British Journal of Pharmacology (2000) 131, 624–630
www.nature.com/bjp

Vagal neurotransmission to the ferret lower oesophageal sphincter: inhibition *via* $GABA_B$ receptors

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1 $GABA_B$ receptors modulate the function of the lower oesophageal sphincter (LOS) *in vivo* by inhibiting neurotransmitter release in the vagal pathway controlling LOS relaxation. We aimed to determine whether this effect was mediated peripherally on vagal motor outflow to the ferret LOS *in vitro*.

2 The LOS, with intact vagal innervation, was prepared from adult ferrets and LOS tension measured. Vagal stimulation (0.5–10 Hz, 30 V) evoked a tetrodotoxin-sensitive, frequency-dependent relaxation.

3 Both GABA (3×10^{-4} M) and (±)baclofen (2×10^{-4} M) inhibited vagally-stimulated LOS relaxation. The potent $GABA_B$ receptor-selective agonist 3-APPA dose-dependently inhibited vagally-stimulated LOS relaxation, with an EC_{50} value of 0.7 μM.

4 Decreased responses following vagal stimulation in the presence of (±)baclofen or 3-APPA were reversed with the potent $GABA_B$ receptor antagonist CGP 62349.

5 Neither CGP 62349 nor muscimol ($GABA_A$ receptor agonist) alone affected LOS responses following vagal stimulation.

6 Agonists of other G protein-coupled receptors (clonidine (α_2-adrenoceptor) (5×10^{-6} M), U50488 (κ opioid) (10^{-5} M), neuropeptide Y (10^{-6} M)) did not affect vagally-mediated LOS relaxation.

7 The present study supports a discrete presynaptic inhibitory role for $GABA_B$ receptors on vagal preganglionic fibres serving inhibitory motorneurones in the ferret LOS.
British Journal of Pharmacology (2000) **131,** 624–630

Keywords: Lower oesophageal sphincter; CGP 62349; 3-aminopropylphosphinic acid; (±)baclofen; vagus nerve; γ-aminobutyric acid

Abbreviations: 3-APPA, 3-aminopropylphosphinic acid; GABA, γ-aminobutyric acid; LOS, lower oesophageal sphincter; NANC, nonadrenergic noncholinergic; NPY, neuropeptide Y; TTX, tetrodotoxin

Introduction

The lower oesophageal sphincter (LOS) is an important component of the antireflux barrier between stomach and oesophagus. Specialized thickened (Seelig & Goyal, 1978) circular smooth muscle of the LOS develops myogenic tone (Daniel, 1992) and receives neurotransmission *via* excitatory and inhibitory motorneurones which are, in turn, coupled to vagal preganglionic neurones (Brookes *et al*., 1996; Yuan *et al*., 1998b). LOS closure is maintained by a combination of physical, myogenic and neurogenic mechanisms (Daniel, 1992). LOS relaxation is elicited following activation of inhibitory motorneurones and occurs to facilitate belching, swallowing and episodes of gastroesophageal reflux. Within the LOS, vagal preganglionic neurones are coupled to enteric inhibitory motorneurones typically through nicotinic cholinergic receptors (Blackshaw *et al*., 1997), but muscarinic M1 and serotonin (5-HT) receptors have also been shown to be involved in vagal synaptic transmission (Gilbert *et al*., 1984; Rattan & Goyal, 1978). Intrinsic reflexes are also involved in coordinated LOS function, recruiting enteric inhibitory motorneurones to relax the LOS (Paterson *et al*., 1992; Schulze-Delrieu *et al*., 1989).

