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CHARACTERISTICS OF THE VAGALLY DRIVEN NON-ADRENERGIC, NON-CHOLINERGIC INHIBITORY INNERVATION OF FERRET GASTRIC CORPUS

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SUMMARY

1. This paper reports a quantitative *in vivo* study on the vagal activation of the intramural non-adrenergic, non-cholinergic inhibitory nerves in the ferret gastric corpus. The nature of the inhibitory neurotransmitter was also investigated.

2. In the atropinized, guanethidine-treated, urethane-anaesthetized ferret, electrical stimulation (10 s at 20 V, 1–20 Hz, 0·5 ms pulses) of the cervical vagi produced a prompt fall in intracorpus pressure that was related to the stimulus frequency. The maximal response was achieved at 10 Hz.

3. The time taken for the intracorpus pressure to return to pre-stimulus levels after a 10 s period of stimulation was related to the stimulus frequency; at 10 Hz the pressure took approximately 11 min to recover.

4. In contrast to studies in the cat (Martinson & Muren, 1963), there was no detectable difference in the electrical threshold for activation of the vagal excitatory and vagal inhibitory fibres.

5. The nature of the vagal non-adrenergic, non-cholinergic inhibitory neurotransmitter was investigated using a variety of antagonists and agonists. Adenosine triphosphate (ATP), adenosine, $\alpha\beta$-methylene ATP and $\beta\gamma$-methylene ATP all contracted the corpus in the presence of vagotomy, atropine, guanethidine and indomethacin. The vagally induced fall in corpus pressure was not blocked by high doses of $\alpha\beta$-methylene ATP.

6. A variety of peptides were investigated for their effects on corpus pressure in the presence of atropine, guanethidine and vagotomy. Bombesin, pentagastrin, substance P, cholecystokinin octapeptide (CCK-8) and bradykinin all produced an increase in intracorpus pressure. Neurotensin and vasoactive intestinal polypeptide (VIP) both decreased intracorpus pressure, and of the two VIP most closely mimicked the response to vagal activation of the non-cholinergic, non-adrenergic inhibitory neurones.

7. The results provide support for the involvement of a peptide (possibly VIP) rather than a purine in the vagally driven decrease in intracorpus pressure in the ferret.

INTRODUCTION

The activity of gastrointestinal tract muscle is regulated by hormones, intramural neural plexuses and the interplay between the sympathetic and parasympathetic divisions of the autonomic nervous system. In recent years it has become clear that the post-ganglionic neurones of the parasympathetic system can either enhance or suppress motor activity in many regions of the gut in a variety of species. The excitatory transmitter to gut muscle is regarded classically as being acetylcholine but the identity of the inhibitory transmitter remains a matter of controversy. Burnstock, Campbell, Satchell & Smyth (1970) have provided substantial evidence that adenosine triphosphate (ATP) or a related purine nucleotide is the transmitter responsible for producing the rapid-onset, short-lasting relaxation of the stomach and intestine in rodents and lagomorphs. The effect of purines on the stomach of carnivores has hardly been investigated in spite of the considerable differences in digestion between carnivores, rodents and lagomorphs. In carnivores a variety of other substances have been suggested as the inhibitory transmitter (e.g. vasoactive intestinal polypeptide (VIP): Fahrenkrug, Haglund, Jodal, Lundgren, Olbe & Shaffalitzky de Muckadell, 1978; dopamine: Valenzuela, 1976). The majority of studies investigating this problem have used a variety of species and experimental techniques and have rarely attempted a quantitative comparison between the neural and agonist-evoked gastric responses.

The ferret is a carnivore in which the vagal and sympathetic influences on gastric motility have been extensively investigated (see, for instance, Andrews & Lawes, 1982; Andrews & Lawes, 1984). Studies *in vivo* have revealed that stimulation of the peripheral cut end of the vagus, in the presence of atropine (0·5–1 mg/kg I.V.), produced a profound decrease in intracorpus pressure which was not blocked by phentolamine or propranolol (Andrews & Scratcherd, 1980).

In view of this demonstration of non-adrenergic, non-cholinergic (n.a.n.c.) inhibitory nerves, it was decided to investigate *in vivo* whether the responses to activation of these nerves was mimicked by purines or antagonized by $\alpha\beta$-methylene ATP, which is reported to desensitize tissues to the action of purinergic nerves (Delbro & Fandriks, 1984). The effect of a number of peptides on gastric motility was also studied to determine whether they might mimic the n.a.n.c. inhibitory response to the stomach.

In addition, a systematic quantitative study of the vagal activation of the n.a.n.c. neurones was undertaken in the hope that this would clarify the nature of the processes underlying their influence on gastric motility.

METHODS

Fifty male and female ferrets weighing between 600 and 1500 g were anaesthetized with an intraperitoneal injection of urethane (1·5 g/kg, 50 % (w/v) in 154 mM-NaCl) after an overnight fast. The trachea was intubated and the right external jugular vein cannulated. Blood pressure was monitored in the majority of animals from the right common carotid artery. Rectal temperature was monitored and maintained at 39 °C by a homoeothermic blanket (Palmer Bioscience Ltd.). The cervical vagi were mobilized and usually left intact initially. The nerves were subsequently sectioned in the neck and prepared for stimulation of their peripheral cut ends, as previously described (Andrew & Lawes, 1982). It is important to note that at least 30 min elapsed after vagotomy before the vagi were stimulated (see Andrews & Lawes, 1984). The stimulus was monitored on an oscilloscope and stimulus parameters are stated at the appropriate points in the text.

A wide-bore, fluid-filled (154 mM-NaCl) cannula was inserted into the stomach via the mouth and oesophagus and secured with a ligature. In the majority of experiments the stomach was divided just proximal to the incisura angularis so that the pressure was only monitored in the corpus, the region in which the vagal stimulation produces the most profound fall in pressure (Andrews & Scratcherd, 1980). The left and right greater splanchnic nerves were sectioned at the level of the crus of the diaphragm in all animals. The pressure was monitored in the distended corpus (20 or 30 ml), or whole stomach (20 or 60 ml) after inflation with 154 mM-NaCl at 37 °C. The outputs from the pressure transducers (Palmer Bioscience Ltd.) were displayed on either a Bryans 28000 flat-bed recorder or a Gould 2400 recorder. In some experiments the gastric pressure was digitized and analysed by computer as previously described (Andrews & Lawes, 1983).

Drugs were administered either into the jugular vein or intra-arterially via a cannula inserted into the aorta so that its tip was in the region of the coeliac axis.

