$	$P = 0.3$	80.7 ± 33.4 vs. 64.5 ± 11.0	$n = 5$	$P = 0.6$
Large intestine						
Circular muscle	6.5 [0.0/7.5] vs. 2.4 [0.0/9.4]	$n = 7$	$P = 1.0$	91.6 ± 19.7 vs. 83.7 ± 15.1	$n = 7$	$P = 0.8$
Longitudinal muscle	19.8 ± 6.1 vs. 31.1 ± 15.6	$n = 6$	$P = 0.3$	26.6 ± 5.9 vs. 30.2 ± 15.8	$n = 6$	$P = 0.8$

Table 18: Effects of STW 5 on human small intestinal circular muscle motility

Conc. [µg/ml]	No.		**Tone** *(mN)*				**Amplitude** *(mN)*				**Frequency** (*peak/min.*)				**Motility index** *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
64	6	Abs.	44.2 [36.3/52.3]	43.9 [35.7/51.8]	43.1 [35.4/51.9]	41.1 [35.4/52.2]	11.2±3.4	11.0±2.9	8.0±1.8	6.3±1.4	9.0±1.6	8.9±1.8	6.3±0.9	5.6±1.1	111.5±40.8	100.3±32.5	48.8±13.2	39.2±15.2
		%		1.3 [-0.6/2.0]	1.0 [0.5/4.9]	1.3 [0.2/4.2]		-8.1±9.9	-28.9±59.1	0.6±42.1		0.5±5.1	18.5±20.0	23.5±23.8		4.3±6.4	51.9±15.4	60.6±17.4
		Sig.		*P = 0.2 §*				*P* = 0.3				*P* = 1.0	*P* = 0.1	*P* = 0.04			*P* = 0.1	
128	5	Abs.	39.6±4.0	39.2±4.2	38.2±4.2	37.8±4.3	12.6±5.7	14.8±6.6	17.8±8.0	18.1±8.3	10.1±2.4	9.4±2.6	8.2±2.0	7.6±1.8	135.6±66.1	145.9±67.0	172.0±81.8	167.4±79.4
		%		1.3±0.9	3.9±1.5	5.0±1.6		-16.4±11.6	-44.1±43.2	-38.4±37.6		16.7±12.4	27.5±15.6	30.7±15.4		1.3±19.6	-14.3±50.9	-8.1±47.4
		Sig.			*P* = 0.01	*P* = 0.002		*P* = 0.3				*P* = 0.1					*P* = 0.5	
256	7	Abs.	39.7±3.5	39.0±3.3	38.3±3.0	37.9±3.0	10.2 [5.9/21.0]	10.6 [4.5/17.9]	10.6 [4.3/18.5]	6.9 [3.2/16.7]	9.6±0.9	8.6±1.4	8.2±0.8	7.2±1.1	139.4±40.4	120.6±42.9	123.7±50.4	102.7±50.0
		%		1.8±0.3	4.0±0.9	5.3±1.2		14.5 [-7.9/33.2]	12.2 [-23.0/46.4]	20.6 [-5.6/68.9]		13.8±11.8	14.1±5.1	27.1±7.4		11.5±21.3	18.8±17.6	42.5±12.8
		Sig.			*P* = 0.04	*P* = 0.006		*P = 0.2 §*				*P* = 0.5	*P* = 0.2	*P* = 0.006			*P* = 0.1	
512	10	Abs.	40.0±2.6	39.1±2.6	37.9±2.5	37.4±2.4	18.9±5.7	16.0±4.7	15.5±4.2	14.0±4.3	9.3±0.7	8.2±0.8	6.8±1.1	5.9±1.1	170.5±49.9	119.9±31.1	128.3±38.4	111.4±37.8
		%		2.4±0.5	5.4±0.8	6.6±1.1		5.1±13.9	-2.7±23.1	19.0±12.4		12.9±5.3	25.6±12.3	33.1±12.9		20.4±9.2	14.7±23.4	41.4±14.5
		Sig.		*P* = 0.08	*P* < 0.001	*P* < 0.001		*P* = 0.1				*P* = 0.9	*P* = 0.09	*P* = 0.02		*P* = 0.03	*P* = 0.03	*P* = 0.01
768	5	Abs.	39.2±3.4	38.1±3.3	37.3±3.5	37.2±3.5	8.9±3.4	9.1±3.4	8.8±3.8	9.9±4.3	10.7±1.3	9.4±0.4	7.8±1.4	7.0±1.6	89.2±30.1	85.0±31.4	77.4±35.7	86.0±41.4
		%		3.3±0.9	5.5±1.1	5.9±1.3		-3.0±15.0	10.0±14.1	0.5±21.7		8.4±8.1	22.5±17.0	28.6±19.4		2.3±21.4	21.3±23.7	15.1±33.9
		Sig.		*P* = 0.02	*P* < 0.001	*P* < 0.001		*P* = 0.8				*P* = 0.2					*P* = 0.9	
1024	6	Abs.	43.6±0.7	42.7±0.6	41.2±0.8	40.5±0.9	9.5±3.6	6.7±2.3	6.2±2.5	4.6±1.5	9.8±1.3	9.5±0.7	7.5±0.9	6.1±1.4	93.1±34.1	60.5±17.4	42.2±12.2	26.3±7.6
		%		2.0±1.5	5.3±1.8	7.1±2.4		17.5±9.4	23.1±14.0	38.1±11.9		-2.6±-.8	17.1±16.4	38.4±12.2		11.6±18.0	34.4±21.5	63.0±9.8
		Sig.		*P* = 0.7	*P* = 0.02	*P* = 0.002		*P* = 0.2	*P* = 0.1	*P* = 0.01		*P* = 1.0	*P* = 0.1	*P* = 0.006		*P* = 0.3	*P* = 0.04	*P* = 0.007

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes mark significant differences from -10min.**

Table 19: Effects of STW 5 on human small intestinal longitudinal muscle motility

Conc. [µg/ml]	No.		**Tone** *(mN)*				**Amplitude** *(mN)*				**Frequency** (*peak/min.*)				**Motility index** *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
64	6	Abs.	41.7±6.9	41.2±6.8	40.6±6.7	41.2±6.7	19.9±6.1	21.3±6.9	19.2±7.5	18.9±6.8	4.2±1.1	4.5±0.9	4.8±0.7	5.7±0.5	47.0 [20.7/130.3]	71.1 [19.6/194.5]	63.9 [21.1/160.8]	78.3 [29.1/174.9]
		%		1.2±1.4	2.3±1.6	0.6±1.8		-1.8±6.8	12.5±15.9	10.1±16.9		-97.1±115.2	-146.7±120.5	-141.6±94.0		5.7 [-190.0/29.4]	-2.3 [-148.3/34.6]	-19.1 [-151.3/12.7]
		Sig.	*P* = 0.4				*P* = 0.7				*P* = 0.6				*P = 0.3 §*			
128	6	Abs.	49.8±6.7	49.4±6.4	47.9±7.1	47.4±7.3	15.4±4.4	16.0±4.7	12.2±5.3	12.3±5.5	4.9±0.8	5.0±1.4	5.2±1.5	4.9±1.6	77.9±25.9	97.0±37.8	75.7±30.1	74.0±32.4
		%		0.1±2.2	5.2±2.4	6.6±3.0		-3.6±11.3	38.4±15.5	37.7±16.6		-0.9±21.5	-18.2±39.4	-11.7±43.1		-4.5±28.5	24.5±18.4	30.8±21.1
		Sig.		*P* = 1.0	*P* = 0.1	*P* = 0.03	*P* = 0.1				*P* = 1.0				*P* = 0.7			
256	7	Abs.	43.2±2.9	42.4±3.2	42.0±3.3	40.8±3.2	13.2±3.2	14.1±3.4	9.9±3.3	9.0±3.1	6.3±1.3	6.8±1.5	7.0±1.3	6.6±1.5	106.5±33.1	114.0±27.5	74.3±21.4	67.3±22.6
		%		1.5±2.7	2.3±5.4	4.6±6.8		1.2±18.7	33.6±17.8	38.3±17.9		-19.4±33.9	-7.5±36.6	1.8±37.3		-43.5±45.9	14.0±33.5	25.1±29.1
		Sig.	*P* = 0.7				*P* = 0.06				*P* = 1.0				*P* = 0.1			
512	7	Abs.	40.3±2.5	38.9±2.9	37.1±3.1	37.0±3.2	12.7±2.7	15.1±4.2	8.1±3.2	7.5±3.2	8.6±0.7	6.0±1.7	7.7±1.6	7.6±1.6	116.3±27.7	122.5±39.4	75.2±28.6	69.4±28.4
		%		3.6±3.1	8.6±3.4	8.8±3.7		-1.2±24.3	41.6±21.5	45.5±21.4		35.0±17.7	16.6±17.1	18.2±17.2		15.1±25.8	40.4±21.5	44.6±21.4
		Sig.		*P* = 0.6	*P* = 0.02	*P* = 0.01	*P* = 0.06				*P* = 0.2				*P* = 0.2			
768	6	Abs.	40.1±4.0	42.4±4.3	35.3±4.2	34.3±4.3	23.2±3.1	24.4±4.7	20.1±7.8	20.2±8.6	7.0±1.3	7.2±1.1	7.1±1.6	6.9±1.6	168.0±37.0	181.9±39.8	148.6±40.4	138.8±38.9
		%		-10.9±12.1	9.8±6.3	12.5±6.0		-5.5±17.8	22.3±24.5	23.2±26.6		-5.6±6.9	0.1±27.9	2.2±28.2		-14.0±26.4	18.3±22.1	23.8±20.3
		Sig.		*P* = 0.6	*P* = 0.01	*P* = 0.01	*P* = 0.8				*P* = 1.0				*P* = 0.3			
1024	7	Abs.	41.9 [19.7/46.8]	39.6 [25.5/52.1]	29.1 [15.3/49.0]	29.2 [10.9/50.4]	31.4 [13.6/41.8]	29.2 [14.2/65.1]	17.7 [0.0/50.5]	16.0 [0/46.9]	6.9±1.0	5.8±1.2	4.7±1.6	4.5±1.6	212.8±50.7	211.2±53.5	132.8±51.2	114.5±49.0
		%		3.2 [-4.3/9.6]	7.3 [-4.9/14.7]*	14.1 [5.7/26.8]*		-4.9 [-28.7/5.7]	48.7 [17.7/100.0]	51.9 [27.9/100.0]*		15.6±11.9	36.5±21.2	40.5±21.7		5.7±10.6	54.1±15.7	61.2±15.1
		Sig.		*P* > 0.05 §	*P < 0.05 §*			*P < 0.05 §*				*P* = 0.6	*P* = 0.04	*P* = 0.02		*P* = 1.0	*P* = 0.02	*P* = 0.005

