VISCERAL PERCEPTION

Gastric hyperalgesia and changes in voltage gated sodium channel function in the rat

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Potential peripheral mechanisms that might contribute to the development of visceral hypersensitivity and ultimately to the altered sensations that characterise functional gastrointestinal disorders were examined by developing experimental models of gastric hyperalgesia. A direct link between alteration in behaviour and potential mechanisms of increased excitability of the gastric afferent innervation was found.

SUMMARY

Functional dyspepsia is a common clinical problem characterised by discomfort and pain associated with the upper gastrointestinal tract. Such functional visceral disorders are considered to represent a visceral hyperalgesia. Because non-human experimental study of this problem is scant, and peripheral contributions to dyspepsia are not understood, we undertook the development of experimental models of gastric hyperalgesia. One model of gastric hyperalgesia was produced by multiple injections of 20% acetic acid (HAc) into the stomach wall; a second model was produced by adding 0.1% iodoacetamide to drinking water. Exaggerated responses to balloon distension of the stomach (that is, hyperalgesia) were present in both models 5–7 days after treatment. HAc treatment produced gastric ulcers and evidence of inflammation. Ingestion of iodoacetamide thickened the stomach wall but produced no evidence of inflammation. Subsequent whole cell patch clamp studies examined voltage gated sodium channels in nodose and dorsal root ganglia neurones. HAc treatment increased peak sodium current, principally in the tetrodotoxin (TTX) resistant sodium current, in nodose ganglion neurones. Iodoacetamide altered sodium currents in dorsal root ganglion neurones by shifting voltage dependence activation to the left. These models of gastric hyperalgesia are associated with an increase in the excitability of the peripheral innervation of the stomach.

INTRODUCTION

It has become generally accepted over the past decade that functional gastrointestinal disorders such as functional dyspepsia and irritable bowel syndrome (IBS) represent a sensory abnormality, specifically hyperalgesia.¹ Since Ritchie first documented a shift to the left in psychophysical function to bowel distension in patients with IBS,² numerous investigators have documented similar sensory dysfunction associated with functional

disorders of the oesophagus, stomach, and gastroduodenum.^{3–5}

Hyperalgesia, defined as an increased response to a stimulus that is normally noxious, was first studied and has been best examined in models of cutaneous hyperalgesia. There are two types of cutaneous hyperalgesia: primary hyperalgesia occurs at the site of injury and secondary hyperalgesia is associated with the injury but occurs in undamaged tissues adjacent to, and at some distance from, the site of the injury.⁶ Conceptually, cutaneous hyperalgesia can be initiated and maintained entirely by peripheral or central nervous system (CNS) mechanisms.

Although the hypersensitivity present in functional gastrointestinal disorders shares many features in common with cutaneous hyperalgesia, there are some important differences. Cutting, crushing, burning, or ulceration of the skin is invariably associated with acute pain as well as the development of hyperalgesia, and the spread of increased sensitivity away from the site of injury (that is, secondary hyperalgesia). However, these same stimuli do not typically produce acute pain when applied to the hollow organs of the gastrointestinal tract. Distension, ischaemia, inflammation, and perhaps muscle dysfunction (spasm) along a length of the organ are adequate stimuli (in the Sherringtonian context) to generate pain in hollow organs. Functional gastrointestinal disorders are not associated with structural or biochemical abnormalities; there is no tissue damage and the relationship between hyperalgesia and tissue injury, which applies to somatic structures, is absent in the viscera. Thus although functional gastrointestinal disorders are closely associated with visceral hyperalgesia, the initiating event(s) in the periphery, which typically leads to the development of cutaneous hyperalgesia, are probably different to those which apply to the viscera.

The objective of our experimental efforts has been to examine potential peripheral mechanisms that might contribute to the development of visceral hypersensitivity and ultimately to the altered sensations that characterise functional gastrointestinal disorders. Our research strategy has been to develop models that provide behavioural evidence that the experimental manipulation (that is, balloon distension) of hollow organs leads to quantifiable and reproducible responses. It is necessary that such responses be

Abbreviations: CNS, central nervous system; HAc, acetic acid; IBS, irritable bowel syndrome; NGF, nerve growth factor; TTX, tetrodotoxin.

