Excitatory and inhibitory motor reflexes in the isolated guinea-pig stomach

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1. We have described and analysed the movements of the isolated stomach during distension by correlating intragastric pressure with video recordings, and investigated the presence of intrinsic inhibitory and excitatory reflexes.

2. Isolated guinea-pig stomachs, placed in an organ bath, were slowly distended with Krebs solution using a syringe pump via a cannula through the pylorus. The changes in intragastric pressure during cycles of distension were monitored by pressure transducers connected to both oesophageal and pyloric cannulae. The resistivity of the gastric wall (change in pressure with volume, $\Delta P/\Delta V$) and the amplitude and frequency of phasic pressure events were calculated from pressure recordings.

3. The movements of the stomach were also recorded onto videotape. The motion of the gastric wall during distension cycles was analysed to establish the patterns of contractions, their propagation and the distribution of fluid in the stomach. During filling, fluid was preferentially accommodated in the fundus. Propagating (peristaltic) contractions, often starting in the fundus, moved aborally towards the pylorus. The peak of the phasic pressure event was observed when a contraction reached the orad antrum. As it reached the pylorus, intragastric pressure was at its minimum.

4. During the initial phase of distension, intragastric pressure increased steeply. Tetrodotoxin and hyoscine reduced both the resistivity and amplitude of phasic pressure events. Hexamethonium had a similar effect. Thus distension appears to activate an excitatory reflex pathway, involving nicotinic ganglionic transmission. This reflex increases wall tension and enhances myogenic peristaltic contractions.

5. In control preparations, with larger distension volumes, the intragastric pressure decreased, despite the continued infusion of Krebs solution. L-NAME and apamin abolished this drop in pressure, indicating that gastric enteric inhibitory mechanisms prevail at larger distension volumes. After blockade of the excitatory reflex, hexamethonium antagonized the inhibitory response, indicating that activation of inhibitory mechanisms involves nicotinic transmission, probably on enteric inhibitory motoneurons.

6. Both the excitatory and inhibitory reflexes in the isolated stomach operate within a physiological range of gastric volumes. The excitatory reflex predominates at small distension volumes, leading to large phasic propagated contractions that mix the contents and may lead to emptying of the stomach. The inhibitory reflex, described previously as adaptive relaxation, can maximally relax the stomach and is activated preferentially at higher distension volumes to accommodate the contents. The interplay of these reflex pathways in the isolated stomach produces a rich repertoire of gastric movements.

7. The isolated stomach preparation, used with a combination of kinematic, kinetic and pharmacological methods, provides a highly suitable means of investigating the mechanisms of gastric motility.

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The movements of the stomach are important in the process of digestion and involve accommodation, mixing and emptying of gastric contents into the duodenum at a controlled rate. The fundus relaxes in response to distension *in vivo*, described by Cannon & Lieb (1911) as receptive relaxation. Increased intragastric pressure *in vitro* also causes a relaxation, described by Paton & Vane (1963) as adaptive relaxation. Gastric contents are mixed by the action of regular propagating contractions which traverse the corpus and antrum. Contractions start in the orad corpus and increase in force and velocity as they approach the pylorus (Cannon, 1911*a*). If the pylorus is open, gastric contents flow into the duodenum, otherwise the contents are retropulsed into the body of the stomach, leading to trituration of large particles.

Gastric motor behaviour is modified by the action of extrinsic neurons in the vagus and splanchnic nerves and by intrinsic (enteric) neurons. Stimulation of the vagus nerve activates both inhibitory and excitatory pathways to the stomach. Inhibitory pathways are involved in receptive relaxation and maintenance of low resting tone of the gastric musculature (Mayer, 1994). Excitatory pathways are involved in peristaltic contractions in the antrum (Langley, 1898) and during vomiting (Grundy & Reid, 1994). Vagal preganglionic neurons have been shown to terminate in most enteric ganglia of the stomach (Berthoud & Powley, 1992) and are functionally connected to a large proportion of gastric neurons (Schemann & Grundy, 1992). The final excitatory and inhibitory motoneurons in these vagal pathways are located in the gastric myenteric ganglia. Sympathetic pathways in the splanchnic nerves exert their inhibitory effect on gastric motor behaviour by acting on vagal preganglionic nerve terminals and enteric neurons (Schemann, 1991).

The gastric motor responses to filling are not severely compromised after acute or chronic lesioning of extrinsic nerve pathways to the stomach (Andrews & Bingham, 1990; Hartley & Mackie, 1991; Grundy, Gharib-Naseri & Hutson, 1993). However, lesioning extrinsic nerve pathways to the antrum appears to decrease gastric emptying of solids (Minami & McCallum, 1984). Thus intrinsic neural connections appear to be able to sustain motor patterns similar to normal. There are at least ten neurochemically defined classes of neurons in the myenteric plexus of the gastric corpus (Schemann, Schaaf & Mäder, 1995), suggesting that there are likely to be other functional classes of neurons in addition to the inhibitory and excitatory motoneurons. Although enteric sensory neurons have not been identified in the gastric myenteric plexus (Schemann *et al.* 1995) distension of the isolated stomach activates inhibitory reflex pathways (Desai, Sessa & Vane, 1991).

In this study, we investigated the possibility that in addition to inhibitory reflex pathways, there are also excitatory reflex pathways in the isolated stomach.

METHODS

Stomach preparation

Guinea-pigs (strain, IMVS coloured; weight, 250–400 g) of either sex were fasted overnight (18 h) with water available *ad libitum*. The following morning (09.00 h) the animals were weighed and then humanely killed by cervical dislocation followed by bleeding from the carotid arteries. The stomach was removed by cutting the oesophagus 10 mm orad to the lower oesophageal sphincter and the duodenum through the duodenal bulb. The gastric contents were flushed out with Krebs solution through a small cannula inserted through the pylorus. The composition of the Krebs solution was (mM): NaCl, 118; KCl, 4·7; NaH_2PO_4, 1·0; $NaHCO_3$, 25; $MgSO_4$, 1·2; D-glucose, 11·0; $CaCl_2$, 2·5; pH 7·4. The isolated stomach was placed in an organ bath as shown in Fig. 1.

A cannula was tied through the lower oesophageal sphincter for measurement of proximal intragastric pressure while a pyloric cannula was used to fill or empty the stomach with simultaneous monitoring of distal intragastric pressure. The pyloric and oesophageal cannulae recorded similar pressure profiles during the infusion period, except during occasional contractions in the antrum which occluded the lumen. Statham (P23X) pressure transducers were connected through MacLab bridge amplifiers (ADI, Sydney, Australia) to a MacLab data acquisition system using Chart v3 software (ADI). The cannulated stomach was placed with the greater curvature down in a organ bath filled with 150 ml of Krebs solution maintained at 37 °C and gassed with 95% O_2–5% CO_2.

Distension cycle

After a 30 min equilibration period, the stomach was emptied by siphoning and then slowly distended with Krebs solution at 4·2 ml min^{-1} to a final volume of 15·75 ml, using a syringe pump (Sage Instruments, 341B). The volume infused was established directly from the duration of infusion. The choice of this volume for distension was based on the normal volume of gastric contents in an unstarved guinea-pig which is approximately 12 ml (see Results). Although the physiological range of liquid filling of the stomach may vary widely, we have chosen an infusion rate which elicited a reproducible pattern of motor activity. Following the ramp infusion, the final volume was maintained for a further 75 s before the stomach was emptied. This distension cycle was repeated every 15 min. The orientation of the stomach in the bath is unlikely to have affected the distribution of infused liquid due to gravity.

After an equilibration period, five control distensions were performed with the Krebs solution changed after an hour. Drugs were added sequentially without washout. The effects of drugs were recorded for two distension cycles.

Analysis

The motor behaviour in response to the distension cycle consisted of reproducible changes in intragastric pressure in which different phases could be identified.

During the initial infusion, intragastric pressure rose rapidly and was associated with large phasic pressure events. Peak basal pressure occurred after infusion of approximately 7 ml of Krebs solution. A second phase could be identified during the infusion where intragastric pressure declined (Fig. 2). When a constant final volume was maintained, intragastric pressure declined to a steady state. The relation between pressure and volume in response to distension was measured as resistivity (change in pressure with

volume, $\Delta P/\Delta V$) and was calculated from pressure recordings taken from the pyloric cannula. The resistivity of the stomach wall was calculated for the initial infusion period (defined as the first 7 ml of infusion) and for the total infusion (15·75 ml of Krebs solution). Resistivity could not be calculated during the constant-volume phase ($\Delta V = 0$), instead the change in pressure over time was calculated ($\Delta P/\Delta T$). The amplitude of the phasic pressure events was measured from trough to peak (in cPa). The period between successive peaks of intragastric pressure was measured in seconds.

Resistivity was compared using repeated-measures ANOVA and amplitude and frequency of phasic pressure events were compared using factorial ANOVA. Scheffé's *post hoc* tests were used. Results are presented as means ± standard errors of the mean for both the initial and total infusion responses, and for amplitude and frequency of phasic pressure events. n refers to the number of animals used. A probability of less than 0·05 was considered significant.

