Characterization of vagal pathways mediating gastric accommodation reflex in rats

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1. We investigated the vagal pathways mediating the gastric accommodation reflex in the rat stomach.
2. Gastric distension (6 ml) evoked an increase of 9·0 ± 1·0 cmH_2O of intragastric pressure *in vivo*. Pretreatment with tetrodotoxin (TTX) caused a significant pressure increase by gastric distension, reaching 17·0 ± 1·7 cmH_2O, suggesting mediation by neural pathways.
3. The pressure increase evoked by gastric distension was significantly enhanced *in vivo* by acute truncal vagotomy (TV), hexamethonium (C6), and N^G-nitro-L-arginine methyl ester (L-NAME), but not by vasoactive intestinal polypeptide (VIP) antiserum, guanethidine, or splanchnicotomy.
4. Gastric distension (6 ml) evoked a much larger intragastric pressure in the denervated, vascularly isolated, perfused rat stomach *in vitro*. Intra-arterial application of TTX and L-NAME did not cause further pressure increases evoked by gastric distension.
5. The pressure increase evoked by gastric distension remained high 2 weeks after TV *in vivo*. However, the accommodation reflex was fully restored 4 weeks after TV *in vivo*. This reflex was antagonized by TTX, C6 and L-NAME, but not by VIP antiserum, guanethidine and splanchnicotomy.
6. Similar to *in vivo* studies, gastric distension caused a smaller increase in intragastric pressure in response to gastric distension in the denervated, vascularly isolated, perfused stomach obtained from rats 4 weeks after vagotomies *in vitro*. The pressure increase evoked by gastric distension was significantly enhanced by L-NAME, hexamethonium and TTX.
7. It is suggested that the vago–vagal reflex plays an important role in mediating the accommodation reflex. This involves a vagal efferent pathway that uses nitric oxide as a final neurotransmitter mediating gastric relaxation in intact rats. It is also suggested that the adaptive mechanism mediating the accommodation reflex following vagotomy occurs in the gastric myenteric plexus.

The accommodation reflex allows the stomach to receive large volumes during food intake with minimal increases in pressure (Canon & Lieb, 1911; Grey, 1917). The vagal nerve has been demonstrated to play an important role in mediating this reflex (Wilbur & Kelly, 1973; Jahnberg, Abrahamsson, Jansson & Martinson, 1977; Andrews, Grundy & Lawes, 1980; Takasugi, Ueda, Kurata, Kodama, Ezaki & Fujii, 1982). Vagal fibres activate postganglionic elements in the gastric wall that use non-adrenergic, non-cholinergic (NANC) neurotransmitters to mediate gastric relaxation (Abrahamsson & Jansson, 1969; Wilbur & Kelly, 1973; Andrews *et al.* 1980; Takasugi *et al.* 1982). However, the specific nature of the neurotransmitters released from the NANC neurons of the gastric myenteric plexus during the accommodation reflex remains to be characterized.

Vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) have been proposed as probable neurotransmitters of NANC neurons. VIP immunoreactivity is present in the intrinsic neurons of the stomach (Larsson, Fahrenkrug, Schaffalitzky, Sundler, Hakanson & Rehfeld, 1976). Vagal stimulation produces a frequency-dependent increase of VIP release into the portal vein (Yasui *et al.* 1987; Reid, Shulkes & Titchen, 1988; Takahashi & Owyang, 1995). These observations support the concept that VIP is an inhibitory NANC transmitter. In studies of cat gastric fundus, the VIP antagonist fails to prevent NANC-induced relaxation and

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there are no similarities between VIP-induced relaxation and NANC-induced relaxation (D'Amato, De Beurme & Lefebvre, 1988). However, in the rat stomach gastric relaxation induced by stimulation of the NANC nerve is significantly antagonized by the NO biosynthesis inhibitors, N^G-nitro-L-arginine (L-NNA) and N^G-nitro-L-arginine methyl ester (L-NAME) (Boeckxstaens *et al.* 1991; D'Amato, Curro & Montuschi, 1992; Shimamura, Fujisawa, Toda & Sunano, 1993; Takahashi & Owyang, 1995). Exogenously applied NO produces relaxation that mimics NANC-induced relaxation in the fundus (Boeckxstaens *et al.* 1991), ileum (Osthaus & Galligan, 1992), and ileo-colonic junction (Bult, Boeckxstaens, Pelckmans, Jordaens, Van Maercke & Herman, 1990). The presence of NO synthase is detected in the myenteric plexus (Bredt, Hwang & Snyder, 1990; Aimi, Kimura, Kinoshita, Minami, Fujimura & Vincent, 1993) and the central nervous system (Bredt *et al.* 1990). The rapid gastric relaxation in response to vagal stimulation is inhibited by L-NAME (Lefebvre, De Vriese & Smits, 1992). These observations suggest that NO may be involved in mediating gastric relaxation. We have previously shown that vagal stimulation produces two different modes of relaxation in the vascularly isolated, perfused rat stomach: a rapid relaxation followed by a prolonged relaxation. The rapid relaxation is antagonized by the NO inhibitor, whereas the prolonged relaxation is blocked by the VIP antagonist. This suggests that different neurotransmitters mediate different modes of relaxation (Takahashi & Owyang, 1995). It is not known which neurotransmitters in the gastric myenteric plexus mediate the accommodation reflex.

Although vagal stimulation has been shown to produce gastric relaxation, normal accommodation reflex has been demonstrated in patients with chronic vagotomies (Hertley & Mackie, 1991). Therefore, the role of the vagal nerve in mediating the accommodation reflex remains controversial.