γ-Aminobutyric acid (GABA) exerts a variety of peripheral effects on gastrointestinal motility and secretion *via* both $GABA_A$ and $GABA_B$ receptors; these are mainly mediated *via* effects on enteric neurones in the small intestine (Ong & Kerr, 1990). However, peripheral spinal afferent and sympathetic efferent transmission (Parkman *et al*., 1993) and vagal afferent activity (Ashworth-Preece *et al*., 1997; Page & Blackshaw, 1999) are also modulated by GABA receptors. Recently, we identified a potential site of action of $GABA_B$ receptor-mediated modulation of vagal motor neurones to the ferret LOS *in vivo*, which suggested a presynaptic inhibition of vagal motor output (Blackshaw *et al*., 2000). As GABAergic pathways have additional broad systemic effects (e.g. acid and somatostatin secretion (Blandizzi *et al*., 1992; Koop & Arnold, 1986; Tsai *et al*., 1987)) which may indirectly alter LOS function, the present study was undertaken to further investigate $GABA_B$ influences on vagal motor control to the LOS in an isolated *in vitro* preparation, with intact vagus nerves serving only the LOS. By a comparison with other G protein-coupled receptor pathways, including α_2-adrenoceptor, κ opioid and neuropeptide Y receptors, we could then investigate the discrete effects of $GABA_B$ receptor ligands on vagally-stimulated responses and explore the pharmacology of this modulation of motor control to the LOS.

Methods

Animal preparation

Experiments were performed on adult ferrets of either sex weighing 400–600 g. None of the female ferrets were in

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oestrous at the time of experimentation. All studies were performed in accordance with the guidelines of the Animal Ethics Committee of the Royal Adelaide Hospital and Institute for Medical and Veterinary Sciences, Adelaide. Animals had free access to water and a standard diet but were fasted from food overnight prior to experimentation. Oesophagogastric tissue was obtained from ferrets following exsanguination under general anaesthesia (sodium pentobarbitone, 60 mg kg^{-1} i.p.).

LOS preparation

The oesophagus and proximal stomach were removed and placed in ice-cold Krebs solution bubbled with carbogen (95% O_2, 5% CO_2). The area encompassing the gastroesophageal junction was located after careful dissection and cleaned of adherent adipose tissue from the proximal stomach, taking care to leave the vagus nerves intact. Entomological pins were then placed around the borders of the circumference of the remaining fundus, which was subsequently removed to leave the oesophagogastric junction and attached oesophagus. A skirt of mucosa and submucosa was then carefully dissected away from the lower oesophageal sphincter and distal oesophagus. The dorsal and ventral vagi were dissected away from their course down the oesophagus to the level of the beginning of the oesophagogastric junction, at the point where the striated oesophageal muscle fibres merge with the smooth muscle of the oesophagogastric junction. The remainder of oesophagus was resected from the oesophagogastric region at this point. The vagus nerves were tied together with a length of cotton suture at the oral end. Ties were then fixed to opposing ends of the circular muscle of the LOS; one end of the tissue was fastened to a support while the other was attached to an isometric force transducer (FTO3, Grass, Quincy MA, U.S.A.). The preparation was placed under a 20 mN preload, which corresponds to a degree of tissue stretch of approximately 200%, within the optimal range for mechanical performance of the tissue. The preparation was allowed to equilibrate for 90 min in 10 ml water-jacketed organ baths containing carbogenated Krebs solution at 37°C of the following composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.6, $MgSO_4$ 1.2, NaH_2PO_4 1.3, glucose 11, $CaCl_2$ 2.5. Pretreatment with atropine (2×10^{-6} M) and guanethidine (3×10^{-6} M) was used to ensure nonadrenergic noncholinergic (NANC) conditions. The LOS developed stable spontaneous tension at rest and exhibited a pattern of relaxation in response to vagal stimulation that was sensitive to hexamethonium (500 μM) and similar to that exhibited in isolated LOS muscle strips of the ferret during electrical field stimulation (Smid *et al*., 1998), indicating tension measurements were recorded from the LOS.

Physiological and pharmacological studies

Vagal stimulation was delivered *via* square wave pulses from a stimulator (S48, Grass, Quincy MA, U.S.A.), with 3 min intervals between each stimulus. Stimulation was applied through the combined ends of the tied vagi, which were lifted out of the organ bath and gently draped over bipolar platinum stimulating electrodes. There was no LOS response when electrodes were applied directly to the bath medium, excluding any residual field stimulation in observed responses. LOS responses to vagal stimulation (0.5–10 Hz, 30 V, 1 ms pulse duration for 5 s) and receptor ligands were measured and recorded onto hard disk using Labview-based software (MAD, Charles Malbert).

