Recording from human gut tissue: a major step towards more efficient drug development?

Michael Schemann

Translational research is a rewarding challenge and of major relevance for the field of gastroenterology, in particular neurogastroenterology, in our endeavour to improve treatment of patients suffering from functional, inflammatory or structural gut disorders. Although in vivo and in vitro animal models have provided insights into potential mechanisms of gut disorders and identified putative drugs targets, these often failed to translate into successful treatments in humans. There are many reasons for this failure such as limited bioavailability, low efficacy of drugs or safety issues. While these obstacles are intrinsic to every drug development programme it is species specific action of endogenous mediators or expression of signalling cascades that restrict translation of data from animal models to human conditions. This, however, is an issue that can be tackled by developing new techniques or adapting existing methods from animal studies to investigate receptor expression, pharmacology of signalling molecules and intracellular pathways involved in drug actions on behaviour of human gut tissue.

Measurements of gastrointestinal functions, in particular motility and transit, are routinely performed in healthy volunteers and patients.[1 2] While such studies provide important information on the nature of motor disorders or the efficacy of a drug to alter gastrointestinal transit they are not suitable to characterise the modes of action of drugs or to identify novel targets to treat a particular pathology. This requires experiments in isolated gut tissue which derives from bowel resections or biopsies obtained during endoscopy.

Recordings from human isolated gut tissues have provided information on the effects of drugs on secretion,[3] motility[4] or enteric nerve activity,[5] to mention only a few examples. Beyond revealing novel insights these types of studies also reveal human tissue specific action of mediators. One striking example is the ability of 5-HT_3 receptor antagonists to modulate peristaltic reflex activity in guinea-pig intestinal preparations, but not human gut, in response to mucosal stroking.[6] Even recordings of human enteric neurons are now routinely possible using calcium[7] or voltage sensitive dyes.[8] Again these studies have revealed distinctive features of human enteric neurons, such as the potent postsynaptic excitatory action of histamine through H3 receptors [8] or the prominent role of protease activated receptor 1.[9]

While our knowledge on secretion, motility and enteric neurobiology in the human gut has advanced over the last decades more research on processing of visceral pain is needed. Visceral pain is not only a focus for drug development but visceral hypersensitivity can also be an adverse effect of drug treatment. While central processing of visceral pain can be visualised by brain imaging,[10] we currently lack information on mechanisms involved in activation and sensitisation of peripheral nociceptors in the human gut. Therefore, one remaining challenge in translational neurogastroenterology is the ability to record from human extrinsic nerves projecting sensory information along the brain gut axis. Lack of such studies has compromised targeted development of anti-nociceptive drugs to relieve visceral pain. In this issue of *Gut*, two papers from two independent groups represent a true breakthrough in that they demonstrate the feasibility of nerve recordings in isolated human gut preparations.[11 12] Both groups have a long-standing history in performing recordings from gut afferents in various animal models.[13] They have now developed techniques to dissect mesenteric nerve bundles to enable direct electrophysiological recordings from human visceral afferents. This potentially has tremendous impact as it is not known whether our concepts on sensory transmission in the gut derived from animal studies apply equally to humans. The studies by Peiris *et al*[11] (***see page 204***) and Jiang *et al*[12] (***see page 281***) describe afferent recordings from distinct preparations of resected human gut. Peiris *et al*[11] utilise mainly the isolated appendix in order to examine the effect of inflammatory mediators on spontaneous afferent firing while Jiang *et al*[12] focused on characterising the mechanosensitivity of flat-sheet preparations of colon. The two groups have to be applauded for their achievement in recording from visceral afferents in isolated viable human gut tissue as it sets the basis for further in-depth studies. Among those are experiments to classify the exact properties of human low and high threshold mechanoreceptors as well as the exact localisations and ramifications of their terminals. Studies on guinea-pig afferents identified intraganglionic laminar endings and intramuscular arrays as mechanosensory transduction sites.[13] It is now possible to study the properties of those transduction sites in human tissue in order to develop new strategies to inhibit their activation in a hypersensitive state. Studies in mouse afferents revealed several subtypes of mechanosensitive afferents [13] and it will be of utmost importance to study the properties of mucosal, muscular, muscular—mucosal, serosal and mesenteric afferents. The study by Jiang *et al* presented preliminary evidence that these sub-populations of afferent exist in human tissue as well.[12] Mucosal stroking, blunt probing of the mucosa and the serosa as well as circumferential and longitudinal tissue stretch all evoked increased nerve discharge. Interestingly, nerve firing ceases in particular after blunt probing of the serosal site and longitudinal stretch reminiscent of the inhibition of muscle activity triggered by an enteric occult reflex evoked by colonic elongation.[14] The techniques employed in both studies may be applied to identify endogeneous mediators that are able to modulate firing in human visceral afferents. It is well known from animal studies that visceral afferents can be sensitised, in particular by inflammatory mediators.[13] Signalling from immune cells to visceral afferents seems also evident in the human gut as demonstrated by Peiris *et al* using a 'soup' of inflammatory mediators to increase the firing rate of appendicular afferents.[11] An interesting topic to address in the future is the sensitivity of afferents to individual inflammatory mediators and characterisation of the receptor mechanisms underlying these responses. Proteases appear of particular