Drugs

The following substances were used: adenosine (Sigma), adenosine triphosphate disodium salt (ATP) (Boehringer Mannheim), adenylyl ($\beta\gamma$-methylene) diphosphonate tetralithium salt (Boehringer Mannheim), $\alpha\beta$-methylene adenosine 5-triphosphate (Sigma), atropine sulphate (BDH), bombesin (Sigma), bradykinin triacetate (Sigma), cholecystokinin octapeptide (CCK-8) (Calbiochem), dipyridamole (Sigma), domperidone (Janssen), guanethidine (Ismelin, CIBA), haloperidol (Haldol, Janssen), methysergide maleate (a gift from Sandoz), naloxone (Narcan, Winthrop), neurotensin (Sigma), noradrenaline (Sigma), pentagastrin (Peptavlon, ICI), phentolamine mesylate (Rogitine, CIBA), propranolol hydrochloride (Inderal, ICI), substance P (Sigma), trazylol (Bayer), trazodone (Molipaxin, Roussel), vasoactive intestinal polypeptide (VIP) (Sigma). Drugs were usually dissolved in 154 mM-NaCl. ATP and its analogues were made up in buffered 154 mM-NaCl to pH 7. Indomethacin was dissolved in a sodium carbonate solution. Responses were compared using a paired sample t test within a group of animals and an unpaired sample t test between groups of animals. Vagal evoked relaxation was compared to the reduction in vagal-evoked contractions (p. 8) using the Pearson product moment correlation coefficient, r. Data are given in the text as mean $\pm$ 1 S.E. of the mean.

RESULTS

Characteristics of the n.a.n.c.-induced relaxation of the corpus

General. In a previous paper, Andrews & Scratcherd (1980) reported that stimulation of the peripheral cut end of the cervical vagus in the urethane-anaesthetized ferret evoked a marked fall in intracorpus pressure in the presence of atropine (1 mg/kg I.V.). The dose of atropine used abolished the increase in intracorpus pressure evoked by vagal stimulation (10 s at 20 V, 10 Hz, 0·5 ms pulses) and by intra-arterial acetylcholine (10 μg; Andrews & Lawes, 1982). As a preliminary to the present study we repeated and extended the above experiments.

The relaxation of the corpus in response to vagal stimulation in the presence of atropine (1 mg/kg I.V.) was unaffected by phentolamine (2 mg/kg I.V.) or propranolol (2 mg/kg I.V.) in combination. In animals treated with guanethidine (5 mg/kg I.V.), this response to vagal stimulation was still present, as it was in animals subjected to acute adrenalectomy and guanethidine (5 mg/kg I.V.) treatment (see Figs. 1 and 7). Guanethidine was without significant quantitative effect on either the fall in intracorpus pressure evoked by vagal stimulation or the time taken for the pressure to return to pre-stimulus levels (control fall in corpus pressure $2{\cdot}9 \pm 0{\cdot}3$ cmH_2O, guanethidine treated $3{\cdot}0 \pm 0{\cdot}2$ cmH_2O; control return time $11{\cdot}5 \pm 1{\cdot}6$ min, guanethidine treated $10{\cdot}7 \pm 1{\cdot}9$ min, $n = 5$ animals).

It could be argued that the fall in intracorpus pressure was secondary to cardiovascular or respiratory changes induced by stimulation of the cervical vagus.

This possibility was excluded by the observation that the response to cervical vagal stimulation was abolished by acute section ($n = 4$ animals) of the dorsal and ventral abdominal vagal trunks as defined by Mackay & Andrews (1983). In the atropinized ferret, stimulation of the peripheral cut ends of the cervical vagus (10 s at 20 V, 10 Hz, 0·5 ms pulses) evoked a transient increase in blood pressure of 53 ± 12 mmHg. In the presence of guanethidine (5 mg/kg I.V.) this was reduced to 14 ± 5 mmHg ($P < 0{\cdot}001$, $n = 4$ animals), whilst the gastric corpus response was unaffected. In addition, frequencies of vagal stimulation that failed to produce a change in blood pressure (e.g. 2 Hz) produced clear falls in intracorpus pressure.

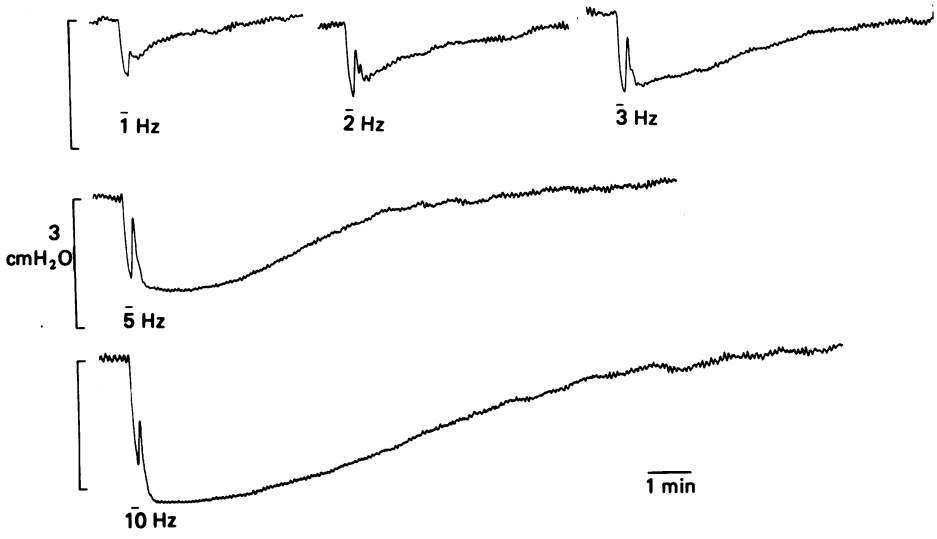

Fig. 1. The effect of variations in vagal stimulation frequency on intracorpus pressure in an atropinized (0·5 mg/kg I.V.) animal treated with guanethidine (5 mg/kg).

In summary, these results show that the fall in intracorpus pressure in response to vagal stimulation was not mediated by a neurotransmitter acting via a muscarinic cholinergic receptor or the conventionally defined adrenergic receptors. The relaxation observed was not secondary to a vagally driven change in respiration or blood pressure. Release of any hypothetical humoral agent from vagally innervated thoracic structures is unlikely to mediate the vagal relaxation of the corpus because the relaxation was abolished by section of the abdominal vagi. In the light of the above observations, a systematic study was undertaken to quantify and characterize further the nature of the vagally driven n.a.n.c. response of the gastric corpus.

