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Perception of gastro-oesophageal reflux

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Gastro-oesophageal reflux disease (GORD) is common and often associated with unpleasant symptoms requiring utilisation of health care resource. While in the majority of patients symptom resolution occurs with acid suppressant therapy, in a proportion this treatment is ineffective in resolving symptoms. This is particularly the case in patients with non-erosive reflux disease (NERD) and functional heartburn (FH). It is increasingly being recognised that the presence of acid in the oesophagus can cause dilated intercellular spaces (DIS) which increases the exposure of the sub-epithelial nerves to the acid. Experimental studies in both animals and humans suggest that a variety of receptors on afferent nerves can be sensitised upon exposure to acid so that there is increased afferent input to the spinal cord dorsal horn neurons which leads to a reduction in threshold of these neurons together with an increase in their receptive field. This increased sensitivity of primary afferent nerves is described as peripheral sensitisation, whereas the consequent increase in sensitivity of the spinal dorsal horn neurons is described as central sensitisation. Once these mechanisms have been established they can cause a long term increase in sensitivity of tissues to previously innocuous stimuli. Furthermore, psychological stress has been shown to increase DIS and may therefore facilitate peripheral sensitisation. Currently peripheral and central sensitisations are considered to be important mechanisms of oesophageal pain hypersensitivity and occurrence of symptoms to even physiological amounts of acid. In these patients treatments aimed at reducing neuronal sensitivity may be effective in the management.

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Background

Gastro-oesophageal reflux disease (GORD) can be broadly divided into erosive oesophagitis (EE) and non-erosive reflux disease (NERD). Symptoms of GORD can be very wide ranging, from the classic feeling of heartburn [1], indigestion, bloating to some very atypical presentations such as recurrent aspiration pneumonia [2] and tooth decay [3]. Heartburn is typically described as burning chest discomfort extending from the upper abdomen, to the chest and sometimes to the jaw. As the name suggests, this symptom is often confused with cardiac chest pain. Regurgitation is another common symptom of GORD, often associated acid taste in the mouth. When heartburn or acid regurgitation dominates the patient's symptoms, they have very high specificity but low sensitivity for GORD [1]. Other common symptoms include; feeling of sickness, bloating, and acidic taste in the mouth.

In patients with EE inflammation and/or erosions are assumed to cause symptoms. However NERD patients have evidence of abnormal acid exposure times but no evidence of erosions. In these patients increased sensitivity to acid is suggested by a significantly shorter lag time and higher pain score to acid infusion in comparison to healthy volunteers [4], Barrett's patients and even EE. This also demonstrates that sensitivity to acid in fact is not dependent on erosive changes in the oesophageal mucosa. Furthermore abnormal response to Bernstein test [5] has been demonstrated in majority of patients who use antacids and report chronic heartburn but have normal acid contact time [5]. This suggests that these patients are hypersensitive to physiological reflux of acid. There are also patients who have all the hallmarks and symptoms of GORD, but do not have objective evidence of abnormal acid exposure. These patients are classified as functional heartburn (FH). Symptoms in FH are considered to be due to excessive sensitivity physiological amounts of acid.

In both presence and absence of inflammation, acid is the main triggering factor in symptom generation in GORD. The corrosive effects of acid can be sensed by sensory nerves and receptors in the oesophageal epithelium. Damage and inflammation will also facilitate dilated intercellular spaces and exposure to acid of the sub-epithelial nerves leading to further symptom generation. Repeated exposure of these nerves could lead to their sensitisation so that symptoms could occur with even minor or physiological reflux episodes; a phenomenon described as oesophageal pain hypersensitivity (OPH). The current chapter will deal with peripheral and central mechanisms of OPH.

