LEADING ARTICLE

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Progress with Novel Pharmacological Strategies for Gastro-oesophageal Reflux Disease

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Abstract

Gastro-oesophageal reflux disease (GORD) is a chronic disorder characterised by an increased exposure of the oesophagus to intragastric contents. Currently, GORD symptoms are maintained under control with antisecretory agents, mainly gastric proton pump inhibitors (PPIs).

Although impaired oesophageal motility may partly underlie the pathophysiology of GORD, the use of prokinetic agents has been found to be unsatisfactory. To date, novel pharmacological approaches for GORD are mainly related to the control of transient lower oesophageal sphincter (LOS) relaxations (TLOSRs). The majority of patients with GORD have reflux episodes during TLOSRs, which are evoked by gastric distension, mainly occurring after ingestion of a meal. Patients with reflux disease with normal peristalsis and without or with mild erosive disease could potentially benefit from anti-TLOSR therapy. This therapy might also be of value to treat some severe forms of esophagitis in combination with PPIs.

GABA-B-receptor agonists are the most promising class of agents identified so far for TLOSR control. The GABA-B-receptor agonist, baclofen, is the most effective compound in inhibiting TLOSRs in humans. Since baclofen has several CNS adverse effects, novel orally available GABA-B agonists are needed for effective and well tolerated treatment of GORD.

Endogenous or exogenous cholecystokinin (CCK) causes a reduction in LOS pressure, an increase in TLOSR frequency and a reduction in gastric emptying. In healthy volunteers and patients with GORD, loxiglumide, a selective CCK_1-receptor antagonist, was found to reduce the rate of TLOSRs, although its effect on postprandial acid reflux may be modest. Orally effective CCK antagonists are not marketed to date.

The anticholinergic agent atropine, given to healthy volunteers and patients with GORD, markedly reduced the rate of TLOSRs. Because of severe gastrointestinal (and other) adverse effects of anticholinergics, including worsening of supine acid clearance and constipation, it is unlikely that this class of drugs will have a future as anti-TLOSR agents on a routine basis.

In spite of their effectiveness in reducing TLOSR rate, untoward adverse effects, such as addiction and severe constipation, currently limit the use of

morphine and other opioid μ-receptor agonists. The same applies to nitric oxide synthase inhibitors, which are associated with marked gastrointestinal, cardiovascular, urinary and respiratory adverse effects.

Animal studies provide promising evidence for the use of cannabinoid receptor 1 agonists, by showing potent inhibition of TLOSRs in the dog, thus opening a new route for clinical investigation in humans.

A better understanding of TLOSR pathophysiology is a necessary step for the further development of novel drugs effective for anti-reflux therapy.

1. Pathophysiology of Gastro-oesophageal Reflux Disease

Gastro-oesophageal reflux is a physiological phenomenon occurring regularly in healthy individuals. Therefore, the term gastro-oesophageal reflux disease (GORD) should be used to include all individuals who are exposed to the risk of complications from gastro-oesophageal reflux or who experience clinically significant impairment of health-related well being (quality of life) because of reflux-related symptoms (mainly heartburn and regurgitation).[1-5]

Current knowledge on the pathophysiology of GORD identifies three major underlying mechanisms, including: (i) the noxious effect exerted by the gastric refluxate (i.e. acid/pepsin and bile) on the oesophageal mucosa; (ii) a defective clearance of the refluxed contents as a result of impaired oesophageal motility; and (iii) abnormalities of the antireflux mechanisms, mainly related to lower oesophageal sphincter (LOS) dysfunction. Given the purpose of the present article, these mechanisms are briefly summarised to provide a conceptual basis for new pharmacological strategies useful in the management of patients with GORD.

1.1 Role of Acid/Alkaline Refluxate

The pathophysiology of GORD involves contact of the oesophageal epithelium with either acid and/or alkaline refluxate, along with impaired antireflux and clearance mechanisms. In this context, rising levels of noxious agents in the oesophageal lumen may cause mucosal damage and inflammation. In the clinical setting, GORD encompasses a wide spectrum of changes, ranging from absence of mucosal injury (non-erosive reflux disease [NERD]) up to severe forms of oesophagitis and related complications. In spite of this wide range of clinical manifestations, symptoms can be experienced both by patients with NERD and those with established GORD, regardless of disease severity. In this respect, patients with NERD may be hypersensitive to acid and other chemical stimuli, that is, their perception of luminal stimuli is enhanced. Indeed, acid exposure results in only 15% of healthy volunteers reporting heartburn compared with 88% of patients with symptoms.[6]

1.2 Oesophageal Motility and Lower Oesophageal Sphincter (LOS) Dysfunction

Oesophageal motor functions are regulated by several mechanisms, including the activity of extrinsic (both parasympathetic and sympathetic) and intrinsic (enteric) innervation widely supplying this viscus, the release of bioactive substances with endocrine and paracrine properties, and by muscular intrinsic electrical activity. In particular, the oesophageal innervation controls motility, including swallowing, peristalsis and sphincter activity, and is also involved in conducting nociceptive stimuli to the CNS. Both central and peripheral nervous systems are functionally integrated with the intrinsic innervation of the gut and exert a pivotal modulatory and regulatory action on the oesophagus.

