Gastro-oesophageal reflux disease (GORD) is characterised by symptoms of recurrent heartburn and/or acid regurgitation. A recent systematic review of the epidemiology of GORD estimated its prevalence at 10-20%, defined by occurrence of symptoms at least weekly\(^1\). It is therefore one of the major clinical problems in our society today. It arises from excessive exposure of the oesophageal mucosa to acidic gastric contents following reflux across the gastro-oesophageal junction. Untreated, it may result in severe erosions of the oesophagus, leading to strictures and metaplasia. The pain associated with reflux disease has a major impact on quality of life regardless of the presence or absence of oesophageal erosions. This review is aimed to provide indicators towards novel therapies for GORD, based on its primary aetiology and knowledge of physiological and pharmacological processes involved in events leading to gastro-oesophageal reflux (GOR) episodes.

GORD is currently managed mainly by inhibition of acid secretion using proton pump inhibitors (PPI). This is despite the fact that gastric acid secretion is actually normal in most reflux disease patients. It is successful because acid is in the wrong place at the wrong time. PPI therapy is unsuccessful in approximately 30% of patients\(^2\), either because of incomplete acid suppression, or because of symptoms produced by non- or weak-acid reflux. A treatment that attacks the fundamental cause of reflux would be expected to provide a better success rate, and of course be more appropriate to the disease aetiology.

**Introduction**

Gastro-oesophageal reflux disease (GORD) is characterised by symptoms of recurrent heartburn and/or acid regurgitation. A recent systematic review of the epidemiology of GORD estimated its prevalence at 10-20%, defined by occurrence of symptoms at least weekly\(^1\). It is therefore one of the major clinical problems in our society today. It arises from excessive exposure of the oesophageal mucosa to acidic gastric contents following reflux across the gastro-oesophageal junction. Untreated, it may result in severe erosions of the oesophagus, leading to strictures and metaplasia. The pain associated with reflux disease has a major impact on quality of life regardless of the presence or absence of oesophageal erosions. This review is aimed to provide indicators towards novel therapies for GORD, based on its primary aetiology and knowledge of physiological and pharmacological processes involved in events leading to gastro-oesophageal reflux (GOR) episodes.

GORD is currently managed mainly by inhibition of acid secretion using proton pump inhibitors (PPI). This is despite the fact that gastric acid secretion is actually normal in most reflux disease patients. It is successful because acid is in the wrong place at the wrong time. PPI therapy is unsuccessful in approximately 30% of patients\(^2\), either because of incomplete acid suppression, or because of symptoms produced by non- or weak-acid reflux. A treatment that attacks the fundamental cause of reflux would be expected to provide a better success rate, and of course be more appropriate to the disease aetiology.

**Key Words:** Lower oesophageal sphincter, Vagus nerves, GABA receptors, Glutamate receptors.
The Lower Oesophageal Sphincter (LOS) and Reflux

The primary aetiology of GORD is disordered control of the GOR barrier. This barrier is comprised of an internal lower oesophageal sphincter (LOS) and an external sphincter formed by the crural diaphragm (CD). The two sphincters briefly and simultaneously relax to allow bolus passage during oesophageal peristalsis. Brief relaxation also occurs prior to GOR, known as transient LOS relaxation (TLOSR). TLOSR normally allow belching in order to vent gas trapped above the gastric contents, during which a little gastric acid may reflux simultaneously, but not normally sufficient to cause oesophageal mucosal damage. TLOSR, unlike swallow-induced relaxations, are independent of oesophageal body motor activity, and are significantly longer in duration. TLOSR range in duration from a few seconds to >30 seconds. Importantly, TLOSR are normally selective for gas reflux. Although this selectivity has long been established, the fundamental mechanisms underlying selectivity remain unknown. TLOSRs account for up to 90% of acid reflux episodes in asymptomatic controls and GORD patients. The development of GORD is therefore dependent upon factors that alter the rate of TLOSR and the physical (liquid vs gas) and chemical (acid vs non-acid) nature of refluxate.

Neural Pathways of TLOSR

Early evidence from a dog model indicated that TLOSRs are mediated via a vagal pathway, based on abolition of TLOSR during vagal cooling. The same model showed that a central mechanism was likely because of the abolition of TLOSR by anaesthesia, and human studies determined that they were inhibited during sleep. It was already known that TLOSR were triggered by meals, but which meal components and the receptor mechanisms responsible remained elusive. The trigger zone for TLOSR was eventually localised specifically within the proximal (cardia) portion of the stomach through elegant partitioning studies of the dog stomach. Around the same time it was shown that refluxing species possessed mechanoreceptors located in this part of the stomach musculature that were responsive to local tension in the smooth muscle, as opposed to its length. These vagal afferents have central terminals in the nucleus tractus solitarius of the brain stem whereupon they synapse with other neurones which constitute a central program generator (Figure 1). It is this program generator that is presumably sensitive to a number of other inputs relating to consciousness and body position. There are several simultaneous outputs from the program: first is a brief and powerful activation of vagal motor neurones (in the adjacent dorsal vagal nucleus) projecting to the LOS, which activate inhibitory motoneurones of the enteric nervous system, leading to smooth muscle relaxation. Second is a suppression of oesophageal body peristalsis presumably due to interruption of excitatory vagal output. Third is a suppression of motor output to the CD leading to opening of the external striated muscle sphincter.