Video analysis

The movements of the stomach were recorded onto a high resolution video recorder (Panasonic AG-7355) using two video cameras arranged perpendicular to one another (JVC TK-1280E/Panasonic WV-CL504) and a video mixer (Panasonic WJ-AVE5) (see Fig. 1). One camera viewed the entire dorsal surface of the stomach while the other was positioned above the lesser curvature. Frames of motion were captured into a Macintosh computer and were analysed with NIH Image 1.61 (NIH, Bethesda, MD, USA). Contractions were visible as indentations in the profile of the stomach. The rate of propagation of contractions was measured in millimetres per second. Volume changes in different regions of the stomach were calculated by determining the external diameters in the longitudinal and circular axis from both top (r_1) and profile (r_2) images of the stomach in 1 mm optical sections (h). The boundary between the fundus and corpus was taken to be circumferential to the oesophagus. The boundary between the

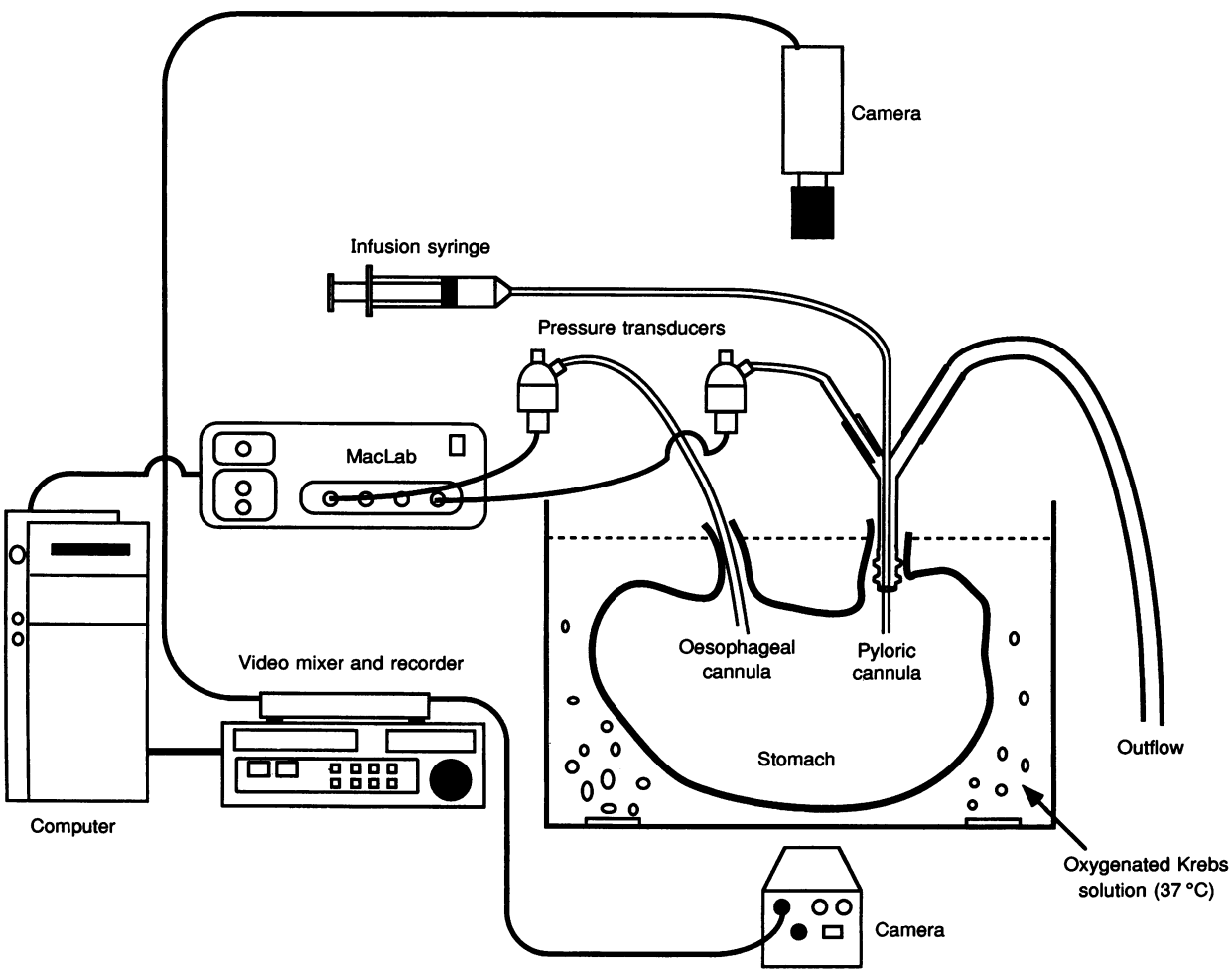

Figure 1. Arrangement of video and intragastric pressure equipment

Intragastric pressure was recorded via a cannula inserted through the lower oesophageal sphincter. A second cannula inserted through the pylorus also recorded intragastric pressure and was used to fill or empty the stomach (Outflow). The pressure transducers were connected to a MacLab data acquisition system. The movements of the stomach were recorded onto a high resolution video recorder using two video cameras arranged perpendicular to one another and a video mixer. Intragastric pressure recordings were stored on a computer.

corpus and antrum was taken to be circumferential to the incisura angularis. Measurements of the diameter were taken when the phasic pressure changes were at their minimum. These external measurements were used to calculate the total volume (i.e. intragastric + gastric wall; V) in each section using the formula, $V = \pi r_1 r_2 h$. The sectional volumes were summed to give the total volume of the stomach. When the stomach is empty, this formula underestimates the total volume. This method was able to detect changes in external diameters of the stomach corresponding to less than 1 ml of infused volume. Rates of expansion were determined by linear regression of the calculated volume data at 30 s intervals. The first and last (30, 240 s) data points were excluded.

Drugs used

Tetrodotoxin (0·6 μM; Sigma) was used to block action potentials in neurons, and papaverine (10 μM; David Bull Laboratories, Melbourne, Australia) was used to maximally relax smooth muscle. Hyoscine (1 μM; Boehringer Ingelheim, Artarmon, Australia) was used to determine the role of cholinergic muscarinic transmission, and hexamethonium (100 μM; Sigma) was used to determine the role of cholinergic nicotinic transmission during distension. These drugs were added to the bath 20 min before test distension cycles. L-Nitro arginine methyl ester (L-NAME, 400 μM; Sigma) and apamin (0·5 μM; Sigma) were used to antagonize inhibitory transmission and were added to the bath 30 min prior to test distension cycles.

RESULTS

Changes in intragastric pressure during control distension cycles

The infusion of the stomach with Krebs solution elicited a reproducible pattern of motor activity (Fig. 2). At the onset of fluid infusion, basal pressure increased steeply and peaked at 7·0 ± 0·3 ml ($n = 57$). Superimposed on this increase in tonic pressure were large-amplitude phasic pressure events which were often irregularly spaced. With further distension, intragastric pressure consistently decreased and the phasic changes in pressure became more regular. At an average infusion of 10 ± 0·6 ml ($n = 57$), the intragastric pressure began to increase again, but at a slower rate than during the initial distension. When the gastric volume was maintained constant for 75 s at the end of the infusion, intragastric pressure decreased. The rate at which intragastric pressure declined was correlated to the pressure at the end of the infusion (linear regression: $y = -0{\cdot}006x + 0{\cdot}015$, $r = 0{\cdot}66$; $P < 0{\cdot}01$, $n = 143$ from 12 animals). Higher pressures at the end of the infusion led to a greater decline in the intragastric pressure, but intragastric pressure remained stable if it was less than 3 cPa at the end of the infusion.

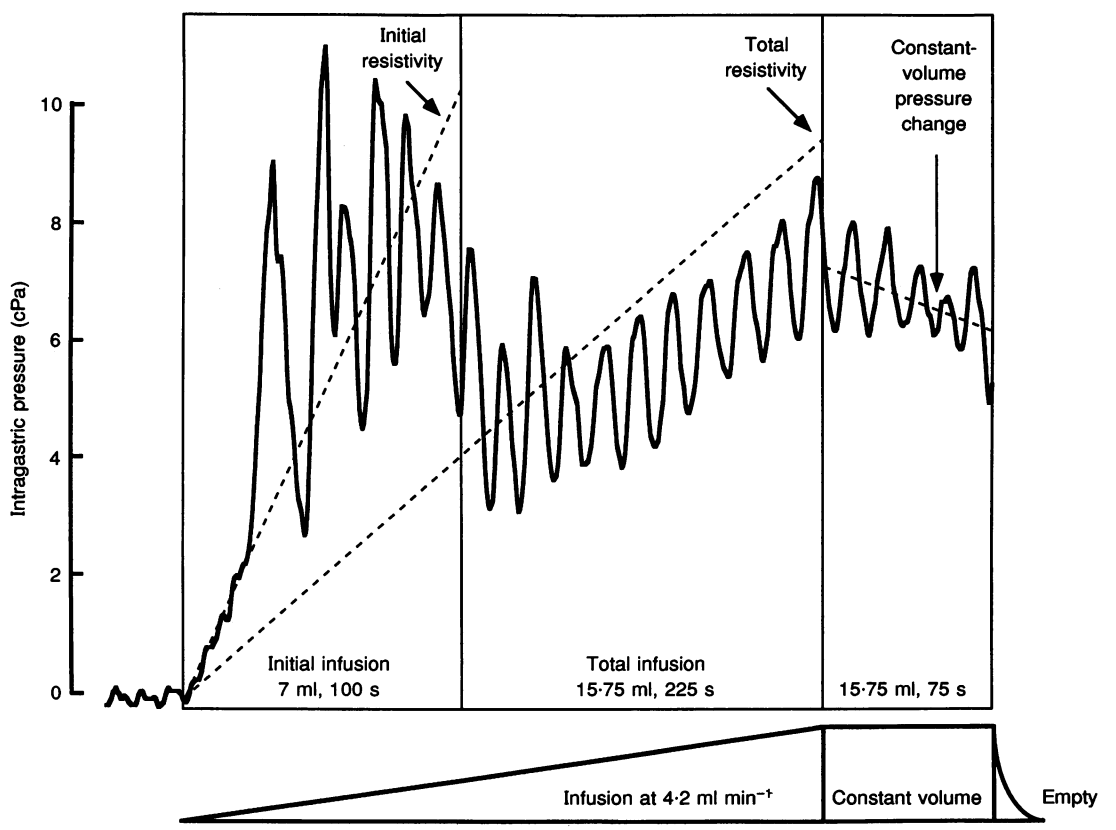

Figure 2. Phases of intragastric pressure changes during the distension cycle

Example of intragastric pressure recording (in cPa) during a distension cycle. The distension cycle is represented at the bottom and consists of a period of constant infusion, a constant-volume period and fast emptying. The dashed lines indicate the segments of trace in which the resistivity (i.e. the slope of the curve) was measured (1 $cmH_2O = 0{\cdot}98$ cPa).