The tonic contraction of the LOS at steady state, together with the contraction of the crural diaphragm, which contributes to the normal functioning of the LOS, are considered the major mechanisms subserving the physiological antireflux barrier.[7] The LOS relaxes in relation to a vagovagal-mediated reflex triggered by swallowing and gastric

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content reflux. LOS relaxation induced by swallowing occurs together with primary peristalsis, whereas isolated (i.e. transient) relaxations of the LOS (TLOSRs) occur independently from this process. The LOS is innervated by parasympathetic (vagal) and sympathetic (mainly splanchnic) nerves, although vagal pathways play a major role during LOS relaxation as well as in TLOSRs. The vagal afferent sensitive nerves of the distal oesophagus and of the LOS end in the nucleus of the solitary tract (nTS), whereas the motor innervation of the LOS is located in the dorsal motor nucleus of the vagus (DMV) [figure 1].[8,9]

LOS tone is of fundamental clinical importance as most of the reflux episodes observed in healthy individuals and in patients with GORD occur during TLOSRs. Nevertheless, low basal LOS pressure alone is usually insufficient to provoke free reflux.

1.2.1 Antireflux Barrier

Clinical experience clearly indicates that patients with GORD manifest more reflux events than healthy individuals.[10] The reasons for a more frequent occurrence of daily reflux episodes in patients with GORD can be ascribed to a wide array of pathogenetic mechanisms, the two most relevant being LOS incompetence and increased frequency of TLOSRs.

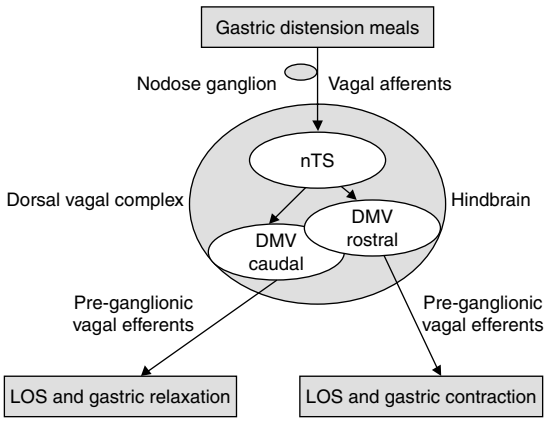

Fig. 1. Schematic representation of the main stimuli and neuronal pathways that mediate lower oesophageal sphincter (LOS) and stomach contraction and relaxation. Pre-ganglionic neurones are identified in the rostral part of the dorsal motor nucleus of the vagus (DMV) and are responsible for LOS (and stomach) contraction, whereas neurones located in the caudal part of the DMV are involved in LOS (and stomach) relaxation. As the vagus releases acetylcholine, the synaptic transmission between pre-ganglionic and post-ganglionic neurones of the myenteric plexus is nicotinic in nature, both for cholinergic motor neurones (releasing acetylcholine and tachykinins) and inhibitory motor neurones, releasing nitric oxide and vasoactive intestinal peptide, which evoke muscle relaxation. **nTS** = nucleus of the solitary tract.

Failure of LOS tonic contraction, which ultimately leads to sphincter incompetence, is a neuromuscular dysfunction, which may be either primary or secondary to acid/pepsin exposure. Experimental data and clinical experience are in support of these two possibilities. Acid exposure of the cat oesophagus, which is followed by reduced cholinergic activity and a consequent decrease in LOS pressure, provides support to the concept that LOS incompetence is secondary to acid/pepsin injury, and can be reversed with antisecretory treatment.[11,12] On the other hand, patients with GORD rarely show reversibility of LOS dysfunction following acid inhibitory treatment, thus indicating that the defect occurring at the LOS level is primary in origin.

The original concept that a defective LOS pressure is a major pathogenetic mechanism in GORD has been substantially revised by the evidence that LOS pressure is in the normal range in a significant proportion of patients with GORD. Indeed, Dodds et al.[13] were the first to show that about 65% of patients with GORD have reflux episodes during TLOSRs. TLOSRs can also occur in healthy individuals, although to a lesser extent than in patients with GORD. As identified by oesophageal manometry, TLOSR is a prolonged, vagovagal reflex-mediated relaxation of the LOS not associated with a swallow (figure 2),[14] which is evoked by free air, intragastric balloon or meal ingestion.[15]

1.2.2 Luminal Acid Clearance Mechanisms

Luminal acid clearance mechanisms include a wide array of factors, such as gravity, oesophageal peristalsis, saliva and oesophageal gland secretion.[16]

Both salivary and oesophageal gland secretion are rich in bicarbonate, which is effective in neutralising luminal acid. However, these mechanisms have not been shown to be altered in patients with GORD. In contrast, acid clearance was observed in

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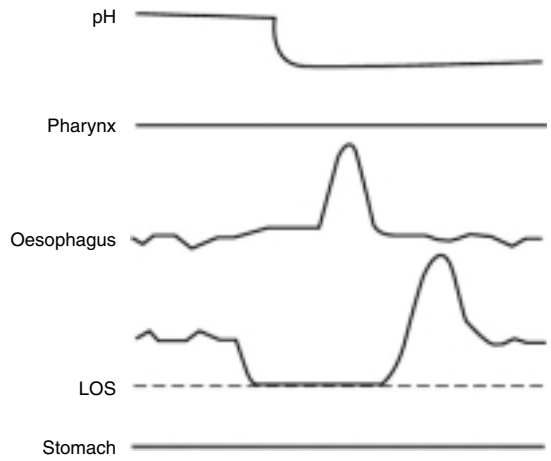

Fig. 2. Episode of gastro-oesophageal reflux during transient lower oesophageal sphincter (LOS) relaxation, showing a sudden drop in LOS pressure in the absence of a pharyngeal contraction and primary oesophageal peristalsis [reproduced from Fang et al.,[14] with permission from the BMJ Publishing Group].

patients with GORD as a result of impaired peristalsis, which encomp a delayedasses manometric abnormalities, such as lower amplitude contractions, aperistaltic contractions, a greater number of failed contractions following swallowing, and early retrograde reflux in patients with hiatal hernia.[16,17] The delay in intraluminal acid/pepsin clearance taken together with the impairment of antireflux mechanisms may underlie the prolonged contact between acid and the oesophageal mucosa.