Peripheral mechanisms

Gastrointestinal pain is mediated by spinal visceral afferent fibres, with a probable important contribution from vagal afferent fibres [6]. When activated, mechanoreceptors and chemo-sensitive receptors (resident in mesentery, serosa and submucosa) depolarise Aδ- and C-fibres. The ability to transduce noxious mechanical, chemical or thermal stimuli into generator currents able to depolarise such fibres is a property of transducer channels such as transient receptor potential (TRP) channels. TRPV1, TRPV4 and TRPA1 channels have been shown to have a role in GI nociception, as have acid sensing ion channels (ASICs) and $P2_x$ purinoceptors [7]. In the presence of tissue inflammation or injury there is an up-regulation of pain transmission. The ability to enhance pain transmission to the brain in these situations is important as heightened bodily awareness can alter behaviour to aid in the protection of injured sites and the promotion of healing. Research in somatic pain has suggested that both peripheral and central mechanisms can increase nociceptive transmission following inflammation or injury to tissues. Peripheral mechanisms include *peripheral sensitisation* (PS), which is an inflammatory mediator-induced facilitation of nociceptor activity in peripheral tissues. *Peripheral sensitisation* causes pain hypersensitivity at the site of injury or inflammation, also known as primary hyperalgesia. Here inflammatory products including bradykinin, histamine, 5HT, prostanoids, proteases and cytokines permit nociceptor firing at reduced thresholds.

Receptors that may be involved in PS are described below

- TRPV channels – Transient receptor potential vanilloid receptors or vanilloid receptor (VR). This is a big group of receptors. They are cation selective ligand-gated ion channel and can be activated by different stimuli. The best studied is the TRPV1 sub-type, also known as capsaicin receptor. TRPV1

is expressed by most small sensory nerves and sensitive to heat, hydrogen ions and capsaicin (and other endogenous capsaicin-like substances) [8]. Whilst immediate sensitisation is mediated by an increased probability of channel opening in response to these stimuli, increased levels of TRPV1 protein have also been observed in the mucosa of patients with some visceral hypersensitivity states with or without evident inflammation [9,10] including the oesophagus of patients with gastro-oesophageal reflux disease (GORD) [11]. Apart from acid, TRPV1 is also responsible for sensation of heat, capsaicin. Most patients with GORD also report sensitivity to hot drinks, and spicy foods.

- Purine receptors-$P2_x$ Purinoceptors are ligand gated membrane cation channels that are ATP dependent. There are currently seven subtypes known. In particular, P2X3 expression in the dorsal root ganglion is increased in rats with chronic oesophageal acid infusion [12].
- ASICs – These 'acid sensing ion channels' are amiloride-sensitive, voltage-insensitive epithelial Na channel. There are subtypes one–three. They are sensitive to acid and possibly mechanical stimuli. They have been shown to be upregulated in mucosal inflammation and stimulated by NGF and serotonin. One in particular, acid sensing ion channel 3 (ASIC3), as the name suggests, has been known to sense acid (fluctuations in pH) but more known to be an important mechanoreceptor [13] and nociceptor in end-organs. It is known to be fairly ubiquitous in the central nervous system (CNS), and exists in sensory nerve endings [14]. However the mechanism of action in its role as a sensory nociceptor has been linked to pH fluctuations [15–17]. In a study in rodents by Akiba et al, demonstrated that luminal CO_2 as a permeant gas rather than H^+ caused hyperaemic responses demonstrated by increase in Doppler blood flow mimicking acid infusion of pH one. In this study use of ASIC inhibitor was shown to inhibit hyperaemic response in the oesophagus [18]. In another study, conducted by Wultsh et al, ASIC3 knockout mice with gastritis did not demonstrate hyper-responsiveness to oesophageal acid infusion compared to wild mice. These animal studies support the role of ASIC3 in sensing oesophageal acid and this receptor may be involved in the development of peripheral sensitisation.