The effect of stimulus frequency. Stimulation of the peripheral cut end of the cervical vagi in the presence of atropine (0·5–1·0 mg/kg) and guanethidine (5 mg/kg I.V.) with supramaximal intensities (20 V, 0·5 ms pulses) produced a prompt fall in intracorpus pressure during application of the stimulus (Fig. 1). On cessation of the stimulus there was a brief contraction which has been referred to as a 'rebound contraction' (Andrews & Scratcherd, 1980). After this rebound contraction the pressure recovered slowly over a period of several minutes. Using periods of stimulation (10 s) which

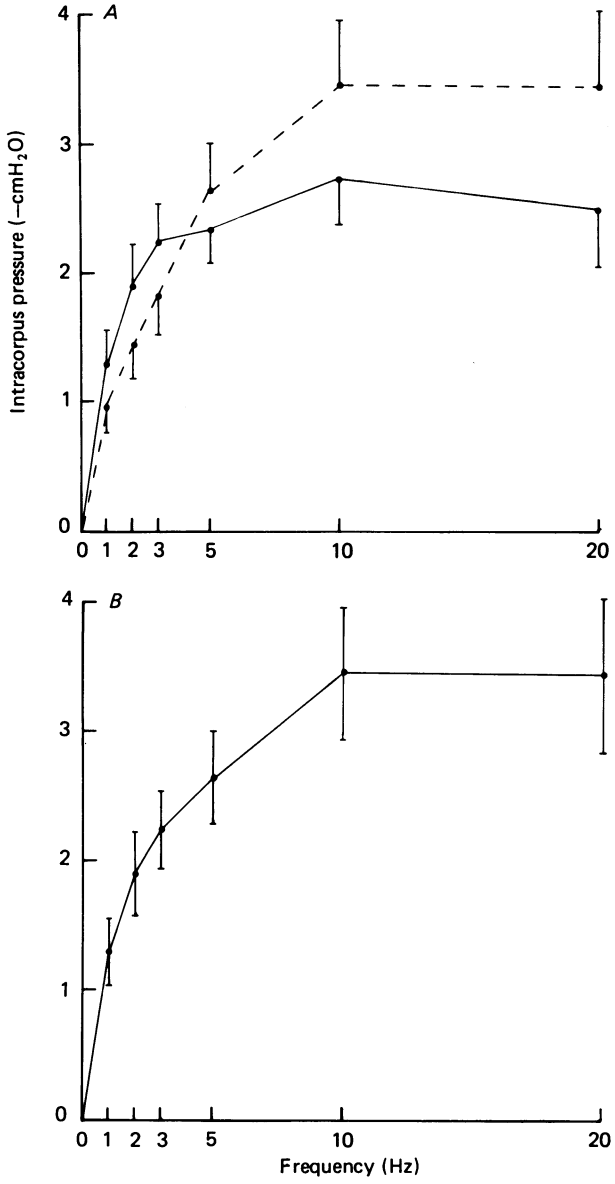

Fig. 2. *A*, frequency–response curve for the effect of vagal stimulation on intracorpus pressure in atropinized, guanethidine-treated animals. Continuous line, the pressure fall recorded on cessation of the stimulus; dashed line, the pressure recorded after the rebound had subsided. Each point is the mean value from five animals. *B*, frequency–response curve as above, but plotting the maximal fall in intracorpus pressure irrespective of whether it occurred at the end of stimulation or after the rebound contraction.

produced a maximal fall in pressure (see below) a frequency–response curve was constructed for the influence of the vagally driven n.a.n.c. nerves on intracorpus pressure (Fig. 2*A* and *B*). At lower frequencies (1–3 Hz) the pressure fell during the stimulation (Fig. 1); once the rebound had subsided the intracorpus pressure began to return to control levels. However, at higher frequencies (5–20 Hz) the fall in

pressure continued after the rebound. The relation between stimulation frequency and the pre- and post-rebound pressures are plotted separately in Fig. 2*A*. It can be seen from this graph that both measures of relaxation are frequency dependent with an optimum of 10 Hz. Both the pre- and post-rebound falls in pressure may be part of a single relaxation interrupted by the rebound, in which case a more representative frequency–response curve is produced by plotting the maximum fall in pressure

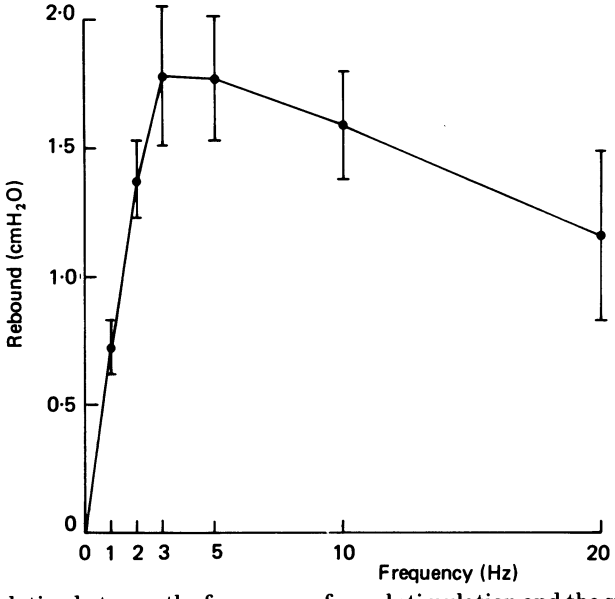

Fig. 3. The relation between the frequency of vagal stimulation and the size of the rebound contraction. Each point is the mean value from five animals.

achieved irrespective of its relation to the rebound (Fig. 2*B*). Alternatively, the two measures may reflect two separate phenomena, such as might be produced by two co-transmitters, one producing an early, brief relaxation, the other a later, more prolonged relaxation (see Discussion).

Characteristics of the rebound contraction. The rebound contraction that follows electrical activation of the n.a.n.c. nerves is a striking feature of the ferret corpus. A rebound contraction was observed at all the frequencies of vagal stimulation used. The relation between stimulus frequency and rebound size is plotted in Fig. 3. The rebound reached a maximum value at a lower frequency (3 Hz) of stimulation than the fall in intracorpus pressure (10 Hz). The size of the rebound contraction was not related to the fall in intracorpus pressure. Rebound contractions of this kind were never seen following splanchnic nerve stimulation, even when this caused relaxation comparable to that produced by vagal stimulation (Andrews & Lawes, 1984). Although it is usually stated that the rebound immediately follows cessation of the stimulus, records taken at high speed showed that there was a delay of 1–2 s during which intracorpus pressure continued to decrease.

We were unable to confirm the observation of Andrews & Grundy (1981) that the rebound contraction was reduced in amplitude by pre-treatment with indomethacin