2. Targets for Pharmacological Intervention

The pharmacological approach to GORD depends on symptom severity, which does not correlate with disease severity, and recurrence. Patients with mild to moderate GORD are usually characterised by having occasional, intermittent reflux symptoms, which are sensitive to empirical therapy (antacids, alginates, histamine H_2-receptor antagonists).[18] Severe disease is diagnosed when symptoms are poorly controlled by empirical therapy or tend to recur after stopping therapy. However, relapse does not necessarily imply severe disease. In addition, all patients should be advised regarding lifestyle changes: these improve symptoms and enhance the efficacy of medical therapy. Pharmacological interventions aim essentially at reducing gastric acid secretion, but may also target LOS motility to achieve a better outcome (table I).

2.1 Gastric Acid Secretion

Over the years, significant experience has been gathered on the use of H_2-receptor antagonists and proton pump inhibitors (PPIs) in the treatment of GORD.[19] H_2-receptor antagonists are effective in relieving reflux symptoms in about 50% of patients but, with regard to healing, they appear to be mainly effective in grades I and II and not in higher grades of oesophagitis.[19] Maintenance treatment with H_2-receptor antagonists is mainly symptomatically effective in patients with mild GORD. PPIs provide significantly higher healing rates of reflux oesophagitis than H_2-receptor antagonists, especially in patients with more severe oesophagitis and Barrett's oesophagus (an epithelial metaplastic condition occurring as a consequence of chronic gastro-oesophageal reflux, which may progress to oesophageal adenocarcinoma).[20] PPIs are also effective in patients with oesophagitis refractory to treatment with H_2-receptor antagonists. PPIs are now the drugs of first choice in healing all patients with more severe forms of reflux oesophagitis. They can now also be considered drugs of first choice for patients with milder forms of oesophagitis. However, in patients with NERD the response rate to a standard dose of PPI is 30% lower than in patients with erosive oesophagitis. In maintenance treatment of GORD, PPIs are the most effective drugs, offering the possibility of keeping nearly all patients in remission with adjusted doses. Current patient data of up to 5 years indicate the safety of this strategy for this period.[21] At present, all but a few patients with GORD can be managed adequately by medical therapy.

Mild-to-moderate forms of the disease may be treated with a 'step-up' or a 'step-down' approach.[22,23] In the step-up strategy, less effective drugs (i.e. H_2-receptor antagonists) are initially used, reserving the more effective PPIs for non-responders. The step-down strategy begins with a

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standard dose of a PPI to bring the patient into remission rapidly, switching to less effective drugs if symptoms permit. A recent approach for mild-to-moderate reflux disease is 'on-demand' therapy with PPIs, which is both efficacious and cost effective in patients with uncomplicated GORD.[24-26] Both omeprazole and esomeprazole 20 mg/day were effective and well tolerated in patients with NERD.[27,28] Because of its faster onset of action (day-1 effect),[29] rabeprazole is suitable for on-demand therapy.

In patients with severe GORD, PPIs are the therapy of choice for rapid symptom relief. A standard daily dose is usually effective in the majority of patients, whereas a standard dose twice daily is required for those patients with Los Angeles grades C and D oesophagitis,[30] those with severe extra-oesophageal disease and those with nocturnal acidity not easily controlled by conventional dosages. A high PPI dosage is also required for patients with Barrett's oesophagus and those with Zollinger-Ellison syndrome.[31]

It is well established that GORD symptoms tend to relapse after discontinuation of drug therapy. In the case of PPIs, one-half of the healing dosage is the usual regimen to maintain healing.[18]

Five PPIs are currently available worldwide: omeprazole, the enantiomerically pure S-isomer of omeprazole, esomeprazole (the last PPI to enter the market),[32] lansoprazole, pantoprazole and rabeprazole. Their suppressant effect on acid secretion stems from a common mode of action, consisting of the inhibition of the enzyme hydrogen-potassium adenosine triphosphatase (H^+, K^+-ATPase, i.e. the gastric proton pump), that in the active parietal cell exchanges intracellular hydrogen for extracellular potassium in an ATP-dependent fashion. Thus, PPIs block the final step of gastric acid secretion at a site where all the other pathways activated by compounds such as histamine, acetylcholine or gastrin converge, hence exerting a superior acid control.[33] For more detailed information on the pharmacokinetics of individual PPIs, readers are referred to the extensive published reviews.[34-38] Although PPIs are similar with respect to their molecular structure, subtle differences in: (i) chemical stability at neutral pH; (ii) activation under acidic conditions; (iii) acid dissociation (pKa) values; (iv) irreversible inhibition of the pump; (v) half-life; (vi) bioavailability; and (vii) metabolism, may be relevant to their clinical efficacy.[39,40]

The clinical endpoint for patients with acid-related disorders is the reduction of acid secretion in order to stabilise the median pH at a value ≥3. Thus, a fast onset of action leading to a more pronounced acid inhibition following the first PPI dose (i.e. a more significant day-1 effect) is important for 'on-demand' or intermittent therapy. Indeed, results from clinical trials and pharmacoeconomic studies have shown that on-demand therapy with PPIs is both efficacious to relieve symptoms, such as heartburn and regurgitation, and cost effective in patients with uncomplicated GORD.[24-26]