We hypothesize that the gastric accommodation reflex is mediated by the vago–vagal reflex that uses NO as the final neurotransmitter to mediate gastric relaxation. Following chronic vagotomy, gastric accommodation reflex returns over time, an adaptation that involves activation of the gastric myenteric plexus to mediate gastric relaxation.

Therefore, the aims of this study were: (1) to characterize the role of the vagal pathway and NANC neurons in the gastric myenteric plexus in mediating the accommodation reflex, and (2) to investigate the adaptive mechanisms that mediate the accommodation reflex following vagotomy.

METHODS

Materials

The following drugs were used: L-arginine, atropine, capsaicin, guanethidine, hexamethonium, phentolamine, propranolol, and tetrodotoxin (Sigma), L-NAME (Research Biochemicals Incorporated, Natick, MA, USA), and VIP antiserum (Peninsula, Belmont, CA, USA).

Animal preparation

To investigate whether the vagal nerve and splanchnic nerve are involved in the mediation of the accommodation reflex, we performed *in vivo* experiments using male Sprague–Dawley rats, weighing 225–250 g. These rats were fasted for 12–16 h and anaesthetized by an intramuscular injection of xylazine and ketamine (13 and 87 mg (kg body wt)$^{-1}$, respectively). If necessary, supplementary doses of ketamine (30 mg kg^{-1}) were administered to maintain adequate anaesthesia. Ketamine has minimal effects on the autonomic nervous system in rats. We have previously reported that cholecystokinin octapeptide (CCK8) mediates pancreatic amylase secretion via the vago–vagal reflex in ketamine-anaesthetized rats (Li & Owyang, 1993). Similar observations were made in conscious rats (Li & Owyang, 1996). A polyethylene catheter was placed in the jugular vein for the infusion of various antagonists. A mid-line incision was made and a pressure transducer (Millar microtip catheter (1 mm o.d.), PC 350, Millar Instruments, Houston, TX, USA) was inserted into the rat stomach through the pylorus to record intragastric pressure. At the same time a polyethylene catheter (3 mm o.d.) was inserted into the stomach through the pylorus to infuse saline for gastric distension and the pylorus was tightly ligated with 3/0 silk. The subdiaphragmatic vagal trunks were carefully exposed halfway between the diaphragm and the gastric cardia. Without damaging the vagal trunks, the oesophagus was ligated 0·5 cm above the cardia to prevent a loss of pressure. The stomach was inflated with prewarmed saline (6 ml) at a rate of 1 ml min^{-1} and the intraluminal pressure was recorded. A volume of 6 ml was chosen because it approximated the volume of the intragastric contents of non-fasted rats (body weight, 225–250 g). In preliminary experiments, the pressure increase evoked by gastric distension (6 ml) was reproducible up to five times when applied every 30 min. Therefore, gastric distension was performed every 30 min and evaluated in triplicate and the mean value was used to calculate the intraluminal pressure. After infusion of various antagonists and extrinsic neural ablation (vagotomy and splanchnicotomy), gastric inflation studies were repeated and the pressure increases were compared with control. Only one antagonist was administered to each rat. At the end of the experiments, rats were killed by an overdose of pentobarbitone given I.V. (200 mg kg^{-1}).

Perivagal application of capsaicin

Capsaicin (10 mg) was sonicated with 0·1 ml Tween 80 for 10 min, made up to 1 ml with olive oil and mixed thoroughly. Rats were anaesthetized by an intramuscular injection of xylazine and ketamine (13 and 87 mg (kg body wt)$^{-1}$, respectively). After anaesthesia, the cervical vagal trunks were exposed as previously described (Raybould & Tache, 1988). A small piece of gauze soaked in capsaicin was placed around the nerve trunk for 30 min. The surrounding area was covered with gauze that was frequently replaced to minimize the spread of capsaicin to surrounding tissues. Additional capsaicin was applied perivagally every 5 min. The maximum dose of capsaicin applied was 0·1 ml (1 mg per rat). The area was thoroughly rinsed with olive oil followed by saline and dried with sterile swabs. The incisions were closed. Experiments were performed 7 days after surgery. Rats in which the vagi were treated with 10% Tween 80 in olive oil served as vehicle controls. A similar method demonstrated that perivagal capsaicin treatment significantly reduced pancreatic enzyme secretion stimulated by physiological doses of CCK infusion (Li & Owyang, 1993).

Surgical treatment

Rats were anaesthetized by an intramuscular injection of xylazine and ketamine (13 and 87 mg (kg body wt)$^{-1}$, respectively. Splanchnic

Table 1. Effects of various neural blocking agents and surgical neural ablation on the intragastric pressure increase evoked by gastric distension (6 ml) in intact rats *in vivo*

Agent (n)	Pressure increase (cmH_2O)	
	Without	With
Phentolamine (3)	9·0 ± 1·2	9·2 ± 1·0
Propranolol (3)	8·9 ± 1·0	9·1 ± 1·3
Guanethidine (4)	9·2 ± 0·9	9·1 ± 1·0
Splanchnicotomy (5)	8·9 ± 1·2	9·1 ± 1·3
Atropine (4)	9·1 ± 1·1	9·1 ± 1·2
VIP antiserum (5)	9·0 ± 1·2	9·1 ± 1·0
Perivagal capsaicin (5)	8·9 ± 1·0	9·1 ± 1·2

The pressure increase evoked by gastric distension (6 ml) was not affected by guanethidine, splanchnicotomy, or VIP antiserum, suggesting that the sympathetic and VIP pathways are not involved. Perivagal treatment with capsaicin did not affect the pressure increase evoked by gastric distension. This suggests that the vagal sensory pathway mediating the accommodation reflex does not involve capsaicin-sensitive afferent fibres in intact rats.

nerve section was performed by deflecting the stomach and spleen to the right of the rat, facilitating identification of the nerves and coeliac ganglion. Splanchnicotomy and abdominal vagotomy were performed 30 min before the stomach inflation studies.