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes mark significant differences from -10min.**

Table 20: Effects of STW 5 on human large intestinal circular muscle motility

Conc. [µg/ml]	No.		**Tone** *(mN)*				**Amplitude** *(mN)*				**Frequency** (*peak/min.)*				**Motility index** *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
64	6	Abs.	48.8±2.8	46.8±2.6	44.5±2.5	44.0±2.5	15.4±3.4	14.5±3.4	12.1±3.7	10.5±3.4	6.4±1.4	6.2±1.5	6.1±1.4	6.3±1.4	111.3±39.5	105.0±40.7	92.3±41.0	82.9±38.4
		%		4.0±1.4	8.6±2.5	9.4±3.2		7.3±2.0	26.8±7.2	37.1±7.0		1.2±6.4	-6.0±17.0	-18.4±30.4		8.5±6.1	22.6±12.5	27.1±15.9
		Sig.		*P* = 0.2	*P* = 0.003	*P* = 0.001		*P* = 0.7	*P* = 0.001			*P* = 0.9				*P* = 1.0	*P* = 0.06	*P* = 0.005
128	6	Abs.	39.6 [32.4/41.2]	31.6 [28.9/43.4]	31.7 [28.6/38.3]	31.8 [27.7/36.2]	19.3±4.1	17.8±4.8	16.6±4.1	15.2±3.9	5.0 [3.3/8.2]	3.9 [2.3/6.8]	4.4 [1.7/7.4]	4.7 [2.6/7.4]	119.4±37.6	95.6±37.8	91.9±35.1	91.9±33.2
		%		4.9 [1.8/16.2]	10.1 [3.4/23.5]	9.2 [6.1/31.4]		12.2±6.9	17.5±7.5	22.5±9.1		15.9 [3.4/33.2]	18.3 [9.5/44.6]	16.5 [2.2/21.0]		28.9±10.0	35.8±9.2	32.7±11.4
		Sig.	§	*p<0.001 §*				*P* = 0.09			§	*P < 0.05 §*				*P* > 0.05	*P* < 0.05	
256	7	Abs.	47.6±5.4	40.6±5.5	37.5±4.7	36.1±4.8	21.6±4.5	18.6±4.1	16.6±4.4	15.7±4.0	8.2±1.0	6.6±1.5	6.7±1.5	6.6±1.5	183.7±45.1	144.4±47.1	140.0±52.8	131.3±48.3
		%		14.8±4.5	20.5±5.1	23.2±6.4		14.2±7.6	27.1±7.3	30.7±9.9		25.6±13.1	23.0±14.2	22.6±15.2		34.3±12.4	40.1±14.6	42.9±13.8
		Sig.		*P* = 0.06	*P* = 0.005	*P* = 0.002		*P* = 0.08	*P* = 0.003	*P* < 0.001		*P* = 0.2				*P* < 0.05		
512	7	Abs.	42.7±2.7	38.6±2.5	34.4±2.3	33.4±2.4	17.6±4.2	17.7±3.8	15.4±3.8	14.5±3.5	10.0 [5.3/10.6]	5.7 [4.9/9.8]	5.8 [4.5/8.3]	6.0 [4.4/7.5]	157.9±37.2	137.4±35.7	110.1±31.1	101.0±30.6
		%		9.6±2.9	18.9±4.2	21.1±5.1		-0.3±5.8	16.2±6.9	18.9±7.4		4.9 [1.8/16.2]*	17.1 [5.1/20.5]*	17.3 [5.4/31.5]*		12.5±8.6	31.9±7.9	35.9±9.1
		Sig.		*P* = 0.1	*P* < 0.001			*P* = 0.09			§	*P* > 0.05	*P < 0.05 §*			*P* = 0.7	*P* = 0.03	*P* = 0.01
768	6	Abs.	36.1 [30.2/48.6]	41.0 [25.5/58.0]	34.6 [23.5/39.7]	31.3 [23.4/38.7]	16.2±4.5	10.5±3.4	7.5±3.8	6.7±3.5	5.4 [2.9/10.7]	4.8 [1.2/11.9]	3.8 [0.5/12.3]	3.3 [0.0/10.5]	100.4±37.0	71.0±43.7	63.2±43.6	50.9±35.3
		%		3.3 [-30.4/12.4]	12.5 [5.2/27.3]	25.6 [7.4/33.5]		21.5±21.5	50.0±17.0	69.1±14.6		27.4 [-50.9/80.5]	39.0 [-67.4/91.7]	52.2 [-34.4/100.0]		47.0±18.7	59.7±18.3	68.8±15.4
		Sig.	§	*P > 0.01*	*P < 0.01 §*			*P* = 0.2	*P* = 0.02	*P* = 0.01	§	*P* > 0.01	*P* > 0.01	*P < 0.01 §*		*P* = 0.1	*P* = 0.04	*P* = 0.005
1024	7	Abs.	42.4 [38.9/52.7]	38.9 [29.5/50.5]	35.3 [27.8/47.2]	32.5 [26.9/45.2]	12.1 [5.9/25.4]	8.9 [2.7/22.1]	6.4 [1.6/22.1]	6.3 [1.7/21.6]	8.5 [4.6/10.4]	7.9 [3.7/10.2]	4.8 [0.8/9.3]	4.9 [0.9/8.1]	94.3 [25.9/208.3]	72.7 [10.9/186.7]	23.6 [4.4/181.1]	8.8 [2.3/170.3]
		%		8.3 [2.2/16.7]	14.4 [5.8/24.6]	16.7 [7.1/30.6]		8.9 [-9.8/30.9]	24.5 [8.1/56.8]	32.9 [19.7/61.9]		1.5 [-5.1/46.5]	5.6 [1.4/86.8]	21.5 [2.9/87.6]		24.1 [0.4/38.7]	24.5 [8.1/56.8]	32.9 [19.7/61.9]
		Sig.	§	*P > 0.05 §*	*P < 0.05 §*		§	*P > 0.05 §*	*P < 0.05 §*		§	*P > 0.05*	*P < 0.05 §*		§	*P > 0.05*	*P < 0.05*	

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes mark significant differences from -10min.**

Table 21: Effects of STW 5 on human large intestinal longitudinal muscle motility