Data analysis

Responses in all experiments were quantified based upon a percentage of the baseline value of LOS tone relative to the nadir of the response. Data were expressed as mean ± s.e.mean of a number of animals ($n=x$). Nonlinear regression of concentration-response data and EC_{50} (effective concentration, 50% of maximum response) calculations for responses to 3-APPA were performed using Prism 2.01 (Graphpad, San Diego, CA, U.S.A.). Statistical analysis was performed using a two-way ANOVA. A P value of less than 0.05 was considered significant.

Drugs

(±)-Baclofen, γ-aminobutyric acid (GABA) and (−)U50488 hydrochloride were obtained from Research Biochemicals International (Natick, MA, U.S.A.). Concentrations of (±)baclofen (2×10^{-4} M) and GABA (3×10^{-4} M) were chosen based on concentrations used in previous studies (Blackshaw *et al*., 2000; Ferguson & Marchant, 1995; Page & Blackshaw, 1999). 3-Aminopropylphosphinic acid (3-APPA) and CGP 62349 were kindly furnished by Dr Anders Lehmann (AstraZeneca R&D, Mölndal, Sweden). Atropine, guanethidine sulphate and muscimol hydrobromide were obtained from Sigma-Aldrich (Sydney, Australia). Clonidine solution (Catapres®: 150 μg ml^{-1}) was obtained from Boehringer Ingelheim. Neuropeptide Y was obtained from Auspep (Melbourne, Australia). All drugs were dissolved in saline. (±)Baclofen stock solution (10^{-2} M) was dissolved in saline and HCl (10^{-4} M). All drugs were allowed to equilibrate with the organ bath for at least 10 min prior to testing preparation responses to other stimuli.

Results

Basal tone and responses to vagal stimulation

After a preload of 20 mN, the LOS developed an additional tone of 6.0 ± 1.7 mN. The response to vagal stimulation consisted of a frequency-dependent relaxation between 0.5–10 Hz, with maximal relaxation of approximately 50% of baseline tone at 5 Hz stimulation, followed by a delay in return to baseline of 1–2 min following stimulus cessation (Figure 1a). Prior treatment with tetrodotoxin increased basal tension (21.0 ± 8.4%) and abolished vagally-mediated LOS relaxation (Figure 1b), indicating solely neurogenic response to vagal stimulation.

Effects of GABA$_B$ receptor-selective ligands

The GABA$_B$ receptor agonist (±)baclofen (2×10^{-4} M) significantly reduced vagally-mediated LOS relaxation without affecting either basal tension or the maximal relaxation ($P<0.05$, (±)baclofen versus control; $n=6$: Figure 2). The effect of (±)baclofen was reversed with the GABA$_B$ receptor antagonist CGP 62349 (2.5×10^{-5} M; $P<0.05$, (±)baclofen + CGP 62349 versus (±)baclofen: $n=6$).

The potent and selective GABA$_B$ receptor agonist 3-APPA (10^{-4} M) did not alter basal tension of the LOS. 3-APPA elicited a dose-dependent reduction in LOS relaxation in response to vagal stimulation. This was seen as significant sequential rightward shifts in the frequency-response curves with increasing concentrations of 3-APPA (3×10^{-7}–