To investigate the effects of chronic vagotomy on the response to gastric distension, Sprague–Dawley rats, weighing 225–250 g, were fasted for 12–16 h and anaesthetized by an intramuscular injection of xylazine and ketamine (13 and 87 mg (kg body wt)$^{-1}$, respectively). The rats underwent either a bilateral abdominal truncal vagotomy or a sham operation. The abdominal wall was closed by 3/0 nylon. No special post-operative care was necessary. The rats were fed the day after the operation. At 2 and 4 weeks after the truncal vagotomy, the stomach was distended with 6 ml of saline and the intraluminal pressure was recorded in the presence and absence of various antagonists.

Antagonist study

To determine the role of cholinergic pathways in the mediation of the gastric accommodation reflex, atropine (50 μg (kg body wt)$^{-1}$) was injected bolus and continuously infused at a rate of 20 μg kg^{-1} h^{-1} for 20 min before the stomach inflation studies. Similar studies were performed with hexamethonium treatment (20 mg (kg body wt)$^{-1}$ bolus plus 10 mg (kg body wt)$^{-1}$ h^{-1} continuous infusion) to determine the role of presynaptic cholinergic neurons in the mediation of the accommodation reflex. For total neural blockade, rats under artificial ventilation received tetrodotoxin (TTX, 36 μg (kg body wt)$^{-1}$), which was injected bolus and also continuously infused at the rate of 2 μg (kg body wt)$^{-1}$ h^{-1} for 20 min before the stomach inflation studies, as previously described (Li & Owyang, 1993). To determine the role of NO and VIP neurons in the gastric myenteric plexus, L-NAME (10 mg (kg body wt)$^{-1}$ bolus) and VIP antiserum (1 : 25 dilution, 40 μl (kg body wt)$^{-1}$ bolus) were infused 20 min before the stomach inflation studies. The specific doses of TTX and hexamethonium were chosen because they are known to abolish vagally stimulated gastric motor responses. The specific doses of L-NAME and VIP antiserum have been shown to abolish the rapid relaxation and prolonged relaxation, respectively, in response to vagal stimulation *in vivo* (Takahashi & Owyang, 1995). VIP antiserum also abolishes exogenously applied VIP (100 pmol)-induced relaxation (Bojo, Lefebvre, Nellgard & Cassuto, 1993). For investigation of the possible involvement of the sympathetic pathway in mediating the accommodation reflex, guanethidine (5 mg (kg body wt)$^{-1}$ bolus), phentolamine (1 mg (kg body wt)$^{-1}$ bolus), or propranolol (1 mg (kg body wt)$^{-1}$ bolus) were infused 20 min before the experiment, as previously reported (Raybould, Roberts & Dockray, 1987).

Isolated perfused rat stomach

To investigate whether the local myenteric plexus is involved in the mediation of the accommodation reflex before and after vagotomy, we performed *in vitro* experiments using a denervated, vascularly isolated, perfused stomach, as previously described (Yokotani, Okuma, Nakamura & Osumi, 1993; Takahashi & Owyang, 1995). Rats were anaesthetized by an intramuscular injection of xylazine and ketamine (13 and 87 mg (kg body wt)$^{-1}$, respectively. After opening the abdomen with a mid-line incision, the abdominal aorta was exposed retro-peritoneally. The coeliac artery was identified and the abdominal aorta was ligated just above the branching of the coeliac artery; a cannula was inserted into the coeliac artery. The stomach was perfused through the coeliac artery with a peristaltic pump (Harvard Apparatus, South Natick, MA, USA) at a constant flow rate of 2 ml min^{-1}. The perfusate was a modified Krebs–Henseleit bicarbonate (KHB) buffer containing (mM): 118 NaCl, 4·8 KCl, 2·5 $CaCl_2$, 25 $NaHCO_3$, 1·2 KH_2PO_4, 1·2 $MgSO_4$, 11·1 glucose; 0·2% (w/v) bovine serum albumin and 4% (w/v) dextran. The perfusate was maintained at pH 7·4 and 37 °C, bubbled with a mixture of 95% O_2–5% CO_2. The oesophagus, duodenum, spleen and pancreas were dissected after the vessels were ligated. The vascularly isolated perfused stomach was kept in a chamber prewarmed at 37 °C. Following isolation of the stomach, rats were killed by an overdose of pentobarbitone given I.V. (200 mg kg^{-1}) (Takahashi & Owyang, 1995). After washing the gastric contents, a cannula and a pressure transducer were inserted into the stomach through the pylorus. The stomach was inflated with saline (6 ml) at the rate of 1 ml min^{-1} and the intraluminal pressure was recorded with and without the pretreatment of TTX (10^{-7} M), hexamethonium (10^{-4} M) or L-NAME (10^{-4} M). TTX, hexamethonium and L-NAME were infused into the coeliac artery for 20 min before gastric inflation. In this preparation, optimal relaxations were observed in response to vagal stimulation, intra-

arterially applied NO, VIP, and the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperizinium (DMPP) (Takahashi & Owyang, 1995).

Statistical analysis

Data were expressed as means ± S.E.M. Statistical analysis was performed on each group using Student's paired t test or a two-way analysis of variance (ANOVA). Significance was accepted at the 5% level.

