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REVIEW

Translational neuropharmacology: the use of human isolated gastrointestinal tissues

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Translational sciences increasingly emphasize the measurement of functions in native human tissues. However, such studies must confront variations in patient age, gender, genetic background and disease. Here, these are discussed with reference to neuromuscular and neurosecretory functions of the human gastrointestinal (GI) tract. Tissues are obtained after informed consent, in collaboration with surgeons (surgical techniques help minimize variables) and pathologists. Given the difficulties of directly recording from human myenteric neurones (embedded between muscle layers), enteric motor nerve functions are studied by measuring muscle contractions/relaxations evoked by electrical stimulation of intrinsic nerves; responses are regionally dependent, often involving cholinergic and nitrergic phenotypes. Enteric sensory functions can be studied by evoking the peristaltic reflex, involving enteric sensory and motor nerves, but this has rarely been achieved. As submucosal neurones are more accessible (after removing the mucosa), direct neuronal recordings are possible. Neurosecretory functions are studied by measuring changes in short-circuit current across the mucosa. For all experiments, basic questions must be addressed. Because tissues are from patients, what are the controls and the influence of disease? How long does it take before function fully recovers? What is the impact of age- and gender-related differences? What is the optimal sample size? Addressing these and other questions minimizes variability and raises the scientific credibility of human tissue research. Such studies also reduce animal use. Further, the many differences between animal and human GI functions also means that human tissue research must question the ethical validity of using strains of animals with unproved translational significance.

LINKED ARTICLE
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Abbreviations
EFS, electrical field stimulation; ENS, enteric nervous system; GI, gastrointestinal; L-NAME, Nω-nitro-L-arginine methyl ester; NK, neurokinin; SCC, short-circuit current; STC, slow transit constipation

Introduction

In biomedical science, the term 'translational' may be applied to studies which compare mRNA expression with distribution of the transcribed protein or more commonly, to animal studies that investigate the functions of the target protein. Increasing emphasis is, however, being placed on measuring the function of the target protein in native human tissues. Arguably, this is the ultimate translational step prior to *in vivo* clinical studies, provided the model is demonstrated to have (patho)physiological relevance. Such models determine the significance of human mRNA or protein expression. They validate functional data using recombinant receptors in host cells and can prove or dismiss the value of animal studies as models of the human condition (Table 1).

This aspiration is easier said than done. It is impossible to organize daily delivery of relevant human tissues to fit with laboratory schedules. Further, although both animal and human studies are regulated by law and ethical reviews, humans have the additional need to give consent before their tissues are used. These and other reasons, discussed next, may explain why experiments with fresh human tissues are relatively uncommon. The scarcity of studies is especially true for complex tissues such as the gastrointestinal (GI) tract where

Table 1
Fundamental reasons why functional responses need to be studied in native human tissues

Translation from host cell studies	Accessing 'difficult to isolate' cell types	Provision of physiologically relevant models	Studying disease	Species-dependent genetics/functions	Reduction in animal use
Studies with host cells and recombinant proteins may be inappropriate because of issues of expression density, cell coupling and/ or passage number (e.g. motilin: Dass *et al.*, 2003) or the absence of receptor activity-modifying proteins (Morfis *et al.*, 2008) which change how GPCRs behave (Bockaert *et al.*, 2004).	In the GI tract, it is relatively simple to isolate muscle or epithelial cells for functional experiments, but not myenteric neurones embedded within the muscle. Instead, the pharmacology of this nervous system can be studied by measuring nerve-mediated changes in muscle movements.	Native human tissues can be used to model physiological events in which different cell types operate together. For example, the human peristaltic reflex, involving sensory and motor nerves operating together with the muscle layers, has rarely been studied (Foxx-Orenstein *et al.*, 1996; Spencer *et al.*, 2012).	Human tissues are removed for therapeutic reasons, enabling disease mechanisms to be studied. For the GI tract, common reasons for surgery are cancer, obesity, chronic inflammation or other disorders.	The jump from molecular or animal research to human studies involves risk, illustrated by several failures of translation to humans (Green *et al.*, 2011). There are examples where the human receptor is absent in rats and mice, or if present, has a different function (Sanger *et al.*, 2011).	There are clear scientific and ethical pressures to reduce our reliance on animals for biomedical research, especially if the animal studies use strains with unknown relevance to the human condition.

muscle, nerve, epithelial and other cell types work together to generate clinically meaningful responses. It is extraordinary to appreciate, for example, that our understanding of intestinal peristalsis is derived almost entirely from animal studies. Thus, only one laboratory has evoked the peristaltic reflex in human isolated jejunum (Grider, 1989; Foxx-Orenstein *et al.*, 1996) while one other observed spontaneous, visibly propagating movements of the human isolated colon, in which only a minority of preparations responded to intraluminal distension by reflex contraction (Spencer *et al.*, 2012). This paucity of data also means that our knowledge of how new drugs, recently discovered endocrine substances or bacteria may modulate peristalsis is based almost entirely on studies with animals or human volunteers.

A human tissue laboratory has to confront the issue of variation in patient ages, gender, lifestyles, genetic backgrounds and disease. Thus, the investigator only has control of the tissue preservation and experimentation. No control of human tissue genotype is possible and there is only limited control on the phenotype and anaesthetics used. Interestingly, sources of variation are also true for animals, but here the issue can be controlled or ignored. For example, a recent study into 17 different mouse genomes, including classical laboratory strains and the progenitors of strains linked to over 5000 different types of knockout mice, identified 56.7 million unique single-nucleotide polymorphisms, 8.8 million unique indels and 0.28 million structural variants (Keane *et al.*, 2011); the translational significance of these variations for any particular strain of mouse (in terms of relevance to human biology) is largely unknown. Similarly, mouse strain-dependent differences can dictate how the GI tract responds to factors such as stress, for example, where clear differences exist in the propensity of different strains to defecate or release colonic 5-HT (Julio-Pieper *et al.*, 2012). Nevertheless, studies with mice can be restricted to one particular strain to minimize data variation. This leads to the provocative hypothesis that while studies with human tissues play an important *supportive* role in translational science (e.g. supporting use of particular strains of animals or studies using host cells transfected with a human receptor), the potential variability means that their use in *primary research* into basic mechanisms should be confirmed by cross-reference back to the control knockout mouse representing a particular phenotype. Clearly, the latter is not a desired outcome, so methods of minimizing variability in human tissue research are vital.

In this review, the use of human tissues for functional studies is examined by reference to studies into the neuromuscular and neurosecretory *functions* of the GI tract. The focus on GI diseases is justified by the enormous healthcare burden such diseases collectively impose. This includes some of the most common human ailments, for example, gastro-esophageal reflex disease, irritable bowel syndrome, chronic constipation, diarrhoea and incontinence. In many of these disorders the enteric nervous system (ENS) is thought to play a major or adjunctive role in mediating the dysfunction. The ENS is embedded within the wall of the GI tract and is so extensive that its number of neurones parallel that of the spinal cord. Further, the diversity of neurotransmitter receptors and channels is comparable with the CNS (Furness, 2006), prompting suggestions that human GI neuropharmacological techniques can also be used to provide some func-

tional characterization of mechanisms relevant to the CNS, such as neurodegenerative changes accompanying Parkinson's disease (Natale *et al.*, 2011). This review addresses the following:

1. procedures for accessing human GI tissues and preserving tissue functions;
2. methods for functional neuropharmacological studies in human GI tissues;
3. basic pharmacological principles for minimizing variation in human tissue research;
4. changed functions caused by the disease for which the tissue was removed; and
5. differences between human and animal GI neuropharmacology.