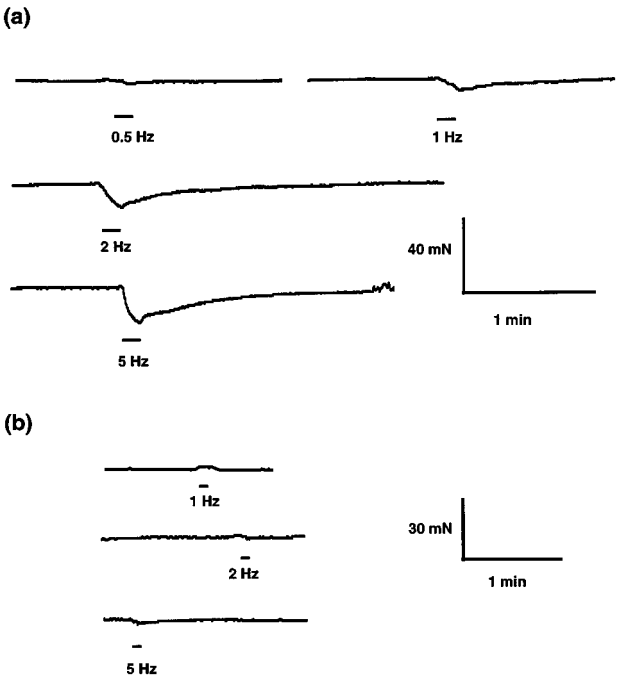

Figure 1 (a) Representative trace of the LOS response to vagal stimulation (0.5–5 Hz, 30 V, 1 ms duration for 5 s) in the ferret LOS preparation (under NANC conditions). The response consists of a rapid and frequency-dependent relaxation during stimulus, with baseline tone restored within 1–3 mins post-stimulus. (b) LOS response to vagal stimulation following tetrodotoxin pretreatment. Relaxation was effectively abolished at all frequencies of stimulation.

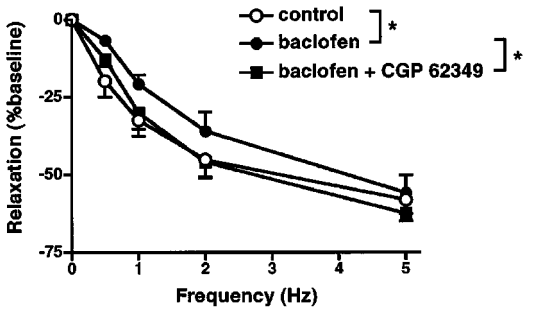

Figure 2 Relaxation of the ferret LOS; frequency-response curve in response to vagal stimulation (under NANC conditions), alone and in the presence of (±)baclofen (2×10^{-4} M) and the GABA$_B$ receptor antagonist CGP 62349 (2.5×10^{-5} M). (±)Baclofen suppressed the relaxation in response to vagal stimulation as shown by a rightward shift in the frequency-response curve (*$P<0.05$, (±)baclofen versus control; $n=6$). The effect of (±)baclofen was reversed in the presence of antagonist (*$P<0.05$, (±)baclofen + CGP 62349 versus (±)baclofen; $n=6$).

3×10^{-5} M; Figure 3). Using a sampled stimulus frequency of 1 Hz, the EC_{50} value for inhibition of LOS relaxation by 3-APPA was calculated to be 6.8×10^{-7} M (Figure 4). The effect of 3-APPA (10^{-4} M) was reversible following treatment with the GABA$_B$ receptor antagonist CGP 62349 ($P<0.05$, 3-APPA (10^{-4} M) versus 3-APPA + CGP 62349 (2.5×10^{-5} M); Figure 5).

When administered alone, the GABA$_B$ receptor antagonist CGP 62349 (2.5×10^{-5} M) did not influence either basal tension or LOS relaxation in response to vagal stimulation (Figure 6).

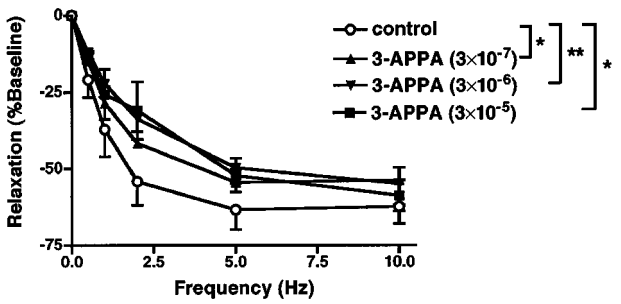

Figure 3 Relaxation of the ferret LOS in response to vagal stimulation (under NANC conditions), alone and in the presence of the GABA$_B$ receptor agonist 3-APPA (3×10^{-7}–3×10^{-5} M). 3-APPA significantly inhibited LOS relaxation at all concentrations and in a concentration-dependent manner up to 3×10^{-5} M (*$P<0.05$, 3-APPA (3×10^{-7} M) versus control, **$P<0.001$, 3-APPA (3×10^{-6} M) versus control and *$P<0.05$, 3-APPA (3×10^{-5} M) versus control: $n=6$).