Conc. [µg/ml]	No.		**Tone** *(mN)*				**Amplitude** *(mN)*				**Frequency** (*peak/min.*)				**Motility index** *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
64	6	Abs.	34.9±2.1	35.3±2.5	35.2±3.0	35.2±3.0	5.6±1.3	5.4±1.7	7.3±2.7	8.2±2.8	8.9±3.8	8.3±3.7	9.1±4.3	9.1±4.2	59.4±29.0	63.6±34.2	132.1±75.4	135.4±72.3
		%		-0.7±2.2	0.3±3.3	0.3±3.3		6.2±13.1	-55.2±47.1	-139.1±129.6		-26.9±44.1	23.3±17.2	21.4±17.1		94.9±10.7	-53.5±47.7	-96.2±97.1
		Sig.		*P* = 0.9				*P* = 0.5				*P* = 0.5				*P* = 0.09		
128	6	Abs.	38.4 [27.6/50.0]	38.2 [27.7/47.6]	38.9 [28.2/47.5]	38.9 [28.3/45.0]	3.8 [2.3/11.0]	5.1 [2.7/10.0]	2.1 [1.8/3.6]	2.3 [1.8/2.9]	1.9 [0.8/4.1]	2.2 [1.1/4.4]	1.8 [0.0/3.5]	1.3 [0.0/3.1]	5.9 [4.4/17.9]	5.7 [3.2/73.8]	4.6 [0.1/12.6]	2.4 [0.1/8.9]
		%		0.6 [-5.6/3.4]	-1.4 [-2.3/3.9]	-1.0 [-2.5/4.2]		7.6 [-14.2/27.2]	14.8 [-3.1/59.5]	28.3 [-8.7/58.8]		7.2 [-11.4/26.5]	10.4 [-15.6/93.9]	27.4 [-7.6/82.0]		12.9 [-25.7/49.9]	17.9 [-17.3/99.2]	48.0 [-17.6/97.4]
		Sig.	§	*P = 0.9* §			§	*P = 0.3* §			§	*P = 0.3* §			§	*P = 0.07* §		
256	7	Abs.	34.8±3.4	34.4±3.5	32.4±3.9	32.5±3.9	10.3 [1.5/27.7]	12.8 [1.8/45.5]	11.0 [2.0/55.9]	10.3 [4.8/53.7]	6.0±2.6	6.6±2.7	6.5±2.6	7.9±3.3	124.8±74.4	133.4±69.5	119.7±63.2	139.4±70.9
		%		-1.0±2.9	6.7±3.4	6.7±3.1		-23.1 [-59.5/-4.9]	-8.1 [-100.1/0.4	0.3 [-93.8/9.5]		-15.1±16.4	-12.5±26.5	-9.4±22.9		-45.4±17.2	-32.9±21.6	-20.2±17.1
		Sig.		*P* = 1.0			§	*P = 0.4* §				*P* = 0.6				*P* = 0.4		
512	7	Abs.	40.7±6.7	40.7±6.7	37.8±6.1	36.5±6.3	19..5 [6.4/28.3]	18.5 [7.5/33.5]	16.5 [5.5/21.6]	15.3 [5.8/25.5]	7.2 [1.9/13.6]	6.5 [2.0/12.7]	2.9 [0.5/14.2]	3.1 [0.6/14.6]	112.5 [6.7/471.6]	192.3 [8.2/418.1]	47.0 [2.7/434.5]	46.8 [3.2/383.6]
		%		-1.2±3.5	6.7±2.8	12.7±1.5		3.4 [-19.9/16.4	2.8 [-34.0/15.4	4.7 [-62.1/21.9]		-6.0 [-26.1/6.8]	5.7 [-28.6/53.9	15.9 [-38.7/65.0		-7.3 [-52.1/16.0	0.9 [-8.7/51.7]	18.7 [-8.3/43.0]
		Sig.		*P* = 1.0	**P = 0.01**	**p<0.001**	§	*P = 0.7* §			§	*P = 1.0* §			§	*P = 0.7* §		
768	7	Abs.	27.4 [22.5/38.0]	26.7 [21.4/41.9]	25.6 [20.4/30.8]	25.0 [19.9/30.5]	24.7±7.3	31.7±9.9	27.8±12.1	12.2±8.8	5.9±1.7	6.3±1.7	5.2±2.1	1.6±1.0	190.9±100.5	175.7±76.6	165.5±90.9	71.7±77.0
		%		0.7 [-4.5/3.1]	8.9 [3.1/13.7]	10.3 [2.6/17.3]		-21.2±23.0	-3.5±29.3	57.1±17.8		-27.7±34.9	1.9±53.9	76.0±9.5		-28.8±34.2	4.7±54.6	81.4±9.2
		Sig.	§	*P = 0.09* §				*P* = 0.1				*P* = 0.06				*P* = 0.1		
1024	7	Abs.	34.6±4.6	32.1±6.0	29.7±5.8	29.3±5.8	21.4±3.1	22.7±4.5	21.0±6.5	16.4±6.5	9.2±2.0	9.0±1.8	6.7±1.9	5.0±1.9	205.0±57.8	229.3±70.9	199.7±83.3	141.3±82.4
		%		11.6±9.9	18.0±9.7	18.6±9.8		-6.4±13.4	4.9±19.6	27.2±21.0		-17.1±21.5	21.9±13.9	40.1±16.5		-18.4±17.1	12.3±20.4	37.1±21.4
		Sig.		*P* = 0.08				*P* = 0.6				*P* = 1.0	*P* = 0.3	**P = 0.01**		*P* = 0.5		

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes mark significant differences from -10min.**

Table 22: Effects of **angelica** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency (*peak/min.*)				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	6	Abs.	44.1±2.1	43.7±2.1	43.4±2.2	43.7±2.2	12.2±3.3	11.7±3.8	9.8±4.8	9.4±4.8	9.1±0.5	8.2±0.6	5.4±1.8	4.9±1.7	109.3±31.1	99.2±36.7	86.2±48.8	81.9±
		%		0.8±0.7	1.4±1.3	0.8±1.3		8.7±5.3	43.9±18.8	46.3±18.9		9.5±3.6	37.6±21.3	43.3±21.9		17.2±6.6	48.3±23.5	51.6±25.1
		Sig.		p=0.3				p=0.2				p=1.0	p=0.08	p=0.04		p=0.5		
5120	7	Abs.	38.6±3.3	37.3±3.3	35.3±3.2	35.1±3.2	25.6±6.9	22.0±7.7	18.2±6.2	15.8±6.1	8.8±0.6	6.6±1.3	6.1±1.4	5.1±1.3	213.3±54.3	175.1±62.1	130.1±51.9	118.6±52.7
		%		3.4±0.8	8.8±1.5	9.4±1.6		32.9±13.6	49.2±11.8	54.2±12.6		25.3±13.8	29.0±15.7	38.4±16.5		38.9±14.6	56.3±12.6	61.5±13.7
		Sig.		p=0.04	p<0.001			p=0.5	p=0.02	p=0.002		p=0.3	p=0.2	p=0.02		p=0.2	p<0.001	
Large intestine_Circular muscle																		
512	8	Abs.	25.5 [23.3/41.6]	25.7 [20.8/41.1]	24.1 [23.9/18.8]	23.8 [18.8/39.6]	11.8 [9.0/19.3]	11.0 [9.1/17.2]	7.5 [6.0/13.7]	6.7 [5.1/13.5]	5.1±0.6	5.1±0.7	4.6±0.9	4.1±0.7	60.6 [32.9/149.6]	47.3 [36.0/140.2]	33.3 [21.0/66.9]	30.2 [16.1/57.0]
		%		1.9 [-0.1/10.6]	4.9 [2.8/27.8]	5.6 [2.7/27.8]		6.8 [-2.1/12.9]	29.1 [20.3/44.1]	31.8 [25.4/56.7]		-1.0±6.5	8.7±10.5	10.9±10.0		8.2 [-7.4/24.1]	32.8 [15.4/58.5]	44.4 [26.0/65.5]
		Sig.	§	*p>0.05*	*p<0.05*		§	*p>0.001*	*p<0.001*			p=0.2			§	*p>0.001*	p<0.001	
5120	8	Abs.	41.1±7.3	33.4±5.6	26.6±4.8	24.8±4.3	23.7±6.7	27.3±8.3	18.7±5.7	18.7±5.2	6.3±0.7	5.0±0.7	3.6±1.1	3.5±1.1	162.8±54.9	146.4±52.5	94.9±39.0	96.7±38.0
		%		17.8±4.1	35.2±5.3	39.0±4.5		-13.1±7.1	15.2±18.2	4.1±27.4		22.2±8.0	44.2±17.5	47.6±17.9		13.0±9.6	40.4±23.1	32.9±34.3
		Sig.		p<0.001				p=0.07				p=0.3	p=0.006	p=0.004		p=1.0	p=0.02	p=0.03

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml)**

Table 23: Effects of **peppermint** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency (*peak/min.*)				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	6	Abs.	44.6±1.5	44.2±1.5	43.9±1.5	44.3±1.6	16.3±2.7	15.2±3.6	14.2±4.4	13.7±4.0	9.6±0.6	8.5±1.7	7.2±1.8	6.8±1.9	163.2±31.5	156.4±38.4	139.3±47.6	131.8±44.3
		%		1.0±0.9	1.7±0.8	0.6±0.9		18.7±16.7	28.7±19.2	30.5±17.9		15.6±16.9	28.4±17.9	31.9±19.3		17.8±16.8	32.9±20.9	35.9±20.3
		Sig.		p=0.1				p=0.3				p=0.2				p=0.3		
5120	7	Abs.	48.3±2.6	46.1±1.5	44.9±1.5	44.6±1.4	19.6±4.3	12.1±3.2	11.7±3.3	10.6±3.3	8.6±0.5	7.9±0.7	7.9±1.1	7.5±1.2	162.1±34.4	99.0±30.9	97.5±30.1	85.5±29.4
		%		3.9±2.1	6.3±2.3	7.1±2.5		29.5±11.5	40.1±15.6	47.7±12.3		8.5±6.3	10.7±10.5	15.7±12.3		31.7±12.6	42.6±14.4	51.8±12.9
		Sig.		p>0.001	p<0.001			p=0.05	p=0.01	p=0.04		p=0.5				p=0.04		p=0.01
Large intestine_Circular muscle																		
512	7	Abs.	31.5±4.4	30.3±3.7	25.6±3.8	25.4±3.6	19.3±2.1	20.5±2.5	18.8±2.8	17.0±2.2	5.9±0.4	5.6±0.5	4.1±0.8	4.0±0.8	113.0±13.9	116.9±18.5	77.9±19.0	69.9±18.4
		%		1.9±3.1	18.9±5.1	19.4±3.2		-5.6±2.4	4.2±8.3	10.9±7.4		4.7±4.0	31.3±8.2	33.7±8.4		-0.9±5.8	34.3±4.5	40.4±9.6
		Sig.		p=1.0	p<0.001			p=0.2				p=1.0	p<0.001			p=1.0	p=0.01	p=0.002
5120	6	Abs.	36.2±6.4	31.2±5.8	24.4±3.9	22.7±3.1	17.1±5.6	18.3±5.9	13.4±6.2	12.9±6.1	5.9±0.8	3.8±1.2	2.5±0.8	1.7±0.6	103.7±33.2	85.2±33.2	43.6±29.5	34.1±24.5
		%		14.6±4.5	31.6±3.0	34.9±4.1		-17.8±20.8	34.6±18.0	34.3±19.0		40.3±17.8	48.4±19.0	62.2±16.1		35.9±18.3	54.6±31.3	62.1±19.9
		Sig.		p=0.2	p<0.001			p=1.0	p=0.4	p=0.01		p=0.2	p=0.02	p=0.003		p=0.8	p=0.005	p=0.002