Access to human GI tissues

Ethic applications

While nearly all patients are happy for redundant surgical tissue to be used for research, the Alder Hey incident in 1999 forced the international research community to significantly tighten research governance in accord with regulatory frameworks, for example, in the UK, the Human Tissue Act 2008. Access to human tissue for research requires ethics documentation and informed consent by the patient for use of their tissue for the research described. Consent also establishes the rights to own data from the experiments, an important principle if the research is sponsored by private industry or leads to patentable discoveries and commercial opportunity. Research ethics applications should be comprehensive to maximize opportunities, minimize paperwork and simplify governance arrangements for future protocol modifications. An ideal application, for example, valid for 20 years, could use a wide range of human GI tissues (biopsies and full thickness), as well as the faeces, blood or urine from the same patient, in a large number of functional and anatomical experiments. As new techniques emerge, amendments can be made to the core ethics agreement. It is standard practice to ensure that there is no direct link between the tissue studied and the identity of the patient.

Teamwork: roles of the surgeon and pathologist

A team of research investigators, surgeons and pathologists are necessary (Figure 1). Surgeons ensure that fresh human tissues are available by asking theatre staff to call researchers to collect tissues promptly, by preventing specimens being placed in formalin prior to tissue collection and by facilitating arrival of research staff in theatre sluice to collect tissues. Together, these actions also help minimize the time taken before the specimen is placed in an oxygenated solution and transported to the laboratory. The team surgeon can also identify the best source of human tissue to answer a specific research question, identify patients for individual studies, carry out consent for research and record the clinical phenotype of patients. Intraoperatively, team surgeons can record

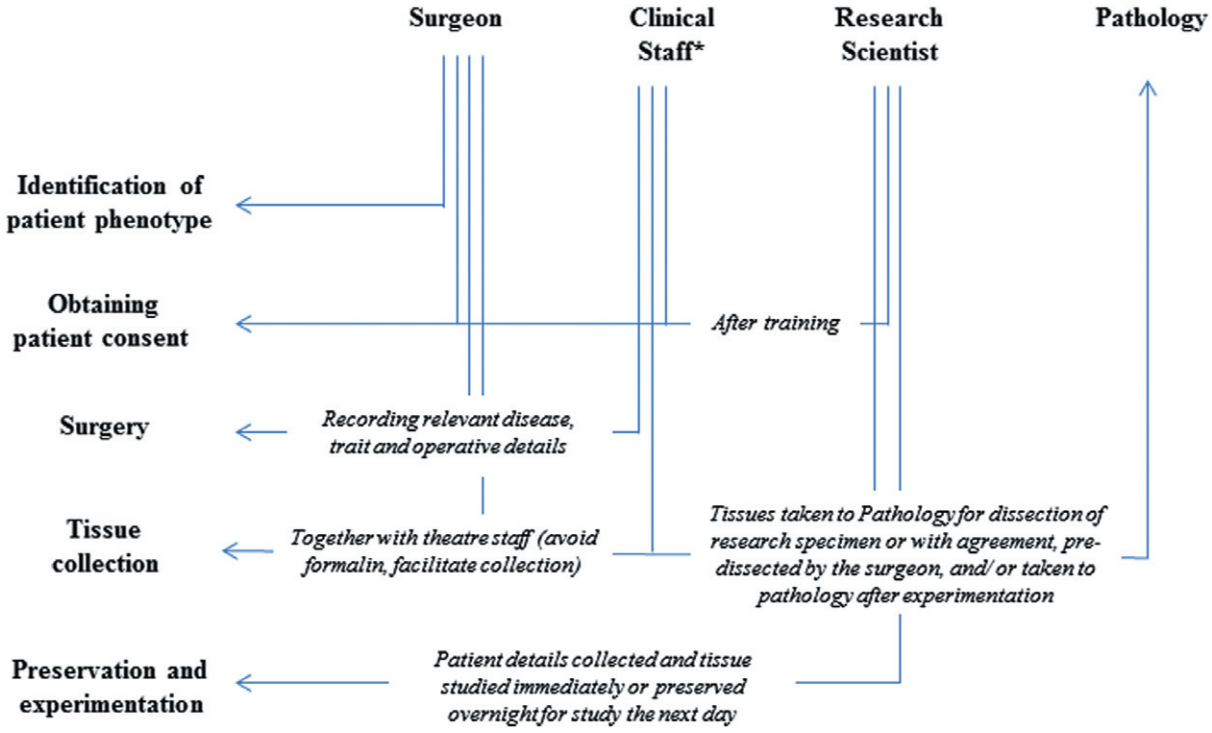

Figure 1

The team required to undertake functional studies with human tissues. Each member of the team has his/her own role, but all are linked together for the benefit of both the patient and the basic scientists. *Clinical staff includes theatre staff and clinical fellows.

anaesthetic details and relevant operative details. Clinical phenotypes or trait factors such as age, gender and body habitus must be recorded. State factors which may modify responses *in vitro* are also recorded. These include preoperative treatments (e.g. medications that modify immune function, opioids that alter peripheral pain transmission, as well as chemotherapy and radiotherapy), the disease process (e.g. peritonitis, obstruction, bowel ischaemia) and the use of anaesthetic drugs such as i.v. opioids and nitrous oxide, known to have profound effects on neurotransmission.

Usually, the pathologist dissects the surgical specimen and provides samples for research. Diagnostic sites, such as site of a tumour, lymph node sampling and resection margins are taken, leaving the remainder potentially available for research. Such an approach allows maximum yield of tissue for research. However, following training and histopathological approval, this role can be performed by the surgeon in certain instances such as elective colonic resections. Here, the surgeon finds the tumour, opens the specimen, washes out faeces, inspects the mucosa and mesentery, and takes samples from non-diagnostic sites. The sites can be marked or repaired so histopathologists do not lose vital information on overall morphology. For some specimens, it is mandatory for pathologists to perform dissection due to disease proximity, for example, oesophagectomy. For others, where the whole specimen is potentially required for diagnosis, it is possible for functional studies to proceed and then the tissue sent to the pathologist after use, for example, appendix in acute appendicitis. Finally, it is worth noting that histopathology departments differ in their practice of retaining specimens for further sampling. Some may give residual non-diagnostic tissue for research in its entirety whereas others require retention of the specimen for up to 1 month. Such matters, of course, require discussion with the local pathology department. Unless tissues are obtained from patients with known transmissible disease, standard containment level 2 safety procedures apply.

Surgical techniques to minimize variation

Modification of operation to optimize tissue collection is rarely considered but its importance is readily demonstrated by comparing the functional viabilities of tissues removed via laparoscopic or an open-modified surgical approach, the latter resulting in shorter tissue ischaemia time prior to collection (sometimes to as little as 5 min if the researcher is waiting; Figure 2) and less peripheral tissue destruction from clamping. Attention to surgical techniques raises a further important point about control tissue which is rarely discussed. Most commonly, the flaw lies with comparing biopsies (representing the disease) with a similar piece of tissue removed from a large resection specimen on the bench (the control). An example is where gastric biopsies, for example, in gastroparesis, are compared with tissue from obese patients undergoing laparoscopic sleeve gastrectomy. Aside from considerations of whether obesity itself could affect function, the former is removed from perfused tissue whereas the latter is removed from tissue that may have been devascularized for several hours. This is relevant as Interstitial cells of Cajal are susceptible to ischaemia (Farrugia, 2008).

Functional GI neuropharmacology

Here the focus is on direct measurements of contractile activity by the *muscularis externa* and on electrogenic secretion by the intestinal mucosa. These functions are controlled or modulated by the ENS, particularly by the myenteric and submucosal plexuses respectively. In normal physiology, both enteric sensory (including mechanosensitive enteric neurones and/or intrinsic primary afferent neurones projecting from the mucosa of the intestine into the nerve plexuses; Furness, 2006; Mazzuoli and Schemann, 2012) and motor nerves are required, so human tissue models of each type of neuronal function are required. Functional studies on other parts of the ENS affecting, for example, the muscularis mucosa (Walder, 1953), are too infrequent to be included. Indirect studies which try to predict functions by looking at protein expression are excluded, along with functional studies on the extrinsic nervous systems affecting human GI physiology (e.g. responses to capsaicin or direct afferent/gastric vagal nerve recordings; Barthó *et al.*, 2002; Lang *et al.*, 2003; Lang and Grafe, 2007; Peiris *et al.*, 2011).