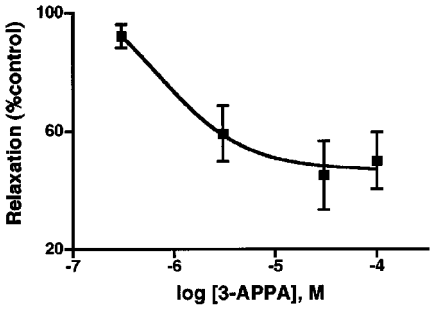

Figure 4 Effects of 3-APPA (3×10^{-7}–10^{-4} M) on inhibition of vagally-mediated LOS relaxation at 1 Hz (under NANC conditions). 3-APPA inhibited LOS relaxation in a concentration-dependent manner up to 3×10^{-5} M, with a calculated EC_{50} value of 6.8×10^{-7} M.

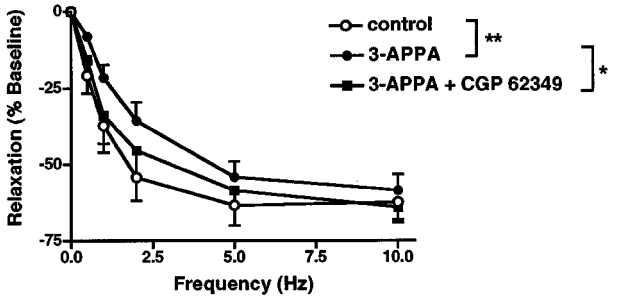

Figure 5 Relaxation of the ferret LOS in response to vagal stimulation (under NANC conditions), alone and in the presence of the GABA$_B$ receptor agonist 3-APPA (10^{-4} M) and the GABA$_B$ receptor antagonist CGP 62349 (2.5×10^{-5} M). 3-APPA significantly inhibited relaxation in response to vagal stimulation (**$P<0.001$ versus control; $n=7$). The effect of 3-APPA was significantly reversed in the presence of CGP 62349 (*$P<0.05$, 3-APPA versus 3-APPA + CGP 62349; $n=7$).

Effects of GABA$_A$ receptor-selective ligands

The GABA$_A$ receptor agonist muscimol (10^{-4} M) did not affect baseline tension or LOS relaxation in response to vagal stimulation (Figure 7). The slight rightward shift in the frequency-response curve was not significantly different versus control ($n=6$).

Effects of GABA

The endogenous nonselective GABA receptor agonist GABA (3×10^{-4} M) decreased vagally-mediated LOS relaxation without affecting basal tension or maximal relaxation, as seen by the significant rightward shift in the linear part of the frequency-response curve ($P<0.05$, GABA versus control; $n=6$: Figure 8). The effect was partly reversed with the GABA$_B$ receptor antagonist CGP 62349 (2.5×10^{-5} M), but was not significant.

Responses to other G protein-coupled receptors

Neither the α_2-adrenoceptor agonist clonidine (5×10^{-6} M), nor neuropeptide Y (NPY; 10^{-6} M) nor the κ opioid agonist (−) U50488 (10^{-5} M) influenced the relaxation in response to vagal stimulation at the frequencies employed (Table 1). All of these agonists increased basal tension of the ferret LOS (Table 1).

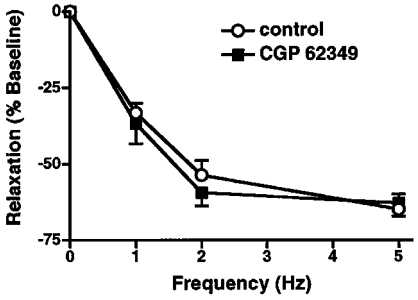

Figure 6 Relaxation of the ferret LOS in response to vagal stimulation (under NANC conditions), alone and in the presence of the GABA$_B$ receptor antagonist CGP 62349 (2.5×10^{-5} M). The antagonist alone failed to alter the relaxation in response to vagal stimulation.