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml).**

Table 24: Effects of **liquorice** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency *(peak/min.)*				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	7	Abs.	41.4±4.0	40.7±4.1	40.2±4.3	39.8±4.3	16.8±3.7	15.2±4.0	12.9±4.8	11.7±4.9	8.7±0.7	7.9±0.8	6.7±1.2	5.9±1.5	152.0±38.5	127.2±41.9	107.9±50.7	99.5±51.0
		%		1.9±0.8	3.3±1.7	4.3±2.3		10.9±7.9	29.7±14.0	36.5±14.9		7.8±7.9	20.7±13.5	28.9±17.0		15.4±12.0	34.9±16.9	41.2±16.4
		Sig.		p=0.1				p=0.5	p=0.06	p=0.01		p=0.09				p=0.6	p=0.08	p=0.03
5120	6	Abs.	42.6±2.0	41.5±2.1	40.2±2.3	39.9±2.4	22.1±5.9	15.7±4.7	9.1±2.8	7.6±2.5	8.8±0.4	8.5±0.5	6.5±1.5	6.4±1.5	187.2±48.6	132.7±40.4	73.7±29.3	64.8±27.2
		%		2.7±1.1	5.7±2.1	6.5±2.4		28.3±7.5	55.6±11.1	60.9±10.2		3.7±2.3	25.7±15.5	26.6±16.7		30.3±8.1	62.9±12.4	67.4±11.2
		Sig.		p=0.3	p=0.005	p=0.002		p=0.3	p=0.009	p=0.004		p=0.2				p=0.3	p=0.005	p=0.003
Large intestine_Circular muscle																		
512	8	Abs.	31.1±5.3	30.7±5.0	30.1±5.2	29.7±5.3	15.9±2.9	15.3±2.9	13.4±2.3	13.9±2.7	5.9±0.9	5.9±0.9	6.1±1.0	5.9±1.0	88.5±19.1	85.7±20.0	78.0±19.0	79.5±19.7
		%		-0.2±2.6	3.4±2.3	5.7±4.2		4.0±3.6	12.5±7.0	11.8±9.1		0.7±2.7	-0.4±6.4	-0.3±7.3		4.5±4.7	11.9±8.7	12.7±8.9
		Sig.		p=0.2				p=0.07				p=1.0				p=0.08		
5120	9	Abs.	42.9±5.3	41.9±5.0	35.1±3.9	32.8±3.8	22.2±6.1	22.7±6.3	23.7±8.2	26.1±9.7	5.3±0.7	5.4±0.7	4.0±1.0	2.6±0.7	130.9±41.6	129.3±40.7	116.2±39.2	99.9±37.5
		%		1.4±2.2	12.6±3.8	15.2±4.3		-0.6±5.3	6.2±16.1	2.3±20.7		-8.4±15.7	34.8±16.5	55.7±10.6		-3.5±8.7	27.6±17.7	48.4±13.9
		Sig.		p=1.0	p=0.03	p=0.003		p=0.7				p=1.0	p=0.09	p<0.001		p=1.0	p=0.2	p=0.001

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml).**

Table 25: Effects of **chamomile** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency *(peak/min.)*				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	6	Abs.	46.9±1.7	46.6±1.7	45.3±1.5	45.4±1.6	12.2±3.9	12.4±3.4	9.7±3.5	8.5±3.9	9.1±0.2	9.4±0.3	8.8±0.5	7.6±1.6	109.9±32.6	113.9±28.0	86.6±30.5	77.4±33.9
		%		0.9±0.4	3.4±0.6	3.3±1.9		-7.1±6.4	19.3±15.7	32.1±19.2		-2.9±1.3	2.7±6.7	15.3±17.8		-10.5±7.4	17.8±19.5	29.4±22.3
		Sig.		p=0.4	p<0.001			p=1.0	p=0.2	p=0.06		p=0.9				p=0.09		
5120	7	Abs.	44.4±1.4	42.9±1.5	40.9±1.7	40.5±1.8	23.6±6.3	20.2±5.4	18.9±6.6	16.4±6.5	9.4±0.4	9.2±0.3	9.1±0.3	7.9±0.7	179.5 [46.4/368.8]	181.0 [47.4/231.9]	150.9 [43.4/237.0]	88.0 [27.1/225.6]
		%		3.2±0.8	7.8±2.3	8.8±2.9		6.3±9.8	27.7±12.2	39.2±13.0		2.8±1.1	2.9±3.7	15.6±6.7		4.2 [-12.5/25.4]	18.1 [0.7/59.1]	45.1 [11.0/76.2]
		Sig.		p=0.3	p=0.002	p<0.001		p=0.07				p>0.05		p<0.05	§	*p>0.05*		*p<0.05*
Large intestine_Circular muscle																		
512	9	Abs.	35.4±4.4	36.7±4.5	34.3±4.3	33.9±4.2	15.6±2.9	15.2±2.6	17.2±3.1	14.8±2.8	6.6±1.0	7.1±1.1	6.5±1.1	6.5±1.0	96.6±16.8	103.0±17.4	97.0±13.1	89.8±16.1
		%		-5.7±3.6	3.3±1.4	3.9±1.9		0.8±5.1	-10.8±7.9	5.0±3.3		-8.8±3.8	4.3±2.4	2.7±2.2		-7.8±6.3	-6.1±7.9	7.6±3.4
		Sig.		p=0.3				p=0.3				p=1.0				p=0.2		
5120	7	Abs.	34.7±5.1	35.5±5.9	32.4±5.8	31.8±5.7	19.0±4.4	19.6±4.8	17.2±5.5	16.3±5.1	8.8 [1.8/8.9]	9.4 [2.1/13.3]	5.3 [0.3/8.5]	5.5 [0.1/8.1]	183.5±58.2	218.1±62.9	138.2±50.1	126.6±46.7
		%		2.1±11.5	11.9±8.4	13.3±7.4		5.0±17.7	16.8±24.0	21.5±21.8		-12.2 [-48.6/-4.6]	11.2 [-6.2/81.5]	27.8 [-10.4/77.4]		-58.9±55.6	20.3±26.3	30.8±25.8
		Sig.		p=0.3	p<0.05			p=0.6			§	*p>0.05*				p=0.08		

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml).**

Table 26: Effects of **lemon balm** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency *(peak/min.)*				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	6	Abs.	38.9±3.6	38.9±8.8	38.4±3.6	38.0±3.5	15.3±5.1	16.3±4.8	13.6±5.1	13.5±4.8	7.9±0.9	8.5±0.9	8.5±0.9	8.3±1.2	129.0±55.8	144.4±53.1	129.9±56.8	129.9±54.3
		%		0.08±0.3	1.3±0.9	2.3±1.7		-15.8±8.5	9.3±15.4	3.6±16.5		-7.9±5.1	-8.3±4.7	-2.5±9.9		-26.3±13.9	-0.8±20.3	-6.1±22.4
		Sig.		p=0.5				p=0.4				p=0.8				p=0.5		
5120	8	Abs.	39.4±3.6	38.4±3.5	37.9±3.6	37.9±3.7	18.5±4.6	17.1±3.3	15.2±3.1	14.9±3.1	8.6±1.0	9.3±1.3	8.9±1.1	9.1±1.1	138.2±35.6	158.6±39.7	138.9±35.1	135.4±35.5
		%		2.4±0.6	3.7±1.4	4.0±1.4		-14.3±15.1	-11.4±19.9	-7.3±19.6		-5.2±9.8	-3.7±5.5	-5.3±5.3		-23.4±22.0	-16.4±22.3	-17.2±23.2
		Sig.		p=0.1	p=0.009	p=0.006		p=0.4				p=0.6				p=0.4		
Large intestine_Circular muscle																		
512	8	Abs.	30.6 [23.6/37.7]	30.2 [23.9/37.9]	28.0 [22.6/32.0]	28.6 [20.9/31.9]	21.9±5.8	20.4±5.2	17.1±3.3	15.1±2.8	5.7 [4.9/7.8]	5.7 [5.1/7.2]	5.8 [4.2/6.8]	5.8 [3.8/6.8]	114.3 [60.6/219.8]	96.0 [55.2/198.2]	99.2 [61.6/120.7]	73.7 [50.1/118.0]
		%		2.3 [-1.6/3.6]	4.8 [-0.5/7.1]	2.9 [-0.7/7.1]		4.3±3.1	9.8±8.8	16.4±9.4		7.9 [-8.4/17.9]	17.7 [-4.5/23.9]	11.6 [-8.5/23.4]		15.8 [-13.9/19.1]	17.7 [-5.9/37.8]	19.1 [-9.7/41.2]
		Sig.	§	*p=0.2*				p=0.2			§	*p=0.09*			§	*p=0.4*		
5120	8	Abs.	35.4±5.9	35.2±6.9	30.7±6.6	29.3±6.2	20.9±4.5	22.3±4.8	21.6±4.4	18.9±4.2	6.7±0.7	5.5±0.9	4.7±0.9	4.7±1.0	136.8±26.9	135.6±32.1	119.9±32.9	106.8±33.8
		%		2.8±5.1	16.3±3.7	19.7±3.9		-4.1±11.8	-0.8±14.9	7.8±16.6		16.8±9.2	30.9±10.3	31.2±11.3		8.8±13.8	26.8±13.2	32.8±13.6
		Sig.		p=1.0	p=0.007	p<0.001		p=0.5				p=0.2	p=0.006	p=0.005		p=0.2		