Neuromuscular pharmacology

In human GI tissues, it is difficult to dissect myenteric neurones away from the surrounding muscle, so direct neurone recordings are unusual. Early intracellular recordings from small numbers of cultured foetal or freshly dissected myenteric neurones (Maruyama, 1981; Brookes *et al.*, 1987) were followed by recordings from primary cultures and freshly dissected human myenteric neurones using voltage-sensitive dyes to image spike discharges evoked by nicotine (Vignali *et al.*, 2010). Nevertheless, these experiments remain the exceptions that are generally the domain of specialized laboratories.

Electrical field stimulation (EFS). Indirect assessments of neuronal functions are made by measuring changes in muscle contractility caused by electrical stimulation of nerves within the muscle; typically, muscle preparations are used in which the mucosa and submucosa have been removed by blunt dissection. The muscle may be cut in parallel to either the outer longitudinal or the inner circular muscle fibres, and different responses to EFS may be obtained using either muscle orientation (see below). Most preparations are 0.3–0.5 cm in width and 1–3 cm long; studies have not been conducted to determine the optimal size which combines the needs for tissue oxygenation with preservation of neuromuscular functions. EFS is likely to activate both intrinsic sensory and motor enteric nerves, but because the submucosa has been removed (severing links to the intrinsic primary afferent neurones) and because the measurements are of muscle contractility, the method is normally considered as a measure of motor nerve functions arising from the myenteric plexus. Nevertheless, at appropriate intensities of nerve stimulation, direct activation of extrinsic nerve terminals embedded within the tissue, followed by release of neurotransmitter to affect myenteric and/ or muscle function, must remain a possibility.

EFS evokes different responses in different regions of the human GI tract (Table 2). In circular muscle preparations of

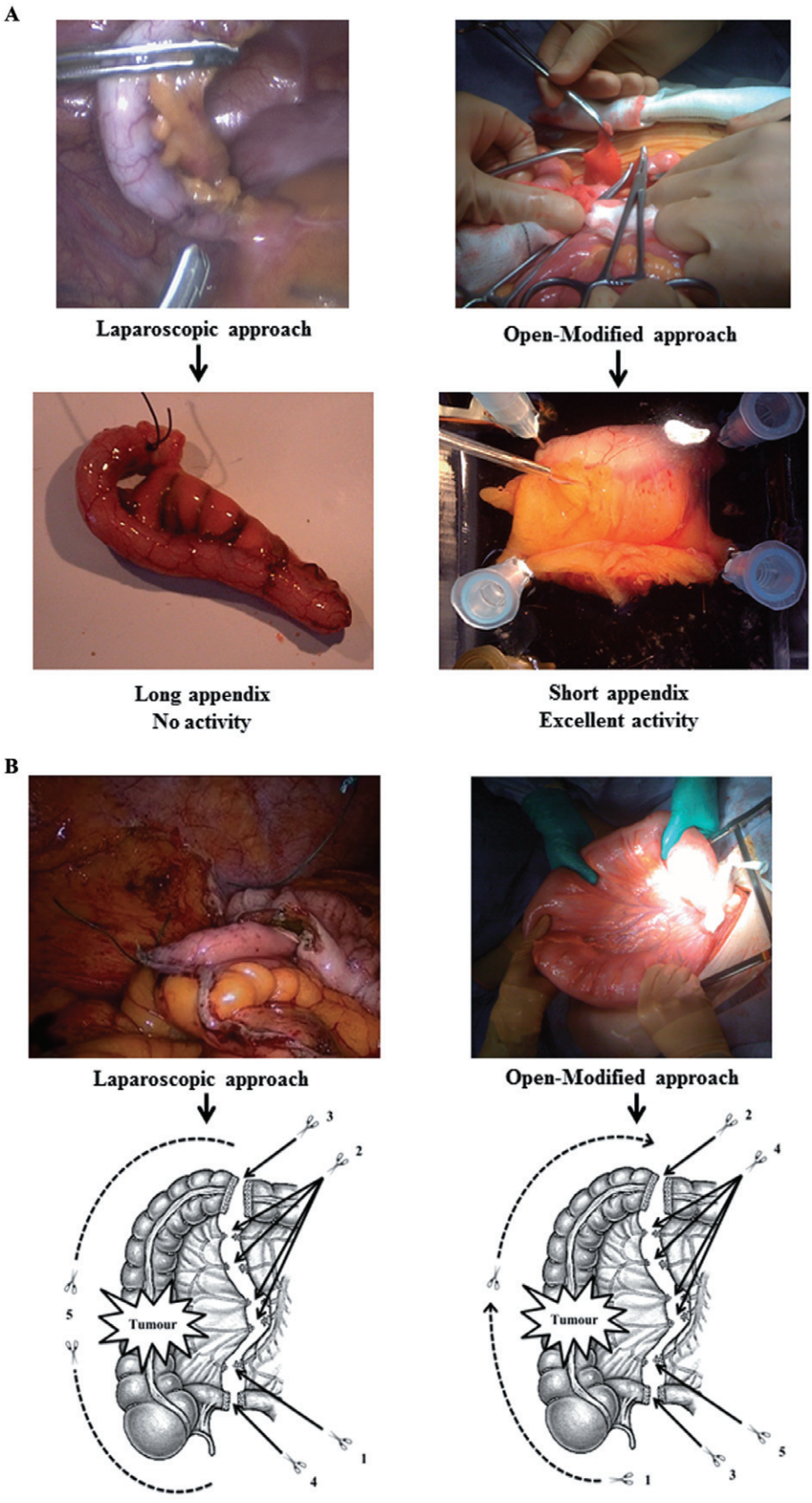
A
Laparoscopic approach
Open-Modified approach
Long appendix
No activity
Short appendix
Excellent activity
B
Laparoscopic approach
Open-Modified approach
Tumour
Tumour

Figure 2

Minimizing ischaemic time by attention to surgical techniques and the instruments used for coagulation. (A) Top panel – laparoscopic versus open (modified) appendicectomy. Both appendices were resected in females 60 to 70 years of age, of thin to modest body habitus. Both were transported to the laboratory in the same media, with less than 20 min travel time. Despite resecting a long appendix using a laparoscopic approach, no activities were recordable in mesenteric afferents. On the other hand, a short appendix resected using an open (modified) approach has excellent recordable activity from mesenteric afferent (Peiris *et al.*, 2011). The difference was due to the need to use harmonic scalpel or diathermy on appendicular mesentery during a laparoscopic appendicectomy, which irreversibly damaged the mesenteric nerve fibres because of the high temperature invoked. (B) Bottom panel – laparoscopic versus open right hemicolectomy. Ischaemic time of the resected specimens may be higher in laparoscopic right hemicolectomy due to the common approach of medial to lateral dissection and early ligation/coagulation of the ileocolic vessel – the dominant vessel to the right colon (for sequence of events see scissors 1–5). In open surgery, ligation of the ileocolic vessel is usually performed last after lateral to medial dissection.