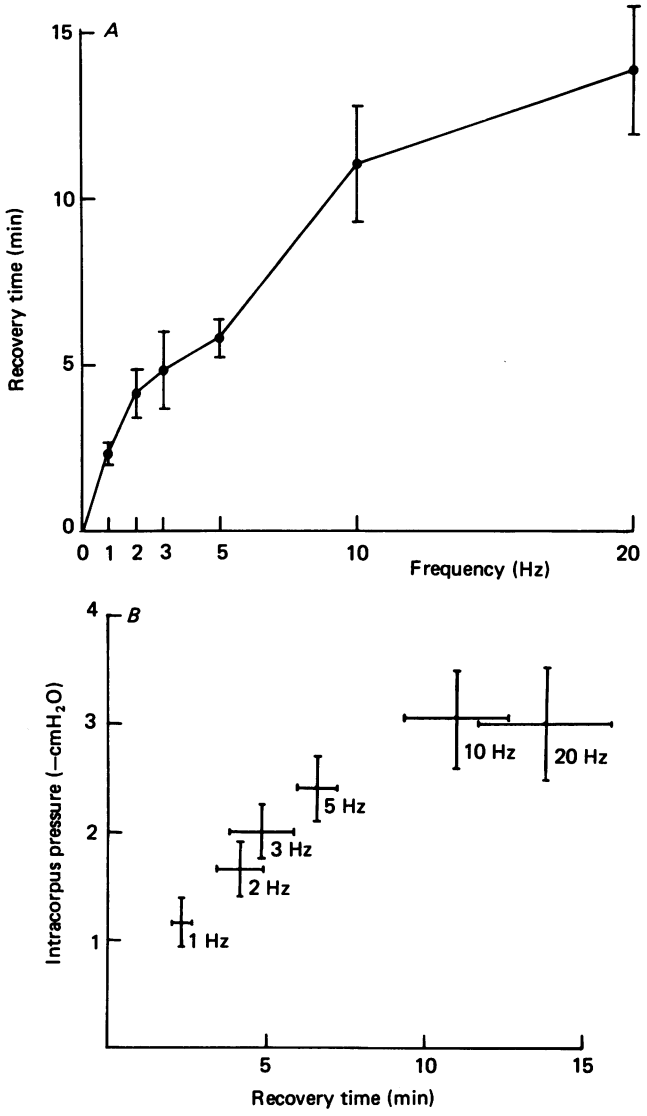

Fig. 4. *A*, the time taken for intracorpus pressure to return to control levels after vagal stimulation (10 s at 20 V, 0·5 ms pulses) at various frequencies in the presence of atropine and guanethidine. Each point is the mean from five animals. *B*, the relation between the recovery time and the fall in intracorpus pressure in response to vagal stimulation at the frequencies indicated (10 s at 20 V, 0·5 ms pulses). Each point is the mean for five animals.

(10 mg/kg I.V.). However, it is difficult to interpret the effect of indomethacin in the ferret as we do not have any independent evidence that it affects prostaglandin synthesis in this species.

The time course of recovery from n.a.n.c. activation. After vagally induced n.a.n.c. activation, the intracorpus pressure recovered slowly over a period of several minutes. Fig. 4*A* shows the relation between the frequency of stimulation and the time taken

for the pressure to return to control levels after stimulation. The recovery time was also related to the fall in intracorpus pressure but when a maximal fall in intracorpus pressure was reached (10 Hz), increasing the frequency of stimulation to 20 Hz only prolonged the recovery time (Fig. 4*B*).

The relative thresholds of vagal excitatory and inhibitory fibres. Martinson & Muren (1963) reported that, in the cat, the vagal inhibitory fibres had a higher threshold to electrical stimulation than the vagal excitatory fibres. Both types of fibre had a higher threshold than the cardio-inhibitory fibres. Their criteria for vagally driven inhibition were: (a) reduction of the maximal height of the contractile response; (b) interruption of the initial rising phase of the response; (c) prolonged depression of the basal tone following stimulation. From their published figures it appears that they used interstimulus intervals of 1 and 2 min and stimulus durations of 1 min.

In the ferret, when the cervical vagi were stimulated twice within a short interval the response to the second stimulus was reduced (Andrews & Scratcherd, 1980), regardless of whether the two stimuli were delivered to the same vagus or not. The duration of this effect (30–120 s) differs from the duration of vagal relaxation (664 ± 102 s). Furthermore, increases in intragastric volume reduced evoked contractions and enhanced evoked relaxations (Andrews & Lawes, 1982). The experiment described below was undertaken to discover whether relaxation and the phenomenon of reduction of contraction were equally affected by changes in volume. The effect of long and short interstimulus intervals on relaxation was also examined.

In one group of animals the left cervical vagus was stimulated once every 600 s (10 s at 6 or 16 V, 10 Hz, 0·5 ms pulses). Half the stimulations were preceded 20 s earlier by stimulation of the right cervical vagus. This caused a reduction in the amplitude of the response evoked by left cervical stimulation. This reduction was almost three times greater when the stomach contained 20 ml ($-6{\cdot}6 \pm 2{\cdot}3$ cmH_2O, $P < 0{\cdot}05$, 16 V) than when it contained 60 ml ($-2{\cdot}3 \pm 0{\cdot}4$ cmH_2O, $P < 0{\cdot}01$, 16 V).

In contrast, the relaxation which followed contraction was twice as great ($P < 0{\cdot}01$) when the stomach contained 60 ml ($1{\cdot}7 \pm 0{\cdot}4$ cmH_2O, 16 V) as when it contained 20 ml ($0{\cdot}8 \pm 0{\cdot}2$ cmH_2O, 16 V).

This experiment was repeated in a second group of animals allowing only 180 s between stimulations of the left cervical vagus. The results were similar, but the relaxation obtained was halved at both volumes (i.e. at 60 ml, $0{\cdot}8 \pm 0{\cdot}1$ cmH_2O and at 20 ml, $0{\cdot}4 \pm 0{\cdot}4$ cmH_2O). This was due to the fact that the pressure was not given sufficient time to recover from the previous stimulation, and relaxation is proportional to the prevailing pressure.

It is clear that reduction in evoked contraction (Martinson & Muren's criterion 'a') and relaxation (their criterion 'c') are two different phenomena. The former was enhanced at low volumes and had a shorter time course. In our experiments the two phenomena were uncorrelated ($r = 0{\cdot}23$). Relaxation was diminished when short interstimulus intervals were used; intervals five to ten times longer than those allowed by Martinson & Muren were necessary to observe full vagal evoked relaxation. It follows that the criteria used by Martinson & Muren (1963) to establish the relative thresholds of excitatory and inhibitory axons were inappropriate. Consequently, the thresholds of the two vagal systems were re-examined more directly.

At a pulse width of 200 μs with a corpus volume of 20 ml, the threshold voltage required to produce cardio-inhibition or an increase in intracorpus pressure (whether or not it was followed by gastric relaxation of any size) was measured. The values obtained were: cardio-inhibition, $2{\cdot}11 \pm 0{\cdot}10$ V; increased intracorpus pressure, $2{\cdot}56 \pm 0{\cdot}14$ V; after relaxation, $2{\cdot}85 \pm 0{\cdot}11$ V. The animals were then atropinized and the threshold for vagal activation of the n.a.n.c. intramural inhibitory neurones was