Second-generation PPIs, namely rabeprazole and esomeprazole, partly overcome the limitations of first-generation agents in that they achieve a more rapid, profound and long-lasting inhibition of gastric acid secretion over 24 hours.[41] This is a clear advantage for on-demand therapy,[26] in which a rapid onset of action is essential for a positive clin-

Table I. Pathophysiological and pharmacological targets in the treatment of gastro-oesophageal reflux disease

Pathophysiological target	Pharmacological target	Therapeutic agents
Secretion	Gastric acid secretion	Proton pump inhibitors, histamine H_2-receptor antagonists
Motility	Oesophageal clearance of refluxed contents	Prokinetics: serotonin 5-HT_4-receptor agonists
	LOS dysfunction	
	decreased LOS tone	Motilides, CCK_1-receptor antagonists
	enhanced transient LOS relaxations	GABA-B-receptor agonists, μ-opioid-receptor agonists, muscarinic-receptor antagonists, CCK_1-receptor antagonists, nitric oxide synthase inhibitors, cannabinoid receptor-1 agonists

CCK = cholecystokinin; **LOS** = lower oesophageal sphincter.

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ical outcome, and to heal moderate or severe oesophagitis in a higher proportion of patients, as observed with esomeprazole.[30,42]

2.2 LOS Motility

Although antisecretory agents are at present the mainstay of pharmacological treatment, drugs targeting motility may have a role in the treatment of gastro-oesophageal reflux by facilitating oesophageal acid clearance (by stimulating oesophageal peristalsis and accelerating gastric emptying), by enhancing LOS tone and, more importantly, by reducing TLOSRs. The compounds acting at these levels are discussed in sections 3 and 4.

3. Agents Targeting LOS Tone

3.1 Serotonin Receptor Ligands

Among serotonin receptor ligands, only serotonin 5-HT_4-receptor agonists and 5-HT_3-receptor antagonists have been tested for their effect on LOS motility.[43]

Cisapride is a 5-HT_4-receptor agonist with moderate 5-HT_3-receptor antagonist properties, whose beneficial effect on oesophageal motility in GORD is still a matter of discussion.

Several studies supported the use of cisapride in GORD for its ability to favour oesophageal peristalsis (and, therefore, to enhance acid clearance from the oesophagus), to increase LOS pressure, and to improve gastric emptying (for a review, see De Ponti and Malagelada[43]). Cisapride was also hypothesised to have oesophagoprotective potential through an enhancement of salivary volume and buffer capacity.[44-46] In the acute treatment of GORD, the prokinetic drug cisapride was shown to be effective in relieving symptoms and endoscopic relapse in patients with mild GORD.[19]

More recently, in a double-blind, placebo-controlled study, Pehlivanov et al.[47] investigated the effect of cisapride on the frequency of nocturnal TLOSRs and oesophageal acid exposure in ten patients with GORD. Patients were randomly assigned to 5-day treatments with cisapride 10mg four times daily or placebo, separated by a 2-day washout period before the treatment crossover. Sleep stages, LOS tone and oesophageal pH were monitored overnight at the end of each treatment regimen. Cisapride decreased the frequency of TLOSRs during sleep, and oesophageal acid exposure. Cisapride also increased LOS tone and decreased heartburn episodes and antacid consumption.

However, these results were not confirmed in a recent study[48] evaluating the effects of cisapride in 30 patients with proven GORD (via endoscopy and 24 hour pH-metry), included in a randomised, double-blind, placebo-controlled study with a crossover design. Cisapride 20mg twice daily for 4 weeks was compared with placebo. At baseline, as well as after 4 and 8 weeks, all patients underwent symptom assessments, sleeve manometry with concomitant oesophageal pH monitoring, and an acid clearance test. Despite adequate plasma levels, cisapride did not significantly affect swallow-induced peristaltic amplitude, duration, propagation speed, the elicitation of secondary peristalsis or acid clearance. Neither the basal tone of the LOS nor the number of TLOSRs induced by gas distension of the stomach was affected by the administered dosage of cisapride. The authors concluded that, although cisapride has been alleged to improve symptoms as well as the oesophagitis in patients with GORD, the compound was devoid of effects on important motor mechanisms involved in the pathogenesis of the disease. Thus, the effect of cisapride on gastro-oesophageal reflux may be less than originally thought. The withdrawal of cisapride because of cardiac adverse effects[49] left some questions unanswered. Theoretically, potent, selective prokinetic agents would be welcome, but are unlikely to be effective as single agents across the range of GORD. However, there is certainly a need for agents acting on motility as adjuncts to acid suppression in patients who fail to respond to the latter.

Second-generation 5-HT_4-receptor agonists, such as tegaserod and mosapride, seem to be devoid of significant cardiac effects.[49-52] Among second generation 5-HT_4-receptor agonists, tegaserod, prucalopride and mosapride have undergone clinical

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trials. Tegaserod and prucalopride are targeted for the irritable bowel syndrome, whereas mosapride is specifically targeted for the treatment of upper gut disorders, such as GORD,[53] and is marketed in Japan. Nevertheless, in one study, the partial 5-HT_4-receptor agonist tegaserod, which, unlike cisapride is devoid of significant effect at 5-HT_3 receptors, is reported to decrease postprandial oesophageal acid exposure because of an increased acid clearance, improved gastric emptying and, particularly, a reduced number of TLOSRs.[54]

Finally, there are some data in experimental animals using selective 5-HT_3-receptor antagonists. One study[55] investigated whether these receptors participate in triggering TLOSRs, independently or in relation to their cholecystokinin(CCK)ergic control. Oesophageal, LOS and fundus pressure were manometrically monitored in five conscious dogs. Gastric distensions with air at a constant pressure were performed under intravenous infusion of CCK8S (0.5 μg/kg/h) or NaCl 0.9% and were preceded by intravenous ondansetron 0.2–500 μg/kg or granisetron 100 μg/kg or NaCl 0.9%. Ondansetron or granisetron dose-dependently reduced the number of TLOSRs induced by gastric distension. Ondansetron did not modify the number of relaxations under a 1.0 kPa gastric pressure, but reduced the increase in the occurrence of relaxations induced by CCK8S under a gastric pressure of 1.0 and 1.7 kPa. The authors concluded that the CCK control in triggering TLOSRs is modulated by serotonin via 5-HT_3 receptor subtypes.