RESULTS

In vagally intact (control) rats, gastric distension caused a linear increase in intragastric pressure, but gastric distension beyond 3–4 ml caused a much smaller increase in intragastric pressure (Fig. 1*A*). Gastric distension with 6 ml of saline infusion for 6 min (1 ml min^{-1}) evoked an increase of 9·0 ± 1·0 cmH_2O of intragastric pressure. A slower rate of gastric distension (0·5 ml min^{-1}) with the same volume of saline (6 ml) evoked a similar increase in intragastric pressure (data not shown). Pretreatment with TTX significantly changed the slope of the volume–pressure curve; and produced a steeper increase in pressure with progressive gastric volume distension. Gastric distension (6 ml) evoked an increase in intragastric pressure to 17·0 ± 1·7 cmH_2O following pretreatment with TTX. This was significantly higher than control (F, 239·9; d.f., 1,48, $P < 0·0001$; ANOVA, where F is the variance ratio and d.f. are degrees of freedom, Fig. 1*A*). These data suggest that the increase in gastric pressure in response to distension is neurally mediated. Truncal vagotomy (TV) also significantly increased the slope of the volume–pressure curve compared with control, reaching 16·8 ± 1·9 cmH_2O when the stomach was distended with 6 ml of saline (F at 104·5; d.f., 1,48; $P < 0·0001$, ANOVA) (Fig. 1*B*). This suggests that the vagal nerve plays an important role in mediating the gastric pressure response to distension.

To investigate the involvement of the adrenergic pathway in mediating the gastric pressure response to distension, we examined the effects of phentolamine, propranolol and guanethidine. In contrast to pretreatment with TTX and TV, an increase in intragastric pressure in response to gastric distension was not affected by phentolamine, propranolol, or guanethidine (Table 1). The slope of the volume–pressure curve evoked by gastric distension was not affected by these antagonists. Similarly, splanchnicotomy did not affect pressure increase by gastric distension (Table 1), suggesting that the sympathetic pathway does not mediate a pressure response to distension. Increase in intragastric pressure by gastric distension (6 ml) was also unaffected by pretreatment with atropine, a muscarinic receptor antagonist (Table 1).

To investigate whether the nicotinic receptor is involved in the gastric pressure response to distension, we studied the effects of hexamethonium. Pretreatment with hexamethonium significantly enhanced the pressure increase evoked by gastric distension compared with control, reaching 14·5 ± 1·8 cmH_2O when the stomach was distended with 6 ml of saline (F at 56·6, d.f. at 1,48, and $P < 0·0001$, ANOVA) (Fig. 2*A*).

Nitric oxide neurons and VIP neurons in the gastric myenteric plexus may mediate gastric relaxation (Larsson *et al.* 1976; Bult *et al.* 1990). The release of these neuro-

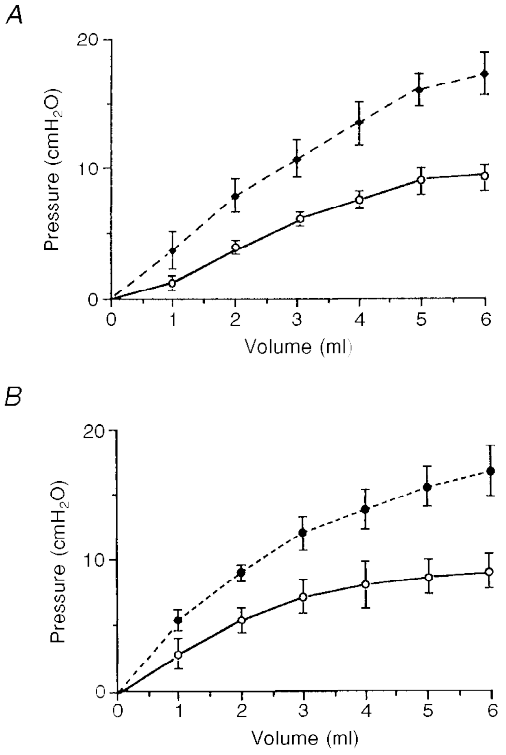

Figure 1. Effects of tetrodotoxin and truncal vagotomy *in vivo*
Effects of tetrodotoxin (◆, TTX; *A*) and truncal vagotomy (●, TV; *B*) on the intragastric pressure increase evoked by gastric distension of the stomach of intact rats *in vivo*. Gastric distension with 6 ml saline evoked an increase from control (○) in intragastric pressure of 9·0 ± 1·0 cmH_2O. TTX and TV caused a significant pressure increase evoked by gastric distension, reaching 17·0 ± 1·7 and 16·8 ± 1·9 cmH_2O, respectively. Means ± S.E.M., $n = 5$.

Table 2. Effects of various neural blocking agents and surgical neural ablation on the intragastric pressure increase evoked by gastric distension (6 ml) in rats with chronic vagotomies (4 weeks) *in vivo*

	Pressure increase (cmH_2O)	
Agent (n)	Without	With
Guanethidine (3)	9·1 ± 0·8	9·1 ± 1·2
Splanchnicotomy (3)	8·9 ± 1·1	9·2 ± 1·4
VIP antiserum (3)	9·0 ± 1·2	9·1 ± 1·5
L-NAME (4)	8·8 ± 1·0	13·5 ± 1·6*
Hexamethonium (4)	8·9 ± 1·0	13·9 ± 1·2*
TTX (4)	8·9 ± 1·0	16·3 ± 2·5*

The pressure increase evoked by gastric distension (6 ml) was not affected by guanethidine, splanchnicotomy, or VIP antiserum, suggesting that the sympathetic and VIP pathways are not involved following chronic vagotomy (4 weeks). However, the pressure increase evoked by gastric distension (6 ml) was significantly enhanced by L-NAME, hexamethonium, and TTX (* $P < 0·05$).