Central mechanisms

Spinal mechanisms

In normal circumstances, the presence of stimuli will activate the peripheral receptors as mentioned above. Action potentials would then be generated via activation of Na/K channels and impulses will be sent to the spinal cord via peripheral afferent nerves. Repetitive stimulation or high intensity stimuli can cause a constant firing of action potential to the spinal cord [19]. Enhanced nociceptor input in turn activates intracellular signalling cascades within spinal dorsal horn neurones, leading to central sensitisation and amplified responses to noxious and innocuous inputs due to facilitated excitatory synaptic responses and depressed inhibition [20–22]. This facilitation is triggered by the pre-synaptic release of neurotransmitters and neuromodulators such as glutamate [23,24], substance P [25,26], brain derived neurotrophic factor (BDNF) [27] and prostaglandins [28,29]. These neurotransmitters and neuromodulators activate ligand-gated ion channels (NMDA-receptor-glutamate) [26], metabotrophic receptors (metabotrophic glutamate receptor (mGluR)–glutamate and NK1–substance P [30]) and tyrosine kinase receptors (Tyrosine Kinase (Trk) B–BDNF [27]) and increase intracellular calcium via release from intracellular stores and calcium inflow [20] (Fig. 1). Consequently, calcium-dependent enzymes such as protein kinase A [31], protein kinase C [32] and tyrosine kinases are activated, leading to phosphorylation of the NMDA receptors [20]. This dramatically changes NMDA-receptor kinetics and reduces its voltage-dependent magnesium block [32], thus augmenting its subsequent responsiveness to glutamate and increasing synaptic strength, enabling previously sub-threshold inputs to activate the cell [20,22,33]. This increase in gain alters receptive field properties and pain sensitivity, causing tissue hypersensitivity far beyond the site of injury.

In addition to producing central sensitisation, which occurs within seconds of appropriate activation of spinal dorsal horn neurones, nociceptive input also generates an activity-dependent change in transcription in dorsal root ganglion and dorsal horn neurones [20,22,33] These changes occur in response to a complex mechanism involving both an increase and a modification of constitutively

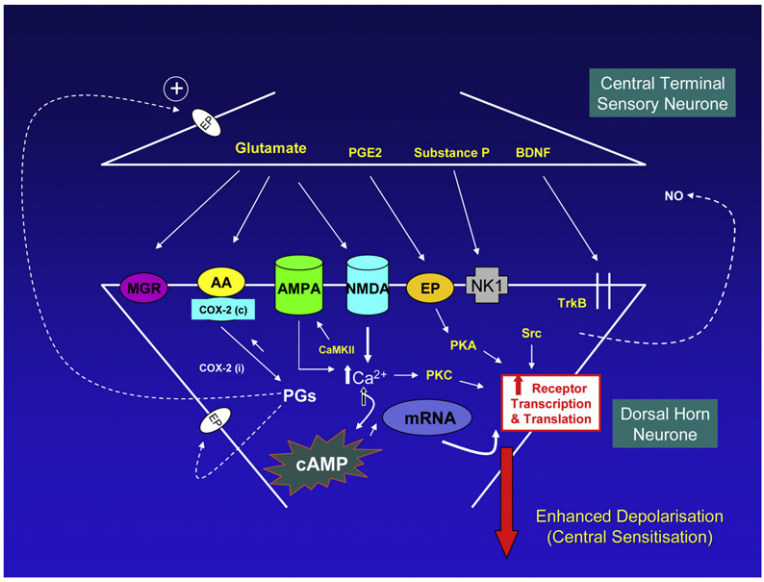

Fig. 1. Shows the receptor mechanisms underlying the induction of central sensitisation. The central terminals of primary nociceptors release glutamate, substance P, brain derived neurotrophic factor (BDNF) and prostaglandin E_2 (PGE_2). Glutamate binds to ionotropic AMPA (Amino-Methylene-Phosphonic Acid) and NMDA (N-Methyl-D-Aspartate) receptors and to metabotropic glutamate receptors (MGR). Substance P and BDNF bind to the G protein coupled neurokinin 1 (NK-1) receptor and the tyrosine kinase receptor B (TrkB) respectively and PGE_2 binds to the EP1 receptor on the postsynaptic membrane. An increase in intracellular Ca^{2+} concentration triggers the activation of protein kinases A and C (PKA & C) and Ca^{2+}-calmodulin-dependent protein kinase (CaMKII). These kinases and tyrosine kinase Src phosphorylate the NMDA and AMPA receptors leading to a potentiation in activity. Nitric oxide (NO) is also produced which has a positive feedback effect on pre-synaptic glutamate release. Central prostaglandin (PG) production is increased via arachadonic acid (AA) by cyclooxygenase-2 (COX-2) induction.

expressed genes and also induction of novel genes. This phenotypic shift results in allodynia (previously non-nociceptive stimuli now induce pain) as well as hyperalgesia (heightened sensitivity to a previously painful stimulus). These changes take hours to manifest but, when established, lead to long-lasting changes in stimulus-response characteristics. Evidence that central sensitisation is a major component of somatic hypersensitivity has already been demonstrated in human somatic pain models [34]. In animal studies direct electrophysiological recordings from spinal neurones also suggest that central sensitisation is important in visceral hypersensitivity [35–37]. However, until recently similar studies in man were not possible due to lack of suitable human experimental models and non-invasive techniques to assess visceral afferent pathways.