Pharmacology of TLOSR pathways

GABA<sub>B</sub> receptors

In order to improve understanding in this field we have established a small animal model of reflux in ferrets, in which similar patterns of oesophageal motility are associated with reflux to those seen in humans. These occur at a similar rate after a gastric nutrient load in ferrets and humans, and show a low level of variability between studies in the same animal. Thus the model is ideal for studies of the effects of experimental interventions on TLOSR, and resulted in the first publication of the inhibitory effects of GABA<sub>B</sub> receptor agonists on reflux, alongside findings from the dog model which has provoked intense clinical and basic interest. These studies showed that a range of GABA<sub>B</sub> receptor agonists inhibited TLOSR, in some cases with complete suppression of TLOSR and thus GOR.

We have in parallel characterised the specific neural reflex pathways in anaesthetised ferrets and in isolated tissue which lead to LOS relaxation. These studies have led to the knowledge that GABA<sub>B</sub> receptors are present at several points along the TLOSR pathway (Figure 1). In vivo and in vitro recordings of vagal tension receptors in the ferret proximal stomach showed that activation of GABA<sub>B</sub> receptors potently inhibited their mechanosensory stimulus response relationships. Therefore these drugs act at the point of initiation of TLOSR by gastric distention. Because GABA<sub>B</sub> receptors are expressed by
vagal afferent cell bodies in the nodose ganglion, we considered the possibility they may also act at the central endings in the brain stem. This was confirmed by recordings of the responses to gastric distension of neurones in the nucleus tractus solitarius and vagal motor neurones. GABAB receptors were also shown immunohistochemically to be present on the vagal motor neurones themselves, suggesting they may influence motor outflow to the LOS directly. This was demonstrated by manometric recordings in vivo and in vitro of LOS responses to vagal efferent stimulation. GABAB receptor agonism did not affect LOS responses to local stimulation, indicating the effect was restricted to the vagal motor neurones and not the enteric nervous system.

Having determined the potential for actions of GABAB receptors at numerous peripheral and central sites along the TLOSR pathway, it remains to be finally understood the location of its main therapeutic action in inhibiting TLOSR. Some clues can be gained by observing the effect of GABAB receptor agonism on TLOSR, where it is clear that the frequency of occurrence is reduced, but not the depth or duration of LOS relaxation. This strongly suggests that the effect lies on the afferent pathway that triggers TLOSR, rather than the motor pathway that relaxes smooth muscle.

Studies in humans began soon after the demonstration that the GABAB receptor agonist baclofen was effective in reducing TLOSR in dog and ferret, helped by its existing clinical use and safety for neurological indications. Potent inhibition of TLOSR and reflux was seen in healthy volunteers, and subsequently in reflux disease patients. Several subsequent trials confirmed these observations in adult patients and children. Baclofen suffers from a number of drawbacks in its use as an anti-reflux agent. It has documented central nervous side effects and cardiovascular contra-indications, which means that it is unlikely to be used as a mainstream or even adjunct treatment for GORD. Clinical trials are currently in progress with novel GABAB receptor agonists whose actions are peripherally restricted, so there is great optimism about the possibility of a “reflux inhibitor” drug being avail-
able soon. What these basic and clinical studies tell us is that vagal mechanoreceptors in the proximal stomach may now be the most important cellular target in this prevalent disease, supplanting or rivalling the parietal acid secreting cells in the gastric epithelium.

Metabotropic Glutamate Receptors (mGluR)