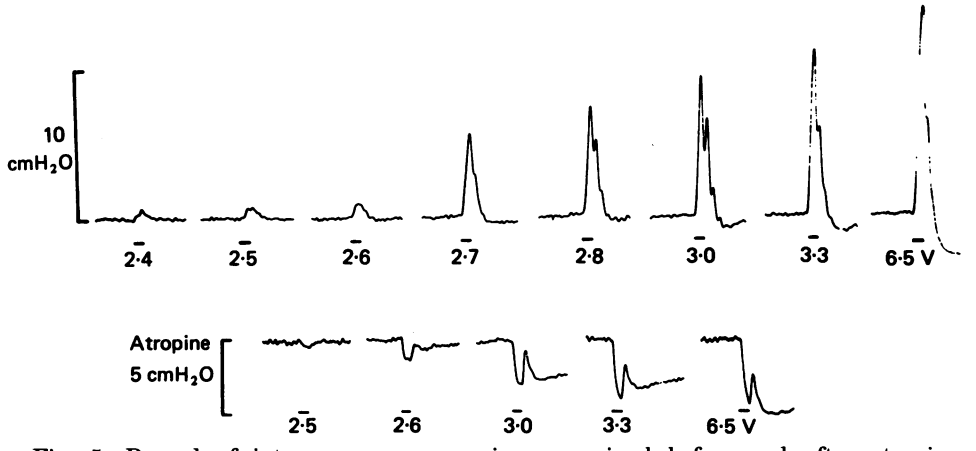

Fig. 5. Record of intracorpus pressure in one animal before and after atropine (0·5 mg/kg I.V.) in response to cervical vagal stimulation (10 s at 10 Hz, 0·2 ms pulses, at the voltage indicated). Note the thresholds for appearance of a contraction (2·4 V), a contraction followed by a relaxation (2·6 V) and a relaxation alone in the presence of atropine (2·5 V).

determined; this was $2{\cdot}67 \pm 0{\cdot}17$ V. There was no statistically significant difference between the thresholds for the fibres evoking an increase in intracorpus pressure before atropine and those evoking activation of intramural n.a.n.c. neurones alone after atropine (Fig. 5). The voltage required to produce a relaxation following a gastric contraction was significantly different in a non-atropinized animal ($P < 0{\cdot}01$) from that required to produce a contraction alone. In view of the threshold data in the presence of atropine it is likely that at these low levels of activation the inhibitory influence of the vagus is obscured by simultaneous activation of the cholinergic excitatory fibres. It is concluded that in this system with 20 ml in the corpus there is no demonstrable difference in the electrical thresholds between the vagal fibres driving the intramural excitatory and inhibitory neurones.

The lower record in Fig. 5 also serves to indicate that stimulus voltage had an effect on the rebound contraction similar to that of frequency of stimulation, namely that the tendency for the intracorpus pressure to continue falling after the rebound contraction depends upon the voltage (number of active fibres) at a fixed maximal frequency (10 Hz) or on the frequency at a supramaximal voltage (20 V, 0·5 ms).

The effect of antagonists on the n.a.n.c.-induced relaxation of the corpus. In an attempt to identify the n.a.n.c. neurotransmitter we have investigated the effect of a number of receptor antagonists. Doses of antagonists that are the same or higher than those reported to be effective in other carnivores (e.g. dog and cat) were used.

Andrews & Scratcherd (1980) have previously reported that mepyramine (2 mg/kg I.V.) and cimetidine (4 mg/kg I.V.) were without effect on the n.a.n.c vagal response in the ferret. Their observation that phentolamine (2 mg/kg I.V.) and propranolol (2 mg/kg I.V.) were also ineffective has been confirmed in the present study. The vagally mediated relaxation of the corpus was blocked by hexamethonium (20–30 mg/kg I.V.) in this series of experiments, confirming the earlier study of this system. The antagonists listed below did not antagonize the relaxation of the gastric corpus produced by maximal (10 Hz) or submaximal (1 Hz) vagal stimulation in the presence of atropine (1 mg/kg I.V.) and guanethidine (5 mg/kg I.V.).

The following drugs were each studied in three to six animals: haloperidol (5 mg/kg I.A.), metaclopromide (10 mg/kg I.A.), domperidone (10 mg/kg I.A.), methysergide maleate (1 mg/kg I.A.), naloxone (400 μg/kg I.A.) and trazodone (15 mg/kg I.A.).

In addition to these receptor antagonists, we also studied the effect of aprotinin (100 000 units/kg I.A.) administered 5–10 min before the vagus was stimulated. This substance did not antagonize the vagal response, nor did indomethacin (10 mg/kg I.A.) administered at least 40 min before vagal stimulation.

These studies indicate that the effect of vagal stimulation in the ferret gastric corpus was not mediated by a 'conventional' neurotransmitter. Studies by Burnstock (1975) have suggested that ATP, or a related substance, may be involved in the neurally mediated inhibition of gastric and intestinal motility in rodents and lagomorphs. We therefore investigated the effect of purines on the ferret stomach to see whether they could mimic activation of the vagal inhibitory nerves.

The effect of purines on the gastric corpus

ATP (100–1000 μg/kg I.A.) and adenosine (100–1000 μg/kg I.A.) administered to vagotomized animals pre-treated with atropine (1 mg/kg I.V.) and guanethidine (5 mg/kg I.V.) produced a small increase in intracorpus pressure (Fig. 6). This response also occurred in the presence of phentolamine (2 mg/kg I.V.) and in animals pre-treated with indomethacin (10 mg/kg I.A.). The amplitude of the response was dose related and the same doses of both purines produced similar responses (Fig. 6). The response consisted of an initial contraction which was usually followed by a slower sustained increase in pressure lasting several minutes (Fig. 6). The latency of the initial response was 3–5 s. A stable analogue of ATP (adenylyl ($\beta\gamma$-methylene) diphosphonate, in the dose range 100–1000 μg/kg I.A.) produced a similar response, as did single doses (100–200 μg/kg I.A.) of $\alpha\beta$-methylene ATP (see below).

Dipyridamole (2 mg/kg I.A.) produced a slow increase in intracorpus pressure which returned to base-line levels after about 10 min. The responses to ATP and adenosine were markedly enhanced after dipyridamole administration, the effect being slightly greater with adenosine (Fig. 6). Dipyridamole had no apparent effect on the inhibitory response to submaximal (5 Hz) vagal stimulation.

