Human visceral nociception: findings from translational studies in human tissue

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Abstract

Peripheral sensitisation of nociceptors during disease has long been recognised as a leading cause of inflammatory pain. However, a growing body of data generated over the last decade has led to the increased understanding that peripheral sensitisation is also an important mechanism driving abdominal pain in highly prevalent functional bowel disorders, in particular irritable bowel syndrome (IBS). As such, the development of drugs that target pain-sensing nerves innervating the bowel, has the potential to be a successful analgesic strategy for the treatment of abdominal pain in both organic and functional gastrointestinal diseases. Despite the success of recent peripherally restricted approaches for the treatment of IBS, not all drugs that have shown efficacy in animal models of visceral pain have reduced pain end points in clinical trials of IBS patients, suggesting innate differences in the mechanisms of pain processing between rodents and humans, and in particular, how we model disease states. To address this gap in our understanding of peripheral nociception from the viscera and the body in general, several groups have developed experimental systems to study nociception in isolated human tissue and neurons; the findings of which we discuss in this review. Studies of human tissue identify a repertoire of human primary afferent subtypes comparable to rodent models including a nociceptor population, the targeting of which will shape future analgesic development efforts. Detailed mechanistic studies in human sensory neurones combined with unbiased RNA sequencing approaches have revealed fundamental differences in not only receptor/channel expression, but also peripheral pain pathways.

Introduction

Abdominal pain is a symptom common to organic (e.g. inflammatory bowel disease; IBD) and functional (e.g. irritable bowel syndrome; IBS) gastrointestinal (GI) diseases (60, 75). Clinically, its management is challenging with many commonly prescribed painkillers showing limited efficacy for the treatment of abdominal pain or contraindicated by GI side effects (52). Pain is a leading cause of long-term disease morbidity in gastroenterology, contributing to significant reduction in patient quality of life, and substantial socioeconomic costs due to increased healthcare seeking and lost productivity (66). The 2016 Global Burden of Disease Study identified that the prevalence of IBD has increased in the last decade with a concomitant increase in years lived with disability (32). Consequently, the development of effective visceral analgesics for the treatment of abdominal pain in GI disease remains a long-standing priority. One therapeutic approach to this problem is to block the activation of pain-sensing nerves, so-called nociceptors, that are responsible for the transduction and transmission of noxious stimuli from the gut to the central nervous system (CNS) (4, 11, 29, 68). Clinical evidence to support the utility of this approach with regard to visceral pain is growing, however not all studies have been successful. For example, peripheral blockade of visceral nociceptors using rectal administration of local anaesthetics has proven effective in ameliorating both spontaneous and stimuli-evoked (e.g. visceral hypersensitivity to intrarectal balloon distension) visceral pain associated with IBS (72, 73). Whilst, more recently, the use of peripherally restricted compounds within well phenotyped patient subgroups (e.g. linaclotide, an agonist of the guanylate cyclase C receptor, in constipation-predominant IBS (16); and

eluxadoline, a μ/κ-opioid receptor agonist and δ-opioid receptor antagonist, in diarrhoea-predominant IBS (26)) has shown efficacy against pain endpoints in clinical trials. These data collectively provide evidence supporting a role of continued peripheral nociceptor input as a key driver in chronic visceral pain. However, not all approaches have been successful, most notably, the NK_3 receptor antagonist SR 142,801 was shown to inhibit the increase in pelvic afferent fibre activity and visceromotor response to colonic distension in rodents (30, 43), but the NK_3 receptor antagonist Talnetant (SB-223412) failed to alter sensory thresholds or response intensity to colorectal distension in healthy human volunteers (40), and lacked efficacy against pain and discomfort endpoints in a multicenter Phase IIB trial of IBS patients conducted by GlaxoSmithKline (ClinicalTrials.gov ID: NCT00101985; EudraCT number: 2004-000848-24).

Design and execution of clinical trials in functional bowel disorders should take into account high placebo responses, patient stratification and the variable and relapsing nature of these conditions (35, 67). Inadequate consideration of these factors has undoubtedly contributed to clinical trial failures in the past. In addition, inconsistencies in the translation of findings from animal models to clinical trials may be attributed to species differences in pain processing between rodent and humans, and a failure of disease models in rodents to fully recapitulate the complex and variable pathophysiology of human disease. In order to bridge this translational gap, a number of new experimental strategies have been applied to investigate human nociceptor function, including microneurography (63), human stem cell-derived sensory neurones (14, 74, 79) and primary cultures of excised human dorsal root ganglia (DRG) neurones (23, 71). Such techniques hold the

promise of directly interrogating native human nociceptor function. However, the application of data generated by these strategies to visceral pain is constrained by the inaccessibility of visceral nerves, scarcity of viscerally-projecting sensory neurones within a given DRG (18) and differences between the molecular mechanisms of stimulus transduction in visceral and somatic nociceptors (8, 36).