transmitters has been shown to be controlled by vagal efferent fibres (Larsson *et al.* 1976; Bult *et al.* 1990; Crist, He & Goyal, 1992; Takahashi & Owyang, 1995). To characterize the vagal efferent pathway mediating the gastric pressure response to distension, we investigated the role of NO and VIP neuropathways. Pretreatment with the NO biosynthesis inhibitor, L-NAME (10 mg (kg body wt)$^{-1}$) significantly enhanced the pressure increase evoked by gastric distension compared with control, reaching 13·8 ± 1·9 cmH_2O when the stomach was distended with 6 ml of saline (F at 40·2; d.f. at 1,48; and $P < 0·0001$; ANOVA) (Fig. 2*B*). This suggests that NO neurons are involved in the mediation of the pressure response to distension. The stimulatory effect of L-NAME on the pressure increase evoked by gastric distension was antagonized by pre-administration of L-arginine (100 mg (kg body wt)$^{-1}$; data not shown). In contrast, administration of rabbit VIP antiserum (1 : 25 dilution, 40 μl (kg body wt)$^{-1}$) had no effect on the pressure increase evoked by gastric distension (6 ml; Table 1).

To characterize the vagal afferent fibres mediating the gastric pressure response to distension, we studied the effects of perivagal capsaicin treatment on the pressure increase evoked by gastric distension. In perivagal capsaicin-

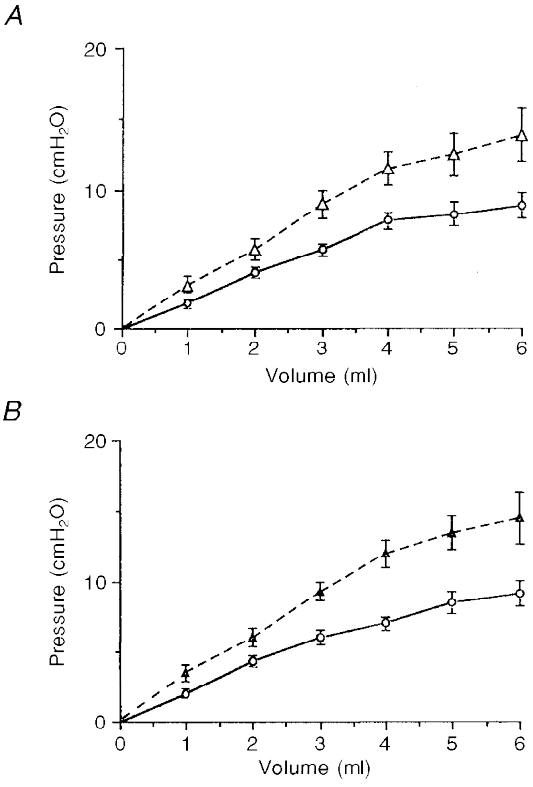

Figure 2. Effects of hexamethonium and L-NAME *in vivo*
Effects of hexamethonium (△, *A*) and the nitric oxide biosynthesis inhibitor, L-NAME; (▲, *B*) on the intragastric-pressure increase evoked by gastric distension of the rat stomach *in vivo*, compared with control (○). Hexamethonium and L-NAME significantly enhanced the pressure increase evoked by gastric distension (6 ml), reaching 14·5 ± 1·8 cmH_2O and 13·8 ± 1·9 cmH_2O, respectively. Means ± S.E.M., $n = 5$.

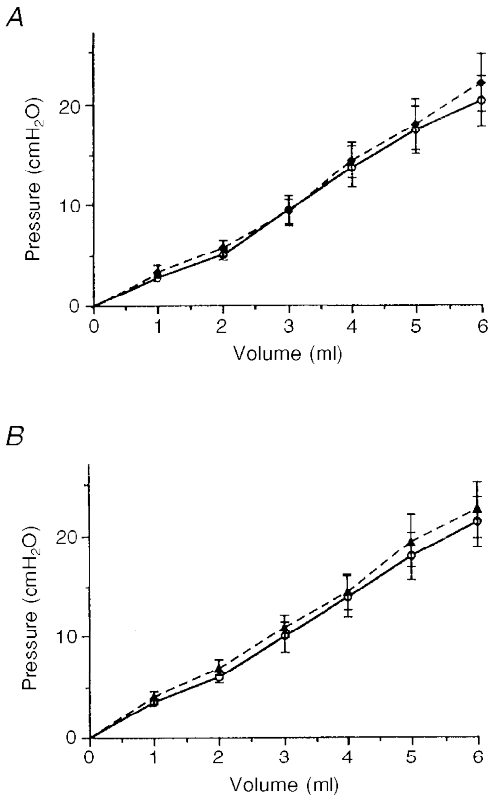

Figure 3. Effects of tetrodotoxin and L-NAME *in vitro*

Effects of TTX (◆; *A*) and L-NAME (▲; *B*) on the intragastric pressure increase evoked by gastric distension of the rat stomach *in vitro*. Intragastric pressure increased linearly with gastric distension reaching more than 20 cmH_2O in the denervated, vascularly isolated, perfused stomach distended with 6 ml of saline. TTX (10^{-7} M) and L-NAME (10^{-4} M) did not cause further pressure increases, suggesting that intramural neural pathways are not involved. Control, ○. Means ± S.E.M., $n = 4$.

treated rats the pressure increase evoked by gastric distension (6 ml) was 9·1 ± 1·2 cmH_2O. This was not significantly different from observations in vehicle-treated rats (Table 1). The slope of the volume–pressure curve was not affected by perivagal capsaicin treatment (data not shown).