Delbro & Fandriks (1984) have reported that the vagally induced n.a.n.c. relaxation of the cat stomach was abolished *in vivo* by desensitization of P_2 purinergic receptors with large doses of $\alpha\beta$-methylene ATP. We have used this substance in the ferret at a higher dose than that used by Delbro & Fandriks (1984). $\alpha\beta$-methylene ATP (200 μg, nine or ten injections) produced contractions in the vagotomized (+1 h)

animals treated with atropine (1 mg/kg) and guanethidine (5 mg/kg I.V.) (Fig. 7). One injection followed another within 1 min and produced an over-all increase in corpus pressure; after five or six injections the pressure reached a plateau. Further injections did not increase the over-all pressure, nor did they manifest themselves as contractions superimposed upon the over-all pressure increase. Stimulation of the

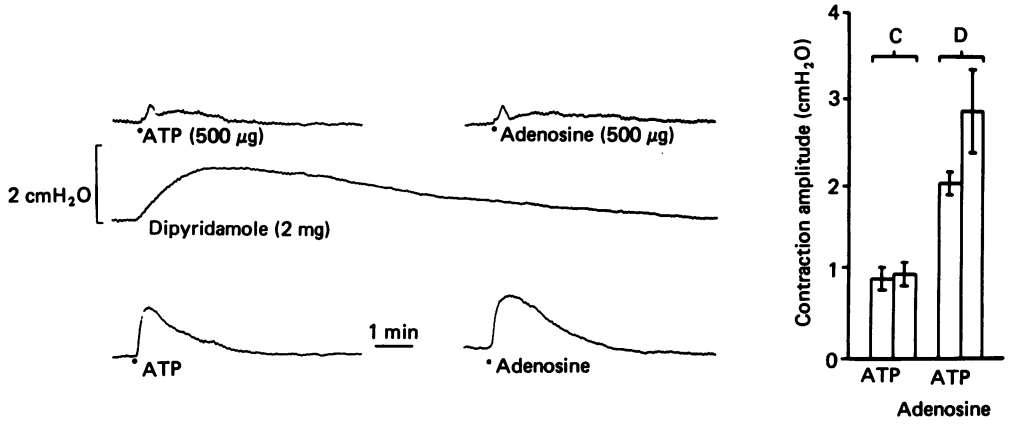

Fig. 6. The effect of ATP (500 μg/kg) and adenosine (500 μg/kg) on intracorpus pressure before (C) and after treatment with dipyridamole (2 mg/kg I.A.) (D). The histogram is based on the results from four animals.

vagus (10 s at 20 V, 1 or 10 Hz, 0·5 ms pulses) within 30 s of the last injection of $\alpha\beta$-methylene ATP produced a fall in intracorpus pressure. The fall in intracorpus pressure was larger than the control response approximately by the degree to which the base-line corpus pressure was increased by the $\alpha\beta$-methylene ATP. Stimulation of the vagus, 10 min after the last injection of $\alpha\beta$-methylene ATP produced a fall in intracorpus pressure similar to the control response. At this time the prevailing intracorpus presure had returned to the control level. $\alpha\beta$-methylene ATP also produced corpus contractions and an increase in the mean pressure after pre-treatment with indomethacin (10 mg/kg I.A.) (Fig. 7). It is of interest that the cardiovascular response to $\alpha\beta$-methylene ATP (an increase in blood pressure) showed signs of desensitization after a similar dose to that at which the corpus pressure began to plateau. The first dose of $\alpha\beta$-methylene ATP (200 μg) increased systolic blood pressure by 50 ± 7 mmHg but after six doses the increase was only 10 ± 2 mmHg ($n = 4$ animals). These increases in blood pressure with $\alpha\beta$-methylene ATP contrast with decreases in systolic (23 ± 3 mmHg) pressure measured in the same animals in response to ATP (500 μg/kg I.A.). The control blood pressures were 130 ± 5 mmHg (systolic) and 81 ± 5 mmHg (diastolic) in these vagotomized, atropine- and guanethidine-treated animals with their greater splanchnic nerves sectioned.

As reported above, ATP and its analogues produced an increase in intracorpus pressure, but in the presence of $\alpha\beta$-methylene ATP (2 mg total I.A.) no contraction was observed. This observation does not provide evidence for desensitization of P_2 receptors, as an increase in intracorpus pressure of itself decreases the amplitude of a corpus contraction evoked by systemic acetylcholine or vagal stimulation (Andrews