Bridging the gap: the use of human tissues to improve preclinical translation

Resected bowel tissues

One alternative method we, and others, have employed to address the challenge of conducting translational studies on visceral pain is to utilise macroscopically normal tissue obtained following pathological inspection from the margins of surgically resected human bowel (42, 51, 54, 56, 80). This is obtained from consenting patients undergoing surgery as part of their clinical treatment for GI diseases, most commonly bowel cancer. Using suction and wire electrode approaches, neuronal activity can be recorded from mesenteric nerves innervating ileum, colon or rectum and receptive fields identified and studied in flat-sheet preparations (54, 56). Alternatively, mesenteric nerve activity can be recorded from the human appendix when cannulated as a tubular preparation, which enables luminal distension in a manner comparable to similar approaches in rodent tissue (36). Distension of the gut evokes pain and is commonly used as a painful stimulus in human experimental medicine studies, for example colorectal distension was classically utilised by Ritchie to demonstrate the presence of visceral hypersensitivity in IBS patients (61). As such, the use of distension as a

stimulus in appendix tissue provides an attractive *ex vivo* preparation to model bowel pain. Both these two approaches (ileum, colon and rectum flat-sheet preparations, and tubular appendiceal preparations) have been successfully exploited to investigate human visceral afferent function in detail (36, 51). As such, these human tissue *ex vivo* preparations represent a translational bridge between animal models and human pain, particularly where pathology is the function of peripheral sensitisation. Importantly, use of human tissue bypasses species-specific issues, such as receptor expression or coupling (although individual variation will still be a factor), and provides a model where the peripheral nerve terminal architecture remains intact. Specifically, the nerve endings studied are surrounded by and interact with supporting cells of the native environment, and function in the presence of endogenous mediators/signalling molecules. These are important considerations that cannot be replicated in pared-down cell-centric models, such as human DRG or human induced pluripotent stem cell (iPSC)-derived sensory neuronal cultures.

Significant progress has been made in the characterisation of visceral nociceptors in rodents using electrophysiological recordings of afferent activity in response to noxious mechanical (e.g. high distending pressures, tissue stretch or blunt probe of receptive fields by von Frey hair filaments) (7, 28), inflammatory (e.g. histamine, proteases, bradykinin and ATP) (41), bacterial (55) and ischaemic stimuli (48). These studies have classified colonic afferents into five major subgroups dependent on the presumptive anatomical location of their receptive field and sensitivity to mechanical stimuli: vascular, intramuscular, intraganglionic, mucosal, and mechanically insensitive ‘silent’ afferents. Additionally, the pelvic nerve

innervating the distal colorectum also possesses muscular-mucosal afferents. These classifications have identified vascular afferents (i.e. those afferents that are closely associated with blood vessels in both the gut mesentery and penetrating intramurally into the gut wall) as a major form of nociceptor by which noxious stimuli are transduced (10). Vascular afferents, unlike muscular or mucosal subtypes, have activation thresholds to mechanical distension (>40 mmHg) that are painful in humans, and they are also activated by algogenic chemical mediators such as capsaicin, bradykinin, and ATP. A recent study by our group using single-cell RNA sequencing of colonic sensory neurones has identified molecular markers for 7 molecularly distinct subtypes, further facilitating the investigation of those fibres responsible for transducing noxious stimuli (38). By understanding which sensory afferents are responsible for transducing pain, we can not only focus drug discovery efforts on mechanisms capable of modulating the neuronal sensitivity in these afferents, but also focus basic research specifically on how these afferents change in disease.

Studies of human bowel afferents have identified, in addition to spontaneous activity, receptive fields responsive to von Frey hair probe (54, 56), light mucosal stroke (42) and circumferential stretch (42, 80) suggesting that a comparable repertoire of afferent subtypes to those found in rodents is also present in humans (Fig. 1). The experimental procedures undertaken in these studies were limited by not insignificant technical and experimental hurdles associated with making these recordings. For example, Jiang *et al*. and Peiris *et al*. report recording success rates of 15% and 48%, respectively, after obtaining tissue from consenting patients (42, 56). Secondly, not all fibres are mechanosensitive, for example, from

20 successful recordings (from 45 specimens), Yu *et al*. only observed 8 sensitive to mechanical or chemical stimuli (80).