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml).**

Table 27: Effects of **caraway** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency (*peak/min.)*				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	6	Abs.	39.0±3.4	38.7±3.4	38.7±3.4	38.9±3.6	27.3±7.3	28.4±6.3	21.7±4.9	15.7±5.0	7.7±1.0	7.9±1.1	7.4±1.4	6.3±1.7	199.8±53.2	217.8±53.5	169.1±60.4	135.6±57.3
		%		0.9±0.4	0.9±0.5	0.5±1.1		-10.8±6.3	10.3±8.1	34.5±14.9		-2.4±4.3	9.1±16.5	21.9±22.3		-13.1±6.9	19.9±16.1	38.2±17.9
		Sig.		p=0.7				p=0.1	p=0.4	p=0.02		p=0.4				p=1.0	p=0.3	p=0.008
5120	7	Abs.	44.1±3.3	43.5±3.3	42.9±3.3	42.6±3.3	16.9±3.9	14.1±3.6	12.1±2.7	11.1±2.9	8.7±0.7	8.5±0.9	8.2±1.1	7.9±1.2	147.7±39.5	120.6±35.1	102.4±30.3	90.9±31.5
		%		1.2±0.3	2.7±0.9	3.5±1.2		14.0±7.8	14.6±22.9	25.7±19.6		3.0±3.1	8.0±7.3	12.0±9.1		16.3±8.6	20.6±23.9	32.6±21.1
		Sig.		p=0.6	p=0.02	p=0.003		p=0.3	p=0.03	p=0.009		p=0.2				p=0.3	p=0.02	p=0.007
Large intestine_Circular muscle																		
512	8	Abs.	27.9±2.9	27.8±2.7	25.7±2.6	25.6±2.5	19.9±4.2	19.4±4.0	16.4±2.6	15.0±2.2	5.5±0.8	5.6±0.7	5.0±0.7	4.8±0.7	116.6±36.6	117.1±34.3	86.9±22.3	77.8±19.2
		%		-0.9±2.9	6.4±4.7	6.3±5.2		-0.9±5.6	11.3±5.1	14.3±10.1		-6.9±5.1	5.9±6.4	4.4±9.9		-6.2±5.4	15.9±8.5	16.4±13. 7
		Sig.		p=0.09				p=1.0	p=0.2	p=0.06		p=0.5				p=1.0	p=0.2	p=0.06
5120	8	Abs.	36.5±3.9	37.7±3.7	34.8±3.7	34.3±3.8	25.6±7.5	25.0±19.5	22.4±7.8	21.4±8.0	6.7±0.7	6.9±0.6	6.3±0.8	5.8±0.9	164.8±44.6	176.0±47.8	150.1±53.5	145.8±55.9
		%		-4.0±2.2	4.1±3.7	5.8±4.2		-0.8±1.8	23.4±7.9	26.4±9.8		-5.8±4.3	4.7±9.1	13.5±8.8		-6.3±3.4	26.2±10.8	32.6±13.9
		Sig.		p=0.6				p=0.06				p=0.8				p=0.1		

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml).**

Table 28: Effects of **greater celandine** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency *(peak/min.)*				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	6	Abs.	43.3±5.9	43.4±5.9	43.5±6.1	43.6±6.1	14.3±4.7	12.9±4.6	12.5±4.8	12.6±4.8	8.5±0.9	7.7±1.0	6.0±1.6	5.9±1.8	134.6±49.3	120.1±122.3	108.9±51.1	113.7±52.7
		%		-0.2±0.5	-0.1±1.3	-0.4±1.5		13.5±3.9	18.5±8.0	19.7±8.6		8.8±8.3	29.9±15.4	31.6±18.2		20.1±9.6	39.2±14.6	38.4±17.9
		Sig.		p=1.0				p=0.2				p=0.06				p=0.06		
5120 D+J	9	Abs.	37.3±3.1	36.9±3.6	35.3±3.2	34.2±3.3	31.3±8.8	42.1±12.5	42.2±12.1	38.5±9.9	8.1±0.7	8.7±0.3	8.5±0.4	8.8±0.4	271.9±86.9	284.8±94.1	352.3±92.8	346.6±93.6
		%		1.4±1.3	5.3±2.6	9.0±2.3		-75.9±39.8	-107.3±79.2	-110.3±94.8		-6.6±8.9	-13.6±14.1	-14.5±10.9		-24.3±17.3	-118.8±72.9	-119.4±85.4
		Sig.		p=1.0	p=0.02	p<0.001		p=0.01	p=0.01	p=0.2		p=0.6				p=1.0	p=0.006	p=0.001
5120 Ileum	7	Abs.	46.7±1.7	44.9±1.9	45.0±2.2	44.5±2.4	22.8±3.9	19.4±3.8	13.7±2.5	13.0±2.6	8.3±0.7	7.5±0.8	7.7±0.9	7.7±1.0	181.9±30.7	137.4±27.6	107.6±23.9	105.3±25.9
		%		3.9±1.2	3.2±1.8	5.0±2.4		7.2±14.0	33.4±12.6	36.2±13.6		9.8±5.1	9.4±3.7	10.9±7.9		18.6±11.3	38.3±11.9	35.5±14.0
		Sig.		p=0.07	p=0.09	p=0.02		p=0.7	p=0.009	p=0.005		p=0.3				p=0.3	p<0.001	p<0.001
Large intestine_Circular muscle																		
512	8	Abs.	33.9±2.1	34.2±2.4	31.7±2.0	32.0±2.2	17.0±7.1	15.7±6.1	16.7±7.3	12.9±4.9	5.3±0.5	5.6±0.4	4.9±0.3	4.9±0.2	35.7 [18.6/154.1]	42.3 [23.2/141.5]	41.0 [22.8/103.9]	38.4 [19.3/92.1]
		%		-0.6±1.1	5.9±3.8	5.3±3.6		-5.2±5.4	-3.4±8.8	8.4±8.7		-8.8±6.7	2.2±10.2	4.9±5.2		0.9 [-20.9/7.8]	0.9 [-17.7/31.2]	4.4 [-5.7/40.5]
		Sig.		p=0.1				p=0.9				p=0.2			§	*p=0.4*		
5120	7	Abs.	37.7±4.6	41.9±4.9	45.2±7.4	45.3±7.8	19.6±6.6	21.4±7.2	17.9±5.3	16.6±4.4	6.5±0.8	7.6±0.9	7.5±1.2	7.6±1.1	198.4±68.8	213.6±66.7	179.4±56.5	161.6±46.7
		%		-12.8±5.4	-17.8±7.3	-17.6±7.7		-12.7±8.6	-1.3±8.9	-4.5±17.3		-18.9±10.2	-13.1±10.9	-16.5±10.0		-24.1±10.5	0.3±6.6	-4.9±15.5
		Sig.		p=0.4	p=0.03			p=0.2				p=0.1				p=0.08		