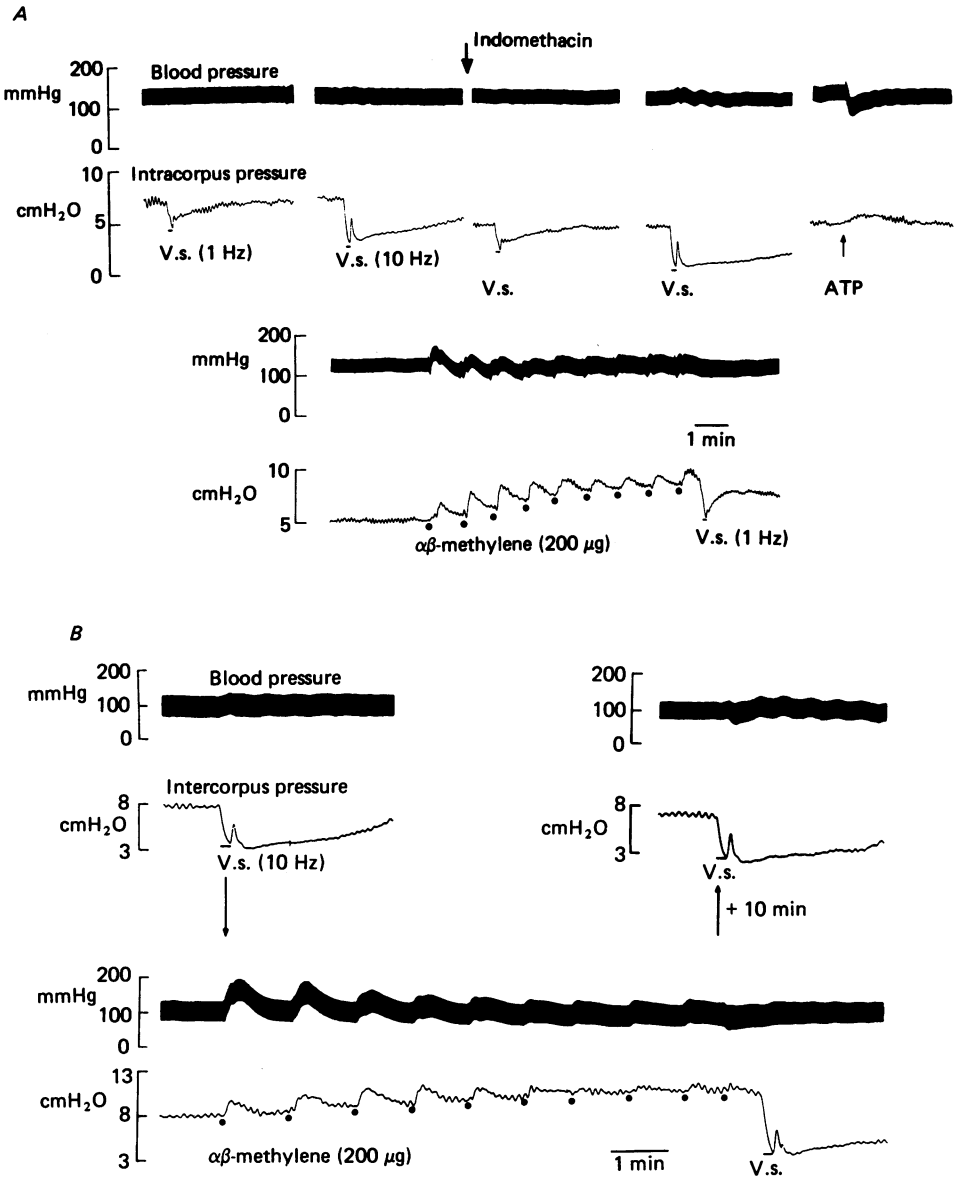

Fig. 7. *A*, blood pressure and intracorpus pressure in a vagotomized, adrenalectomized, atopine- and guanethidine-treated animal. The records show the responses to vagal stimulation (10 s at 20 V, 1 and 10 Hz, 0·5 ms pulses) (v.s.) before and 45 min after administration of indomethacin (arrow). Also shown are the responses to ATP (500 μg/kg I.A.) and nine 200 μg/kg I.A. injections of $\alpha\beta$-methylene ATP. Note the response to 1 Hz vagal stimulation in the presence of $\alpha\beta$-methylene ATP. *B*, blood pressure and intracorpus pressure in a vagotomized, atropine- and guanethidine-treated animal. The records show the response to vagal stimulation (10 s at 20 V, 10 Hz, 0·5 ms pulses) before, during and after administration of $\alpha\beta$-ATP (10 × 200 μg/kg). Note the enhancement of the response to 10 Hz vagal stimulation in the presence of $\alpha\beta$-methylene ATP.

& Lawes, 1982, 1984). $\alpha\beta$-methylene ATP (total dose 2 mg I.A.) also failed to antagonize the response to VIP (1 μg/kg I.A.) and neurotensin (1 μg/kg I.A.).

The above data do not provide any evidence to support the hypothesis that ATP or a closely related compound is involved in the vagally mediated decrease in intracorpus pressure in the anaesthetized ferret.

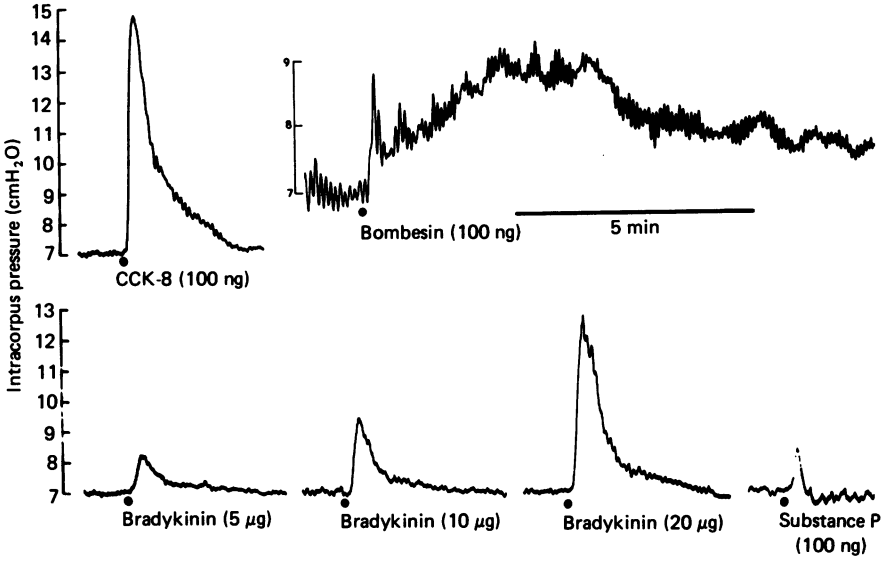

Fig. 8. The effect of CCK-8, bombesin, bradykinin and substance P on the intracorpus pressure in the presence of atropine and guanethidine in a ferret with cut cervical vagi.

The effect of polypeptides on the gastric corpus

The aim of these experiments was to investigate which of a range of peptides could mimic the vagally driven relaxation of the gastric corpus. It should be noted that we do not have any data about the blood or lymphatic levels of these substances in this species and as a result cannot comment on how close our doses are to the physiological levels. In addition, since our experiments aimed to mimic the burst of inhibitory neurotransmitter which is presumed to occur in response to vagal stimulation we administered the peptides as a bolus injection rather than as an infusion.

The effects of the peptides were investigated under a variety of conditions: atropine (1 mg/kg I.V.) and guanethidine (5 mg/kg I.V.); phentolamine (2 mg/kg I.V.), propanolol (2 mg/kg I.V.) and atropine (1 mg/kg I.V.); adrenalectomy, guanethidine (5 mg/kg I.V.) and atropine (1 mg/kg I.V.). In all animals both cervical vagi and both the greater splanchnic nerves were sectioned. No substantial differences were observed in the gastric responses to the peptides under these different conditions. The responses fell into the following two broad groups.

Peptides increasing intracorpus pressure (*Fig 8*). Bradykinin (5–100 μg/kg I.A.), CCK-8 (10–100 ng/kg I.A.) and substance P (10–500 ng/kg I.A.) evoked rapid-onset

contractions of the corpus which reached a peak after similar intervals (bradykinin, 14·2±0·8 s, $n = 4$ animals; CCK-8, 10·5±2·5 s, $n = 4$ animals; and substance P, 12·3±1·3 s, $n = 6$ animals). Substance P produced the smallest-amplitude and shortest-duration response (0·9±1 cmH_2O, 52±11 s duration), whereas CCK-8 and bradykinin produced greater responses (CCK-8, 6·4±0·7 cmH_2O, 130±13 s duration; bradykinin, 6·3±0·4 cmH_2O, 137±9 s duration).

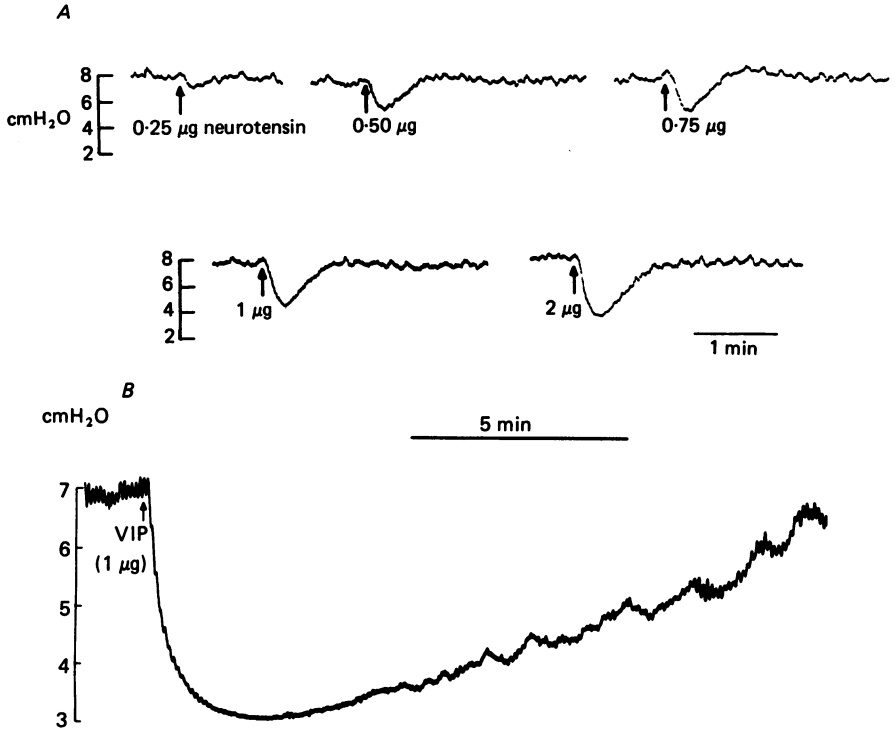

Fig. 9. *A*, the effect of increasing doses (0·25–2 μg) of neurotensin on intracorpus pressure in the presence of atropine and guanethidine in a ferret with cut cervical vagi. *B*, the effect of VIP (1 μg) on intracorpus pressure studied under the same conditions.