The average period of the phasic pressure events was 12 s. Successive large-amplitude phasic pressure events occurred at a slower frequency, shown by the positive correlation between the amplitude and the period of phasic pressure events (linear regression: $y = 0{\cdot}183x + 12{\cdot}895$, $r = 0{\cdot}37$; $P < 0{\cdot}01$, $n = 263$ from 12 animals).

Movements of the stomach

Propagating contractions were visible in video recordings of the stomach as rings of indentation of the stomach wall, particularly on the greater curvature. Some contractions appeared to start at the apex of the fundus and propagated a short distance aborally. As a contraction reached the antrum, it caused a deep indentation of the gastric wall and accelerated along the greater curvature, but not along the lesser curvature, thus preserving a ring of contraction that pivoted around the incisura angularis. The contraction then continued into the distal antrum and terminated at the pylorus. Two or three waves of contraction were usually visible simultaneously in different parts of the stomach (Fig. 3). Detailed analysis of the site of initiation and propagation of the small contractions in the fundus was difficult, because movement of fluid displaced by the large antral contractions tended to ablate the other indentations. When indentations were easily identifiable, the average rate of propagation of contractions along the greater curvature in the corpus was $1{\cdot}3 \pm 0{\cdot}05$ mm s^{-1} compared with $2{\cdot}8 \pm 0{\cdot}09$ mm s^{-1} ($n = 4$) in the antrum.

The phasic pressure events generated by the propagating contractions are shown in Fig. 3. The peak of the phasic pressure change occurred when a propagating contraction reached the orad antrum; as it reached the pylorus, intragastric pressure was at its minimum.

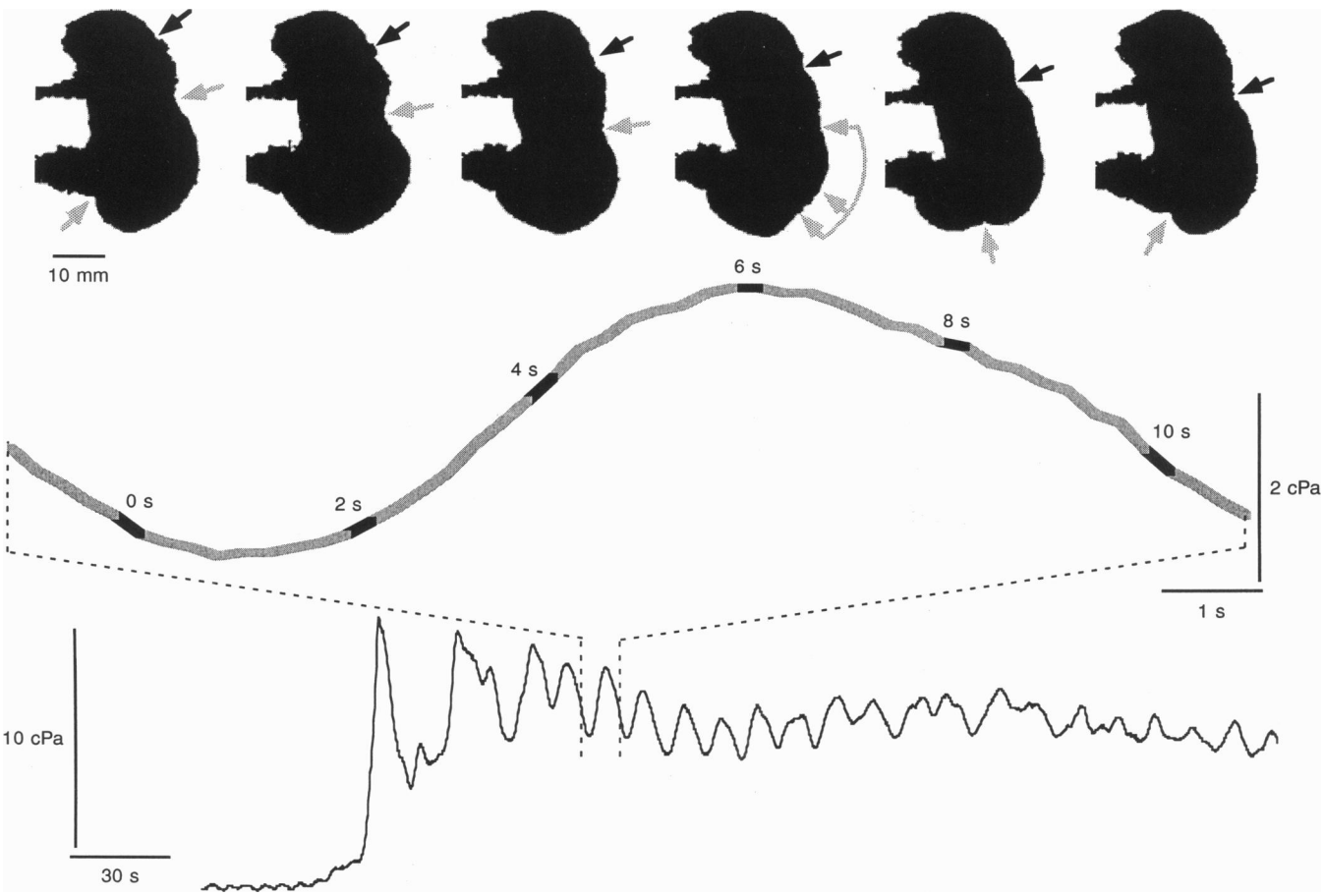

Figure 3. Relationship between intragastric pressure and wall motion during a single phasic pressure event

Bottom trace shows an example of an intragastric pressure recording during a distension cycle. The time scale of one of the phasic pressure events is expanded into the middle trace. Silhouettes of the stomach corresponding to the numbered points on the expanded intragastric pressure trace are shown above (fundus topmost). Note the presence of multiple contractions at any single time (single arrows) with propagation towards the pylorus (grey arrows). The peak pressure coincides with a broad contraction (triple arrow) of the orad antrum.

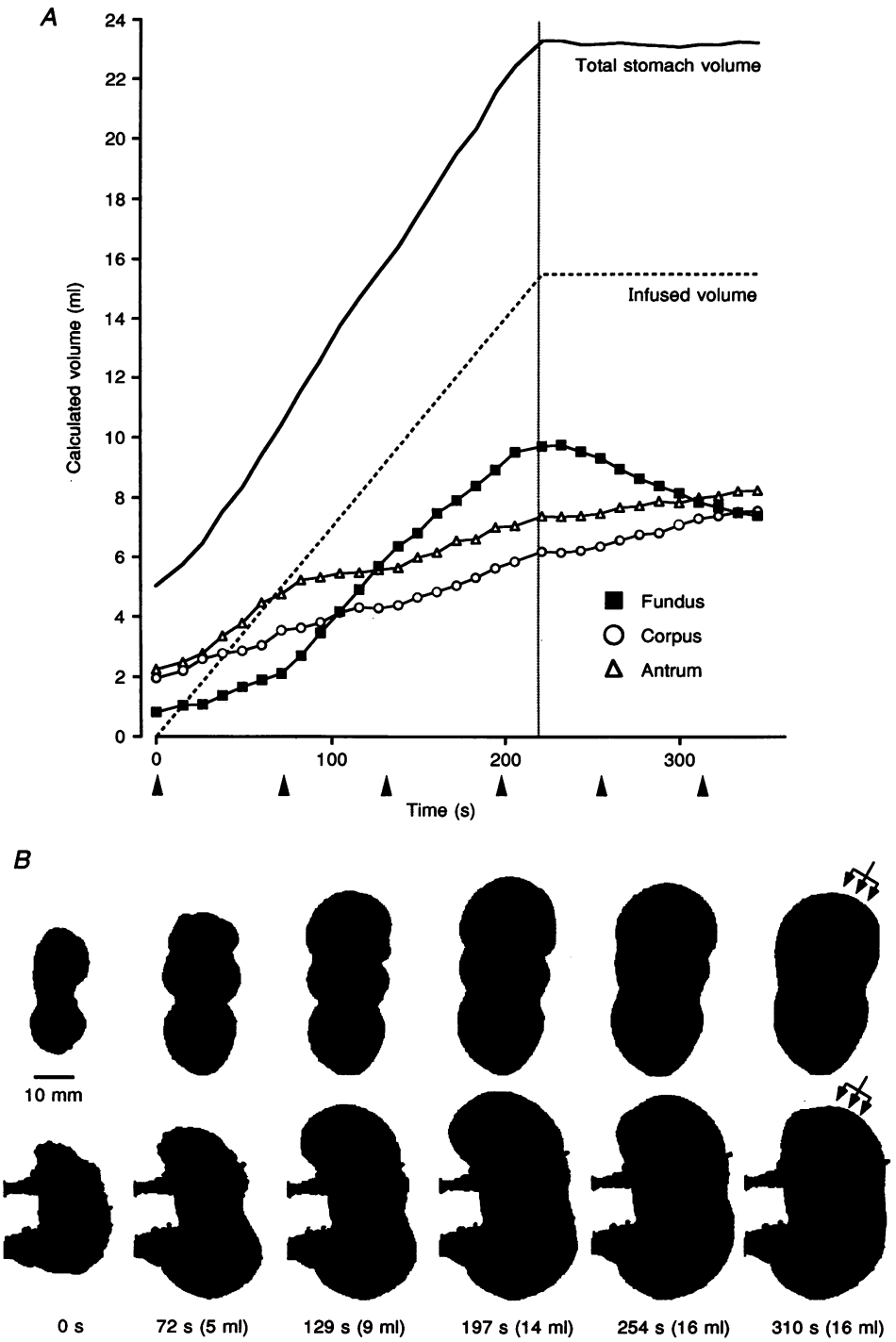

Figure 4. Example of an experiment showing volumes in different regions of the isolated stomach during a distension cycle