Just over a decade ago it was found that the cloned GABA_B receptor shared many features with mGluR. They both belong to family 3 of the G-protein coupled receptors, and possess large extracellular domains35. There was evidence they had similar effects on synaptic transmission in the nucleus tractus solitarius36, so we embarked on a study of the effects and localisation of mGluR along TLOSR pathways. One of the major differences between GABA_B receptors and mGluR is the existence of several distinct subtypes of mGluR encoded by different genes. Thus group I mGluR are in fact excitatory, comprising mGluR1 and mGluR5, whereas group II and III more closely resemble GABA_B receptors, being inhibitory. Group II comprises mGluR2 and 3, and group III comprises mGluR4, 6 and 8. Using RT-PCR we found all of these were expressed in the brain and nodose ganglion of several species, although human and ferret lacked expression of mGluR3 and 6 in the nodose ganglion based on use of conserved sequences for detection37. Similar findings were made in ferret nodose ganglion using immunohistochemistry and retrograde labelling of gastric vagal afferents37. Application of selective agonists to group I, II or III mGluR revealed that group II and III activation inhibited gastric mechanoreceptors to a similar extent to that we observed with GABA_B receptor agonists. A group I agonist was without effect. It became clear that tonic endogenous activation of mGluR took place on vagal afferents, because a group III antagonist alone was able to potentiate vagal afferent mechanosensitivity37. Conversely, we found that group I (mGluR5) antagonists potently reduced mechanosensitivity38,39. Both group III and group I effects in vitro translated to corresponding responses in vivo. Group III agonists caused inhibition of TLOSR in ferrets (notably mGluR8), and group I (mGluR5) antagonists potently inhibited TLOSR in both dogs and ferrets40,41. Experiments using nucleus tractus solitarius recordings in vivo indicate that mGluR5 effects are most likely on the peripheral endings of vagal afferents, but some central actions along excitatory pathways are evident38. These results in animal models in turn have translated to early findings in humans, which demonstrate the possibility for clinical use of mGluR5 antagonists in treatment of GORD42.

Nitric Oxide

Interest in the role of NO in the enteric nervous system has been intense over the past 20 years or so, and its role in LOS relaxation is important in a number of species. Effects of NO synthase inhibitors on TLOSR have been observed in dogs43 and humans44, but their site of action is not yet known. It is worth noting that they do not affect the depth of LOS relaxation at the doses used in humans, and may therefore act on the trigger mechanism rather than the motor pathway as may be presumed. The clinical utility of NO synthase inhibitors in GORD is questionable due to the presence of side effects on cardiovascular function and elsewhere in the GI tract.

Cholecystokinin

CCK is traditionally thought to mediate effects of fat and protein in the small intestine on food intake and gastric emptying via activation of vagal afferents. Since this is comparable with the pathway involved in TLOSR (albeit from a different location), it made sense to investigate the effect of blockade of vagal CCK receptors on TLOSR. Studies in both dogs43,45 and humans46 showed that a significant decrease in TLOSR occurred after CCK1 receptor antagonism. Further development of CCK1 receptor antagonists for GORD has not been evident, presumably because of their side effects on other systems relying on CCK signalling.

Cannabinoids

Cannabinoids have been established for some time as anti-emetics, which was recently shown to be attributable to their actions on central vagal pathways47. This prompted us to investigate if cannabinoid type 1 (CB1) receptor activation was effective in inhibiting TLOSR in dogs. Potent inhibition of TLOSR was observed below the threshold for central nervous system side effects, and this was not attributable to effects on vagal afferents48. Although cannabinoids are promising for treatment of emesis and pain, it remains to be seen if they have potential in the treatment of GORD.
Other Transmitters

5-Hydroxytryptamine (5-HT), opioid, and ionotropic glutamate receptors of the NMDA type have all been shown to play a role in the modulation of triggering of TLOSR\textsuperscript{30-32}, but either the effects are marginal, or the target is associated with too many side effects. The site of action to inhibit TLOSR of drugs acting on these receptors is not known, but if it would be feasible to direct treatments peripherally this could improve their profile in some cases. In this context there are documented actions of 5-HT3 and NMDA receptors at vagal afferent endings\textsuperscript{39,53} as well as in the central nervous system.

Conclusions

It is hoped that there are several key points that are worth noting from this review. Firstly that vagal afferents are key targets in GORD. Secondly that agonists at GABA\textsubscript{B} receptors and Group III mGluR or antagonists of Group I mGluR reduce TLOSR and reflux, and they may do this by inhibiting mechanosensitivity of vagal sensory endings, and therefore have a peripheral action. Thirdly, that they may also inhibit vagal transmission in the nucleus tractus solitarius, and therefore also have some central actions. Finally, that these actions offer an exciting new avenue in the treatment options for GORD.

Acknowledgements

The author is indebted to several of his colleagues and collaborators for producing many of the data on which this review is based. Most notably the Nerve-Gut Research Laboratory, from which John Dent, Claudine Frisby, Amanda Page, Richard Young, Esther Staunton, Tracey O’Donnell and Nicole Cooper have contributed a great deal. Also Anders Lehmann and his colleagues at AstraZeneca Måland have made considerable contributions to the realisation of a “reflux inhibitor” drug, and who funded much of our work. The National Health and Medical Research Council of Australia funded many of the fundamental studies described here, and the University of Adelaide supported many of the students involved.

References


22) Smid SD, Young RL, Cooper NJ, Blackshaw LA. GABA(B)R expressed on vagal afferent neurones inhibit gastric mechanosensitivity in ferret proximal stomach. Am J Physiol Gastrointest Liver Physiol 2001; 281: G1494-G1501.


New insights in the neural regulation of the lower oesophageal sphincter


42) Adex-Pharmaceuticals. ADX10059 may be a potential treatment for patients with gastroesophageal reflux. Inpharma 2007; 1: 10-10.