More recently, McGuire *et al*. expanded greatly on these studies and fully characterised the mechanosensitivity of 46 human colonic afferent fibres from just under one hundred tissues identifying two main subtypes of afferent (51). Specifically, approximately half of the fibres recorded were sensitive to low weight (< 0.6 g) von Frey hair probing of the serosal surface, but not stretch or mucosal stroking, and were classified as serosal nociceptors following responses to bradykinin and ATP. By contrast, fibres sensitive to stretch were unresponsive to low weight von Frey hair probing of the serosal surface and algogenic chemical mediators, and were subsequently classified as muscular afferents. Although less frequent, receptive fields in the mesentery and some sensitive to mucosal stroke were also observed (37, 51). Additionally, a mechanically insensitive ‘silent’ population, which became mechanosensitive only after bradykinin application, was also identified in human bowel in agreement with the presence of such a population in rodent studies (28). The identification of silent nociceptors in human bowel tissue is extremely important, as such neurones are believed to contribute greatly to inflammatory pain and visceral hypersensitivity observed in patients (58). It seems apparent that detailed descriptions of rodent colonic afferent neuroanatomy are comparable to that observed in human tissues. This not only provides a rational criterion for identifying and studying nociceptors in human tissue, but also lends support to the translational validity of observations on peripheral pain processing in rodent tissue.

In addition to mechanosensitivity, human afferents are sensitive to a diverse range of algogenic and inflammatory mediators comparable to findings in rodent tissue. Initially confirmed using an experimental inflammatory soup (consisting of bradykinin, 5-HT, histamine and prostaglandin E_2) (56), the individual constituents of which have subsequently been shown to evoke action potential discharge in human tissues (e.g. histamine (51), PGE_2 (51), bradykinin (51, 56, 80) and lastly, 5-HT (51, 80); Fig. 1). In addition, transient receptor potential (TRP) channel agonists, capsaicin (TRPV1) (42, 51, 54, 56, 80) and AITC (TRPA1) (80); and purinergic receptor agonists, ATP (51), ADP and UTP (37) have also all been shown to excite variable proportions of human visceral afferents. Whilst the mechanosensitivity of those fibres responding to individual chemical stimuli has not been comprehensively characterised in all cases, in the examples where it has, serosal nociceptors are sensitive to inflammatory stimuli (e.g. ATP and bradykinin), but muscular afferents are not (51).

The reproducibility of responses to both mechanical stimuli (such as von Frey hair probe and ramp distension of the appendix (36, 51)) and some chemical mediators (including bradykinin and ATP (51)) has enabled mechanistic interrogation of human afferent, and specifically nociceptor function, within individual specimens thus countering some of the inherent variability observed in the diverse population from which these tissues are sourced. Whilst desensitisation of responses to repeated 5-HT, histamine and experimental inflammatory soup application may limit pharmacological investigation in some signalling pathways (51, 56), critical translational studies are achievable using this approach. Indeed, data supporting the observed modulation of serosal nociceptor mechanosensitivity by TRPV4 in

rodent studies (8, 17) was demonstrated by the TRPV4 antagonist HC067047 inhibiting human nociceptor firing to repeated von Frey hair probing (51). Additionally, the anti-epileptic drug retigabine, which augments the function of voltage-gated potassium channels of the K_V7 family, inhibits the colonic afferent response to bradykinin in mouse and human tissue (57). Furthermore, the unexpected finding that both genetic loss and pharmacological block (by PF-5198007) of $Na_V1.7$ in mice does not alter colonic afferent firing to noxious stimuli, was confirmed in human colonic afferents using ramp distension of human appendix in the presence of $Na_V1.7$ blocked with PF-5198007 (36). These studies build confidence in the efficacy, or lack thereof, of novel visceral analgesic pharmacophores identified through pre-clinical animal studies capable of modulating nociceptor function. Importantly, human tissue studies can also be utilised to identify the mechanism of action for clinically effective compounds. For example, tegaserod (5-HT_4 receptor agonist), a clinically effective treatment of abdominal pain in IBS-C patients, reduces rectal sensitivity in healthy subjects and pain scores in IBS patients (22, 53). Rodent studies suggest that this is mediated by a direct inhibition of visceral afferent firing (62). Using human bowel tissues, we were able to show an attenuation of serosal nociceptor mechanosensitivity and validate this mechanism of action (51), therefore bolstering the translatability of this approach.