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml).**

Table 29: Effects of **Milk thistle** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency *(peak/min.)*				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	6	Abs.	42.5±3.1	42.7±3.2	42.3±3.4	42.1±3.5	21.8±6.0	20.8±5.9	17.4±4.4	17.5±4.9	8.6±0.8	8.7±0.8	8.7±1.3	8.2±1.6	180.9±52.1	174.7±51.3	144.5±44.5	130.7±52.2
		%		-0.3±0.3	0.9±1.6	1.4±2.6		3.5±5.3	11.5±11.2	14.4±13.2		-1.5±0.7	1.4±9.5	11.2±15.4		1.9±5.6	10.4±18.3	27.1±17.8
		Sig.		p=0.8				p=0.2				p=0.8				p=0.08		
5120	6	Abs.	38.2±3.1	37.9±3.1	37.6±3.0	37.6±2.9	16.7±7.1	16.8±7.6	12.2±5.0	11.3±4.3	7.7±0.8	7.1±1.1	7.1±1.2	7.7±0.9	116.9±44.2	112.0±43.4	93.7±48.2	90.8±37.8
		%		0.9±0.8	1.5±1.4	1.5±1.6		2.6±8.8	22.6±12.6	24.2±12.6		5.6±8.7	2.1±11.4	-3.9±9.6		8.9±12.0	25.4±12.7	19.8±14.9
		Sig.		p=0.5				p=0.07				p=0.7				p=0.1		
Large intestine_Circular muscle																		
512	8	Abs.	33.3 [26.8/39.3]	32.4 [26.7/38.7]	29.8 [25.0/38.6]	29.7 [23.9/39.2]	13.7 [8.5/35.5]	13.3 [8.1/31.3]	13.9 [7.4/27.9]	14.5 [8.0/28.2]	6.3±0.6	6.5±0.5	6.1±0.6	5.8±0.6	94.2 [47.9/227.2]	86.1 [49.8/240.1]	79.4 [46.2/200.8]	71.9 [46.4/200.1]
		%		0.9 [-0.1/3.9]	4.5 [1.4/13.9]	3.4 [0.2/7.8]		4.3 [-0.8/6.3]	4.7 [-7.7/22.5]	2.9 [-10.3/21.9]		-4.3±3.7	3.1±6.6	6.1±8.9		-0.4 [-6.8/9.1]	14.7 [-5.0/16.1]	12.1 [-4.4/28.9]
		Sig.	§	*p=0.07*			§	*p=0.7*				p=0.3			§	*p=0.4*		
5120	7	Abs.	39.2±2.7	39.8±2.9	36.7±3.1	36.1±3.3	34.5±10.4	34.5±10.6	34.3±11.4	31.0±9.4	6.5±0.6	6.9±0.7	5.7±0.7	5.8±0.6	207.8±52.8	215.2±50.4	190.6±55.2	183.6±50.1
		%		-1.4±1.6	6.4±3.8	8.0±4.7		-0.4±3.9	6.3±9.2	10.7±8.6		-6.9±3.6	12.6±7.5	8.7±9.2		-6.8±2.9	16.6±10.2	17.8±10.2
		Sig.		p=1.0				p=0.2				p=0.5				p=0.3		

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml).**

Table 30: Effects of **bitter candytuft** on human intestinal circular muscle motility

Conc. [µg/ml]	No.		Tone *(mN)*				Amplitude *(mN)*				Frequency (*peak/min.)*				Motility index *(mN/min.)*			
			Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.	Bef.	0min.	30min.	50min.
Small intestine _Circular muscle																		
512	6	Abs.	38.5±4.0	38.3±3.9	38.0±3.9	37.8±3.9	19.1±4.4	19.8±4.8	17.9±4.2	16.8±4.2	9.1±0.7	9.1±0.8	8.7±1.1	8.6±1.3	180.1±49.4	188.2±53.4	169.9±50.5	160.7±48.4
		%		0.2±0.4	1.0±0.8	1.3±0.8		-2.8±8.8	6.5±4.1	13.7±8.9		0.6±1.6	6.2±7.7	8.7±10.8		-2.6±10.1	11.3±9.8	16.8±13.9
		Sig.		p=0.1				p=0.2				p=0.3				p=0.2		
5120	6	Abs.	39.3±3.9	38.8±4.1	38.3±4.3	38.3±4.3	16.4±7.1	16.0±7.0	9.7±2.6	8.2±2.5	7.3±0.7	7.1±0.8	6.1±1.1	5.7±1.5	117.4±46.6	107.9±43.3	63.8±24.1	56.3±26.0
		%		1.9±0.8	3.4±4.9	3.7±1.9		6.8±6.8	17.2±14.6	23.9±14.4		5.8±3.6	19.8±10.6	27.3±15.6		11.1±9.0	28.3±17.2	36.4±18.1
		Sig.		p=0.7				p=0.3				p=0.1				p=0.2		
Large intestine_Circular muscle																		
512	6	Abs.	27.7±2.7	27.9±2.7	27.2±2.9	26.5±3.0	15.3±3.7	15.9±3.2	15.4±3.5	15.3±3.6	4.9±1.4	4.9±1.4	4.7±1.2	4.7±1.2	76.2±31.6	84.2±32.0	76.2±27.9	69.3±24.1
		%		-0.9±0.9	1.9±2.3	4.5±3.6		-10.3±6.8	-2.5±5.3	-1.9±4.6		6.9±11.6	6.6±8.4	3.5±4.2		-4.5±15.2	2.8±11.7	0.9±8.0
		Sig.		p=0.2				p=0.8				p=0.6				p=0.3		
5120	8	Abs.	39.4±4.2	39.6±4.0	36.9±4.1	36.6±4.2	36.4±9.7	36.0±9.6	30.7±8.9	30.1±9.0	5.7±0.8	6.3±0.7	5.9±0.8	6.2±0.9	217.8±57.6	230.9±60.4	196.7±54.8	202.3±58.9
		%		-1.8±3.3	5.7±3.3	6.4±4.5		-0.2±1.8	14.4±4.7	16.1±5.1		-14.5±5.2	-7.0±8.8	-7.9±9.3		-14.8±5.9	10.4±4.8	11.2±7.3
		Sig.		p=0.2				p=0.1				p=0.4				p=0.7		

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values), black boxes marked significant differences from -10min.**
- **Extract was applied in concentrations corresponding to their concentrations in STW 5 (512 and 5120 µg/ml).**

Table 31: Changes in control human intestinal motility patterns

No.		**Tone** *(mN)*				**Amplitude** *(mN)*				**Frequency** *(peak/min.)*				**Motility index** *(mN/min.)*			
		Bef.	**0min.**	**30min.**	**50min.**	**Bef.**	**0min.**	**30min.**	**50min.**	**Bef.**	**0min.**	**30min.**	**50min.**	**Bef.**	**0min.**	**30min.**	**50min.**
Small intestine _Circular muscle																	
12	**Abs.**	46.4 [41.2/49.6]	46.2 [40.8/49.9]	46.4 [40.3/49.7]	46.5 [40.9/49.4]	6.5 [4.9/12.0]	6.2 [5.3/12.5]	6.7 [4.5/13.3]	6.1 [4.9/13.6]	9.8 [6.9/2.0]	9.6 [6.7/1.9]	9.7 [6.6/1.9]	9.7 [6.9/2.0]	70.8±18.4	71.2±17.8	77.8±21.0	80.5±22.5
	%		0.1 [-0.2/0.5]	0.1 [-0.8/1.6]	-0.2 [-0.5/0.6]		-2.2 [-8.7/8.1]	1.9 [-15.0/10.9]	-4.7 [-10.6/10.8]		1.9 [-11.4/11.1]	0.9 [-9.3/10.2]	0 [-6.4/12.8]		-4.4±8.7	-4.9±12.3	-1.8±11.0
	Sig.		p=0.7§				p=0.3§				p=0.9§				p=0.2		
Large intestine _Circular muscle																	
12	**Abs.**	34.1 [24.5/50.6]	33.9 [22.8/50.8]	34.4 [24.0/47.3]	33.0 [24.2/41.6]	25.0 [4.6/30.5]	25.4 [4.1/29.9]	24.7 [3.5/29.5]	21.2 [5.9/28.7]	7.1±1.0	6.8±1.0	6.7±1.1	6.9±1.1	161.3±36.3	153.6±37.5	153.7±37.3	145.4±31.6
	%		2.2 [0.3/7.1]	0.2 [-1.7/6.2]	2.7 [-0.9/9.5]		-0.6 [-2.3/5.3]	-0.2 [-5.8/18.9]	-2.2 [-40.4/14.1]		3.2±1.1	3.9±2.6	5.8±3.1		0.9±1.4	3.3±4.4	2.9±1.1
	Sig.		p=0.2§				p=0.8§				p=0.6				p=0.3		

- **Normally distributed data presented as mean ± SEM and tested by RM ANOVA with Bonferroni's *post-hoc* test.**
- **Non-normally distributed, marked by (§), presented as median [75 /25] percentile and tested by RM ANOVA on Ranks with Dunn's *post-hoc* test.**
- **(Abs.) is the measured absolute values. (%) is the percentage reduction in response relative to (-10min) before application.**
- **(No.) is the number of muscle strips (equal to number of patients)**
- **Statistical tests were done with absolute values (not with percent values)**

Table 32: Effects of STW 5 on EFS-evoked contraction in small intestinal circular muscle