Table 2

Neuromuscular responses of human GI tissues to EFS

Region	Response to EFS	References
Distal oesophagus	Frequency dependent, cholinergically mediated contractions during EFS and again on termination of EFS; the amplitudes enhanced by inhibition of NOS, indicating simultaneous activation of inhibitory nitrergic neurones	Richards *et al.*, 1995, González *et al.*, 2004. Tøttrup *et al.*, 1990 and Preiksaitis *et al*, 1994 observed after-contractions only
	Circular muscle strips tended to contract at low frequencies of EFS (0.1–0.5 Hz), with relaxations and after-contractions appearing at the higher frequencies; in longitudinal muscle EFS predominantly evoked contraction	Bennett & Stockley 1975
Lower oesophageal sphincter	Muscle cut adjacent to the esophagogastric junction relaxed in response to EFS whereas strips cut progressively more rostral showed a biphasic response, consisting of an after-contraction	Burleigh 1979
Stomach	*Fundus and antrum, circular muscle:* frequency-dependent, monophasic contractions, predominantly cholinergic but with simultaneous nitrergic inhibitory influence suppressing amplitude of contractions	Bennett & Stockley 1975; Broad *et al.*, 2012
	Fundus and antrum, circular muscle: during EFS the nitrergic motor function activated more readily by EFS than the cholinergic system	Tonini *et al.*, 2000
	Fundus and antrum, longitudinal muscle: monophasic contractions, predominantly cholinergic but with simultaneous activation of an inhibitory transmitter	Sanger 1985
	[^{3}H]-acetylcholine release: from human proximal stomach previously incubated with [^{3}H]-choline during nerve stimulation	Leclere & Lefebvre 2002
	Pyloric sphincter: cholinergically mediated contraction mostly in the proximal region of the sphincter and a neuronally mediated inhibitory response mostly in the more distal region of the sphincter	Tomita *et al.*, 2007
Duodenum and terminal ileum	*Circular muscle preparations:* nitrergically mediated relaxations usually occurred at lower frequencies of stimulation, with cholinergically mediated contractions at higher frequencies	Bennett & Stockley, 1977; Maggi *et al.*, 1990; Broad *et al.*, 2012
Ascending or descending colon	*Longitudinal or circular muscle:* contractions or relaxations occur during EFS mediated by, respectively, cholinergic and nitrergic neurones. Termination of EFS is then usually followed by a relatively large contraction of the muscle (the 'after-contraction'), abolished by a combination of atropine plus NK_1, NK_2 and NK_3 receptor antagonists, suggesting cholinergic and tachykinergic involvement.	e.g. Bennett & Stockley 1975, Boeckxstaens *et al.*, 1993, Cao *et al.*, 2000, Prins *et al.*, 2000; Tavares & Rennie, 2001, McKirdy *et al.*, 2004, Cellek *et al.*, 2006, Hinds *et al.*, 2006, Auli *et al.*, 2008; Gallego *et al.*, 2011; Broad *et al.*, 2012
Internal anal sphincter	In strips cut in circular muscle direction, EFS caused muscle relaxation.	Burleigh *et al.*, 1979

gastric fundus and antrum, EFS usually evokes frequency-dependent, monophasic and predominantly cholinergically mediated contractions, although simultaneous activation of inhibitory, nitrergic neurones suppresses the amplitude of contractions (revealed by facilitation of contraction amplitudes in the presence of an inhibitor of NOS) and sometimes the nitrergic influence dominates so muscle relaxations are observed (Table 2). These observations are consistent with the dominance of myenteric neurones in human gastric fundus labelled by antibodies against ChAT and NOS, with smaller numbers of neurones labelled by antibodies to vasoactive intestinal peptide and substance P (Pimont *et al.*, 2003). It should also be noted that the fundus and antrum respond in a different manner. For example, in circular muscle preparations, ACh tended to induce or increase phasic 'spontaneous' contraction frequency and amplitude, whereas in the corpus and fundus, ACh increased basal muscle tension (Sinn *et al.*, 2010). These differences relate to the different functions of these areas of the stomach.

In other regions of the human GI tract, the effects of EFS are more complex. Muscle contraction and/or relaxation may be evoked *during* EFS and contraction may follow the termination of EFS (*after-contraction*). This has been observed in circular muscle preparations from distal oesophagus and lower oesophageal sphincter, duodenum, terminal ileum, and ascending or descending colon (Table 2). For example, in the colon, contractions or relaxations occur during EFS mediated by, respectively, cholinergic and nitrergic neurones. Termination of EFS is then usually followed by a relatively large after-contraction, abolished by a combination of atropine plus neurokinin $(NK)_1$, NK_2 and NK_3 receptor antagonists, suggesting cholinergic and tachykinergic involvement (Cellek *et al.*, 2006; Broad *et al.*, 2012). These responses are consistent with high numbers of cholinergic and nitrergic motor- and inter-neurones found in human colon (Porter *et al.*, 2002). In one laboratory, small purinergic activation was also observed at low frequencies of EFS (Auli *et al.*, 2008; Gallego *et al.*, 2008; 2011).

The peristaltic reflex. Enteric sensory nerve activity can be studied indirectly by measuring reflex functions such as peristalsis, in which both sensory and motor nerve transmissions are involved. Burleigh *et al.* (1984) used human colon in which the arterial supply was perfused with oxygenated Krebs-Dextran solution (with venous drainage) and the tissue kept in a functional state by placing in a warm, humidified chamber. Intraluminal pressure transducers recorded changes in movements caused by neostigmine or bethanechol and seen as 'waves of contraction', sometimes expelling mucus from the lumen. However, peristalsis induced by stimuli such as intraluminal distension was not attempted. More recent studies used the entire colon from patients with slow-transit constipation (STC), mounted longitudinally in an organ bath; changes in circular muscle tension were recorded from multiple sites along its length. It was argued that the use of long segments make it possible to study long enteric nerve pathways, thought to occur in mice. The results were compared with those obtained using descending or partial transverse colon from cancer patients. Each preparation was mounted within 10–20 min after surgery and studied immediately, before being sent for histopathology. In most non-STC patients, cyclical motor complexes (CMCs) began spontaneously 30–45 min after establishing the preparation and were seen to propagate; they were considerably faster than those recorded by others *in vivo* and were less frequent in colons from patients with STC. Intraluminal balloon distension of the ascending or descending colon evoked an ascending excitatory reflex contraction (or evoked CMC) in 8 of 30 trials from 3 of 8 control colons but not from colons from STC patients (Spencer *et al.*, 2012).

An alternative method measured the peristaltic reflex in human isolated jejunum, obtained from patients undergoing gastric bypass surgery for morbid obesity (Grider, 1989; Foxx-Orenstein *et al.*, 1996). After opening the intestine, flat sections of intestine were placed in tissue chambers, with mucosa uppermost. The chambers were subdivided into two compartments by vertical partitions sealed with vacuum grease. Thus, in one compartment a stimulus could be applied to the tissue and in the other, the response measured, thereby separating the sensory and motor components of the peristaltic reflex. Using this apparatus, ascending excitatory and descending inhibitory movements of the circular muscle (typically found in the intestine of laboratory animals and integral to peristalsis; Furness, 2006) were evoked by stroking the mucosa with a brush. Nevertheless, although these experiments demonstrated (for the first time) that a peristaltic reflex can be induced in human isolated intestinal tissues, the pharmacological phenotype appears not to fully translate in terms of activities observed in human volunteers. Thus 5-HT_4 receptor antagonism greatly inhibited the reflex in human isolated jejunum (Foxx-Orenstein *et al.*, 1996) whereas *in vivo*, antagonism at this receptor had no effects on gastric emptying or small intestinal transit and only a modest, dose-independent tendency to reduce colonic transit times (Bharucha *et al.*, 2000). More recently, a different pattern of reflex movements has been induced in human isolated *taenia coli* (Broad *et al.*, 2011). These thickened bands of longitudinal muscle run the length of the human colon and aid mixing and movements of colonic contents (*taenia* are absent in the colon of rodents, although these structures occur in the caecum, a vestigial area in humans; Langer and Takács, 2004). Mucosal compression evoked muscle contraction in both oral and aboral directions, implying different reflex mechanisms to those mediating the ascending contraction and descending relaxation in the human jejunum studies above and by implication, different from animal intestine where *taenia* are absent.