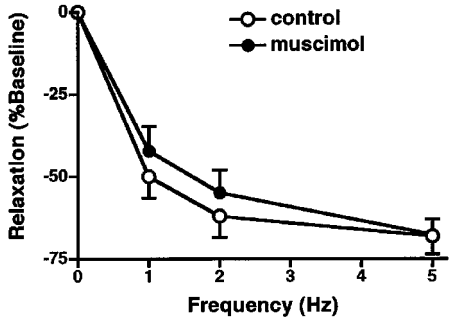

Figure 7 Relaxation of the ferret LOS in response to vagal stimulation (under NANC conditions), alone and in the presence of the GABA$_A$ receptor agonist muscimol (10^{-4} M). Muscimol failed to alter the relaxation in response to vagal stimulation.

Discussion

The present study has identified a selective and reversible inhibitory action of GABA$_B$ receptors on vagal motor pathways to the LOS. This is in contrast to other distinct G protein-coupled receptor pathways that had no influence on vagally-mediated LOS relaxation.

While it is well established that GABA$_B$ receptors operate *via* both postsynaptic and presynaptic mechanisms to inhibit neurotransmitter release in the central nervous system (Malcangio & Bowery, 1996; Misgeld *et al*., 1995; Pozza *et al*., 1999), presynaptic GABA$_B$ receptor-mediated neuronal inhibition in the periphery is less common. Typically, inhibition occurs *via* GABA$_B$ receptors located prejunctionally, for example in the guinea-pig and rat ileum (Krantis & Harding, 1987; Ong & Kerr, 1990), guinea-pig lung (Chapman *et al*., 1991), rat anococcygeus muscle (Hills *et al*., 1989) and mouse and guinea-pig vas deferens (Bowery *et al*., 1981). These effects are commonly targeted to inhibiting the release of excitatory neurotransmitters, such as acetylcholine (Chapman *et al*., 1991; Hills *et al*., 1989), but also excitatory nonadrenergic noncholinergic (NANC) transmission (Belvisi *et al*., 1989). Immunohistochemical studies have suggested that GABA, colocalized with nitric oxide synthase in motorneurones of the guinea-pig ileum, may also regulate nitric oxide release (Williamson *et al*., 1996). Functional studies suggest that both GABA$_A$ and GABA$_B$ receptors have the capacity to inhibit NO release in myenteric neurones of the guinea-pig ileum (Kilbinger *et al*., 1999). Presynaptic inhibition of acetylcholine release *via* GABA$_B$ receptors occurs at lumbar preganglionic splanchnic nerves synapsing within the

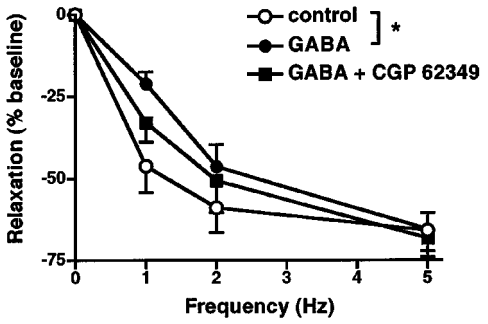

Figure 8 Relaxation of the ferret LOS in response to vagal stimulation (under NANC conditions), alone and in the presence of GABA (3×10^{-4} M) and the GABA$_B$ receptor antagonist CGP 62349 (2.5×10^{-5} M). GABA inhibited relaxation in response to vagal stimulation (*$P<0.05$, GABA versus control; $n=6$), which was partly (but not significantly) reversed following antagonist treatment.