STW 5 (µg/ml)	**On response**			**Off response**		
	Before vs. after	**n**	***P* value**	**Before vs. after**	**n**	***P* value**
64	21.7 ± 14.9 vs. 18.2 ± 21.0	*n* = 8	*P* = 0.7	50.7 ± 14.4 vs. 66.1 ± 10.2	*n* = 8	*P* = 0.1
128	8.9 [0.4/37.9] vs. 2.4 [0.0/17.7]	*n* = 8	*P* = 0.2	40.1 ± 14.9 vs. 45.1 ± 8.8	*n* = 8	*P* = 0.6
256	3.1 [1.8/17.9] vs. 2.1 [-1.5/22.3]	*n* = 10	*P* = 0.6	18.9 [0.0/68.1] vs. 11.5 [0.0/37.4]	*n* = 10	*P* = 0.3
512	17.4 [5.9/60.2] vs. 3.1 [0.0/28.2]	*n* = 8	*P* = 0.2	43.3 ± 16.1 vs. 49.6 ± 12.4	*n* = 8	*P* = 0.6
768	6.8 ± 4.9 vs. 5.4 ± 3.3	*n* = 3	*P* = 0.5	8.6 ± 8.6 vs. 8.9 ± 8.8	*n* = 3	*P* = 0.5
1024	4.6 ± 1.4 vs. 3.5 ± 0.7	*n* = 3	*P* = 1.0	35.5 ± 18.3 vs. 36.8 ± 18.6	*n* = 3	*P* = 0.5
5120	93.3 ± 46.9 vs. 93.5 ± 49.1	*n* = 5	*P* = 0.9	99.9 ± 12.9 vs. 107.5 ± 11.6	*n* = 5	*P* = 0.4
Control	12.4 ± 5.2 vs. 11.3 ± 4.3	*n* = 4	*P* = 0.3	47.4 ± 34.5 vs. 36.4 ± 24.4	*n* = 4	*P* = 0.4

Values are delta muscle tones and expressed in mN. (n) is the number of muscle strips (equal to number of patients)

Table 33: Effects of STW 5 on EFS-evoked contraction in small intestinal longitudinal muscle

STW 5 (µg/ml)	**On response**			**Off response**		
	Before vs. after	**n**	***P* value**	**Before vs. after**	**n**	***P* value**
64	81.5 ± 22.3 vs. 67.7 ± 26.8	*n* = 8	*P* = 0.5	26.5 ± 6.9 vs. 37.3 ± 12.3	*n* = 8	*P* = 0.3
128	84.9 ± 24.0 vs. 63.9 ± 25.4	*n* = 9	*P* = 0.2	30.5 ± 10.4 vs. 37.4 ± 15.9	*n* = 9	*P* = 0.4
256	12.6 [3.1/59.9] vs. 69.7[44.9/125.7]	*n* = 10	*P = 0.6*	26.4 ± 8.9 vs. 16.1 ± 7.4	*n* = 10	*P* = 0.3
512	109.1 ± 20.9 vs. 76.2 ± 28.2	*n* = 8	*P* = 0.1	36.0 ± 12.1 vs. 18.1 ± 18.6	*n* = 8	*P* = 0.1
768	47.7 ± 26.8 vs. 50.8 ± 25.6	*n* = 3	*P* = 0.2	37.1 ± 13.0 vs. 33.9 ± 6.5	*n* = 3	*P* = 0.7
1024	86.1 ± 0.4 vs. 78.6 ± 17.1	*n* = 4	*P* = 0.7	24.2 ± 17.3 vs. 29.7 ± 10.0	*n* = 4	*P* = 0.8
Control	124.4 ± 14.7 vs. 124.5 ± 10.2	*n* = 2	*P* = 1.0	33.9 ± 33.9 vs. 31.3 ± 31.3	*n* = 2	*P* = 0.5

Values are delta muscle tones and expressed in mN. (n) is the number of muscle strips (equal to number of patients)

Table 34: Effects of STW 5 on EFS-evoked contraction in large intestinal circular muscle

STW 5 (µg/ml)	**On response**			**Off response**		
	Before vs. after	**n**	***P* value**	**Before vs. after**	**n**	***P* value**
64	69.2 ± 47.0 vs. 11.3 ± 9.0	*n* = 5	*P* = 0.2	23.9 ± 13.5 vs. 83.5 ± 35.9	*n* = 5	*P* = 0.2
128	10.0 [0.5/186.3] vs. 0.0 [-26.8/3.8]	*n* = 5	*P* = 0.3	38.2 ± 20.8 vs. 75.7 ± 27.2	*n* = 5	*P* = 0.4
256	13.1 ± 6.2 vs. 10.3 ± 5.7	*n* = 7	*P* = 0.3	69.0 ± 15.0 vs. 65.3 ± 12.1	*n* = 7	*P* = 0.7
512	23.3 ± 13.6 vs. 25.1 ± 13.3	*n* = 5	*P* = 0.7	53.4 ± 16.6 vs. 53.2 ± 16.1	*n* = 5	*P* = 1.0
768	0.0 ± 0.0 vs. 1.2 ± 1.2	*n* = 3	*P* = 0.3	60.7 ± 20.4 vs. 39.7 ± 10.7	*n* = 3	*P* = 0.5
1024	7.4 ± 3.2 vs. 8.0 ± 15.2	*n* = 4	*P* = 1.0	55.0 ± 19.1 vs. 61.6 ± 19.3	*n* = 4	*P* = 0.6
5120	22.8 ± 5.8 vs. 25.8 ± 10.8	*n* = 4	*P* = 0.7	190.2 ± 47.9 vs. 171.8 ± 40.0	*n* = 4	*P* = 0.5
Control	13.9 [3.2/43.1] vs. 16.5 [9.3/45.2]	*n* = 4	*P* = 0.3	123.7 ± 55.1 vs. 120.1 ± 47.1	*n* = 4	*P* = 0.4

Values are delta muscle tones and expressed in mN. (n) is the number of muscle strips (equal to number of patients)

Table 35: Effects of STW 5 on EFS-evoked contraction in large intestinal longitudinal muscle

STW 5 (µg/ml)	**On response**			**Off response**		
	Before vs. after	**n**	***P* value**	**Before vs. after**	**n**	***P* value**
64	15.7 ± 17.9 vs. 13.0 ± 2.2	*n* = 4	*P* = 0.9	31.1 ± 17.3 vs. 28.6 ± 10.5	*n* = 4	*P* = 0.8
128	44.1 ± 17.7 vs. 15.5 ± 5.9	*n* = 4	*P* = 0.2	33.3 ± 17.9 vs. 35.6 ± 9.3	*n* = 4	*P* = 0.9
256	42.9 ± 16.2 vs. 41.4 ± 16.3	*n* = 9	*P* = 0.8	26.7 ± 6.5 vs. 29.2 ± 6.4	*n* = 9	*P* = 0.7
512	31.3 ± 13.8 vs. 34.3 ± 18.1	*n* = 6	*P* = 0.6	22.1 ± 5.5 vs. 25.1 ± 9.1	*n* = 6	*P* = 0.6
768	46.1 ± 29.1 vs. 31.1 ± 9.2	*n* = 3	*P* = 0.6	47.8 ± 31.0 vs. 51.0 ± 17.8	*n* = 3	*P* = 0.9
1024	137.3 ± 28.9 vs. 125.4 ± 43.2	*n* = 3	*P* = 0.6	95.1 ± 34.3 vs. 106.7 ± 1.5	*n* = 3	*P* = 0.8
Control	51.5 ± 40.0 vs. 54.8 ± 30.4	*n* = 2	*P* = 1.0	33.9 ± 33.9 vs. 31.3 ± 31.3	*n* = 2	*P* = 0.5

Values are delta muscle tones and expressed in mN. (n) is the number of muscle strips (equal to number of patients)

Table 36: Effects of STW 5, its components and their combinations on EFS-evoked secretion and human tissue resistance

	Response to EFS (µA / cm²)		***P* value**	**Resistance (Ω / cm²)**		***P* value**
	Before application	**After application**		**Before application**	**After application**	
Peppermint (P) as in 5120µg/ml	53.5[20.5/62.0]	34.9[26.1/75.1]	*P* = 0.9	79.6±17.4	76.8±17	*P* = 0.4
	n = 12			*n* = 6		
Angelica (A) as in 512µg/ml	36.4 ± 6.1	33.8 ± 5.9	*P* = 0.5	50.8±9.3	51.6±9.2	*P* = 0.9
	n = 18			*n* = 8		
Angelica (A) as in 5120µg/ml	21.3 [7.7/31.7]	18.6 [9.0/34.6]	*P* = 0.5	87.9±21.1	75.8±17.1	*P* = 0.07
	n = 14			*n* = 6		
Lemon balm (L) as in 5120µg/ml	34.0 ± 5.2	26.5 ± 3.1	*P* = 0.1	56.2±8.5	54.1±8.6	*P* = 0.2
	n = 12			*n* = 9		
APL[1] as in 512µg/ml	32.2 ± 7.5	23.8 ± 5.0	*P* = 0.2	67.3±11.3	61.6±8	*P* = 0.2
	n = 11			*n* = 6		
APL[1] as in 5120µg/ml	23.1 ± 4.8	15.8 ± 2.9	*P* = 0.06	58.3±9.3	57.7±12.1	*P* = 0.9
	n = 11			*n* = 7		
STW 5 512µg/ml	22.8 ± 6.8	26.2 ± 8.1	*P* = 0.7	85.9±20.2	80±18.2	*P* = 0.1
	n = 12			*n* = 6		
sSTW 5 512µg/ml	27.8 ± 4.2	28.4 ± 3.3	*P* = 1.0	61.4±14.4	52.3±15.7	*P* = 0.2
	n = 16			*n* = 6		
Chamomile as in 5120µg/ml	27.2 ± 7.8	28.1 ± 7.9	*P* = 0.7	62.1±8.3	55.7±9.3	*P* = 0.2
	n=10			*n* = 7		
Liquorice as in 5120µg/ml	25.5 ± 4.9	21.5 ± 4.8	*P* = 0.2	55.4±5	53.7±6.7	*P* = 0.6
	n = 15			*n* = 12		
Caraway as in 5120µg/ml	27.5 ± 6.3	24.6 ± 6.6	*P* = 0.6	71±9.5	69.5±10.1	*P* = 0.6
	n = 14			*n* = 11		
Milk thistle as in 5120µg/ml	22.7 ± 4.3	18.3 ± 4.4	*P* = 0.4	51.4±7.1	46.5±7.5	*P* = 0.1
	n = 12			*n* = 6		
G. celandine as in 5120µg/ml	22.5 ± 7.3	21.5 ± 5.5	*P* = 0.9	63.9±11.3	54±9.3	*P* = 0.1
	n = 10			*n* = 12		
Bitter candytuft as in 5120µg/ml	23 ± 5.2	18.5 ± 3.6	*P* = 0.3	41.2±6.6	38.1±5.4	*P* = 0.5
	n = 13			*n* = 7		
STW 5 (5120µg/ml)	**46.8 ± 8.9**	**12.4 ± 3.9**	***P* = 0.002***	77.2±14.8	71.68±15.1	*P* = 0.5
	***n* = 6**			*n* = 7		