To investigate whether the local myenteric plexus is involved in the mediation of gastric pressure response to distension, we performed *in vitro* experiments using a vascularly isolated perfused rat stomach. In the vascularly isolated perfused rat stomach, we have previously demonstrated that electrical stimulation of the vagal trunk produced a triphasic response which comprised a rapid transient relaxation (first phase) followed by a phasic contraction (second phase) and a delayed prolonged relaxation (third phase). Intra-arterial infusion of L-NAME (10^{-4} M) significantly antagonized the rapid relaxation, suggesting the mediation of NO release from the gastric myenteric plexus (Takahashi & Owyang, 1995).

Compared with the *in vivo* studies, gastric distension caused a much larger increase in intragastric pressure, reaching 20·3 ± 2·5 cmH_2O, when 6 ml of saline was administered to the denervated stomach. Intra-arterial application of TTX (10^{-7} M) and L-NAME (10^{-4} M) did not cause any further pressure increases evoked by gastric distension (6 ml; Fig. 3*A* and *B*). This suggests that an intramural pathway does not mediate gastric relaxation in an extrinsically denervated stomach.

To investigate whether adaptive changes in the mediation of accommodation reflex occur after vagotomy, we performed gastric inflation studies using rats with a chronic vagotomy.

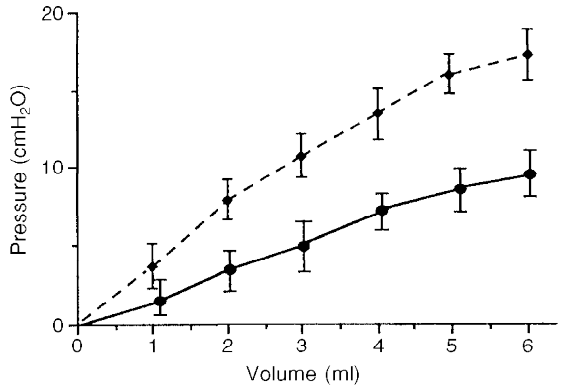

Figure 4. The intragastric pressure increase evoked by gastric distension of the rat stomach 2–4 weeks after vagotomy *in vivo*

Gastric distension with 6 ml saline evoked an increase in intragastric pressure of 16·0 ± 2·1 cmH_2O, 2 weeks after vagotomy (◆), similar to the increase after acute vagotomy. The gastric accommodation reflex was fully restored 4 weeks after vagotomy (●), producing a volume–pressure curve similar to that observed in intact rats or sham-operated rats. Means ± S.E.M., $n = 4$.

Table 3. Effects of various neural blocking agents and surgical neural ablation on the increase of intragastric pressure evoked by gastric distension (6 ml) in denervated, vascularly isolated, perfused stomach obtained from rats 4 weeks following vagotomy

	Pressure increase (cmH_2O)	
Agent (n)	Without	With
L-NAME (4)	$10{\cdot}3 \pm 1{\cdot}3$	$14{\cdot}6 \pm 2{\cdot}0$*
Hexamethonium (4)	$10{\cdot}3 \pm 1{\cdot}6$	$14{\cdot}8 \pm 1{\cdot}8$*
TTX (4)	$10{\cdot}2 \pm 1{\cdot}5$	$18{\cdot}9 \pm 2{\cdot}8$*

The pressure increase evoked by gastric distension (6 ml) was significantly enhanced by L-NAME, hexamethonium, and TTX in rats with chronic vagotomies (4 weeks) *in vitro* (* $P < 0{\cdot}05$).

Sham-operated animals served as controls. There was no significant difference in body weight gain between TV and sham-operated animals 2–4 weeks after the operation. Two weeks after TV, intraluminal pressure increase in response to gastric distension (6 ml) remained high *in vivo* (Fig. 4) and was the same as that observed after acute vagotomy (Fig. 1*B*). In these rats, the intraluminal-pressure increase evoked by gastric distension was not affected by TTX (data not shown), indicating the loss of naturally mediated gastric relaxation.

However, 4 weeks after vagotomy the intraluminal pressure increase evoked by gastric distension was similar to that observed in the intact rats *in vivo* (Fig. 4). The pressure increase evoked by gastric distension was significantly enhanced by L-NAME, hexamethonium and TTX in these rats, also similar to the intact rats or sham-operated rats (Table 2). To see if the splanchnic nerve is involved in the adaptive changes following chronic vagotomy, we studied the effects of guanethidine and splanchnicotomy. In contrast to pretreatment with L-NAME and hexamethonium, the intraluminal pressure increase evoked by gastric distension was not affected by guanethidine or by splanchnicotomy, suggesting that the sympathetic pathway is not involved in the mediation of the accommodation reflex following vagotomy. Administration of rabbit VIP antiserum also had no effect on the accommodation reflex in rats with a chronic vagotomy (i.e. 4 weeks post vagotomy) *in vivo* (Table 2).

To investigate whether the local myenteric plexus is involved in the mediation of the accommodation reflex following chronic vagotomy (4 weeks post vagotomy), *in vitro* experiments were performed using a denervated, vascularly isolated, perfused stomach. Similar to the *in vivo* studies, gastric distension caused a smaller increase in intragastric pressure, reaching $10{\cdot}3 \pm 1{\cdot}3$ cmH_2O, when 6 ml of saline were administered to the isolated stomach obtained from chronically vagotomized rats. This increase was significantly lower than that observed in stomach obtained from sham-operated rats (F at 40·9, d.f. at 1,48 and $P < 0{\cdot}001$, ANOVA; Fig. 5). The pressure increase evoked by gastric distension (6 ml) was significantly enhanced by L-NAME (10^{-4} M), hexamethonium (10^{-4} M) and TTX (10^{-7} M) in rats with chronic vagotomies (4 weeks) *in vitro* (Table 3). This suggests that an intramural pathway within the gastric wall mediates gastric relaxation in stomach following chronic vagotomy.