As well as investigating those mechanisms capable of modulating human afferent sensitivity, direct interrogation of human tissues has shed light on disease processes and the contribution of receptor-ligand interactions occurring at the peripheral terminal. For example, bradykinin-mediated excitation of human

sensory nerves occurs via B_2, but not B_1, receptors (51). The blockade of adenosine receptors by CGS15943 and inhibition of $P2X_{2/3,3}$ receptors by R04, failed to greatly attenuate afferent firing in response to ATP therefore highlighting the importance of P2Y receptors to purinergic signalling in human afferents (37, 51). In single fibre studies, afferent firing in response to capsaicin was blocked by treatment with the TRPV1 antagonist ABT-102 (51). In contrary to its canonical blockade of sodium-hydrogen antiporter NHE3, such TRPV1 antagonism was recently reported to underpin the analgesic properties of Tenapanor, a novel therapy under investigation for the treatment of constipation-predominant IBS (20, 46).

The diversity of source tissues enables investigation of how nociceptor function is dependent upon sex, age and disease state. Whilst the vast majority of work conducted so far has been on ‘healthy’ tissues isolated away from cancer margins, differences in afferent sensitivity to noxious stimuli (e.g. bradykinin) with age have been suggested (80). Although these were not supported at the level of an individual nerve fibre in the much larger sample size study of McGuire and colleagues (51), it may be that a reduction in nociceptor innervation as opposed to function in remaining fibres accounts for age related changes in nociception. In addition to resections for cancer, human bowel tissue is available following colectomy for Crohn’s disease (predominantly ileocecal) and ulcerative colitis (predominantly rectum, descending and sigmoid colon) providing a model for chronic inflammation. Alternatively, access to appendicitis resections enables direct comparison of acute inflammatory processes with those of a chronic state. Initial retrospective analyses have suggested no significant difference in

responses to noxious stimuli in chronically inflamed IBD tissues (37, 51), however more detailed studies are required to test specific hypotheses. Direct investigation of human tissues also enables the study of human-specific variants of receptors/channels; examples of such differences are discussed in more detail below.

Whilst providing an economical and potentially valuable stepping stone between animal models and clinical trials, the use of resected human bowel tissues is not without its limitations. One potential caveat in these studies is the risk that hypoxic conditions may alter nociceptor function. Ideally studies should start as soon as possible after surgery, however for human GI tissues there are several lines of evidence suggesting that longer post-surgery times are sometimes acceptable. Often tissues are not available until late in the day requiring experiments to be conducted in the evening or after overnight storage at 4°C in pre-oxygenated Krebs buffer. Both contractile responses and neuronally mediated responses to electrical field stimulation (EFS) in neuromuscular studies of isolated GI tissue strips (6, 9) were unaffected by short-term storage (< 24 hrs) at 4°C. Studies using the more fragile mucosa tissues also suggest that overnight storage at 4°C does not alter responses to 5-HT or forskolin (12). Whilst no comprehensive study of the effects of hypoxia on human primary afferent function has been conducted, researchers have sought to minimise the risk of hypoxia-mediated changes and maintain tissue health. Specifically, Jiang *et al*. report pinning tissues mucosa-side up to ensure good perfusion rates of the oxygenated Krebs buffer with the degradation prone mucosa (42). In post-hoc analysis of single unit responses of human visceral afferents to both mechanical and chemical (bradykinin and ATP)

stimuli, no significant difference was observed between those stored overnight at 4°C compared to those used straight from surgery (51). Importantly, all groups report immediate extraction from surgery into pre-oxygenated Krebs buffer for transport to laboratories and gross dissection (42, 51, 54, 56, 80). Whilst the effects of cessation of blood supply on the tissues cannot be directly evaluated, providing that control studies are conducted within the same preparation or on tissue treated in a comparable fashion (e.g. entering oxygenated buffer as soon as possible), the risk of hypoxia-mediated changes significantly influencing conclusions drawn from such experiments should be ameliorated.