A, the volumes were calculated as described in the Methods section. The vertical dotted line separates the infusion period from the constant-volume period of the distension cycle. The difference between the continuous line (total volume of the stomach calculated from its external dimensions) and the dashed line (volume actually infused; also represented in Figs 2, 5, 8, 10 and 11) corresponds to the volume of the wall of the stomach. The individual points represent the times (see Methods) at which images were analysed. The arrowheads at the bottom mark the times of the corresponding silhouettes of the stomach shown in *B*. *B*, top row of images are views from the lesser curvature (fundus topmost); bottom row of images are viewed from the dorsal aspect (fundus topmost, lesser curvature left). Values below images indicate time from the beginning of infusion and, in parentheses, the volume infused. The last two silhouettes were taken during the constant-volume period. Note the small contraction of the fundus during this period (triple arrows).

Regional accommodation

The volume of the contents of the normal unstarved guinea-pig stomach was 12·0 ± 0·9 ml ($n = 57$). Following overnight starvation, this volume decreased to 5·5 ± 0·3 ml ($n = 76$).

During the initial period, infusion of Krebs solution distended the fundus, corpus and antrum to a similar extent. As the infusion volume increased, fluid preferentially distended the fundus, as shown by the greater rate of expansion of its external dimensions (Figs 4*A* and 6). The dimensions of the corpus and antrum increased linearly, at a slower rate than the fundus during the infusion period (Fig. 6). An example of the actual changes in shape of the different parts of the stomach is shown in Fig. 4*B*. At the end of infusion, when the intragastric volume was held constant for 75 s, the fundus gradually contracted (arrows in Fig. 4*B*) and reduced its volume, displacing fluid into the corpus and antrum (Fig. 4*A*).

Involvement of neurons

To determine the role of neuronal activity during the distension cycle, the sodium channel antagonist tetrodotoxin (TTX, 0·6 μM) was used. Intragastric pressure rose more slowly during the infusion and the characteristic drop in intragastric pressure at moderate infusion volumes observed in controls was not present (Fig. 5). Resistivity was significantly decreased during initial and total infusion periods. The amplitude of the phasic pressure events was greatly reduced and a small increase in frequency was observed (Table 1).

The distribution of Krebs solution in the fundus, corpus and antrum was also altered by TTX (Fig. 6). The expansion of the fundus was significantly decreased compared with controls, and the corpus expanded at a higher rate. The volume accommodated in the antrum was not affected by TTX. Unlike the control infusions, when nerves were active, there was no redistribution of fluid between different regions of the stomach when the volume was maintained constant at the end of the infusion in the presence of TTX.

In some experiments, small phasic changes in pressure remained after addition of TTX and were probably due to contractions propagated over short distances, as visualized by video analysis. To establish whether indeed contractions remaining after TTX were propagated, the muscarinic agonist carbachol was added in increasing concentrations to directly enhance muscle activity. At carbachol concentrations

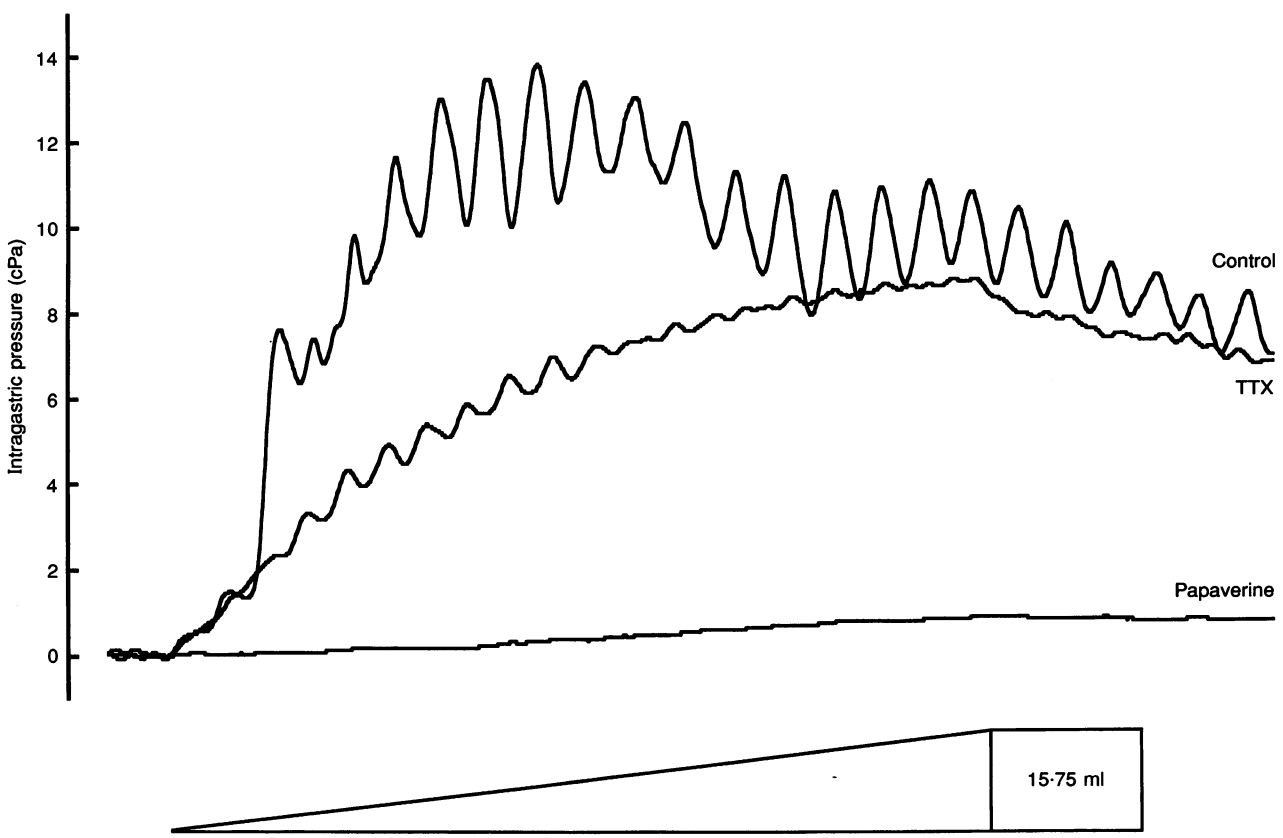

Figure 5. Neurogenic and myogenic components of the gastric motor behaviour

Example of traces from one experiment in which TTX (0·6 μM), to block neuronal activity, and papaverine (100 μM), to fully relax the smooth muscle, were added sequentially after control distension cycles. The distension is represented by the ramp at the bottom. Resistivity was reduced by TTX and dramatically lowered by the further addition of papaverine.

Table 1. Effect of drugs on motor behaviour of the isolated stomach

Drugs	Initial resistivity (cPa ml^{-1})	Total resistivity (cPa ml^{-1})	Amplitude of PPEs (cPa)	Period of PPEs (s)
Control	0·51 ± 0·07 (14)	0·29 ± 0·02 (19)	2·82 ± 0·34 (18)	12·10 ± 0·11 (12)
TTX	0·32 ± 0·03**	0·21 ± 0·03**	0·90 ± 0·18**	11·54 ± 0·12*
Papaverine	—	0·04 ± 0·002†† (5)^{f}	n.a.	n.a.
Control	0·56 ± 0·06 (14)	0·36 ± 0·30 (14)	3·34 ± 0·39 (10)	n.a.
Hyoscine	0·14 ± 0·03**	0·13 ± 0·02**	0·34 ± 0·07**	n.a.
Control	0·59 ± 0·10 (6)	0·31 ± 0·05 (6)	3·11 ± 0·19 (6)	11·76 ± 0·19 (6)
L-NAME	0·85 ± 0·12*	0·57 ± 0·07**	5·16 ± 0·35**	10·54 ± 0·17*
L-NAME + apamin	1·00 ± 0·19**	0·63 ± 0·08**	6·31 ± 0·45**	12·60 ± 0·49††
Control	0·25 ± 0·03 (5)	0·21 ± 0·03 (5)	1·76 ± 0·20 (5)	11·76 ± 0·41 (5)
Apamin	0·41 ± 0·12	0·35 ± 0·08*	4·11 ± 0·48**	13·53 ± 0·58*
Apamin + L-NAME	0·59 ± 0·12*	0·58 ± 0·12**	13·57 ± 0·80*††	15·90 ± 0·37*††
Control	0·42 ± 0·11 (10)	0·27 ± 0·04 (10)	2·65 ± 0·31 (10)	10·80 ± 0·17 (6)
Hexamethonium	0·18 ± 0·04	0·17 ± 0·03**	0·17 ± 0·24**	11·28 ± 0·24
Hyoscine	55·2 ± 6·8% TTX (5)	51·3 ± 6·2% TTX (5)	—	—
Hyoscine + hexamethonium	73·4 ± 7·5% TTX**	69·5 ± 7·4% TTX**	—	—
Hexamethonium	0·18 ± 0·04 (5)^{f}	0·17 ± 0·03 (4)^{f}	0·93 ± 0·24 (5)	—
Hex + L-NAME/apamin	0·51 ± 0·11**	0·55 ± 0·03*	5·37 ± 1·67**	—
TTX	0·56 ± 0·12 (4)	0·51 ± 0·09 (4)	1·10 ± 0·30 (4)	—
L-NAME	0·76 ± 0·10	0·68 ± 0·03	2·10 ± 0·89	—
L-NAME + apamin	0·87 ± 0·05*	0·73 ± 0·07	2·65 ± 0·28	—
TTX	0·79 ± 0·08 (5)	0·64 ± 0·05 (8)	0·79 ± 0·10 (8)	—
Apamin	1·09 ± 0·08**	0·80 ± 0·04	1·84 ± 0·51	—
Apamin + L-NAME	1·20 ± 0·05*	0·86 ± 0·08*	1·94 ± 0·59*	—