Correspondance to Professor Dr Michael Schemann, Human Biology, Technische Universität München, Liesel-Beckmann-Strasse 4, 85350 Freising, Germany; schemann@wzw.tum.de

interest as proteases present in fecal samples from colitis ulcerosa or irritable bowel syndrome patients induce hypo- or hypersensitivity, respectively, in animal models.[15] Peiris *et al*[11] and Jiang *et al*[12] both demonstrated sensitivity to capsaicin which indicates that human visceral afferents express TRPV1 (transient receptor potential vanilloid 1) receptors which studies in knockout mice have shown plays a role in modulating mechanosensivity and can encode noxious stimuli.[16] It will be interesting to learn whether capsaicin pretreatment sensitises afferents to certain stimuli and how TRP channel antagonists can reverse sensitisation of mechano- or chemosensitive afferents. Recordings from human afferents will not only shed light on their sensory functions but may also reveal any efferent functions of axon collaterals within the gut wall which may provide motor input to targets like enteric neurons, smooth muscle, epithelium, immune cells or blood vessels.

Admittedly, the success rate for viable afferent recordings was rather low in both studies, especially in the Jiang *et al*[12] study in which single unit recordings were obtained. While these pilot studies prove feasibility, a comprehensive assessment of the response pattern of human visceral afferents needs further efforts to increase the yield. As acknowledged in both papers, working with human gut tissue also means making compromises as the conditions are never as well controlled as in experiments with animal tissue. Specimens often suffered from ischaemia due to cutting mesenteric blood supply prior to bowel resection and this has to be kept to a minimum. Due to the thickness of the tissue oxygen supply is a critical issue and the mucosal epithelium is most vulnerable. These limitations can be overcome by appropriate adjustments of the experimental conditions. Further improvement of the viability of full thickness preparations may be achieved by vascular perfusion in order to assure sufficient oxygen and energy supply. Despite some inherent limitations related to tissue supply and handling, there is no doubt that results obtained with human gut tissue are extremely valuable. Studies in human tissues are more than self-serving endeavours as they will also help to decide on appropriate animal models.

There are exciting times ahead of us because recordings of human visceral afferents will not only advance our knowledge of their basic properties but even more importantly reveal novel targets that allow more efficient treatment of visceral hypersensitivity as a hallmark of many gut diseases. Moreover, the scene is set to screen novel drugs, also those developed against non-gastrointestinal diseases, for possible adverse effects on visceral sensation. Today we look on a rather impressive list of functional recordings in human gut tissue, such as mucosal ion fluxes, mucosal barrier function, smooth muscle and interstitial cells of Cajal, enteric nerve activity, visceral afferent nerve discharge as well as approaches using genomics and proteomics. Some of these studies recorded effects of human samples on human tissue behaviour with the idea to use such approaches as biomarkers.[17 18] Peiris and colleagues propose that the in vitro human tissue model may be used to aid disease mechanistic studies.[11] While this seems futuristic because intact extrinsic nerve supply to the gut requires full thickness biopsies which are for ethical reasons difficult to obtain, techniques may become available to record from terminals of extrinsic afferents in mucosal biopsies. There are certainly challenges that remain to be met in the future: let's be realistic and demand the impossible.

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