When using human tissue, the effects of age, ethnicity and sex of the patient from which the tissue is acquired must also be monitored. Importantly, prior patient treatment (e.g. steroids and/or anti-tumor necrosis factor (TNF) antibodies) may also impact afferent signalling. The complexity of the system (with multiple cell types, signalling cascades and interactions) is both an advantage and a disadvantage. Human bowel tissues represent a powerful tool to investigate peripheral afferent sensitivity *in situ*, however detailed mechanistic studies are challenging to perform and risk influence from unforeseen cell-types present in the bowel. To combat this, academic (e.g. in the UK, CRACK-IT DRGNet), not for profit (e.g. in the US, National Disease Resource Interchange and in the Netherlands, the Netherlands Brain Bank) and commercial (e.g. Anabios) infrastructures have arisen recently to provide reliable access to human sensory neurones isolated from DRG and trigeminal ganglia (TG) of healthy donor patients, with the promise of aiding investigation of sensory processing in health and chronic pain.

Cultured human DRG neurones

Access to human DRG neurones has greatly facilitated translational research with initial studies exploiting avulsion and ganglionectomies in chronic pain patients or following removal from foetuses (2, 50). Fundamental differences in expression and function of ion channels and receptors have been identified between rodent, non-human primate and human DRG neurones. These include differing biophysical properties contributing to the excitability of DRG neurones with a greatly reduced input resistance and higher action potential threshold of human, compared to rat DRG neurones (23, 33). Once the threshold for action potential firing is reached however, human DRG neurones tend to fire more action potentials, that are wider and at a greater frequency (33). It is perhaps not surprising then that Na_V channels contributing to both electrogenesis and to the regulation of resting membrane potential in human DRG neurones differ to those of rodents. Both tetrodotoxin (TTX)-resistant Na_V channels $Na_V1.8$ and $Na_V1.9$ exhibit altered biophysical characteristics, with human $Na_V1.8$ possessing enhanced persistent and ramp currents capable of elongating the action potential and increasing firing rates (33), whilst human $Na_V1.9$ can open in response to a weaker stimulus compared to rodent $Na_V1.9$ (25). Of the Na_V channels, $Na_V1.8$ and $Na_V1.9$ in particular can regulate inflammatory and visceral pain pathways and have been proposed as pharmacological targets for intervention in visceral hypersensitivity (27, 39).

Human nociceptors are far more promiscuous in their sensitivity to chemical stimuli than their rodent counterparts with significantly larger numbers responding

to the TRPA1 agonist AITC (5, 23) and the inflammatory mediators bradykinin (5, 34) and histamine (34). The contribution of multiple TRP channels to visceral hypersensitivity is well established (4) with histamine also sensitising TRPV1 in IBS (77). In contrast to both rodent and primate isoforms, human TRPA1 is sensitive to acidic pH (44), suggesting that its already diverse range of stimuli modalities (e.g. noxious cold, noxious heat, mechanical, irritants and bacterial lipopolysaccharide) may be expanded in humans. Furthermore, human DRG neurones possess differential GABA receptor pharmacology with GABA antagonists bicuculline and picrotoxin unable to block native GABA-sensitive Cl^- currents (70). Lastly, purinergic signalling, an important contributing pathway to visceral mechanosensitivity (13), in human DRG neurones differs compared to rodent, with an absence of the $P2X_2$ receptor subtype and also altered potency of $P2X_3$ receptor antagonists (64).

Beyond functional differences, an understanding of species-specific expression is vital for successful translation from model species to human. As just one example, although many others exist, mouse DRG neurones express more than 20 MAS-related G-protein coupled receptors (MRGPRs), several of which are involved in pruriception and nociception, whilst human DRG neurones only possess 4 at high levels (3). Recent comprehensive RNA sequencing screens of both human and mouse DRG neurones are filling in the gaps left by other comparative studies (59).

Of course, a caveat of such studies where adult DRG have been removed for medical reasons is that they may not be representative of healthy tissue. The development of robust surgical resection protocols (71) has enabled a more

comprehensive characterisation of human sensory neurones isolated from healthy adult donors lacking chronic pain (typical examples of cause of death include head trauma, stroke and anoxia). Indeed, many recent studies have shed significant light on mechanisms regulating neuronal excitability in humans, with important species-specific differences identified in the biophysics of $GABA_A$ channel function (81) and both Na_V channel kinetics and sensitivity to the chemotherapeutic paclitaxel, an agent that can produce chemotherapy-induced neuropathic pain (19, 82). Parallel studies have also identified toll-like receptor-4 (TLR4) as an important effector of paclitaxel capable of modulating TRPV1 in human sensory neurones: the combined effects of which likely contribute to chemotherapy-induced neuropathic pain (47).