Human oesophageal models of peripheral and central sensitisation

Specific experimental models of OPH in the human oesophagus to acid infusion have been described. Sarkar et al, using either saline or acid infusion in a double blind randomised manner to the distal oesophagus demonstrated a drop in pain threshold to electrical stimuli within the exposed distal and non-exposed proximal oesophagus after the acid but not the saline infusion in healthy volunteers [38] (Fig. 2). In addition a decrease in patient threshold has also been demonstrated on the anterior chest wall after oesophageal acidification. Although the primary hyperalgesia/allodynia at the site of infusion is believed to be due to PS, the secondary hyperalgesia/allodynia in the proximal oesophagus and anterior chest wall, distant from the acid infusion, is believed to be due to the sensitisation of spinal dorsal horn neurones [38–40].

Sarkar et al demonstrated that in patients with gastro-oesophageal reflux disease, oesophageal pain hypersensitivity to experimental acid infusion can be reversed by acid suppression with proton pump

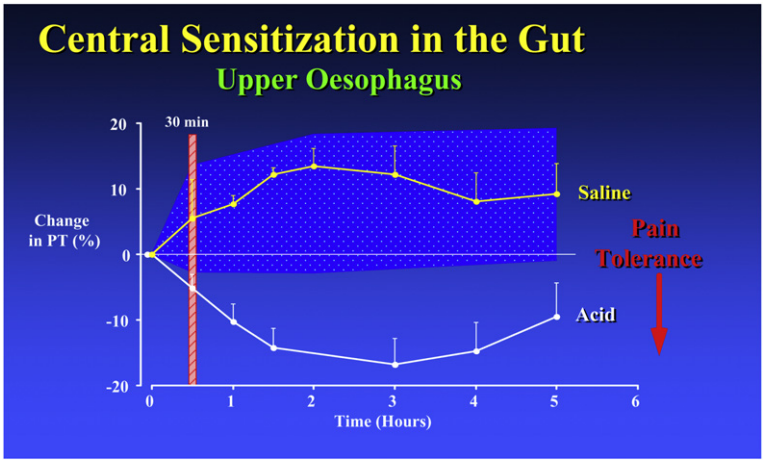

Fig. 2. Shows the change in pain threshold on the upper oesophagus after acid/saline infusion in the lower oesophagus. (Reproduced from Sarkar et al Lancet 2000) [38].

inhibitors [41]. The mechanism of this reduced sensitivity to acid may be related to the fact that treatment of acid reflux removed the peripheral afferent input to the spinal cord which may in turn have reduced the degree of resultant central sensitisation. Patients with functional heartburn have also been speculated to have heightened sensation to other peripheral inputs, such as bile reflux [42]. However, pain sensitivity to oesophageal chemical stimuli is far from understood, especially when one considers that even in patients with documented acid reflux on pH studies, the majority of events are not perceived [43].

Reproducibility studies with the model of acid induced OPH have demonstrated variation in the magnitude of sensitisation between subjects [44]. Recently state anxiety has been implicated as a factor that may modulate the degree of sensitisation to oesophageal acidification within this model [45,46]. It can be speculated that psychological state modulates pain sensitivity and the degree of central sensitisation in response to injury by moderating descending spinal inhibitory and facilitatory influences.

Drewes et al demonstrated that the induction of pain hypersensitivity to acid infusion is also associated with enhanced oesophageal contractions [47]. The same group also demonstrated that acid sensitisation of the oesophagus made it more sensitive to heat rather than cold [48]. This may be related to peripheral up-regulation of receptors such as TRPV1. Other variations to the acid perfusion model were explored and gender differences were observed. For instance, females were shown to report a wider anatomical area of pain referral over the anterior chest wall after acid infusion in comparison to males whereas males reported increased sensitivity to chemical and mechanical stimuli [49]. This is important in clinical context in explaining gender differences in reporting of symptoms. It has also been demonstrated that sensitisation can occur in different areas of the gut after acid induced sensitisation of distal oesophagus. The oesophagus, duodenum and rectum all were more sensitive to mechanical distension after acid sensitisation rather than saline [50]. As the innervation of these organs overlaps at the level of the spinal cord it is likely therefore that this cross sensitisation is related to central sensitisation of spinal dorsal horn neurons caused by the acid infusion.