3.2 Motilin Receptor Agonists

The reviews by Peeters[56] and Itoh[57] provide full coverage of the history of erythromycin and its derivatives as prokinetics. It is now well established that erythromycin is a potent motilin receptor agonist and displays prokinetic effects. The ability to interact with motilin receptors is shared, although to a lesser extent, by other antibacterial macrolides with a 14-member ring structure, such as clarithromycin, oleandomycin, roxithromycin, troleandomycin, but not by those derivatives with a 16-member ring structure, such as josamycin, midecamycin, miokamycin, rokitamycin and spiramycin.[58] Recent research into this area has developed erythromycin derivatives with no antibacterial activity but preserved or even higher motilin-like properties – the so-called motilides, such as alemcinal (ABT-229) and mitemcinal (GM-611).[59,60]

The use of erythromycin and its derivatives as upper gut prokinetics finds a rationale from the observation that there is a gradient of motilin receptors from stomach to terminal ileum, with the highest density in the upper gut. Erythromycin can stimulate gut motility both through a direct action on smooth muscle motilin receptors and probably also through neural receptors.[56,61-63] After intravenous injection, erythromycin dramatically improves gastric emptying in diabetic gastroparesis.[64] However, trials with alemcinal in functional dyspepsia were unequivocally disappointing for symptom improvement, regardless of gastric motor function.[65,66]

Alemcinal was also tested for its potential in GORD, but the results were not encouraging because of the limited effect on reflux.[67-69] Since motilin receptor agonists appear to have only a minor effect on LOS tone (not in all studies) and do not affect TLOSRs, they have a limited, if any, clinical utility in the treatment of GORD.

4. Agents Targeting Transient LOS Relaxations

As mentioned in section 1.2, TLOSRs are the key mechanism underlying the vast majority of reflux episodes. They account for virtually all episodes in healthy individuals and most (up to 80%) reflux episodes in patients with GORD. Since PPIs control acid but not non-acid reflux, the possibility to reduce TLOSRs pharmacologically in order to control both reflux components has been repeatedly explored during the last 10 years. Several drugs have been shown to reduce the rate of TLOSRs and concomitantly the number of reflux episodes, thus disclosing a new target for the pharmacological treatment of GORD. Agents investigated in humans include GABA-B-receptor agonists (baclofen), muscarinic-receptor antagonists, opioid μ-receptor agonists (morphine), CCK_1- (formerly CCK_A) receptor ant-

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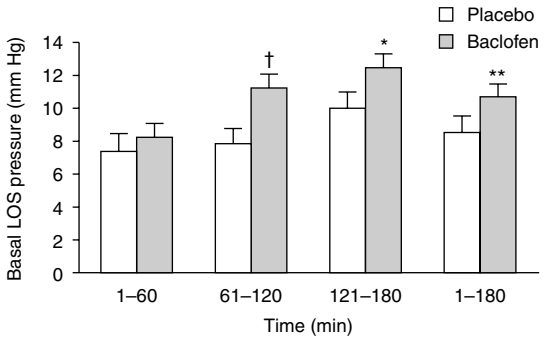

Fig. 3. Enhancement of basal lower oesophageal sphincter (LOS) pressure by oral baclofen 40 mg/day in healthy individuals. Data (mean ± SEM) refer to each postprandial hour and to the whole 3-hour (1–180 minutes) postprandial period (reproduced from Lidums et al.,[74] with permission from the American Gastroenterological Association). * $p < 0.01$; ** $p = 0.001$; † $p < 0.0001$, vs placebo.

agonists, nitric oxide (NO) synthase inhibitors and a series of other compounds (discussed in section 4.6).

4.1 GABA-B-Receptor Agonists

GABA is a fundamental inhibitory neurotransmitter in the central and peripheral nervous system, with a prominent function in modulating TLOSRs. GABA exerts its inhibitory effect on LOS by preventing neurotransmitter release in the vagal pathways controlling LOS relaxation. Indeed, GABA-B receptors are present on vagal pre-ganglionic fibres in rats, and a high concentration of GABA-B receptors has been observed in LOS-projecting pre-ganglionic neurones in ferrets.[70,71] Furthermore, GABA-B receptors have been identified, by means of retrograde labelling, in vagal afferent neurones in the nodose ganglia.[72] These observations, together with the evidence that GABA-B receptors are also present in the nTS and DMV strongly support the role of GABA as a main modulator of TLOSRs.[73] As a consequence, GABA-B agonists have been used to reduce TLOSR events in an attempt to prevent gastro-oesophageal reflux. In healthy volunteers, baclofen, a GABA-B-receptor agonist administered orally as a single 40mg daily dose, significantly decreased gastro-oesophageal reflux by increasing the basal LOS pressure and reducing the rate of a meal-induced TLOSRs by >50% compared with placebo (figure 3 and figure 4).[74] Interestingly, baclofen can decrease the rate of TLOSRs and increase the basal LOS pressure without altering the meal-induced fundus accommodation.[75]