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significantly enhanced after organ insult (that is, organ insult should contribute to hyperalgesia) and that the consequences of the insult can be examined electrophysiologically, by examining the behaviour of afferent fibres and their sensory cell bodies in the absence and presence of visceral insult.

EXPERIMENTAL MODELS OF HYPERALGESIA

Two models of gastric hyperalgesia have recently been characterised in the rat. One model is produced by multiple injections of 20% HAc (or saline as a control) into the stomach wall.⁷ This procedure produces multiple ulcers in the stomach, a significant increase in tissue myeloperoxidase activity, and enhanced responses to balloon distension of the stomach 5–7 days after HAc treatment. A second model is produced by adding 0.1% iodoacetamide to the animal's drinking water.⁸ When examined seven days after initiation of this treatment, responses to balloon distension of the stomach were significantly increased. This occurs in the absence of stomach ulceration or an increase in tissue myeloperoxidase activity. Both models are associated with exaggerated responses to balloon distension of the stomach (that is, gastric hyperalgesia) although they differ in the magnitude of gastric insult produced by the experimental manipulation. The HAc model of gastric ulceration produces clear evidence of tissue damage and inflammation. Ingestion of 0.1% iodoacetamide for seven days is not associated with obvious tissue damage or inflammation. However, the stomach wall appears slightly thickened in iodoacetamide treated animals and it would be inappropriate to characterise this model as having no effect on stomach architecture.

MECHANISMS OF HYPERALGESIA

Peripheral mechanisms

If these models represent visceral hyperalgesia, what peripheral mechanisms could explain the exaggerated response to gastric distension? One would expect that gastric vagal or gastric splanchnic afferent fibres, or both, would exhibit sensitised responses to gastric distension. It is well documented that primary cutaneous hyperalgesia develops as a consequence of nociceptor sensitisation. Sensitisation of nociceptors is expressed as an increase in response magnitude, a decrease in response threshold, or significant increase in resting spontaneous activity.

Visceral afferent fibres typically have no spontaneous activity, or very low rates of spontaneous activity (for example, ≤ 1 Hz). While it is clear how increased responses to stimulation or reduced response thresholds could contribute to increased responses to peripheral stimuli, it should also be appreciated that a significant increase in spontaneous activity contributes to increased afferent input into the CNS which represents a change in excitability and afferent fibre sensitisation.

Gastric vagal and splanchnic afferent fibres have been shown to become sensitised after an acute insult is applied to the otherwise normal stomach.^{9–10} In these experiments, infusion of the inflammatory mediator, platelet activating factor, via the gastric artery can significantly increase spontaneous activity of gastric vagal afferent fibres.⁹ Similarly, intragastric infusion of a mixture of inflammatory mediators increases the mechanosensitivity of gastric splanchnic afferent fibres.¹⁰ It is likely that HAc injection into the stomach wall or ingestion of iodoacetamide in drinking water sensitises gastric vagal as well as gastric splanchnic afferent fibres. While in the healthy gastrointestinal tract vagal afferent fibres are not generally considered to contribute to sensations of discomfort and pain, we are testing the hypothesis that they may do so under circumstances of chronic gastrointestinal insult. These experiments are in progress.

Sodium channels

Sodium channels are known to play an important role in the excitability of peripheral nerves, especially in the presence of

Figure 1 Sample traces (A) and mean data (B) of the effect of acetic acid (HAc) injected into the stomach wall on voltage sensitive sodium current recorded from nodose gastric sensory neurones. The peak tetrodotoxin (TTX) resistant current was significantly greater ($p < 0.05$) in cells harvested from HAc treated stomachs than in cells from saline injected stomachs (control). The TTX sensitive peak current was unaffected by HAc treatment.

peripheral tissue insult. We have therefore examined the behaviour of voltage gated sodium channels in gastric nodose ganglion and dorsal root ganglion neurones. At least nine distinct voltage gated sodium channels have been identified in mammals and functionally expressed in recombinant systems.¹¹ Most voltage gated sodium channels exhibit nM sensitivity to tetrodotoxin (TTX) but several TTX resistant channels (that is, μ M sensitivity to TTX) have also been characterised. Two of the TTX resistant sodium channels, $Na_v 1.8$ and $Na_v 1.9$, are present only in sensory neurones, particularly those with small diameter cells that originate in the dorsal root ganglia. These sodium channels are responsible for the TTX resistant current found in nodose and dorsal root ganglia neurones.