Cortical mechanisms

A major limitation of most visceral hypersensitivity studies is that they rely on subjective methods of reporting sensation intensity [51]. To overcome this, a commonly used neurophysiological technique, cortical evoked potentials (CEP), has been adopted for use as a more objective correlate of oesophageal sensation. CEP allow recording of cortical neuronal electrical fields generated in response

to a peripheral nerve stimulus. Signal averaging of cortical electrical activity of up to 200 oesophageal stimuli is conducted to generate an optimal signal to noise ratio and a temporal pattern of cortical activation is obtained. Because of the excellent temporal resolution of this technique (one ms) it is possible to study the conduction velocity of afferent neuronal transmission from the oesophagus to the cortex. Using this technique before and after oesophageal acid infusion a consistent reduction in CEP latency to oesophageal electrical stimulation has been described which demonstrates that facilitation of afferent pathway conduction accompanied the CS (Fig. 3) [52]. In a recent study in NERD and functional heartburn patients [53] there was a correlation between pain threshold and acid exposure, with increased oesophageal sensitivity being associated with lower DeMeester score. Thus reflux negative (functional heartburn) patients had lower pain thresholds when compared to both reflux positive patients and controls. Cortical evoked potentials were normal in reflux negative patients but significantly delayed in the reflux positive group. This suggests that increased oesophageal pain sensitivity in functional heartburn patients is associated with heightened afferent sensitivity as normal latency evoked potential responses could be elicited with reduced afferent input. Similar differentiation in the afferent response using cortical evoked potentials has also been shown in subgroups of patients with Non Cardiac Chest Pain [54].

Functional Magnetic Resonance imaging has also been used to study the brain processing of acid induced oesophageal hypersensitivity. Lawal A et al studied the brain processing to mechanical stimulation of the proximal oesophagus following infusion of acid or control buffer solution into the distal oesophagus [55]. Following distal oesophageal acid infusion, both subliminal and liminal levels of proximal oesophageal distentions, caused a significant increase in brain activity in both the cingulate and the insular cortices in comparison to the control buffer solution [55]. This suggests the development of acid-induced sensitisation of the oesophagus to mechanical distention and indicates that this sensitisation is accompanied by increased activity in brain areas processing both sensory (insular cortex) and cognitive (cingulated cortex) aspects of sensation.

In a recent fMRI study in GORD patients [56], acid induced heartburn was associated with an increase in activity in the sensory/motor, parieto-occipital, cingulate and prefrontal regions, and the