Values are expressed as means ± S.E.M. of a number of preparations from different animals (n). Values expressed as '%TTX' refer to resistivity measured before and after TTX, taking the value after TTX as 100% (see also text). PPEs, phasic pressure events; n.a., not applicable. * $P < 0·05$ compared with controls; ** $P < 0·01$ compared with controls; † $P < 0·05$ compared with previous drug; †† $P < 0·01$ compared with previous drug; f, factorial analysis.

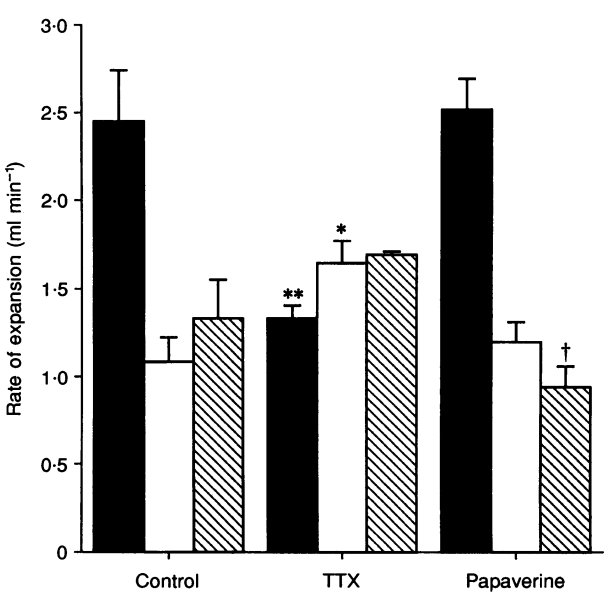

Figure 6. Effect of tetrodotoxin and papaverine on rates of expansion in different regions of the stomach during the distension cycle

The rates of expansion in the different parts of the stomach were calculated as described in the Methods. TTX significantly decreased the rate of expansion in the fundus (■; $P < 0·01$, $n = 4$) but increased expansion in the corpus (□; $P < 0·05$, $n = 4$), with no change in the antrum (▨). Papaverine restored the pattern of filling to control values. * $P < 0·05$, ** $P < 0·01$, significantly different from controls. † $P < 0·05$, significantly different from the effects of TTX.

above 0·013 μM, the amplitude of the phasic pressure events increased in a concentration-dependent manner, reaching a maximum at 0·33 μM. At higher concentrations of carbachol (> 1·3 μM), the amplitudes of phasic pressure events declined. The period of phasic pressure events decreased at carbachol concentrations between 0·03 and 0·13 μM. At high concentrations of carbachol (1·3–13·3 μM), the period increased from approximately 12 to 16 s, and antiperistaltic and non-propagated contractions were common (Fig. 7). Co-ordinated propagating contractions were evident in the distal antrum with carbachol concentrations between 0·033 and 1 μM. Propagating contractions often terminated before they reached the pylorus.

Myogenic activity

To determine the tension generated by smooth muscle during the distension cycle, the cAMP phosphodiesterase inhibitor papaverine (10 μM) was added to the organ bath after TTX. Papaverine fully relaxed the smooth muscle, thus greatly reducing total resistivity (see Fig. 5 and Table 1). Papaverine abolished all phasic activity. The resistivity of the stomach following addition of TTX and papaverine represents a measure of the passive viscoelastic properties of the stomach wall.

After addition of papaverine, the Krebs solution was accommodated predominantly in the fundus with very little

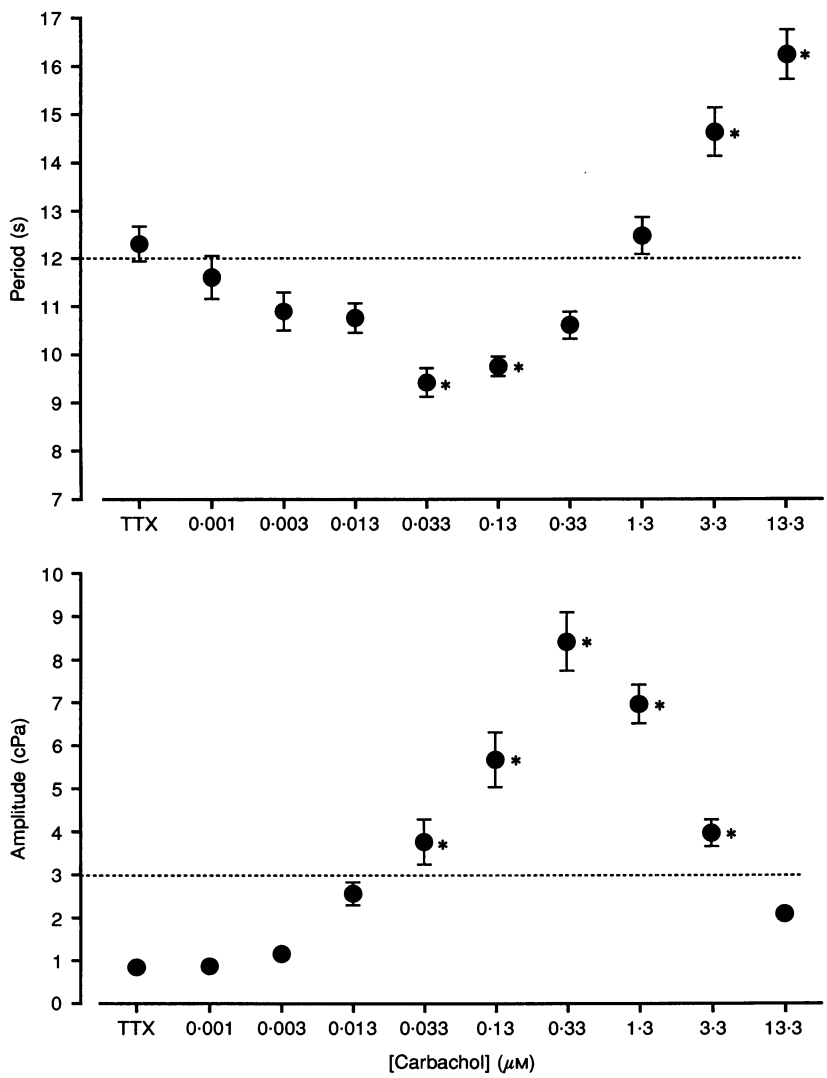

Figure 7. Effect of muscarinic stimulation on gastric myogenic activity

The graph shows the effect of carbachol on the period (top panel) and the amplitude (bottom panel) of phasic pressure events after nerve activity was blocked with TTX. The period changed from 12·4 ± 0·4 s with TTX to 9·4 ± 0·3 s ($P < 0·01$, $n = 4$) at a carbachol concentration of 0·033–0·13 μM. With increasing concentrations of carbachol the period increased up to a maximum of 16·2 ± 0·5 s at 13·3 μM ($P < 0·05$, $n = 4$). The amplitude of phasic pressure events increased 10-fold at a carbachol concentration of 0·33 μM compared with TTX alone (8·4 ± 0·6 *vs.* 0·85 ± 0·1 cPa; $P < 0·01$, $n = 4$). Asterisks show a statistically significant difference compared with TTX ($P < 0·05$). Dashed lines represent the mean control values prior to TTX.

of the infused solution being located in the antrum. As with TTX, there was no further redistribution of fluid when infusion was stopped, and intragastric volume was maintained constant (see Fig. 6).

Excitatory neuronal mechanisms

The decrease in resistivity and amplitude of phasic pressure events following the addition of TTX, particularly during the initial infusion period, suggested that excitatory neuronal mechanisms that contract gastric smooth muscle had been activated during the control infusions. Neuronal excitation of gastric smooth muscle has been shown to be predominantly mediated by the action of acetylcholine on muscarinic receptors (Christensen & Torres, 1975). Hyoscine (1 μM) was used to antagonize this transmission. Both the total resistivity and amplitude of phasic pressure events were greatly decreased by hyoscine compared with controls (Fig. 8 and Table 1). The effects of hyoscine were particularly evident on initial resistivity, which decreased to 14·6% of control values. Total resistivity was reduced to 33% of controls. Small phasic pressure events (< 1 cPa) were occasionally detected at high distension volumes in the presence of hyoscine.