Mechanisms important for regulating visceral pain have been investigated in human DRG neurones including proteinase-activated receptor (PAR) activity (24) and the analgesic properties of α-Conotoxin Vc1.1 from the marine cone snail *Conus victoriae* via $GABA_B$ receptors (15). Whilst clearly invaluable in providing translational validation of mechanism in native tissue, human DRG neuronal cultures do possess experimental limitations. Principally, as with protocols for isolating rodent DRG neurones, enzymatic dissociation, mechanical trituration and time in culture are likely to impact the expression and function of ion channels and receptor signalling pathways. Secondly, a limitation specific to working with human DRG neurones is that at present we have no method of isolating human neuronal populations that innervate a specific target organ, i.e. the GI tract. This point is especially pertinent considering that it is becoming increasingly apparent that neuronal populations isolated from differing spinal segmental regions and

innervating different tissues possess distinct phenotypes (49, 57, 58, 65, 78). Thus, comparisons between rodent colorectal sensation mediated by pelvic afferents originating from sacral DRG and unlabelled human sensory neurones isolated from non-sacral DRG should be undertaken with care. RNA sequencing studies of both mouse (21, 45, 69) and human sensory neurones (1, 31, 49) have elucidated their transcriptomic diversity. Our recent extension of this to single-cell resolution of a population of sensory neurones in mouse targeting the colorectum provides a clearer phenotype for those cells relevant to gastrointestinal pain (38). However, until a molecular fingerprint or panel of marker genes is identified that can differentiate visceral from other neuronal subtypes, studies of human DRG neurone studies may only provide significant translational insight into basic pain mechanisms, with additional parallel strategies required to fully interrogate visceral pain physiology.

Conclusions

In order to harness the translational utility of human tissue to develop more effective drugs, researchers must combine methodologies to investigate both detailed cellular mechanism and visceral pain physiology (Fig. 2). Bringing to bear powerful new techniques to selectively ablate/modulate/excite specific neuronal populations using expression of tools such as optogenetics and chemogenetics will undoubtedly progress our understanding of visceral pain pathways. The establishment of adeno-associated viral (AAV) vectors capable of transducing human sensory neurones is an important contributor to harnessing such tools (76). Finally, in comparison to the isolation of human DRG, resected colonic tissues are

a widely-accessible resource removed as part of routine surgical treatment and thus represent an ideal translational model in order to study, not only visceral afferent physiology, but also peripheral sensitisation in human more broadly.

Figure 1 Legend

Summary of human visceral afferent subtypes and signalling pathways investigated in resected human bowel tissues to date showing the specific receptor agonists, antagonists and ligands used to conduct these studies. A Human visceral afferent fibres are sensitive to a diverse range of chemical mediators, which have been used to confirm the expression of peripheral pain pathways. Arrows represent receptor activation/agonism. Blunt arrows represent receptor inhibitors/antagonism. B Mechanosensitivity to von Frey hair probe, stretch and mucosal stroke is used to classify fibres into subtypes. Serosal and mesenteric fibres (which collectively can be classed as vascular) are proportionally more sensitive to algogenic mediators (bradykinin and ATP), indicating a greater role in visceral nociception. ✔✔✔, high response frequency; ✔, low response frequency; ✖, no response; -, not tested. 5-HT, 5-hydroxytryptamine; 5-HTR, 5-HT receptors; AR, adenosine receptors; AITC, allyl isothiocyanate; ATP, adenosine-5-triphosphate; ADP, adenosine-5-diphosphate; B_2R, bradykinin receptor B_2; EPR, prostaglandin E_2 receptors; HR, histamine receptors; PGE_2, prostaglandin E_2; UTP, uridine triphosphate; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1; TRPV4, transient receptor potential vanilloid 4.

Figure 2 Legend

Interrogating abdominal chronic pain using human tissues. Peripheral sensitisation of nociceptors innervating the bowel contributes to abdominal pain in organic and functional GI disorders with inflammatory mediators, and bacterial and dietary metabolites acting on a diverse range of signalling pathways and ion channels (including Transient Receptor Potential (TRP) channels). Using *ex vivo* electrophysiological recordings of mesenteric nerves isolated from resected human bowel tissues it has been possible to validate regulators of neuronal excitability in humans such as voltage-gated potassium channel K_V7 and the metabotropic serotonin receptor 5-HT_4. Such findings can be further investigated at the level of the cell body using primary cultures of human DRG and utilising patch-clamp electrophysiology and Ca^{2+} imaging techniques. The integration of, and access to, these two models of peripheral pain pathways can inform our understanding of clinical phenotypes including visceral hypersensitivity and chronic abdominal pain in conditions such as irritable bowel syndrome (IBS; constipation- (C) or diarrhea(D)-predominant or mixed (M); or post-infectious IBS).