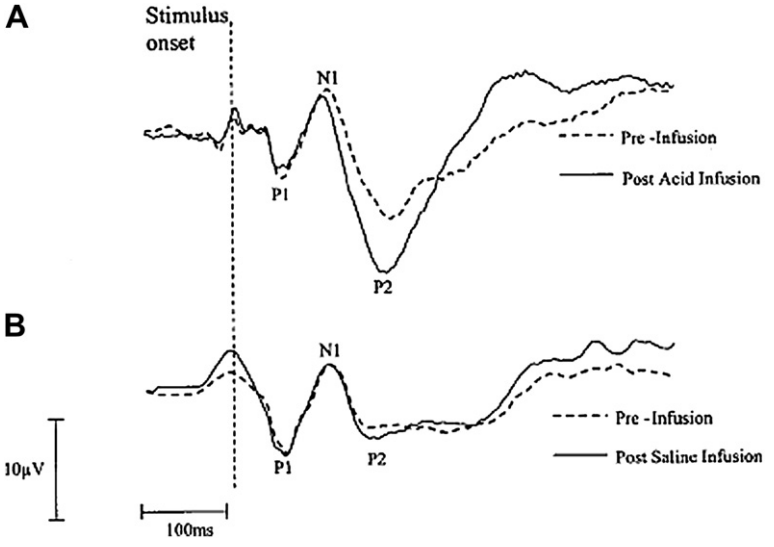

Fig. 3. Shows increased proximal esophaesophageal afferent pathway sensitivity following distal esophaesophageal acidification. Distal esophaesophageal acid infusion is associated with a reduction in latency of the evoked potential response from the non-acid exposed proximal oesophagus. No change in latency occurs following saline infusion. These results support the involvement of central sensitisation in the mediation of OPH. (Reproduced with permission from Sarkar S, Hobson AR, Furlong PL, Woolf CJ, Thompson DG, Aziz Q. Central neural mechanisms mediating human visceral hypersensitivity. Am J Physiol Gastrointest Liver Physiol 2001; 281(5):G1196–202) [55]. American Physiol Soc, with permission.

insula. Activation of similar areas was also observed in healthy subjects who did not experience heartburn to the administered acid infusion. However, activity in these regions occurred more rapidly and with greater intensity in GORD patients than in healthy controls in response to the acid exposure. This suggests the presence of heightened sensitivity of afferent pathways in the patients.

Autonomic nervous system

The role of the ANS in modulating oesophageal sensitivity has also been demonstrated. For instance, increased basal sympathetic activity and lower vagal activity, as measured by power spectral analysis of heart rate variability, are associated with increased sensitivity to oesophageal acid perfusion in patients with NCCP compared with healthy controls [57]. In addition, the heightened sensitivity to oesophageal acid infusion in GORD patients as compared to healthy controls has been shown to be associated with reduced vagal tone during the infusion [58]. These data support the concept of neuro-humoural influences on the susceptibility to symptoms such as heartburn.

Stress and hormones

Animal studies have shown that stress (early maternal separation) induces visceral hyperalgesia, colonic dysmotility, and anxiety-like behaviour [59,60]. Stress could affect OPH by modulating ANS as described above. In addition, stress is also associated with activation of the hypothalamic pituitary axis and Corticotrophin-releasing factor (CRF) is believed to play an important role [60] in altering physiology, permeability and secretory functions of the gut [61] during stress. Induction of stress is associated with increased paracellular permeability and dilated intercellular spaces (DIS) in the oesophagus [62]. Previous links between DIS with NERD [63] and development of symptoms of acid reflux [64] have been demonstrated. Stressful situations often also alter cognitive interpretation of stimuli and alter pain reporting [65].

Conclusion

Perception of symptoms in GORD is a multi-factorial process. The origin of symptoms in GORD is obviously acid in the oesophagus. Acid is corrosive and the normal human oesophagus could sense acid but is also very resilient to it. While in most individuals, intermittent acid exposures can cause the occasional heartburn that we all experience from time to time, in some individuals, OPH could be triggered due to peripheral and central sensitisation of primary afferent and spinal dorsal horn neurons causing more persistent and chronic symptoms. The understanding and future treatment of symptoms of GORD patients require comprehensive understanding and profiling of all the factors influencing OPH including ANS dysfunction, emotions, stress and cognition.

Practice points

- Symptoms in gastro-oesophageal reflux disease can occur in the absence of objective evidence of acid reflux.
- Oesophageal pain hypersensitivity is likely to be an important mechanism in these patients.
- Both peripheral and central mechanisms play a role in the development of oesophageal pain hypersensitivity.
- Psychological stress can exacerbate oesophageal pain hypersensitivity by enhancing both peripheral and central mechanisms.
- Neurophysiological techniques are now available that can help in the identification of the mechanisms of oesophageal pain hypersensitivity.
- In future treatments that reduce peripheral and central mechanisms of oesophageal pain hypersensitivity may be effective in patients refractory to acid suppression therapy.

Research Agenda

- Role of stress in mediating oesophageal peripheral and central sensitisation needs elucidation.
- Molecular makers of afferent receptor sensitisation in oesophageal mucosal biopsies need to be identified.
- Use of neurophysiological techniques to identify subgroups of patients with true afferent nerve hypersensitivity or psychological causes such as hypervigilance needs to be explored.
- Treatment with anti neuropathic pain drugs needs to be explored in patients with oesophageal pain hypersensitivity refractory to acid suppression.

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