Neurosecretory pharmacology

Submucosal nerve activity. Compared with myenteric neurones, access to submucosal neurones can be more easily achieved after removal of the mucosa. Submucosal nerve recordings have therefore been achieved by measuring changes in intraneuronal free $[Ca^{2+}]$ in submucosal plexus dissected from Roux-en-Y jejunum specimens, following 3 s fibre tract stimulation (Wunderlich *et al.*, 2008); in these experiments, the tissue was kept in 2 L of Krebs solution at room temperature for 1 h to remove anaesthetics, and activity was sensitive to inhibition by purine receptor antagonists. Similarly, calcium imaging techniques have measured submucosal nerve activity in duodenal biopsies (removed during endoscopy for presumed functional disorders) in response to

high-potassium, nicotine or 5-HT receptor stimulation or by electrical stimulation of interganglionic fibre tracts (Cirillo *et al.*, 2012). Finally, stimulation of submucosal nerve activity in human intestine (measured by fast imaging of voltage-sensitive dyes) has been demonstrated in response to histamine (Breunig *et al.*, 2007), to activation of protease-activated receptor-type 1 (Mueller *et al.*, 2011), by a mast cell mediator cocktail (from patients undergoing surgery for cancer, in which mast cells were obtained after enzymatic tissue dispersion and enrichment, followed by culture and stimulation to release their contents; Schemann *et al.*, 2005) or by supernatants of colonic biopsies from patients with constipation- or diarrhoea-predominant irritable bowel syndrome (containing a higher density of mast cells); the latter activity was prevented by antagonism at H_{1-3} or 5-HT_3 receptors, or by protease inhibition (Buhner *et al.*, 2009). In these experiments, the use of the human tissue was optimized by applying drugs locally to each ganglion so that different drugs could be used in each preparation. The same techniques were used to show inhibition of human submucosal nerve firing by β3-adrenoceptor activation (Schemann *et al.*, 2010).

Ussing chambers. This technology measures the potential difference and changes in short-circuit current (SCC) across intestinal mucosa, attributed to increased electrogenic chloride secretion and reduced electroneutral sodium absorption; changes in water movement across the mucosa are assumed to follow. Human mucosa may be obtained from surgical specimens or smaller preparations from biopsies and the neuropharmacology studied by investigating changes in SCC following application of ligands which stimulate submucosal neurones (e.g. substance P or NO donors: Stack *et al.*, 1996; Riegler *et al.*, 1999) or by release of endogenous mediators from mucosal endocrine cells to exert similar neuronally mediated actions (e.g. stroking human jejunal mucosa to release 5-HT: Kellum *et al.*, 1999; but see Burleigh & Borman 1993 and Borman and Burleigh, 1996, who found no such activity after application of exogenous 5-HT to either human colon or ileum), by direct nerve stimulation with EFS in ileum (e.g. Burleigh and Borman, 1993) and jejunum (Wunderlich *et al.*, 2008) or by distension of the mucosa (evoked by removal of fluid for 30 s from the submucosal compartment: Wunderlich *et al.*, 2008). Interestingly the 5-HT_3 receptor agonist 2-methyl-5-HT did not evoke chloride secretion in human small/large intestine, but did transiently activate enteric submucosal neurones (an effect blocked by the 5-HT_3 receptor antagonist tropisetron) (Michel *et al.*, 2005); these data caution against the assumption that these two activities are necessarily linked. Finally, Krueger *et al.* (2010) found that hydrogen sulfide evoked mucosal secretion in human isolated mucosa by activating TRPV1-expressing afferent neurones via release of substance P acting at NK_1 and NK_2 receptors in human but via NK_1 and NK_3 in guinea pig.

Functional studies with human tissues: basic pharmacological principles

At the outset of any study with human tissues, certain basic questions need to be asked.

What is the control tissue?

Because the tissue has been removed for a medical reason, it cannot be 'normal'. Nevertheless, it is reasonable to make certain assumptions about what is relatively normal, provided the source of tissue is acknowledged. For example, in neuromuscular studies, it is common to regard tissue removed away from the tumour in cancer patients as control tissue. Thus, when removing the tumour, it is necessary to remove material from either side of the tumour, at least up to the margin affected by stopping the blood supply to the region of bowel containing the tumour. This macroscopically normal material becomes the surrogate control tissue for basic pharmacology studies and/or for comparison with other diseased tissues. This compromise does, however, have limitations. A notable example occurs when studying tissues from children where the incidence of cancer, or resection for any other indication, is low.

When is it best to use the tissues?

Ideally, studies should begin as soon as possible after surgery, and for cardiac tissues the timing is critical (Hillier and Bunton, 2007). However, for human GI tissues, longer post-surgery times are sometimes acceptable. This is important because tissues are often not available until late in the day, when experiments must be carried out in the evening or after overnight storage in pre-oxygenated physiological solution at 4°C.

In neuromuscular studies, few investigators have provided objective measurements of the recovery of functions when tissues are used on the day of surgery or after overnight storage. Tonini *et al.* (2000) reported that at least 90 min was allowed before starting experiments with human stomach muscle removed that day, during which muscle tone increased in all preparations not rejected. Richards *et al.* (1995) observed that spontaneous, repetitive phasic contractions of human isolated oesophagus began approximately 60 min after equilibration in the tissue bath. Gagnon *et al.* (1972) observed that it took 90 to 120 min before spontaneous activity developed fully and the baseline longitudinal or circular muscle tension become stable in human colon stored overnight. In another example, patterns of spontaneous muscle contractions were simply investigated after 1 h of recovery (e.g. Auli *et al.*, 2008). However, none of these examples actually measured how long it took before neuronal functions had recovered. Broad *et al.* (2012) showed that motor nerve functions were slower to recover in all regions of the human GI tract. The median time before consistent responses to EFS were obtained was ~2.5 h for tissues used on the day of surgery or ~3.5 h for tissues stored overnight, irrespective of GI region; attention to this detail led to a reduction in variability between different experiments. Notably, the phenotype of responses to EFS after recovery appeared consistent between the tissues used immediately or after overnight storage. Others who have successfully used tissues stored overnight to study neuronal function include Leclere and Lefebvre (2002), who successfully used human stomach strips within 24 h (or in one case, 36 h) after surgery. In other studies, storage of human colon in Krebs solution for up to 24 h at 4°C did not modify neuronally mediated responses evoked by EFS in a range of different GI tissues (Bennett and Stockley, 1975) or the response to adrenaline

(Gagnon *et al*., 1972). Finally, Guagnini *et al*. (2006a) incubated sections of human small intestine for 48 h at 18°C in aerated DMEM containing 10% foetal calf serum, penicillin and streptomycin. In these experiments, cholinergically mediated contractions evoked by EFS were similarly inhibited by the cannabinoid receptor agonist (+)WIN 55,212-2, providing a method to study drug tolerance or self-desensitization.

The mucosa is generally regarded as more fragile and is usually studied immediately. Nevertheless, Burleigh and Borman (1993) used sheets of mucosa plus submucosa on the day of surgery or after overnight storage at 4°C in DMEM plus Ham's F12 medium (1:1) with 10% FBS added; responses to EFS (10 Hz) were smaller after storage but responses to serosal application of 5-HT or forskolin were similar. In other Ussing chamber experiments, University of Wisconsin (UW) solution was found to minimize damage in human colon but not the ileum during 6–48 h of ischaemia (Kawashima *et al*., 1999). Similarly, circular muscle preparations of human ileum responded to EFS with neurogenic contractions and relaxations, but whereas the excitatory innervation appeared resistant to ischaemic damage, the inhibitory innervation declined over time with storage at 4°C in UC solution, with significant differences after 24 h storage (Zorychta *et al*., 1993).