DISCUSSION

In this study, the pressure increase evoked by gastric distension was significantly enhanced by truncal vagotomy (TV) of the rat stomach, confirming previous reports that the vagal nerve plays an important role in the mediation of the accommodation reflex in ferrets and dogs (Wilbur & Kelly, 1973; Andrews *et al.* 1980; Takasugi *et al.* 1982). Gastric distension induced-pressure increase after TV was

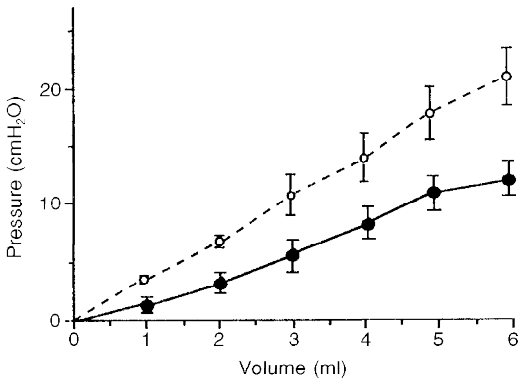

Figure 5. Increase in intragastric pressure evoked by gastric distension in the denervated, vascularly isolated, perfused stomach obtained from rats 4 weeks after vagotomy

Gastric distension caused a smaller increase in intragastric pressure, reaching $10{\cdot}3 \pm 1{\cdot}3$ cmH_2O, when 6 ml of saline was administered to the isolated stomach obtained from chronically vagotomized (●) rats. This increase was significantly lower than that observed in stomach obtained from sham-operated (○) rats. Means ± S.E.M., $n = 4$.

similar to that observed in rats pretreated with TTX, suggesting that the vagal pathway is the predominant neural pathway mediating the intragastric pressure in response to gastric distension of the rat stomach.

It has been demonstrated that the mechanoreceptors on vagal afferents have low thresholds, and that the nerve fibres transmitting from high threshold mechanoreceptors project to the spinal cord. Spinal afferents transmit signals of noxious events; vagal fibres monitor peristalsis and trigger secondary peristalsis. In contrast to the relatively high threshold for the activation of splanchnic afferents, vagal afferents respond to more physiological stimuli (Grundy, 1988). Our studies confirmed this concept by demonstrating that the intragastric pressure increase evoked by gastric distension was unaffected by guanethidine or splanchnicotomy. Electrical stimulation of the peripheral end of the splanchnic nerves evokes gastric relaxation through activation of adrenoreceptors located on the muscle cells (Kreulen, Muir & Szurszewski, 1983). However, our present study suggests that the splanchnic neural pathway does not play a significant role in the regulation of intragastric pressure if the physiological level of distension is employed.

We recently demonstrated that the preganglionic fibres in the vagal trunk are connected via nicotinic synapses to at least three types of postganglionic neurons that contain acetylcholine, NO, and VIP and serve as neurotransmitters to mediate gastric contraction and different relaxation modes in the rat stomach (Takahashi & Owyang, 1995). We also demonstrated that stimulation of the vagal nerve with low and high frequencies preferentially releases NO and VIP, respectively. The frequency-dependent release of neurotransmitters in response to nerve stimulation has been demonstrated previously. Vagal nerve-stimulated ACh release has been shown to be maximum at 5 Hz (Yokotani *et al.* 1993), whereas higher frequencies (10–50 Hz) stimulate VIP release (Agoston, Conlon & Whittaker, 1988). Recently we showed that vagal stimulation evoked the maximum NO release at 2·5 Hz (Takahashi & Owyang, 1995).

In the present study, neither rabbit VIP antiserum nor atropine affected the pressure increase induced by gastric distension. In contrast, the NO biosynthesis inhibitor, L-NAME, significantly enhanced pressure increases induced by gastric distension. Therefore, it seems that the vagal inhibitory and excitatory fibres are not simultaneously activated by gastric distension. We propose that gastric distension preferentially stimulates NO neurons in the gastric myenteric plexus. Consistent with our results, Grundy, Gharib-Naseri & Hutson, 1992 have shown that gastric pressure increase in response to 20 ml of ramp distension is not affected by VIP immunoneutralization in the ferret stomach. They concluded that the NANC inhibitory mechanism regulating corpus tone to accommodate gastric distension does not involve VIP neurons.

Hexamethonium also significantly enhanced the pressure increase induced by gastric distension, producing an effect similar to that observed with L-NAME. We have previously shown that in the isolated perfused rat stomach, the nicotinic receptor agonist, DMPP (10^{-6} to 10^{-4} M), significantly increased NO production in a dose-dependent manner, which was abolished by hexamethonium and TTX (Takahashi & Owyang, 1995). These observations confirmed that vagal stimulation of NO release from the gastric myenteric plexus is mediated by nicotinic synapses.

The effects of hexamethonium on the pressure increase evoked by gastric distension were less than those of TTX and TV. This suggests that neural pathways other than nicotinic transmission may be involved in mediating the pressure response to gastric distension. In addition, since the effects of L-NAME on the pressure increase evoked by gastric distension were also less than those of TTX and TV, we cannot exclude the possibility that other inhibitory neurotransmitters are involved in mediating the pressure response to gastric distension in the rat stomach. It has been suggested that along with NO and VIP, adenosine triphosphate (ATP) may be a NANC neurotransmitter in the gastrointestinal tract (Crist *et al.* 1992; Keef, Du, Ward, McGregor & Sanders, 1993). More studies are needed to determine the role of ATP neurons in the mediation of this reflex.