To determine whether changes in voltage gated sodium channels contribute to gastric hyperalgesia, the stomach was exposed and the dicarbocyanine dye DiI was injected in multiple locations into the gastric wall. DiI was incorporated into the lipid bilayer of nerve processes close to the site of injection, and transported to the cell body (nodose or dorsal root ganglia) without transfer to adjacent cells. After allowing time for the dye to be retrogradely transported to the cell body, nodose, and dorsal root ganglion, cells were harvested. Those containing the dye have been shown by fluorescence techniques to innervate the stomach.

In studies of gastric sensory neurones isolated from adult nodose ganglia, the peak amplitude of TTX resistant currents was greater in neurones recorded from stomachs treated with HAc than in neurones recorded from stomachs treated with saline (fig 1A, 1B).¹² Interestingly, the TTX sensitive current was unaffected in these experiments. Neurones from HAc treated stomachs also exhibited slower inactivation kinetics and more rapid recovery from inactivation. Similarly, DiI labelled gastric sensory neurones isolated from T9 and T10 dorsal root ganglia exhibited an accelerated recovery from inactivation of sodium currents compared with neurones harvested from saline treated control animals.

The TTX resistant sodium channel has a slower onset and faster recovery from inactivation compared with other sodium channels. The observed increase in TTX resistant sodium current partially accounts for the more rapid recovery from inactivation. Other properties of TTX sensitive currents were also altered. TTX sensitive currents in neurones from HAc treated

Figure 2 Effect of nerve growth factor (NGF) on the tetrodotoxin resistant sodium current. Sodium currents were triggered by depolarisation from -70 mV to various test potentials and plotted as a function of the test potential for cells cultured for 24 hours in the presence of NGF (100 ng/ml) or a neutralising antibody (anti-NGF 1:2500).

stomachs exhibited accelerated recovery from inactivation compared with saline treated control neurones. These changes in voltage dependence and recovery from inactivation probably translate into a lower threshold for action potential generation and higher spike frequency. This finding may explain how gastric insult leads to the development of gastric hyperalgesia, which in the periphery is clearly associated with a change in the excitability of the afferent innervation of the organ.

In contrast with the effects of HAC injected into the stomach wall, luminal exposure to iodoacetamide in drinking water did not alter the peak sodium current in gastric neurones from either nodose or dorsal root ganglia. However, in preliminary experiments with dorsal root ganglion neurones from iodoacetamide treated animals, voltage gated sodium currents became activated at potentials that were approximately 10 mV more negative compared with control conditions.¹³ These shifts in voltage dependence activation may alter the excitability of these afferent neurones, as discussed previously.

Biochemical mediators

Increases in the excitability of vagal and splanchnic nerve sensory fibres that arise from alterations in the function of voltage gated sodium channels are almost certainly associated with changes in one or more potential mediators (for example, biogenic amines, cytokines, eicosanoids, neuropeptides, or neurotrophins). Given the established role that neurotrophins play in maintenance and modulation of nervous system function in adults, the contribution of these mediators in sodium channel function has been studied. It is possible that by modulating the function of sodium channels, and possibly expression of different sodium channels present in gastric sensory neurones, the neurotrophins released as a consequence of gastric insult may contribute in a significant manner to the development of gastric hyperalgesia.

Experimental studies have shown that gastric ulceration produced by HAC injection into the stomach wall increases immunoreactivity for nerve growth factor (NGF). This response is most pronounced in the submucosa and muscularis. Intraluminal administration of NGF was found to sensitise responses of gastric vagal afferent fibres to gastric distension, confirming an earlier report that instillation of NGF into the urinary bladder sensitises pelvic nerve afferent fibres to bladder distension.¹⁴ There is also preliminary evidence that exposure to NGF significantly increases TTX resistant sodium currents in gastric nodose neurones (fig 2). This finding is consistent with the observed changes in excitability of gastric afferents.