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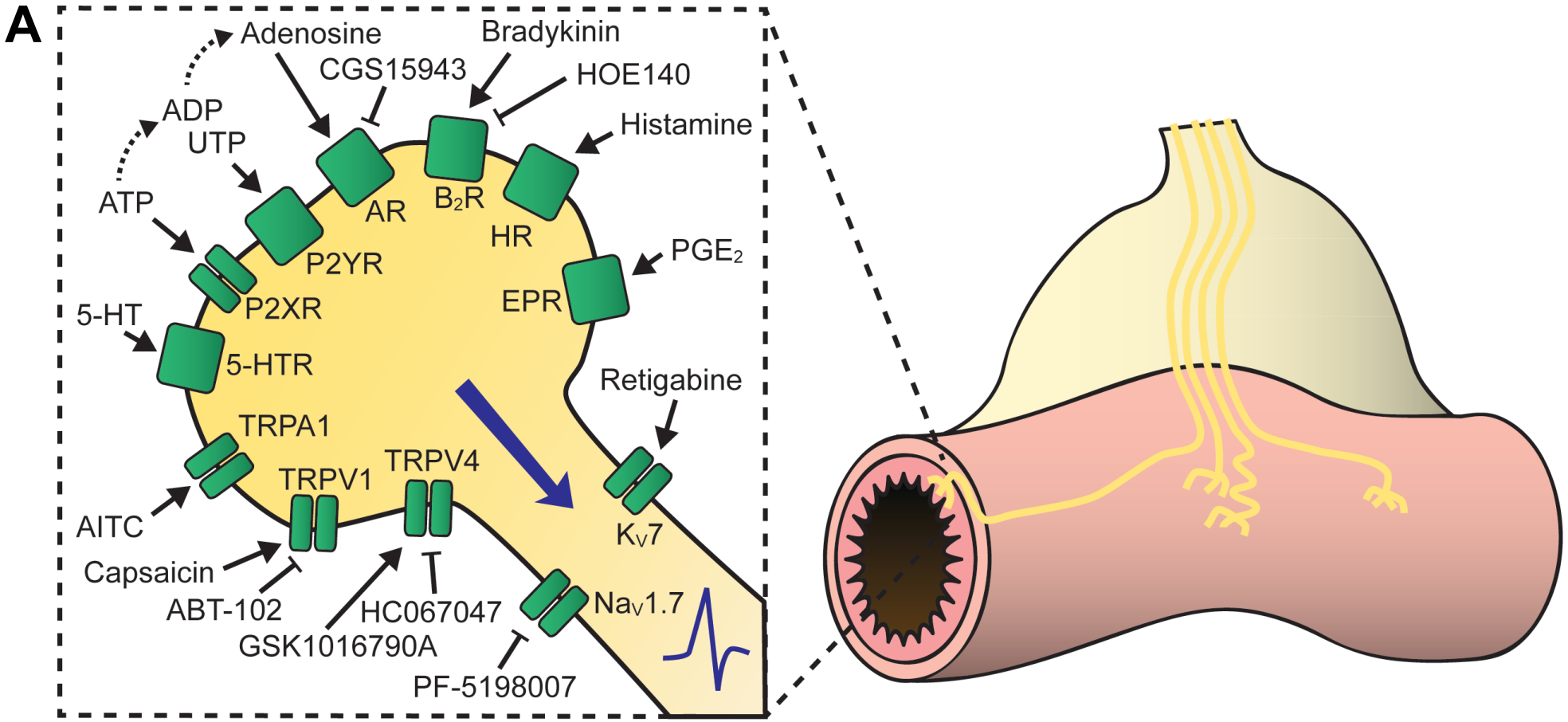
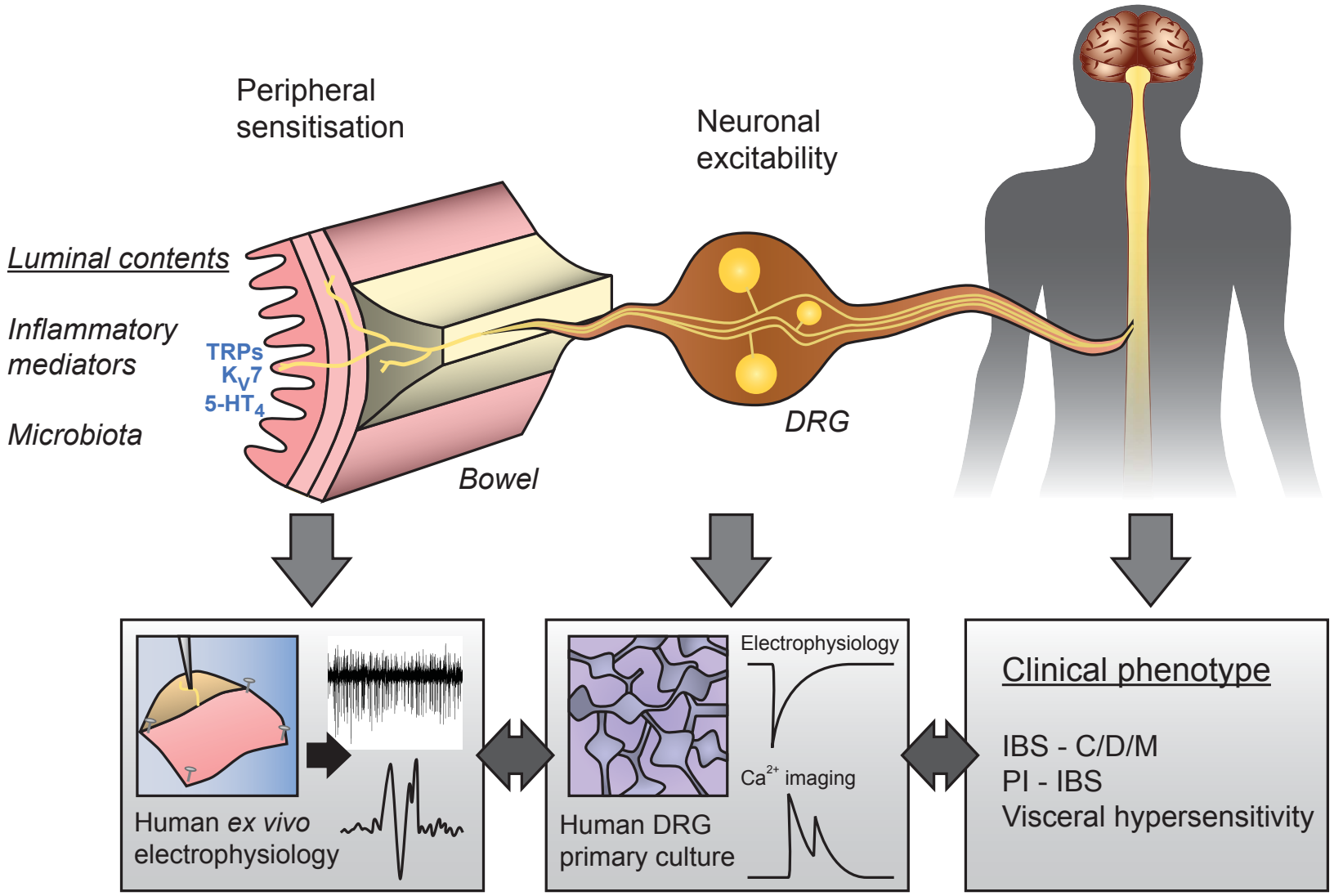


B

	Mechanosensitivity			Chemosensitivity	
	Probe	Stretch	Stroke	Bradykinin	ATP
Vascular					
Serosal nociceptor	✓✓✓	×	×	✓✓✓(43%)	✓✓✓(40%)
Mesenteric	✓✓✓(mesentery)	×	×	✓✓✓(100%)	✓✓✓(100%)
Muscular	×	✓✓✓	×	×(0%)	✓(11%)
Muscular-Mucosal	×	✓✓✓	✓	×	×
Mucosal	✓	×	✓	-	-


Peripheral sensitisation
Neuronal excitability
Luminal contents
Inflammatory mediators
Microbiota
TRPs
K_V7
5-HT_4
Bowel
DRG
Human ex vivo electrophysiology
Human DRG primary culture
Electrophysiology
Ca^{2+} imaging
Clinical phenotype
IBS - C/D/M
PI - IBS
Visceral hypersensitivity