Inhibitory neuronal mechanisms

As described above (see Fig. 2), control distensions showed a decrease in intragastric pressure after approximately 7 ml of Krebs solution had been infused into the stomach. This decrease was an indication that the muscle became actively relaxed despite the continuing distension by Krebs solution. In order to establish whether this relaxation was mediated by enteric inhibitory motoneurons, a combination of (L-NAME; 400 μM) and apamin (500 nM) was used. L-NAME significantly increased both initial and total resistivity (Fig. 9 and Table 1). After addition of L-NAME, the second phase of decline in intragastric pressure was abolished. The amplitude of phasic pressure events was enhanced with L-NAME and the period was decreased. The sequential addition of apamin after L-NAME caused no further change in initial or total resistivity or the amplitude of phasic pressure events, but did increase the period of phasic pressure events compared with the effect of L-NAME alone (Table 1).

When apamin was added before L-NAME, there was a significant increase in the amplitude and frequency of phasic pressure events and total resistivity. The subsequent

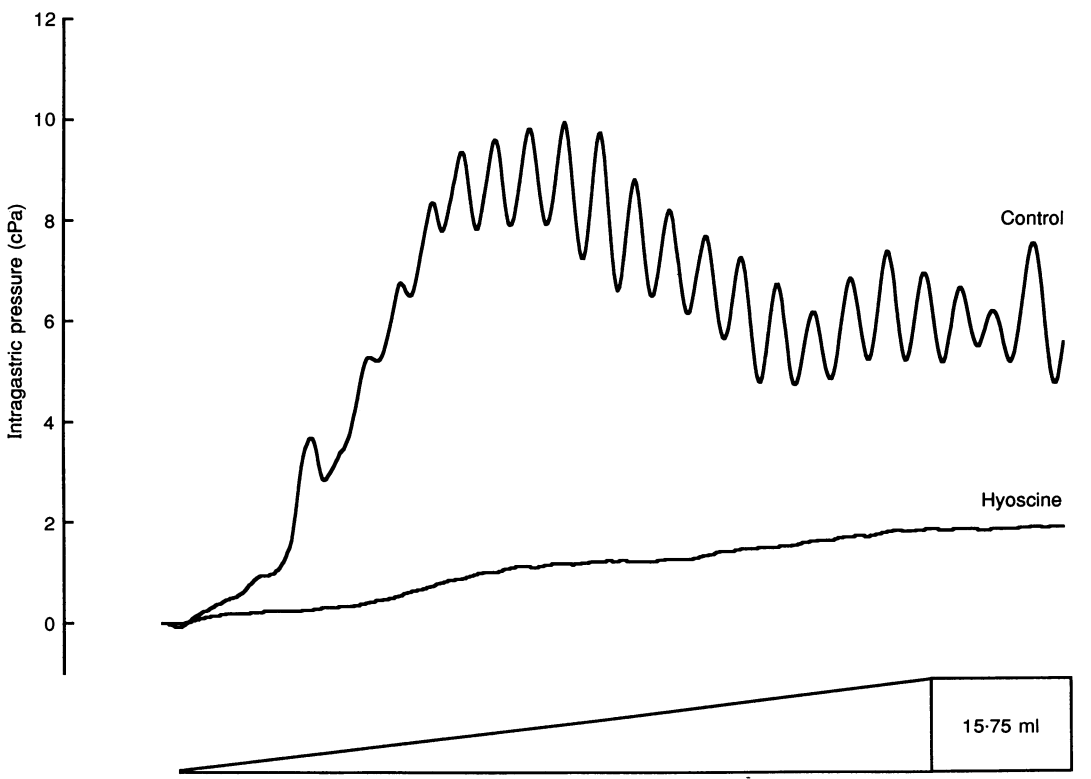

Figure 8. Effect of blockade of cholinergic excitatory transmission on motor behaviour of the isolated stomach

Example of a trace from one experiment in which hyoscine (1 μM) was added after control distension cycles. The distension cycle is represented by the ramp at the bottom. Hyoscine greatly reduced the intragastric pressure and the amplitude of the phasic pressure events during the infusion.

addition of L-NAME dramatically increased the amplitude and period of phasic pressure events and enhanced both initial and total resistivity (Table 1).

Excitatory reflex pathways

In order to investigate whether the neuronal mechanisms described above were due to activation of reflex pathways via nicotinic receptors, hexamethonium was used. Hexamethonium (100 μM) significantly decreased total resistivity and the amplitude of phasic pressure events (Fig. 10 and Table 1). Subsequent addition of hyoscine after hexamethonium caused a further significant decrease in total resistivity (Fig. 10) and amplitude of phasic pressure events (1·14 $\pm$ 0·11 cPa ($n = 6$) *vs.* 0·15 $\pm$ 0·01 cPa ($n = 4$); $P < 0{\cdot}001$), indicating that excitatory motoneurons remained active during distension, and suggests the existence of both nicotinic and non-nicotinic transmission in excitatory motoneurons.

Inhibitory reflex pathways

In order to reveal possible inhibitory reflex mechanisms involving nicotinic transmission, we first blocked excitatory transmission to gastric smooth muscle with hyoscine. Hexamethonium (100 μM) added after hyoscine significantly increased both initial and total resistivity (Fig. 11 and Table 1). This indicates the presence of an inhibitory reflex pathway involving nicotinic transmission.

In a separate series of experiments, when apamin and L-NAME were added after hexamethonium, both initial and total resistivity were significantly increased. The amplitude of phasic pressure events was significantly increased compared with the effect with hexamethonium alone (Table 1). This suggests that inhibitory reflex pathways in the stomach also involve non-nicotinic transmission.

Other sites of action of drugs

In order to establish whether L-NAME and apamin may act via non-neuronal mechanisms, TTX was added first and then either apamin or L-NAME was subsequently added. There was a significant increase in initial resistivity with apamin but not with L-NAME. The amplitude of phasic pressure events was not significantly increased by apamin or L-NAME. However, with both drugs in the bath, both initial and total resistivities and the amplitude of phasic pressure events were significantly increased compared with

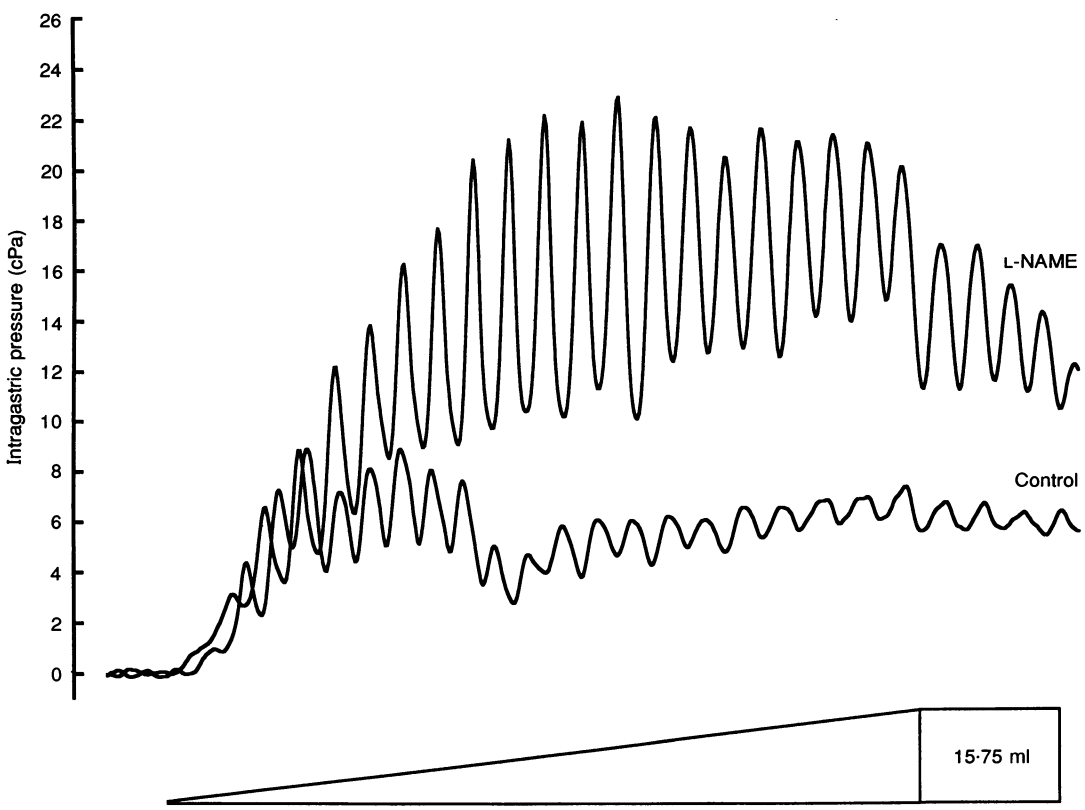

Figure 9. Effect of antagonists of inhibitory transmission on motor behaviour of the isolated stomach

Example of a trace from one experiment in which L-NAME (400 μM) was added after control distension cycles. The distension cycle is represented by the ramp at the bottom. Inhibition of nitric oxide synthesis greatly increased the resistivity of the gastric wall and the amplitude of the phasic pressure events during the distension cycle.

values obtained with TTX alone (Table 1). When added in the reverse order, L-NAME, by itself had no significant effects on the parameters measured. The subsequent addition of apamin significantly increased initial resistivity (Table 1).