Table 1 Effects of various agonists of G protein-coupled receptors on vagally-mediated LOS relaxation (1–5 Hz) in the ferret (under NANC conditions). $n=7$ for all experiments

	Δtension (%baseline)	*1 Hz* (%baseline)	*2 Hz* (%baseline)	*5 Hz* (%baseline)
Control		10.6±4.2	19.6±5.3	46.8±4.5
U50488	28.5±4.1	12.5±2.3	26.4±3.2	51.8±1.4
Control		13.9±2.9	25.5±6.1	52.3±6.6
NPY	14.0±3.1	13.2±1.8	32.9±5.4	52.2±6.0
Control		19.7±2.7	33.0±5.8	49.2±4.9
Clonidine	14.0±4.0	8.6±1.8	27.0±6.1	48.9±5.4

Values given as per cent baseline tension. Representative agonists: U50488 10 μM (kappa opioid receptor), NPY 1 μM (neuropeptide Y receptor), clonidine 5 μM (α_2 adrenoceptor). All of the above agonists increased basal tension in the LOS but did not significantly affect vagally-mediated relaxation of the LOS.

inferior mesenteric ganglion of the guinea-pig (Parkman *et al*., 1993). The present study demonstrates a similar effect for vagal preganglionic nerves serving inhibitory motorneurones of the ferret LOS. We can exclude a postganglionic or prejunctional site of action for $GABA_B$ receptors in this preparation, as (±)baclofen failed to modify LOS relaxation in response to direct activation of inhibitory motorneurones in isolated muscle strips of ferret LOS (Blackshaw *et al*., 2000). Thus, evidence from this study and previous studies in our laboratory shows that $GABA_B$ receptors may be activated at the level of the vagal preganglionic neurone to inhibit activation of enteric inhibitory motorneurones to the LOS.

Due to its greater potency and ease of removal by washout in this tissue compared with (±)baclofen, 3-APPA was selected to compare the $GABA_B$ receptor profile in this vagal motor pathway to the LOS with that of other known peripheral $GABA_B$ receptors. The EC_{50} value for inhibition of vagally-mediated LOS relaxation was 0.7 μM in the present study, which compares favourably with an EC_{50} value of 0.8 μM for 3-APPA's inhibition of the cholinergic twitch response in the guinea-pig ileum (Ong *et al*., 1990a) and 0.9 μM in rat anococcygeus muscle (Hills *et al*., 1989). 3-APPA has been shown to be a 7–11 fold more potent inhibitor of cholinergic twitch contractions in the guinea-pig ileum than baclofen (Hills *et al*., 1989; Ong *et al*., 1990a), but has found to have varying potency in peripheral and central tissues believed to be partly due to expression of $GABA_B$ receptor subtypes in the CNS (Bowery, 1989; Ong *et al*., 1999) and uptake by GABA transporters in the CNS (Ong & Kerr, 1998). In addition, $GABA_A$ receptor agonist properties of 3-APPA have been reported, also in the CNS (Vigot & Batini, 1999). These factors are unlikely to be considerations in our isolated *in vitro* preparation. The concentrations of GABA and (±)baclofen used in the present study are comparable with supramaximal concentrations used in previous studies in *in vitro* settings (Ferguson & Marchant, 1995; Gentilini *et al*., 1995; Page & Blackshaw, 1999).

In the presence of the $GABA_B$ antagonist CGP 62349 alone, there was no change in the LOS relaxation to vagal stimulation. This suggests either receptor redundancy, or that endogenous GABA was not recruited under the prevailing experimental conditions of electrical stimulation of the vagus nerves. This situation has been shown to occur within the inferior mesenteric ganglion of the guinea-pig, where baclofen and GABA inhibited acetylcholine release from stimulated splanchnic preganglionic nerves, but the $GABA_B$ receptor antagonist phaclofen failed to alter stimulated transmitter output alone (Parkman *et al*., 1993). This study evidenced the likely source of GABA from colonic mechanosensory nerves synapsing within the ganglion, activating splanchnic presynaptic $GABA_B$ receptors and thus providing for a negative feedback control of central outflow to lumbar colonic nerves (Parkman *et al*., 1993). A similar interaction occurring between vagal afferent and efferent pathways to the LOS is unlikely, as supranodose vagotomy, which selectively denervates vagal efferent but not afferent fibres, abolished the LOS relaxation to vagal stimulation completely (Blackshaw *et al*., 2000). This suggests no functional influence on the LOS in response to antidromic stimulation of viable extrinsic vagal afferent fibres. Thus, to have physiological relevance, intrinsic gastric afferent nerves or interneurones would need to be the source of GABA within the vagal motor pathway. From studies in the intestinal tract, most GABA immunoreactivity is in Dogiel type I neurones (Williamson *et al*., 1996) and has been shown to form pericellular networks in myenteric ganglia, suggestive of at least some neuro-neuronal connections of GABAergic fibres in the gut (Hills *et al*., 1987).