In selected patients with GORD (the majority of whom had oesophagitis of various degrees), the same dose of baclofen reduced the rate of TLOSRs by approximately 40% during the 3-[76] to 12-hour postprandial period.[77] Furthermore, baclofen was found to decrease acid and non-acid postprandial gastro-oesophageal reflux (measured by combined multichannel intraluminal impedance and pH) in both healthy volunteers and patients with heartburn.[78] An elegant study with multiple oral dosages of baclofen (up to four 10mg divided doses per day) was recently carried out in healthy individuals and patients with GORD to obtain an adequate 24-hour drug coverage, since peak plasma levels of baclofen after a single oral daily dose occur 2 hours after administration, with disappearance from blood 4–6 hours thereafter.[79] Under these conditions, baclofen reduced 24-hour gastro-oesophageal reflux and increased pH in both patients and healthy controls. When given for 1 month to patients with GORD, baclofen reduced oesophageal acid refluxes and improved symptoms significantly. Interestingly, the anticonvulsant gabapentin, a compound with a complex pharmacological profile, including some

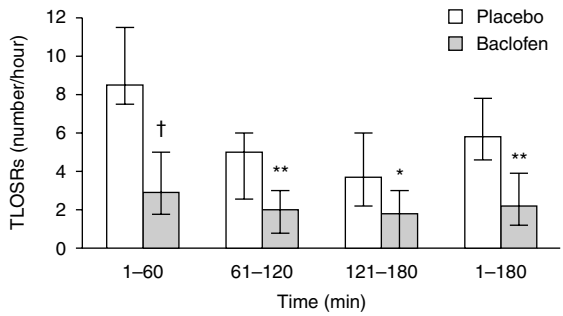

Fig. 4. Inhibitory effect of oral baclofen 40 mg/day on postprandial transient lower oesophageal sphincter relaxation (TLOSR) frequency in healthy individuals. Data are expressed as median (interquartile range) for each postprandial hour and for the whole 3-hour (1–180 minutes) postprandial period or recording (reproduced from Lidums et al.,[74] with permission from the American Gastroenterological Association). * $p < 0.002$; ** $p < 0.0001$; † $p = 0.0001$.

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agonist properties at a subset of GABA-B receptors and blocking properties at the $\alpha_2\delta$ neuronal calcium channel subunit,[80] did not affect the TLOSR rate in dogs.[81]

On the basis of these results, agonism at GABA-B receptors may have therapeutic benefit in GORD by reducing the triggering of TLOSRs. Since baclofen has CNS adverse effects (e.g. nausea, sleepiness, dizziness and tiredness), novel orally available selective GABA-B agonists are required for an effective and well tolerated treatment, particularly in patients with persistent symptoms despite adequate acid suppression.

4.2 Opioid Receptor Agonists

Opioid-containing nerves have been demonstrated in the myenteric plexus of human LOS,[82] while opioid receptors are widely distributed in nerves supplying the gut and the CNS. One of the main mechanisms of opioid μ-receptor agonists (e.g. morphine) is to suppress neuronal excitability both in the central and peripheral (including enteric) nervous systems.[83]

Recently, Penagini and colleagues[84] have investigated for the first time the effect of morphine on the oesophageal motility of healthy individuals, showing that morphine decreases the duration and amplitude of LOS relaxation induced by swallowing. This effect is likely to be mediated by opioid μ-receptor-induced inhibition of vagovagal reflexes responsible for LOS relaxations. Naloxone, an opioid μ-receptor antagonist, completely reversed the effect of morphine on TLOSRs.[84] Furthermore, when morphine was given to patients with GORD, a significant decrease in the number of TLOSRs was observed (figure 5).[85] The effect of morphine is presumably exerted at a central site, since the peripherally acting opioid receptor agonist loperamide did not affect the rate of TLOSRs.[86]

In spite of their effectiveness in reducing the TLOSR rate, untoward adverse effects such as addiction and severe constipation are limiting factors to the use of morphine and other opioid receptor agonists in the clinical setting.

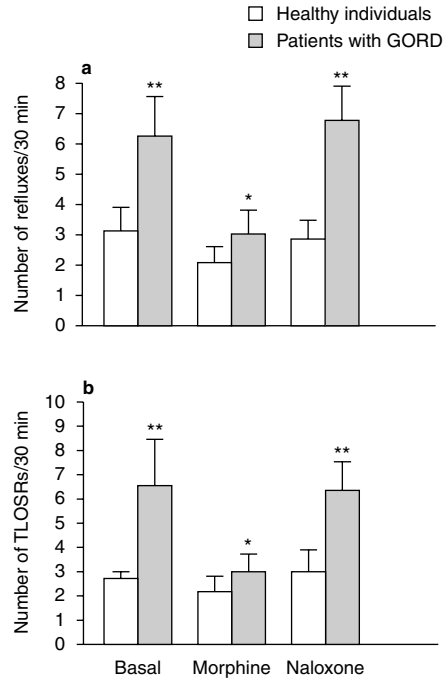

Fig. 5. Effects of intravenous morphine 100 μg/kg on reflux episodes (**a**) and transient lower oesophageal sphincter relaxation (TLOSR) frequency (**b**) in healthy individuals and patients with gastro-oesophageal reflux disease (GORD). Note that morphine significantly inhibited both reflux and TLOSR episodes in patients with GORD through a mechanism completely reversed by the opioid receptor antagonist naloxone (80 μg/kg intravenously). * $p < 0.05$ vs basal and naloxone; ** $p < 0.02$ vs healthy individuals (reproduced from Penagini and Bianchi,[85] with permission from the American Gastroenterological Association).