DISCUSSION

In this study, we have described and analysed the movements of the isolated stomach during distension, by correlating intragastric pressure and video recordings. This combination of recording methods allows both accurate descriptions of gastric motor patterns and quantitative analysis of mechanisms contributing to smooth muscle activity, measured as resistivity. These studies revealed the presence of both inhibitory and excitatory neuronal reflex pathways within the isolated stomach.

Patterns of motor activity of the isolated stomach

This work shows that the motor activity of the isolated guinea-pig stomach displays motor patterns similar to those observed *in vivo*. Our results confirm the well-documented differences in motor activity in different regions of the stomach *in vivo*, with peristaltic waves being prominent in the corpus/antrum and the fundus being mainly involved in the accommodation of contents.

The pattern of gastric wall motion has been well described *in vivo* using a number of different techniques including X-rays (Cannon, 1898; Seide & Ritman, 1984), magnetic resonance imaging (Fraser, Schwizer, Borovicka, Asal & Fried, 1994) and ultrasound (Brown, Schulze-Delrieu, Schrier & Abu Yousef, 1993). Original work by Cannon, (1911*a*) describes peristaltic waves that originated in the corpus and propagated toward the pylorus. As the time taken for a contraction to travel to the pylorus was longer than the interval of contractions, Cannon reported several indentations present on the gastric wall at any one time. The final stage of the propagating contraction was characterized by a pronounced indentation of the antral wall. Rings of contraction press on the gastric contents causing phasic changes in intragastric pressure and trituration of gastric contents (Schulze-Delrieu, Cook, Herman, Shirazi & Brown, 1996) during pyloric closure.

However, there are few accounts of gastric wall motion in isolated preparations and those few only provided qualitative descriptions. In 1911, Cannon pointed to the importance of relating wall motion to manometric recordings of intragastric pressures (Cannon, 1911*b*). Our work represents a step in this direction. The ramp distension mimicked slow filling of the stomach. By correlating video

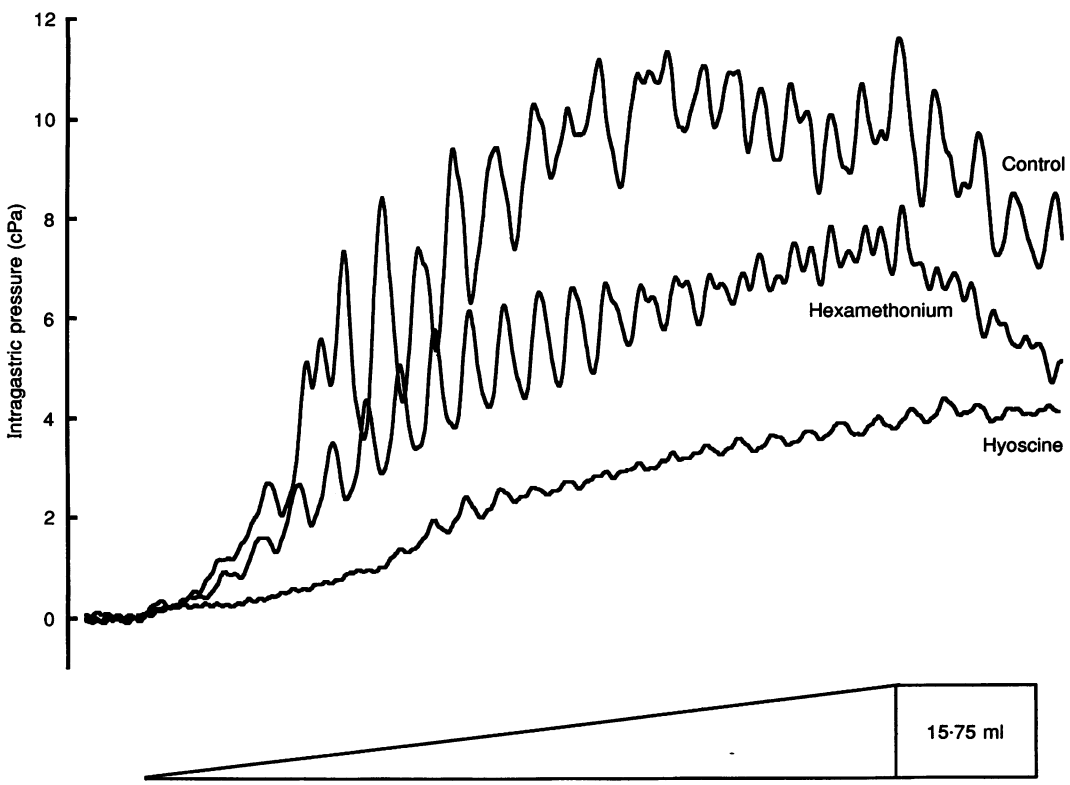

Figure 10. Role of muscarinic cholinergic transmission after nicotinic blockade on gastric motor behaviour

Example of traces from one experiment in which hexamethonium (100 μM) and hyoscine (1 μM) were added sequentially after control distension cycles. The distension is represented by the ramp at the bottom. Hyoscine, after nicotinic blockade, further reduced the total resistivity of the gastric wall during distension ($P < 0{\cdot}05$, $n = 5$), suggesting a non-nicotinic activation of excitatory cholinergic motoneurons.

analysis and intragastric pressures during the slow distension of the isolated stomach, we were able to determine the relation between changes in intragastric pressure and the motion of the wall of the stomach. The peak of the phasic increase in intragastric pressure occurred during a broad wave of contraction in the orad antrum. In these experiments the stomach was not free to empty and thus our experimental set-up is more likely to represent the movements of the stomach when the pylorus is closed.

We also observed short, shallow, propagating contractions in the fundus. In the interdigestive period *in vivo*, pulsatile activity in the proximal stomach has been recorded at the highest distension levels (Azpiroz & Malagelada, 1984; Gill, Pilot, Thomas & Wingate, 1985). Previous work using recordings by balloon or by pouches constructed from the wall of the proximal stomach shows mainly tonic changes in pressure (Haffner & Stadaas, 1972; Schulze-Delrieu & Wall, 1985). The nature of the rhythmic contractions we observed in the guinea-pig fundus is unknown but it is likely that, in this species, the slow waves that underlie gastric peristalsis may start in the upper fundus. These results suggest that the fundus has a greater repertoire of motor activity than is commonly believed.

Phases of motor patterns

Two phases could be readily distinguished during the slow filling cycles. At lower distension volumes there was an overall increase in the tension of the stomach wall, while at the higher volumes tension decreased. We have provided evidence that these two phases are due to differential activation of excitatory and inhibitory reflex pathways. The gastric muscle layers are innervated by both excitatory and inhibitory gastric enteric motoneurons (Hennig, Brookes & Costa, 1994) and electrical stimulation evokes both inhibitory and excitatory neuromuscular transmission to gastric smooth muscle (Mayer, 1994).

The existence of intrinsic reflex pathways in the stomach has been suggested by experiments which have shown that the stomach can perform a variety of motor functions, including filling and emptying, after it has been disconnected from the central nervous system (Armitage & Dean, 1966; Campbell, 1966; Schuurkes, Van Nueten, Van Daele, Reyntjens & Janssen, 1985; Desai *et al.* 1991).

We have recently demonstrated, by making intracellular recordings from gastric smooth muscle, that localized distension of the gastric wall elicits intrinsic inhibitory and excitatory reflex responses (Yuan, Brookes & Costa, 1997).

Evidence for excitatory reflex pathways in the intact isolated stomach

The resistivity and amplitude of phasic pressure events during the distension cycles were reduced by hyoscine or TTX, particularly during the initial infusion. This suggests

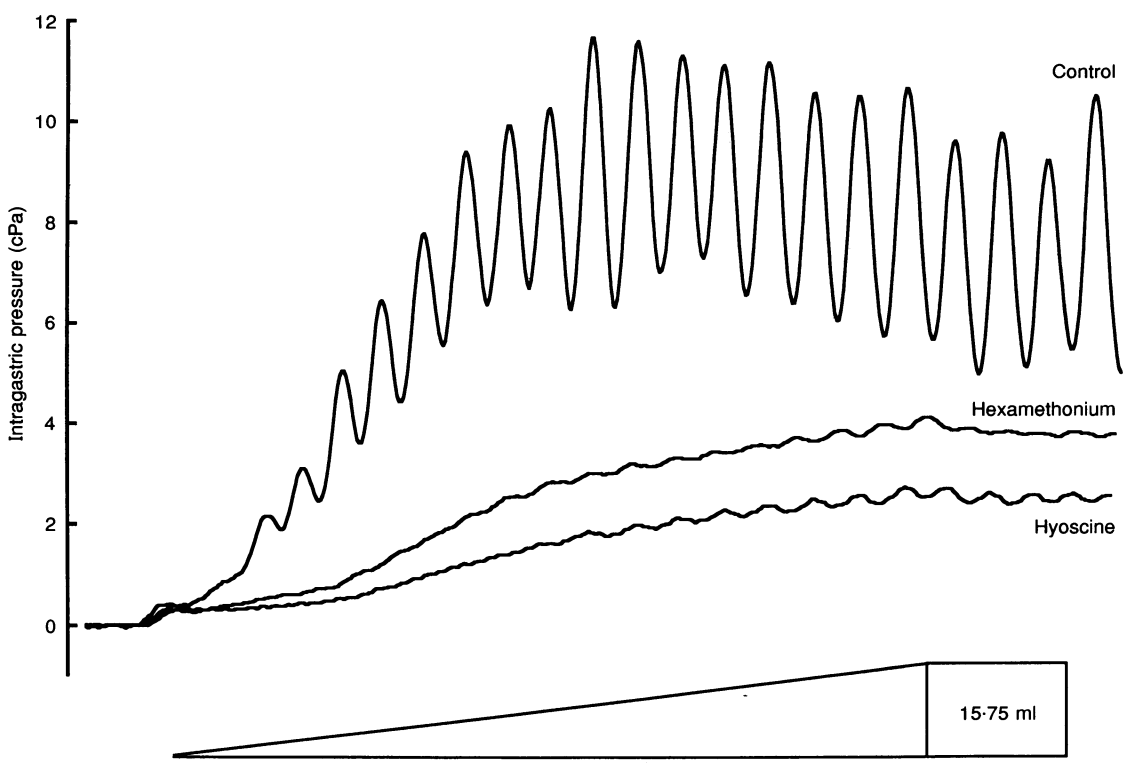

Figure 11. Role of nicotinic transmission after muscarinic blockade on gastric motor behaviour