While $GABA_A$ receptors have been shown to be involved in tonic inhibition of LOS motility from within the central nervous system (Washabau *et al*., 1995), a role for peripheral $GABA_A$ receptors in LOS function in the ferret is lacking, as muscimol exerted no influence on basal and vagally-mediated LOS tone and has been shown to lack an effect on basal or stimulated LOS motility in vagotomized ferrets *in vivo* (Blackshaw *et al*., 2000). $GABA_A$ receptors mediate a variety of effects on motility and secretion from within the gut, mainly confined to direct influences on enteric motorneurones (Ong & Kerr, 1983; 1990), but also to modulate sensory input (Ashworth-Preece *et al*., 1997; Parkman *et al*., 1993). This marks the ability of GABAergic pathways to modulate both efferent and afferent transmission peripherally in the gut. The dose employed in this study was substantial in comparison to studies in which muscimol successfully altered gastrointestinal sensory activity (Parkman *et al*., 1993; Yuan *et al*., 1998a).

While $GABA_B$ receptor-mediated effects were reversible with CGP 62349, the effects of GABA itself were not. The reasons for this difference are not apparent, but recent evidence of a $GABA_C$ receptor-mediated inhibition of rat enteric synaptosomes (Sennefelder *et al*. unpublished observations) suggests that a role for $GABA_C$ receptors cannot be excluded.

In contrast to the $GABA_B$ receptor agonists, neither clonidine, neuropeptide Y nor (−) U50488 altered the LOS relaxation in response to vagal stimulation. Each of these agonists increased basal tension in the LOS, implying that the doses used exerted a pharmacologic effect. Preliminary results (not shown) indicated that this was tetrodotoxin-insensitive, suggesting receptors on smooth muscle were the likely site of action of each of these agents. Together with the $GABA_B$ receptor, α_2-adrenoceptor, NPY and κ opioid receptors all belong to the superfamily of G protein-coupled receptors (Herzog *et al*., 1992; Rohrer & Kobilka, 1998) and there is evidence to suggest that activation of the latter three receptors can all inhibit NANC bronchoconstriction in the guinea-pig airway (Matran *et al*., 1989). An effect on NANC pathways was not observed in the ferret LOS, highlighting the discrete function of $GABA_B$ receptors on vagal transmission in this model.

$GABA_B$ receptor-mediated inhibition of vagal preganglionic nerves serving the LOS is supported by our previous results using an *in vivo* model, in which (±)baclofen was able to both enhance basal LOS pressure *via* a vagal pathway and enhance a contractile component to vagally-stimulated LOS pressure in the ferret (Blackshaw *et al*., 2000). Baclofen and other $GABA_B$ ligands were also found to reduce the frequency of transient LOS relaxations and concomitant reflux episodes in healthy humans (Lidums *et al*., 2000), dogs (Lehmann *et al*., 1999) and ferrets (Blackshaw *et al*., 1999), suggestive of a therapeutic application for $GABA_B$ receptor agonists in the treatment of gastroesophageal reflux disease. This effect has been shown to be due almost entirely to the pharmacologically active R-enantiomer of baclofen (Lehmann *et al*., 1999). It is possible that $GABA_B$ receptors on vagal preganglionic neurones may be of relevance to the effects of these ligands observed both clinically and in experimental models.

In conclusion, the present study has provided evidence of a presynaptic inhibitory role for $GABA_B$ receptors on vagal preganglionic fibres serving inhibitory motorneurones in the ferret LOS. This may be a useful site in which to further test

the pharmacology of $GABA_B$ receptors and may have applications in the use of $GABA_B$ ligands as therapeutic tools for the treatment of gastrointestinal disease related to LOS dysfunction.

AstraZeneca R&D are gratefully acknowledged for their financial support of this project.

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(Received June 7, 2000
Revised July 12, 2000
Accepted July 14, 2000)