Are responses affected by drugs given during surgery?

Remarkably, this has been little studied. In early experiments on neuromuscular functions of human GI tissues, no effects of drugs used in surgery could be demonstrated (Fishlock and Parks, 1963; Bennett and Stockley, 1975). Modern anaesthesia typically uses a combination of a volatile gas, for example, isoflurane, sivoflurane or desflurane and a mix of nitrous oxide and oxygen. Although not specifically studied, it is assumed that tissue recovers function in accord with the patient recovering consciousness, that is, rapidly after cessation of administration. Volatiles are not metabolized so accurate half-life data are unavailable; however, newer generation volatiles have a shorter half-life (minutes rather than hours) than halothane. In the author's practice of colorectal surgery, the shortest acting volatile – desflurane – is used without need for nitrous oxide. Of perhaps more concern is the combination of anaesthesia with strong analgesia, the latter almost always including i.v. opioids, for example, morphine or fentanyl. There is little that can be done to eradicate this concern other than ensuring that the state-phenotype of disease and control tissues is similar in terms of duration and type of anaesthesia/analgesia.

To what extent do age- and gender-related differences affect data variability?

Age-related changes in the presence of Interstitial cells of Cajal have previously been noted in human GI tissues (e.g. stomach; Gomez-Pinilla *et al*., 2011) but the functional consequences have not been studied. In addition, immunohistochemical studies with adult human colon, all from cancer patients, suggest that enteric cholinergic neurones decrease with advanced age, whereas nitrergic neurone numbers are unchanged (Bernard *et al*., 2009). However, as noted by the authors, imbalanced nerve phenotypes may not necessarily translate into changes in functions, given the large enteric nerve 'reserves' that are potentially available. Studies are therefore required to determine if these findings are functionally important.

Surprisingly, gender-related differences have rarely been addressed using human GI tissues, although here it should be noted that most female cancer patients will be of postmenopausal age. In one study, Maselli *et al*. (2011) suggested that circular muscle of sigmoid colon from female cancer patients contracted to a greater extent in response to carbachol, compared with tissues from male patients, whereas contractions evoked by EFS were more pronounced in tissues from elderly males. However, the number of observations represented the number of muscle strips studied and not the number of patients. As certain patients generated more muscle strips than others, the potential to artificially skew the data must therefore be high. Broad *et al*. (2012) found no obvious differences in neuromuscular responses of the human stomach or colon to EFS between male and female patients, and in the stomach, the ability of motilin receptor agonists to facilitate cholinergic function did not appear dependent on the sex of the patient.

What is the sample size?

A primary caveat included in many experiments with human tissue is that the sample size is too low to overcome the inherent variability of non-normalized data and thereby generate statistical power. For example, although Broad *et al*. (2012) were unable to show significant difference in the neuromuscular response to EFS in human gastric antrum used immediately or following overnight storage, the tissues stored overnight seemed to generate less tension in response to EFS than tissues used immediately (0.45 ± 0.08 g vs. 0.65 ± 0.12 g, $n = 9$ and 18 obese patients respectively; $P = 0.17$). As *post hoc* power analyses are not desirable (Goodman and Berlin, 1994), it is not possible to know if this study was sufficiently powered to show any effect of overnight storage. Using these data, given the difference in means of 0.2 g and a standard deviation of 0.35 g, to achieve statistical power of 80%, 49 stomachs would need to be studied in each category (fresh and overnight), and to achieve statistical power of 90%, 65 stomachs would require study (Lenth, 2006-9).

An alternative approach is to use a standard stimulus (e.g. EFS or maximal response to carbachol) in every tissue examined and by reference to this response, determine the magnitude of responses to drugs or disease-associated changes. Inherent in the responses to these stimuli is a measure of the health of the tissue (arguably more important than the response expressed per weight of tissue), so reducing at least some of the impact of the variable nature of human tissue research.

Do the models have translational value?

Translational value may be demonstrated by showing that a drug which changes GI motility *in vivo* has an equivalent activity *in vitro*, at concentrations found in blood plasma after a therapeutic dose. For example, low concentrations of the 5-HT_4 receptor agonist prucalopride facilitate cholinergic and nitrergic activity evoked by EFS in human isolated colon (Cellek *et al*., 2006), suggesting that this model predicts the

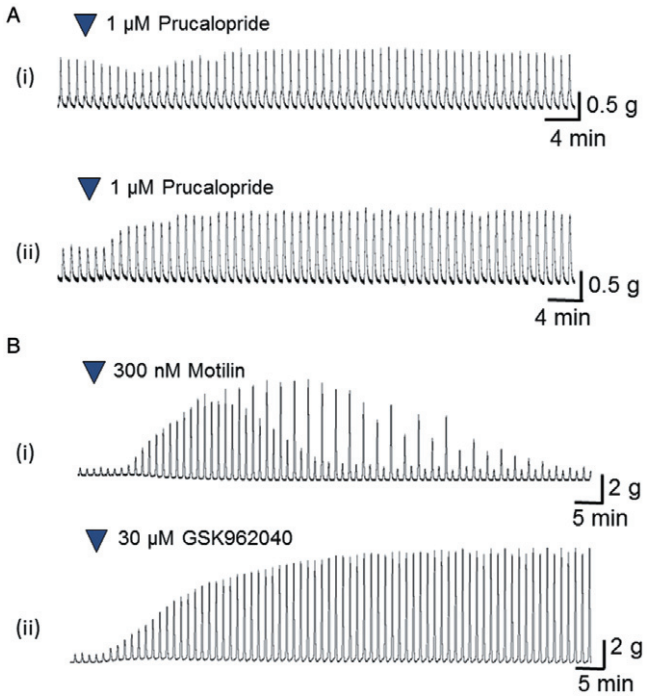

Figure 3

Translational studies with the selective 5-HT_4 (prucalopride) and motilin (GSK962040) receptor agonists, showing enhanced cholinergic activities in human isolated colon and stomach circular muscle strips, consistent with their prokinetic activities *in vivo*. (A) Representative trace examples showing the effects of 1 μM prucalopride on neuronally mediated contractions (predominantly cholinergically mediated) evoked by EFS in human colon in (i) the absence and (ii) the presence of the NOS inhibitor L-NAME 300 μM. Note the two different actions of prucalopride. (B) Representative trace examples showing the enhancement of electrically evoked, cholinergically mediated responses in human gastric antrum following addition of (i) 300 nM motilin and (ii) 30 μM GSK962040. Note the agonist-specific differences in the durations of action. Methods of tissue preparation and EFS are in Broad *et al.*, 2012.

ability of prucalopride and related drugs to promote colonic motility *in vivo* (Figure 3). Similarly, motilin and the motilin receptor agonist GSK962040 facilitate cholinergic activity in human isolated stomach (Broad *et al.*, 2012), suggesting that this model is predictive of the ability of GSK962040 and related drugs to promote gastric emptying *in vivo* (Figure 3). By contrast, higher concentrations of motilin receptor agonists directly contract the muscle and this is less likely to translate into meaningful clinical activity at therapeutic doses.

To some extent, the situation is clearer with Ussing chamber experiments, with drugs known to promote intestinal secretion *in vivo* having similar ability to promote SCC in human isolated intestinal mucosa (Sun *et al.*, 2011). Nevertheless, similar arguments can apply if novel substances are simply examined for their ability to change baseline SCC without regard to any potential ability to modulate ongoing neuronal control of secretory functions.

Changes associated with GI disease

Most studies with human GI tissues involve the use of macroscopically normal specimens from cancer patients. However, perhaps with the exception of avoiding a combination of experiments on inflamed and non-inflamed GI tissues, it is common for studies to be reported using tissues from different types of patients without regard to the possible existence of disease-specific differences. This section highlights some of the more obvious differences in disease-related GI neuromuscular functions.