CONCLUSIONS

Although these investigations are ongoing and the results preliminary, the data presented are among the first to directly link an alteration in behaviour with potential mechanisms of increased excitability of the gastric afferent innervation. In a clinical context, extrapolation of such findings suggest that increased excitability of the afferent innervation of hollow organs can lead to altered perception of otherwise normal intraluminal events.

These and similar results also provide clear evidence that peripheral insult can alter the excitability of the afferent innervation of hollow organs. Experimentally, this increased excitability is consistent with events associated with the development of cutaneous hyperalgesia. Although functional gastrointestinal disorders are not commonly associated with a known structural or biochemical explanation for the symptoms that characterise such disorders, this does not exclude the possibility that excitability of peripheral innervation is altered in these patients. Indeed, there is no reason why increased excitability of the peripheral innervation cannot exist in the absence of structural organ damage.

The lack of a biochemical explanation for the symptoms either implies that there is none or, more likely, that the appropriate marker or markers have yet to be discovered. The intrinsic primary afferent neurones of the enteric nervous system in the walls of organs may be the source of a sensitising chemical/mediator leading to the excitability of the extrinsic innervation, and to development and maintenance of altered sensations that arise from the gastrointestinal tract. It is also possible that hypersensitivity, which characterises functional gastrointestinal disorders, is maintained after an initiating event entirely by central mechanisms. These mechanisms may include alterations in descending endogenous modulatory systems, dorsal root reflexes, or efferent projections to organs which contribute to the neurochemical milieu at sensory receptors located in the tissue.

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REFERENCES

- 1 **Mayer EA**, Gebhart GF. Basic and clinical aspects of visceral hyperalgesia. *Gastroenterology* 1994;**107**:271-93.
- 2 **Ritchie J**. Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. *Gut* 1973;**14**:125-32.
- 3 **Lemann M**, Dederding JP, Flourie B, et al. Abnormal perception of visceral pain in response to gastric distension in chronic idiopathic dyspepsia: the irritable stomach. *Dig Dis Sci* 1991;**36**:1249-54.
- 4 **Richter JE**, Barish CF, Castell DO. Abnormal sensory perception in patients with esophageal chest pain. *Gastroenterology* 1986;**91**:845-52.
- 5 **Talley NJ**, Stranghellini V, Heading RC, et al. Functional gastroduodenal disorders. *Gut* 1999;**45**(suppl II):1137-42.
- 6 **Hardy JD**, Wolff HG, Goodell H. Experimental evidence on the nature of cutaneous hyperalgesia. *J Clin Invest* 1950;**29**:115-40.
- 7 **Ozaki N**, Bielefeldt K, Gebhart GF. Characterization of visceromotor responses to gastric distension in normal and gastric ulcer induced rats. *J Pain* 2001;**2**(suppl 1):26.
- 8 **Dial EJ**, Hall LR, Romero JJ, et al. Rats with gastritis have increased sensitivity to the gastrin stimulatory effects of luminal ammonia. *Gastroenterology* 1996;**110**:801-8.

- 9 **Ozaki N**, Sengupta JN, Gebhart GF. Mechanosensitive properties of gastric vagal afferent fibers in the rat. *J Neurophysiol* 1999;**82**:2210–20.
- 10 **Ozaki N**, Gebhart GF. Characterization of mechanosensitive splanchnic nerve afferent fibers innervating the rat stomach. *Am J Physiol* 2001;**281**:G1449–59.
- 11 **Goldin AL**. Resurgence of sodium channel research. *Annu Rev Physiol* 2001;**63**:871–94.
- 12 **Bielefeldt K**, Ozaki N, Gebhart GF. Experimental ulcers alter voltage-sensitive sodium currents in rat gastric sensory neurons. *Gastroenterology* 2002;**122**:394–405.
- 13 **Bielefeldt K**, Ozaki N, Gebhart GF. Mild gastritis alters voltage-sensitive sodium currents in gastric sensory neurons. *Gastroenterology* 2002;**122**:752–61.
- 14 **Dmitriev AN**, McMahon SB. Sensitization of visceral afferents by nerve growth factor in the adult rat. *Pain* 1996;**66**:87–97.