Example of traces from one experiment in which hyoscine (1 μM) and hexamethonium (100 μM) were added sequentially after control distension cycles (represented by the ramp below). The increase in resistivity produced by hexamethonium after muscarinic blockade suggests that inhibitory pathways involving nicotinic transmission were active during the distension cycle.

that cholinergic motoneurons are responsible for these effects. Many neurons with choline acetyl transferase immunoreactivity have been demonstrated in the stomach myenteric ganglia (Schemann *et al.* 1995). After addition of TTX, the amplitude of phasic pressure events could be restored by directly enhancing the contractility of the muscle with carbachol. Video analysis revealed that these carbachol-enhanced contractions propagated aborally. These results suggest that, in the isolated stomach, excitatory cholinergic motoneurons are activated by distension and increase both the overall tension of the gastric wall and the amplitude of phasic contractions. The phasic contractions are initiated and propagated by non-neuronal mechanisms (Sanders & Publicover, 1989).

The evidence for an intrinsic excitatory reflex pathway being activated during distension is based on the action of the nicotinic antagonist hexamethonium. Hexamethonium decreased resistivity and reduced the amplitude of the phasic pressure events, especially at low infusion volumes, indicating that small distensions activate excitatory neurons via nicotinic input. It has been shown by Schemann & Wood (1989*a*, *b*) that the majority of synaptic transmissions between gastric neurons are mediated by nicotinic fast excitatory synaptic potentials.

There also appears to be a non-nicotinic component to this reflex pathway. The evidence is based on the observation that hyoscine significantly lowered resistivity when added after hexamethonium. This implies that there is a hexamethonium-resistant neuronal component to the reflex pathway. However, the nature of the transmitter involved in this non-nicotinic transmission remains to be established.

Evidence for inhibitory reflex pathways in the intact isolated stomach

At higher volumes of infusion, the resistivity of the gastric wall decreased, indicating the activation of inhibitory mechanisms. This corresponds to the mechanism of adaptive relaxation described by Desai *et al.* (1991) and Mayer (1994). The reduction of wall tension is likely to be due to the activity of gastric inhibitory motoneurons. Blocking the synthesis of nitric oxide (NO) with L-NAME increased both resistivity and the amplitude of phasic pressure events. This confirms previous work by Desai *et al.* (1991), who showed that inhibitors of nitric oxide synthase (NOS) antagonized the adaptation to increasing intragastric pressure. Additional evidence for an inhibitory mechanism in our experiments is the observation that TTX increased resistivity when added after hyoscine. This indicates that after blockade of transmission from the excitatory motoneurons with hyoscine, inhibitory motoneurons must have been active since resistivity remained low during the distension.

Apamin also increased resistivity and the amplitude of the phasic pressure events. Apamin has been shown to antagonize fast inhibitory junction potentials (IJPs), which are probably mediated by ATP (Zagorodnyuk & Maggi, 1994). In the stomach, electrical stimulation of the enteric inhibitory motoneurons has been shown to elicit apamin-sensitive IJPs (Komori & Suzuki, 1986). Thus, in the guinea-pig stomach there are at least two mechanisms of inhibitory neuromuscular transmission, one mediated by NO and the other by ATP or a related purine. Is unlikely that vasoactive intestinal polypeptide, which is also present in gastric inhibitory motoneurons (Schemann *et al.* 1995), is also involved in distension-induced gastric accommodation in guinea-pig (Desai, Warner, Bishop, Polak & Vane, 1994). Our results also indicate that apamin may partially act directly to excite the gastric smooth muscle, since, in the presence of TTX, apamin caused an increase in resistivity. Apamin is known to block calcium-dependent potassium channels, and electrophysiological recordings have shown an effect on smooth muscle resting membrane potential (Maas, Den Hertog, Ras & Van den Akker, 1980).

By analogy with excitatory reflex pathways, the question arises of whether the inhibitory motoneurons are activated by a reflex pathway during distension. After the excitatory transmission to gastric smooth muscle had been reduced by hyoscine, addition of hexamethonium increased resistivity. This suggests that an inhibitory reflex pathway, utilizing nicotinic transmission, is activated during distension.

We also observed a decrease in intragastric pressure during the distension cycle which is similar to the adaptive relaxation response reported by Desai *et al.* (1991), although they found no evidence for nicotinic transmission in the response. This may be due to a difference in sensitivity between the two experimental arrangements; our apparatus was very sensitive to small changes in wall tension.

There may also be a substantial non-nicotinic component to the inhibitory reflex pathway since L-NAME and apamin increased resistivity after nicotinic transmission had been blocked with hexamethonium. There are a number of potential transmitters localized within enteric neurons which may mediate non-nicotinic transmission between neurons (Schemann *et al.* 1995). It is possible that a decrease in cholinergic activity may contribute to adaptive relaxation and is likely to be mediated by nitric oxide and apamin-sensitive mechanisms, probably acting presynaptically.

A recent report suggests that there is an on-going release of NO in the stomach which keeps smooth muscle relaxed (Ozaki, Blondfield, Hori, Publicover, Kato & Sanders, 1992). In our experiments, after blocking neuronal activity with TTX, inhibition of NO synthesis did not significantly alter the resistivity or the amplitude of the phasic pressure events. This would suggest that in our experiments NO was only released from active neurons during distension.

Intracellular recordings from gastric enteric neurons do not show any spontaneous activity or pacemaking characteristics (Schemann & Wood, 1989*a*; Tack & Wood, 1992). These results, taken together, indicate that neural activity is likely to be initiated by sensory stimulation.

Sensory neurons of the reflex pathways

Sensory neurons involved in excitatory and inhibitory gastric enteric reflex pathways have not yet been identified anatomically. Sensory neurons in the small intestine have a distinct morphology (Dogiel II) and after-hyperpolarization, (AH) and most contain the calcium-binding protein, calbindin. In the stomach, there appear to be no neurons with Dogiel II morphology or containing calbindin (Schemann *et al.* 1995), and AH neurons have not been identified, except in the antrum (Tack & Wood, 1992).

It is possible that reflex pathways may involve axon collaterals of extrinsic sensory neurons. There is ample and well-accepted evidence for extrinsic vago-vagal inhibitory and excitatory pathways which operate in similar conditions to those described in this work (Mayer, 1994). There is also some evidence for axon collaterals of extrinsic sensory neurons being functionally connected to enteric motoneurons (Delbro, Fandriks, Lisander & Andersson, 1982). Tracing studies demonstrate that vagal afferents to the muscle have collaterals in the myenteric ganglia (Berthoud & Powley, 1992). These collaterals would probably be functional in the acutely isolated stomach.

However, following chronic vagotomy, adaptive relaxation to intragastric distension still occurs, indicating that there must be intrinsic sensory neural mechanisms (Andrews & Bingham, 1990; Grundy *et al.* 1993). It is of course possible that both intrinsic and extrinsic sensory neurons mediate local reflex responses. In any of the above cases, the intrinsic reflex pathways provide greater local control of gastric motor functions.

Role of the gastric enteric reflexes in gastric motility

The slow infusion of liquid in the isolated guinea-pig stomach simulates what may happen during feeding as the stomach is slowly distended by the ingested food. The guinea-pig stomach, when full, contains approximately 12 ml of contents. This means that the distension cycle in our experiments is close to the physiological range of distensions that may occur in real life.

The excitatory gastric enteric reflex pathway is involved in the contraction of the fundus and in the increase in force of the myogenic contractions of the corpus and antrum. This pathway is active with small distensions and would result in the mixing of gastric contents.

The inhibitory gastric enteric reflex pathway becomes prominent during middle to large distensions, acting mainly on the fundus to accommodate the contents (adaptive relaxation), may also inhibit myogenic peristalsis in the distal stomach, and counteracts the on-going cholinergic activity. The inhibitory reflex is very powerful and is able to relax the fundus completely under physiological conditions. This is demonstrated by the similar degree of expansion of the fundus during control distensions and after complete relaxation with papaverine.

Once infusion was stopped, the fundus contracted slightly, redistributing the contents to the other regions of the stomach. This indicates that the inhibitory reflex is less active during constant-volume distension than during filling and suggests that it has a dynamic component. In the presence of TTX, the distribution of fluid in the fundus was similar to controls at the end of the constant-volume period. This suggests that the reduction of accommodation by the fundus may be due to a decrease in the inhibitory reflex activity, rather than an increase in excitation.

It appears that the apparent complexity and variety of movements of the stomach may be due to the interplay of the two reflex pathways which affect the various portions of the organ differently. Since many of these motor patterns are maintained in the isolated stomach, the strategy used in this work of correlating wall motion and intragastric pressure changes with pharmacology will provide deeper understanding of the neuronal and non-neuronal mechanisms underlying the motor behaviour of the stomach.

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