Inflammation

Colonic circular muscle from patients with ulcerative colitis has been found to contract similarly in response to EFS, compared with preparations from cancer or acute diverticulitis patients. However, the amplitude of after-contractions was reduced, as were the contraction amplitudes to bethanechol (Snape *et al.*, 1991). Decreased force of colonic contraction has previously been noted in patients with ulcerative colitis (Cao *et al.*, 2000). Among the mechanisms proposed to explain reduced colonic contractility in inflammatory bowel disease (Ohama *et al.*, 2007) are an increased availability of NOS in the myenteric plexus and nerve fibres of the circular muscle (Crohn's ileum; Belai *et al.*, 1997) and raised production of hydrogen peroxide in the muscle (ulcerative colitis; Cao *et al.*, 2004). Such changes may have contributed to the ability of NOS inhibition to increase spontaneous contractility of ileum from eight children (8 ± 13 months) with ulcerative colitis and other disorders, but not from two adults with cancer and other disorders (Wittmeyer *et al.*, 2010). Nevertheless, these experiments need to be treated with caution because of the low n-values and the failure to match the reasons for resection. More recently, Broad *et al.* (2010) observed that in patients with ulcerative colitis or Crohn's disease, the circular muscle generally contracted less well in response to EFS, compared with macroscopically normal colon from cancer patients. Together, these observations point to marked changes in neuromuscular functions during inflammation of the intestine.

Obesity

After correction for differences in tissue weights, Gallagher *et al.* (2009) found increased contraction amplitude to a maximally effective concentration of carbachol in circular muscle from small intestine removed from obese patients during Roux-en-Y gastric bypass, compared with intestine from non-obese patients undergoing resection for benign disease. Contractions evoked by $PGF_{2\alpha}$ or substance P were also increased, an effect prevented by atropine and guanethidine; relaxations evoked by NO were unchanged. These differences were argued to be consistent with increased intestinal transit during obesity. Broad *et al.* (2012) found no difference in responses to EFS in gastric fundus from obese patients or from gastro-esophageal cancer patients.

Diverticular disease (DD)

In longitudinal strips of colon (*taenia*), responses to EFS were similar in both cancer and DD; the contractions were

abolished by atropine in both groups but whereas tetrodotoxin abolished contractions in the cancer patients, these were only reduced in DD (Maselli *et al.*, 2004). In longitudinal strips of colon (inter-*taenia*), EFS evoked similar responses in disease and control strips, although a greater number of disease specimens failed to respond (consistent with degeneration of the myenteric plexus; Deduchovas *et al.*, 2008), with the remainder requiring more time for the response to become stable. In both types of tissue, the contractions were abolished by atropine whereas terodotoxin abolished contractions in the control group but potentiated contractions in the disease group, an effect inhibited by the NK_1 receptor antagonist SR140333 (Guagnini *et al.*, 2006b). Finally, experiments with cannabinoid receptor agonists and antagonists suggest that endocannabinoids exert a strong inhibitory control, explaining a generally lower sensitivity to the cannabinoid receptor agonist (+)WIN 55,212-2 (100× more potent in inhibiting EFS-evoked contractions in controls than in patients with DD) and the ability of the CB_1 receptor antagonist SR141716 to potentiate responses to EFS in diverticular patients but not in controls (Guagnini *et al.*, 2006b).

Slow transit constipation (STC) and faecal incontinence

In patients with severe STC, the percentage of NOS-only myenteric neurones was raised compared with control tissues from cancer patients, with numbers of ChAT-containing neurones reduced (Wattchow *et al.*, 2008). Consistent with these observations, EFS more readily evoked relaxations of circular muscle from colon of patients with STC, an activity attenuated by inhibition of NOS (Tomita *et al.*, 2002). Finally, movements of the entire colon from patients with STC have been examined after mounting longitudinally in an organ bath, with changes in circular muscle tension recorded along its length (see earlier: 'The peristaltic reflex'). In colons from most non-STC (cancer), cyclical motor complexes propagated more frequently than in colons from patients with STC. Further, in the descending colon of controls, many of these consisted of ascending excitatory and descending inhibitory responses. Intraluminal balloon distension of ascending or descending colon evoked ascending excitatory reflex contraction in 8 of 30 trials from 3 of 8 control colons, but not from colons from STC patients (Spencer *et al.*, 2012). Finally, transverse strips of internal anal sphincter from patients with neurogenic faecal incontinence showed decreased sensitivity to the relaxant effect of carbachol (pD_2 values respectively, 6.0 vs. 5.4 in the control cancer patient and incontinent group; Speakman *et al.*, 1992).

Diabetes

In circular muscle strips of colon from patients with diabetes (colon removed for polyps or malignancy), EFS-evoked contractions were significantly impaired, compared with similar tissues from non-diabetic patients; the increase in contractions caused by NOS inhibition was also lower in diabetics, consistent with both enteric nitrergic and cholinergic loss (Chandrasekharan *et al.*, 2011).

Differences between humans and laboratory animals

The basic functions of the GI tract are similar but there are a surprising number of anatomical and functional differences between rodent and human GI tracts (Sanger *et al.*, 2011). Most obvious is the inability of rodents to vomit, a rare peculiarity within the mammalian kingdom and associated with structural, hormonal and genetic variations. Structural changes include marked differences in gastro-esophageal anatomy and distinct gastric muscle slow wave activities. Thus, the gastric fundus is relatively quiescent in the mouse and guinea pig, whereas slow-wave activity of ~5 cycles per min (cpm) is found in fundus and corpus of macroscopically normal tissues from patients with gastric cancer, rising to >7 cpm in the antrum (Rhee *et al.*, 2011). Hormonal differences are exemplified by large rises in blood vasopressin during nausea in humans, whereas rodents given the same emetogenic stimuli experience a rise in oxytocin, not vasopressin (see Sanger *et al.*, 2011). Genetic changes are exemplified by the absence of certain $5\text{-}HT_3$ receptor subunits in rodents, which are otherwise expressed by humans and other species capable of emesis (Holbrook *et al.*, 2009). Finally, the marked difference between the affinity of NK_1 receptor ligands (also involved in emesis) in rodents and humans suggests a link with the structural changes associated with the inability of rodents to vomit (see Sanger *et al.*, 2011). Most recently, the motilin system (a hormone released from the upper gut of humans during fasting to mediate phase III of the migrating motor complex and possibly facilitate the sensation of hunger) has been shown to be functionally absent in rodents (He *et al.*, 2010; Sanger *et al.*, 2011), a deletion again somehow linked to loss of the emetic reflex.

Marked differences between lower bowel functions also exist between rodents and humans. Most obvious is the large caecum in rodents (degenerated to appendix in humans) and the relatively shorter colon which packages faeces into discrete pellets prior to defecation. Particularly noticeable is the arrangement of the longitudinal muscle of human colon into three discrete bands known as *taenia*, running along the outer surface of the circular muscle; *taenia* exist within the rodent caecum but not the colon. It therefore seems reasonable to suppose that further differences will be identified between rodent and human colon neuromuscular control mechanisms (Sanger *et al.*, 2011). Finally, positive selections of genes during evolution are thought to confer human-specific pathophysiological variations which cannot satisfactorily be replicated by animal models (Vamathevan *et al.*, 2008). It should, therefore, be no surprise that these differences are sometimes translated into genetic and pharmacological differences and this can be illustrated by neuropharmacological studies with human tissues.

Opioid

Benko *et al.* (2010) found that morphine reduced contractions evoked by EFS in the longitudinal muscle of guinea pig ileum but had no effects on EFS-evoked, cholinergically-mediated contractions of the longitudinal muscle of human ileum.