4.3 Muscarinic-Receptor Antagonists

Several lines of evidence are consistent with the notion that, in humans, acetylcholine released by post-ganglionic cholinergic nerves contributes to the regulation of peristalsis in the smooth muscle portion of the oesophageal body and to LOS tone.[87] Indeed, anticholinergics were found to reduce the incidence of complete peristaltic sequences after swallows, and to also decrease LOS pressure.[87] For these reasons, anticholinergics were believed to exacerbate gastro-oesophageal reflux. Currently, it is widely accepted that a low basal LOS pressure does not predispose to reflux episodes in the vast majority of individuals.[88-90]

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Evidence in healthy individuals indicates that atropine (a cholinergic muscarinic-receptor antagonist) reduced the frequency of gastro-oesophageal reflux by inhibiting the frequency of TLOSRs to gastric distension by a meal.[90,91] More recently, another anticholinergic agent, dicycloverine (dicyclomine), caused different effects on gastro-oesophageal reflux depending on body position and whether the individual was in the fasted or fed state. This drug inhibited the early postprandial upright reflux episodes, but enhanced the percentage reflux and time with pH <4 during the first 2 hours supine.[92] Similar to observations made in healthy individuals, in patients with GORD atropine was found to reduce gastro-oesophageal reflux by inhibiting TLOSRs.[93]

However, none of these findings helped elucidate whether the site of action of atropine is at the level of the CNS or in the periphery (i.e. through a local action in the gut). The use of methscopolamine bromide, a quaternary ammonium anticholinergic derivative that does not cross the blood-brain barrier, allowed establishment that the effect of atropine on TLOSRs is mediated through a central cholinergic blockade, since methscopolamine bromide was ineffective in patients with GORD (figure 6).[14] A study aimed at investigating the effect of atropine on fasting gastric compliance and postprandial gastric tone using an electronic barostat in healthy individuals, strengthened the conclusion that the inhibition of TLOSRs caused by atropine is unlikely to be mediated by an effect on the proximal stomach, but rather by a blockade of muscarinic receptors located on the integrated structures controlling LOS tone in the brainstem.[94] Indeed, a peripheral site of action, such as that achieved with the use of scopolamine butylbromide (hyoscine N-butylbromide), a compound that, similar to methscopolamine bromide, does not cross the blood-brain barrier, enhanced the total number of oesophageal acid refluxes in patients with GORD and in healthy individuals.[95] This suggests that the sole local action of anticholinergics in the gut may worsen reflux episodes by inhibiting LOS tone, oesophageal peristalsis and chemical neutralisation of the oesophageal acid residue, because of a reduction of saliva production. Because of severe intestinal (and extra-intestinal) adverse effects of anticholinergics, including worsening of supine acid clearance and constipation, it is unlikely that this class of drugs will have a future as anti-TLOSR agents on a routine basis.

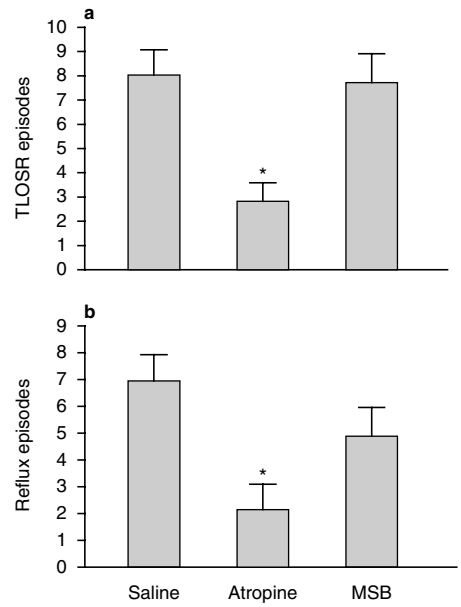

Fig. 6. Effects of intravenous (15 μg/kg bolus, 4 μg/kg infusion) atropine and oral (5mg four times daily) methscopolamine bromide (MSB) administration on the number of transient lower oesophageal sphincter relaxation (TLOSR) [**a**] and reflux episodes (**b**) in patients with gastro-oesophageal reflux disease. Note that atropine significantly (* $p < 0.05$) inhibited both TLOSRs and reflux episodes, whereas MSB, a quaternary anticholinergic ammonium derivative that does not cross the blood-brain barrier, was ineffective (reproduced from Fang et al.,[14] with permission from the BMJ Publishing Group).

4.4 Cholecystokinin Receptor Antagonists

CCK is released by endocrine duodenal and jejunal mucosa T cells, especially in response to a variety of fat nutrients, and its secretion is associated with gall bladder contraction, pancreatic enzyme secretion, inhibition of gastric emptying and a fall in LOS pressure and rise in TLOSR frequency.[96] Two CCK receptor subtypes (CCK_1 and CCK_2) have been characterised in the alimentary canal. Interestingly, Gonzalez and colleagues[97] have shown that CCK exerts a direct excitatory effect on human

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isolated LOS circular muscle by activating muscular CCK_1 receptors, a response antagonised by loxiglumide (a selective CCK_1-receptor antagonist) and lintitript (SR-27897; a nonselective CCK-receptor antagonist), but not by selective CCK_2-receptor antagonists.