[1] **APL is mixture of angelica, peppermint and lemon balm. (*n*) is the number of tissues. * marks significant reduction.**

Table 37: Effects of STW 5, its components and their combinations on the resistance of T84 cells

	T84 cells resistance (Ω / cm²)		*P* value
	Before application	**After application**	
STW 5 [512µg/ml]	152.0 ± 38.1	217.9 ± 72.4	*P* = 0.5
	n = 6		
sSTW 5 [512µg/ml]	307.0 ± 72.1	310.7 ± 52.0	*P* = 0.9
	n = 6		
STW 5 [5120µg/ml]	234.9 ± 55.7	156.6 ± 66.2	*P* = 0.4
	n = 6		
Peppermint (P) as in [5120µg/ml]	229.6 ± 55.1	193.7 ± 43.5	*P* = 0.3
	n = 6		
Angelica (A) as in [5120µg/ml]	132.1 [6.4/184.8]	151.6 [73.8/172.8]	*P* = 0.8
	n = 6		
Lemon balm (L) as in [5120µg/ml]	307.9 ± 114.5	233.6 ± 49.1	*P* = 0.5
	n = 6		
APLC[1] as in [512µg/ml]	289.5 [174.2/353.7]	181.8 [126.6/299.1]	*P* = 0.1
	n = 9		
APLC[1] as in [5120µg/ml]	298.8 ± 26.3	312.1 ± 26.2	*P* = 0.8
	n = 6		
Chamomile (C) as in [5120µg/ml]	200.7 ± 50.3	206.5 ± 58.7	*P* = 0.9
	n = 6		
Liquorice as in [5120µg/ml]	228.2 ± 44.4	208.5 ± 49.0	*P* = 0.4
	n = 6		
Caraway as in [5120µg/ml]	114.2 [65.4/326.7]	81.2 [59.9/187.9]	*P* = 0.4
	n = 6		
Milk thistle as in [5120µg/ml]	176.5 ± 21.3	181.6 ± 21.2	*P* = 0.8
	n = 6		
Greater celandine as in [5120µg/ml]	103.8 ± 28.5	115.9 ± 48.2	*P* = 0.8
	n = 6		
Bitter candytuft as in [5120µg/ml]	164.8 ± 32.9	104.2 ± 29.1	*P* = 0.2
	n = 6		

[1] APLC is mixture of angelica, peppermint, lemon balm and chamomile. (*n*) is the number of T84 cell filters.

Table 38: The percentage reduction in the prossecretory responses of sSTW 5, its prosecretory components and their combination

	In MDL 12,330A 10 µM		**In $CFTR_{inh}$-172 20 µM**		**In SITS 1 mM**		**In $CFTR_{inh}$-172 20 µM + SITS 1mM**
	Human tissue	**T84 cells**	**Human tissue**	**T84 cells**	**Human tissue**	**T84 cells**	**T84 cells**
STW 5[1] (512 µg/ml)	**70%** $P = 0.001$	**51%** $P = 0.02$	**63%** $P = 0.02$	**59%** $P = 0.003$	**42%** $P = 0.03$	**43%** $P = 0.001$	**65%** $P = 0.001$
sSTW 5 (512 µg/ml)	**81%** $P \leq 0.001$	**56%** $P = 0.004$	**79%** $P \leq 0.001$	**79%** $P = 0.002$	**70%** $P \leq 0.001$	**55%** $P = 0.004$	**67%** $P = 0.009$
Angelica (A) as in 512 µg/ml	**80%** $P \leq 0.001$	-	**82%** $P \leq 0.001$	-	**89%** $P \leq 0.001$	-	-
Angelica (A) as in 5120 µg/ml	**88%** $P \leq 0.001$	**47%** $P = 0.01$	**53%** $P = 0.002$	**67%** $P = 0.01$	**78%** $P \leq 0.001$	**48%** $P = 0.006$	**72%** $P \leq 0.001$
Peppermint (P) as in 5120 µg/ml	**86%** $P \leq 0.001$	**86%** $P = 0.003$	**91%** $P \leq 0.001$	**85%** $P = 0.003$	**83%** $P \leq 0.001$	**54%** $P = 0.003$	**86%** $P = 0.002$
Lemon balm (L) as in 5120 µg/ml	**98%** $P = 0.01$	**47%** $P = 0.007$	**56%** $P \leq 0.001$	**89%** $P \leq 0.001$	**49%** $P = 0.01$	**68%** $P \leq 0.001$	**51%** $P = 0.001$
Chamomile (C) as in 512 µg/ml	-	**96%** $P = 0.004$	-	**78%** $P = 0.005$	-	**68%** $P = 0.004$	**79%** $P = 0.002$
Chamomile (C) as in 5120 µg/ml	-	**64%** $P = 0.002$	-	**74%** $P \leq 0.001$	-	**57%** $P = 0.005$	**79%** $P \leq 0.001$
APL(C)[2] as in 512 µg/ml	**70%** $P = 0.009$	**62%** $P = 0.005$	**69%** $P = 0.009$	**81%** $P = 0.005$	**83%** $P = 0.009$	**62%** $P = 0.005$	**65%** $P = 0.02$
APL(C)[2] As in 5120 µg/ml	**58%** $P = 0.01$	**74%** $P \leq 0.001$	**61%** $P \leq 0.001$	**94%** $P \leq 0.001$	**75%** $P \leq 0.001$	**48%** $P \leq 0.001$	**96%** $P \leq 0.001$

[1] STW 5 data are from our previous study (Krueger *et al*., 2009). [2] APL(C) is a combined application of a mixture from angelica (A), peppermint (P) and lemon balm (L) in human intestine. In T84 cells, chamomile (C) was added to the mixture. (-) the component had no effect by itself and consequently, no pharmacology had been done.

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Abbreviations

$[Ca^{2+}]_i$	Intracellular calcium concentration
µg	Microgram
µM	Micromolar
5-HT	5-hydroxytryptamine (serotonin)
Ca^{2+}	Calcium
CaCl	Calcium-activatd chloride channel
CaMKII	Calmodulin kinase II
CFTR	Cystic fibrosis trans-membrane conductance regulator
Cl^-	Chloride
CM	Circular muscle
EFS	Electrical field stimulation
FD	Functional dyspepsia
FGIDs	Functional gastrointestinal disorders
GERD	Gastro-esophageal reflux disorders
Hz	Hertz
IBS	Irritable bowel syndrome
IBS-C	Constipation-predominant IBS
IBS-D	Diarrhoea-predominant IBS
IBS-M	Mixed-predominant IBS
IBS-U	Un-subtyped IBS
IP_3-R	Inositol triphosphate receptor
I_{SC}	Short circuit current
K^+	Potassium
LM	Longitudinal muscle
MI	Motility index
ml	Millilitre
mM	Millimolar
mN	Millinewton
MT	Muscle tone
Na^+	Sodium
nM	Nanomolar

PKA	Protein kinase A
PKC	Protein kinase C
PKG	Protein kinase G
RyRs	Ryanodine receptors
SERCA	Sarcoplasmic endoplasmic reticulum calcium ATPase pump
SOC	Store-operated calcium channel
SR	Sarcoplasmic reticulum
STW 5	Iberogast®
STW 5-KII	Peppermint
STW 5-KIII	Chamomile
STW 5-KIV	Liquorice
STW 5-KIX	Greater celandine
STW 5-KVIII	Lemon balm
STW 5-KV	Angelica
STW 5-KVI	Caraway
STW 5-KVII	Milk thistle
STW 6	Bitter candytuft
TRP channels	Transient receptor potential channels
TRPA channels	Ankyrin transient receptor potential channels
TRPC channels	Canonical transient receptor potential channels
TRPV channels	Vanilloid transient receptor potential channels
TTX	Tetrodotoxin
V	Volts

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