Cannabinoid

Cholinergically mediated contractions of guinea pig isolated ileum were enhanced by the cannabinoid (CB) CB_1 receptor antagonist rimonabant, but in similar experiments with human ileum these contractions were unchanged unless the preparations were made tolerant to the inhibitory activity of the CB receptor agonist (+)WIN 55212-2 (Guagnini *et al.*, 2006a). These experiments suggest marked species-dependent differences in the role of endogenous cannabinoids in GI functions (Sanger, 2007).

5-HT_3 and 5-HT_4

Activation of the 5-HT_3 receptor by 2-methyl-5-HT evoked chloride secretion in guinea pig isolated intestine but not in the human intestine (Michel *et al.*, 2005). In this activity, the significance of the limited expression by rodents of the different 5-HT_3 receptor subunits (Holbrook *et al.*, 2009) is unknown.

In circular muscle of human isolated colon, in which EFS activates both cholinergically mediated contractions and nitrergically mediated relaxations (see above), the selective 5-HT_4 receptor agonist prucalopride had little effect or reduced cholinergically mediated contractions. However, after NOS inhibition, any inhibitory activity of prucalopride was replaced by clear facilitation of cholinergic activity (Cellek *et al.*, 2006; Figure 3), suggesting that 5-HT_4 receptor activation facilitates both nitrergic and cholinergic activity in human colon; such actions are consistent with an ability to facilitate, respectively, the ascending cholinergic and descending nitrergic reflex pathways in peristalsis. However, in guinea pigs, only facilitation of cholinergic activity has been described (e.g. Rizzi *et al.*, 1992). This difference appears small and the reason is unclear, but it serves to illustrate potential risks of relying on rodent models of GI functions to predict activity of novel substances in humans.

In longitudinal muscle from human ascending or sigmoid colon (*taenia coli*) or from rectum (patients with cancer, STC or DD), prucalopride facilitated cholinergically mediated contractions evoked by EFS during NOS inhibition. In similar experiments in dogs, prucalopride had similar activity in the colon but not the rectum (Prins *et al.*, 2000). These data again point out human–animal species differences and also suggest differences in actions between the two muscle layers.

Motilin

Motilin, a hormone present in endocrine cells of the human stomach and duodenum (but notably absent in rodents), may be released with ghrelin during hunger to regulate gastric movements and possibly, facilitate hunger (Sanger, 2008). Immunoreactive motilin receptors have been identified in both muscle layers of the human stomach (fundus and antrum), with smaller distribution to neurones of the myenteric plexus (Takeshita *et al.*, 2006; Broad *et al.*, 2012). This distribution of immunoreactive receptors suggests that the predominant activity of motilin is achieved via receptors on the muscle, and indeed there have been many studies which have examined the abilities of motilin receptor agonists to cause GI muscle contraction (e.g. Lödtke *et al.*, 1989). More recent studies have, however, examined the ability of motilin receptor agonists to facilitate cholinergic activity evoked by EFS in rabbit (Dass *et al.*, 2003; Jarvie *et al.*, 2007; Sanger *et al.*, 2009) and human (Broad *et al.*, 2012) isolated stomach. In these studies, different motilin receptor agonists (motilin, erythromycin, GSK962040) facilitated cholinergic activity at concentrations less than those which directly contracted the muscle. In human stomach, enhancement of cholinergic activity was considerably greater in the antrum compared with the fundus (e.g. ~810% facilitation with GSK962040 in the antrum, compared with ~98% in the fundus; Broad *et al.*, 2012), consistent with the need for the gastric antrum to generate more powerful phasic muscle contractions during gastric emptying. These studies are also consistent with the ability of erythromycin 40 mg to stimulate gastric motility in human volunteers in a manner prevented by atropine, whereas the excitatory action of a higher dose (200 mg) was unaffected by atropine (Coulie *et al.*, 1998). As receptor function depends on efficiency of coupling to downstream effector mechanisms, these data suggest that motilin receptors expressed by GI cholinergic neurones are better coupled than those on the muscle and play a greater role in mediating the GI actions of motilin. They also caution against over-reliance on immunohistochemistry studies and suggest the need for further translation by functional studies in native tissues.

A second translational issue is highlighted by the ability of motilin and motilin receptor agonists to rapidly desensitize the motilin receptor transfected into host cells (Thielemans *et al.*, 2005). Thus, the desensitization liability does not clearly translate into short-lasting prokinetic activity of all motilin receptor agonists in clinical studies *in vivo* (see Westaway and Sanger, 2009). Further, whereas the ability of motilin to facilitate cholinergic activity in rabbit and human isolated stomach is not sustained during its continuous presence, long-lasting facilitatory activity was observed with erythromycin and GSK962040 (Dass *et al.*, 2003; Broad *et al.*, 2012; Figure 3), consistent with their ability to stimulate human gastric emptying. These experiments point towards cell- and ligand-dependent differences in the ways in which motilin receptor agonists evoke responses in both native human and host cells.

Histamine

Using a potentiometric dye and fast imaging techniques to assess neuronal excitability in human submucosal plexus (using macroscopically normal areas of small and large intestine from patients with different disorders, including cancer, diverticulitis, Crohn's and ileus), Breunig *et al.* (2007) showed that histamine excited neurones via histamine H_1, H_2, H_3 and H_4 receptors, whereas in similar experiments with guinea pig intestine, predominantly H_2 receptors were activated. To optimize use of the human tissue, drugs were applied locally to each ganglion so different drugs could be used in each preparation. Interestingly, in Ussing chamber experiments with human isolated colon mucosa, histamine stimulated electrogenic chloride secretion via H_1 receptors to increase eicosanoid release, via a tetrodotoxin-resistant pathway. These data contrast with the involvement of H_2 and H_3 receptors in similar experiments with guinea pig mucosa (Keely *et al.*, 1995).

Protease-activated receptor (PAR)

Mueller *et al.* (2011) used voltage- and calcium-sensitive dye recordings to study the effects of PAR ligands on neurones and glia in the submucosal plexus from guinea pig colon and human small and large intestines. The latter were macroscopically normal areas from patients with different disorders, including cancer, diverticulitis, Crohn's and ileus. In summary, PAR1 rather than PAR2 or PAR3 activation, stimulated both human nerves and glia, whereas PAR2 activation was more effective in guinea pig colon. These data again illustrate the need for caution when translating novel data obtained using rodents into human intestinal biology.

Conclusions

The need for translational studies which measure physiological and clinically relevant functions is not questioned. However, relatively little work seems to involve measurements of functions in human isolated tissues. This is surprising, given the additional need to reduce reliance on animal research and increasing recognition of marked species differences in physiology and receptor pharmacology.

It is important to recognize that compared with animal tissue research, certain pharmacological principles differ. Foremost is the recognition that although animals used in research can be tightly controlled (but without necessarily knowing the clinical or translational relevance of the strain or genetically modified animal used), this is not possible with human tissues. In one respect, this is an advantage, enabling animal studies to be properly 'translated'. However, by attention to surgical techniques, methods of collection and storage of human tissues, the use of appropriate sample sizes and attention to obvious potential variables such as age, sex and disease, variation can be minimized and both translational and innovative data generated. As with all *in vitro* experiments, the model should be benchmarked against a clinically relevant drug, to prove the translational value of the model.

A side benefit of human tissue research is that there are no animal costs. Moreover, the existence of species-, cell-, method- and sometimes ligand-dependent variations in functions can even question the ethical use of animals without first validating the relevance to human physiology. It is recognized, however, that for this conclusion to be valid, it is important that studies with human tissues address the basic issues of potential variability addressed herein. Only in this way can human tissue research become the prime method for functional translational studies.

Conflict of interest

JB and VK disclose no conflict of interest. GJS has received funding from GlaxoSmithKline, Shire Pharmaceuticals and Probiotics International, and has undertaken consultation for Theravance and SK Life Science. CHK has received speaker fees from Shire and Medtronic.

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