The involvement of an intramural NANC pathway in mediating gastric relaxation in response to distension has been demonstrated in the isolated guinea-pig stomach *in vitro* (Desai, Sessa & Vane, 1991). To investigate the involvement of an intramural pathway in mediating the accommodation reflex in the rat stomach, we performed *in vitro* experiments using a vascularly isolated, perfused rat stomach. Intragastric pressure increased linearly with gastric distension reaching over 20 cmH_2O when 6 ml of saline was administered. TTX (10^{-7} M) and L-NAME (10^{-4} M) did not cause any further pressure increases evoked by gastric distension *in vitro*, suggesting lack of involvement of a local reflex pathway in the rat stomach. The importance of the vagal nerve in mediating balloon-induced lower oesophageal sphincter (LES) relaxation has been demonstrated in the dog (Price, El-Sharkawy, Mui & Diamant, 1979). Similar to our results, they showed that intramural neural pathways are not involved in mediating LES relaxation (Price *et al.* 1979).

Vagal afferent fibres may terminate in the mucosa or muscle layer of the gastrointestinal tract (Grundy, 1988). These afferent receptors transmit sensory information to the central nervous system and play an important role in the vago–vagal reflex. Generally, tension receptors are located in the serosa and/or muscle layers and mechanoreceptors are found in the mucosa (Grundy, 1988). However, the specific nature of the sensory receptors that mediate the accommodation reflex remains to be determined. Capsaicin is a neurotoxin that impairs the function of sensory C fibres

and if applied to the vagus nerve, inhibits the action of CCK on gastric motility and emptying (Raybould & Tache, 1988). However, in our study, perivagal capsaicin treatment failed to affect the intragastric pressure increase evoked by gastric distension. This observation is consistent with the previous report that vagal discharge following gastric distension was not affected by perivagal capsaicin treatment (Raybould & Davison, 1989). In our laboratory we demonstrated that perivagal capsaicin treatment markedly reduced the labelling of neurons in the nodose ganglia if the dye was applied to the mucosa but it had little effect on the labelling if the dye was injected into the serosa and/or muscle layer (Wang, Li & Owyang, 1994). These observations suggest that vagal afferent fibres that transmit sensory information from the tension receptors in the muscle layer are capsaicin insensitive.

As previously demonstrated, vagal efferent pathway utilizes nicotinic receptors to stimulate NO release from the gastric myenteric plexus (Takahashi & Owyang, 1995). The intragastric pressure increase in response to saline infusion was significantly increased by hexamethonium or L-NAME treatment. This strongly suggests that the vagal efferent pathway mediating gastric accommodation reflex involves the release of NO from the gastric myenteric plexus via nicotinic receptor stimulation. It has been demonstrated that the vago–vagal reflex plays an important role in mediating gastric relaxation during food intake (Wilbur & Kelly, 1973; Andrews *et al.* 1980; Takasugi *et al.* 1982). However, patients with a vagotomy show normal gastric relaxation (Hertley & Mackie, 1991). Therefore, the role of the vagal nerve in mediating gastric relaxation remains controversial. We hypothesize that adaptive changes may occur after chronic vagotomy to mediate gastric relaxation.

To investigate this possibility, we performed gastric distension studies over time following vagotomy. Two weeks after TV, the intraluminal pressure increase evoked by gastric distension (6 ml) remained high. This was not affected by TTX, indicating a loss of the accommodation reflex *in vivo*. However, 4 weeks after vagotomy, the accommodation reflex was fully restored *in vivo*. Intraluminal pressure increase evoked by gastric distension was significantly enhanced by L-NAME, hexamethonium and TTX *in vivo*, suggesting the mediation of the NO pathway via nicotinic synapses following chronic vagotomy. In a manner similar to *in vivo* studies, gastric distension caused a smaller increase in intragastric pressure when 6 ml of saline was administered to the isolated stomach obtained from chronically vagotomized rats. The pressure increase evoked by gastric distension (6 ml) was significantly enhanced by L-NAME, hexamethonium and TTX. This suggests that nicotinic synapses and the intramural NO pathway in the gastric myenteric plexus are involved in the mediation of gastric relaxation following chronic vagotomy. These observations support and extend findings by Grundy *et al.* who reported that gastric accommodation reflex returns to normal following chronic vagotomy and this was mediated by intrinsic neural pathway (Grundy, Gharib-Naseri & Hutson, 1993). This may explain the observation that patients following chronic vagotomy have a normal accommodation reflex (Hertley & Mackie, 1991).

In contrast to pretreatment with L-NAME and hexamethonium, the intraluminal pressure increase evoked by gastric distension was not affected by guanethidine or splanchnicotomy *in vivo* suggesting that the sympathetic pathway is not involved in the mediation of gastric relaxation following vagotomy. Administration of rabbit VIP antiserum also had no effect on the pressure increase evoked by gastric distension in these rats *in vivo*.

In conclusion, our studies show that the vago–vagal reflex plays a prominent role in mediating the accommodation reflex in the rat stomach. It involves a capsaicin-insensitive vagal afferent pathway that transmits sensory information from tension receptors located in the serosa and/or muscle layers. The vagal efferent pathway, which utilizes NO as a final neurotransmitter, mediates gastric relaxation in intact rats. The accommodation reflex is fully restored 4 weeks after chronic vagotomy, a result of adaptive changes in the gastric myenteric plexus that release NO via nicotinic synapses.

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