In healthy humans, gastric distension with a barostat enhanced TLOSR frequency, as did infusion with CCK. Either mechanical or chemical induction of TLOSRs was antagonised by loxiglumide, indicating the participation of CCK_1 receptors in both types of stimulation.[98] Similar results were obtained by distending the stomach with 400mL of air.[99] The effect of loxiglumide was also investigated on postprandial LOS function and fundic tone. After an oral meal or duodenal infusion of a meal, loxiglumide significantly reduced (oral) or totally abolished (duodenal infusion) the increase in TLOSRs, reduced LOS pressure following duodenal infusion and markedly inhibited fundic relaxation.[100] A recent study carried out in healthy volunteers and patients with GORD has shown that loxiglumide attenuated TLOSRs caused by meal ingestion, although its effect on gastro-oesophageal acid reflux was quite modest.[101]

Inhibition of gall bladder emptying associated with gall stone formation is a potential adverse effect of CCK antagonists, but long-term clinical studies have excluded this effect for dexloxiglumide, the active enantiomer of the parent racemic compound loxiglumide.[102]

4.5 Nitric Oxide Synthase Inhibitors

Nitrergic neurones are part of the intrinsic inhibitory pathways innervating the LOS, where NO is the major post-ganglionic inhibitory neurotransmitter regulating TLOSRs. Indeed, Hirsch et al.[103,104] have demonstrated that treatment of healthy volunteers with L-NMMA (N^G-monomethyl-L-arginine), an NO synthase inhibitor, evokes a significant reduction (>75%) of gastric balloon distension-induced TLOSRs. In contrast, intravenous infusion in humans of arginine, an NO precursor, does not cause variations of LOS activity.[105]

NO synthase inhibitors are known to markedly alter several gastrointestinal, cardiovascular, urinary and respiratory functions.

4.6 Miscellaneous Agents

An attractive field for drug development is the cannabinoid receptor system. Cannabinoid receptors, currently subdivided into two groups, cannabinoid receptor 1 (CB_1 receptors, mainly localised to neurones) and cannabinoid receptor 2 (CB_2 receptors, mainly localised to immunocompetent cells) have many functional similarities with GABA-B receptors. A well known agonist of cannabinoid receptors is Δ-9-tetrahydrocannabinol, the principal psychoactive substance of marijuana. Other agonists include endogenous ligands (endocannabinoids), such as anandamide and 2-arachidonyl glycerol.[106]

A preliminary investigation in the dog indicates that activation of the CB_1 receptor by exogenous agonists and endogenous ligands (endocannabinoids) may affect TLOSR frequency. Indeed, the intravenous administration of the CB_1 agonist, WIN 552122, potently and dose-dependently inhibited TLOSRs (up to 80% with the highest dose of 57 nmol/kg), with a significant reduction in reflux episodes, although this was not clearly dose dependent.[107] The effect of WIN 552122 was antagonised by the CB_1-selective antagonist rimonabant (SR-141716A), which stimulated TLOSR incidence *per se*, shortened the latency to the first TLOSR, and increased the number of reflux episodes. The latter evidence implies the participation of an endocannabinoid in ongoing functional suppression of TLOSRs. Apparently, the site of action of CB_1 ligands is the CB_1 receptor located on the dorsal vagal complex.

Recently, a nonpharmacological approach to treating TLOSRs and reflux episodes has been published.[108] It consists of the delivery of radiofrequency energy to the LOS and gastric cardia, which causes a circumscribed thermal coagulative necrosis of tissues, which heals by necrosis. In patients with GORD, the use of this technique reduced postprandial TLOSRs by approximately 25% and reflux episodes by 50%.[108]

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5. Conclusions

GORD is a common condition characterised by heartburn and acid regurgitation, symptoms that are generally controlled with antisecretory agents (mainly gastric PPIs). Although impaired oesophageal motility may partly underlie GORD pathophysiology, the use of prokinetic agents has been found to be unsatisfactory to prevent reflux episodes, with some exceptions in patients with NERD or dyspepsia associated with delayed gastric emptying.

To date, novel pharmacological approaches for GORD are mainly targeted at the control of TLOSRs. Indeed, the majority of patients with GORD have reflux episodes during TLOSRs (evoked mainly by gastric distension), such as that occurring after ingestion of a meal. It is likely that control of TLOSRs is applicable to the majority of patients, especially those with NERD or minimal to mild erosive disease, but also in combination with PPIs to treat more severe forms of oesophagitis.

GABA-B-receptor agonists are the most promising class of agents identified so far for TLOSR control. Currently, only the GABA-B-receptor agonist baclofen, which is the most effective compound in inhibiting TLOSRs in humans, is available for oral therapy. Since baclofen has CNS adverse effects (e.g. nausea, sleepiness, dizziness, tiredness), novel orally available GABA-B agonists are needed for the effective and well tolerated treatment of GORD.

The rationale for proposing selective CCK_1-receptor antagonists, such as loxiglumide, stems from the fact that enteric CCK, once secreted in response to ingestion of fat nutrients, causes a reduction in LOS pressure, an increase in TLOSR frequency and a reduction in gastric emptying. In healthy volunteers and patients with GORD, loxiglumide was found to reduce the rate of TLOSRs, although its effect on postprandial acid reflux may be modest. Whether CCK antagonists will prove to be useful agents in the management of reflux episodes in GORD awaits further studies.

Clinical investigations with anticholinergics, opioid μ-receptor agonists and NO synthase inhibitors have demonstrated varying degrees of effectiveness in controlling TLOSR generation in healthy individuals and patients with GORD. However, the well known occurrence of serious adverse reactions, at both the intestinal and extra-intestinal level, is probably an insurmountable obstacle for their routine use in a chronic disorder such as GORD.

CB_1 agonists represent an attractive target for drug development. Animal studies have provided promising results that will open new routes to clinical investigation.

Lastly, data concerning the pharmacological control of TLOSRs were derived from short-term clinical investigations. Studies with prolonged pharmacological treatment are required to ascertain the real therapeutic potential of currently available or future agents targeted at controlling the rate of TLOSR in patients with GORD.

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