Pentagastrin (1–5 μg/kg I.A.) and bombesin (1–2 μg/kg I.A.) produced long-lasting (< 10 min) small (2–3 cmH_2O) increases in the over-all corpus pressure (e.g. Fig. 8). These responses were not systematically studied.

Bombesin, pentagastrin and CCK-8 produced no consistent marked change in blood pressure. Substance P decreased blood pressure reproducibly (systolic decrease 30±7 mmHg, diastolic 28±2 mmHg, $n = 6$ animals, dose 100 ng/kg I.A.). Bradykinin produced a biphasic cardiovascular response consisting of an initial decrease followed by a transient increase. This was not systematically studied. The cardiovascular responses were studied only in atropine- and guanethidine-treated animals with the vagi and greater splanchnic nerves sectioned.

Peptides decreasing intracorpus pressure (*Fig. 9*). Only two of the eight peptides studied decreased intracorpus pressure: neurotensin and VIP. Both peptides produced

dose-related (neurotensin 0·25–2 μg/kg I.A., VIP 0·2–5 μg/kg I.A.) relatively rapid decreases in intracorpus pressure (Fig. 9*A* and *B*). The rate of fall in intracorpus pressure that most closely mimicked vagal stimulation was produced by doses of VIP of 1 μg/kg I.A. or greater and by doses of neurotensin of 500 ng/kg I.A. or greater.

The response to neurotensin lasted approximately 1 min even at the highest doses, whereas the response to VIP lasted several minutes, depending on the dose. In addition to their effect on over-all intracorpus pressure, both peptides suppressed the

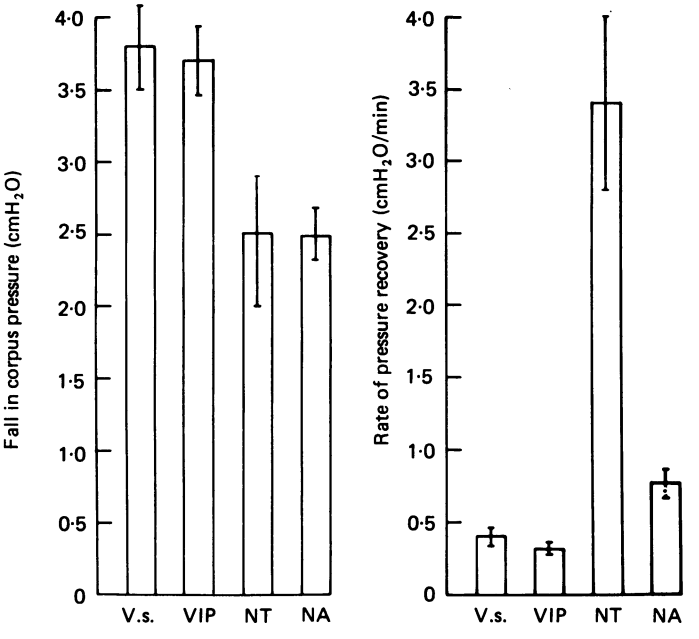

Fig. 10. Histograms showing the fall in intracorpus pressure (cmH_2O) and the rate of recovery (cmH_2O/min) of pressure after vagal stimulation (v.s., 10 s at 10 Hz, $n = 10$), VIP (1 μg/kg, $n = 10$), neurotensin (NT, 1 μg/kg, $n = 6$) or noradrenaline (NA, 10 μg/kg, $n = 4$).

spontaneous contractions of the corpus. The responses to VIP and neurotensin were quantified at a dose of 1 μg/kg I.A. The responses were analysed for the over-all fall in intracorpus pressure and the rate of recovery. The same parameters were measured for the responses to vagal stimulation (10 s at 20 V, 10 Hz, 0·5 ms pulses) and noradrenaline (10 μg/kg I.A.) tested under the same conditions. These results are plotted in Fig. 10. It can be seen that VIP (1 μg/kg I.A.) produced a fall in corpus pressure similar to that obtained from vagal stimulation. Neurotensin (1 μg/kg I.A.) and noradrenaline (10 μg/kg I.A.) evoked smaller responses of similar magnitude to each other. The rate of recovery of the corpus pressure was significantly greater for neurotensin and noradrenaline when compared to vagal stimulation and VIP ($P < 0·02$) (Fig. 10). The rate of recovery from vagal stimulation and VIP were not significantly different. It can also be seen from this histogram that the rate of recovery is not a simple function of the magnitude of fall in intracorpus pressure.

The corpus responses to neurotensin and VIP were present in animals treated with hexamethonium (20 mg/kg I.V.) in addition to those treated as described on p. 13. VIP produced small but consistent decrease in blood pressure (systolic decrease 10 ± 3 mmHg, diastolic decrease 13 ± 4 mmHg, $n = 5$ animals) in the presence of atropine, vagotomy, guanethidine and greater splanchnic nerve section. In contrast, neurotensin at low doses (< 1 μg/kg I.A.) usually produced no change in blood pressure but at higher doses (> 1 μg/kg I.A.) a small delayed, transient increase in blood pressure was observed.

DISCUSSION

The present study demonstrates that the ferret gastric corpus contains potent intramural inhibitory nerves that can be activated by vagal preganglionic fibres. The *in vivo* pharmacological studies (discussed below) provide evidence that the post-ganglionic fibres are non-adrenergic and non-cholinergic (n.a.n.c.) and are also unlikely to be purinergic. Our results indicate that vasoactive intestinal polypeptide (VIP) and neurotensin may be involved in the vagally mediated relaxation of the gastric corpus.

The discussion below deals with the characteristics of the n.a.n.c.-induced relaxation of the corpus and the nature of the neurotransmitter.

Characteristics of the n.a.n.c.-induced relaxation of the corpus

The results show that stimulation of the vagus in the presence of atropine and guanethidine produces a frequency-dependent fall in intracorpus pressure. In common with vagally evoked gastric contractions in the cat and ferret, the full response range was obtained with frequencies between 1 and 10 Hz (Martinson, 1965; Andrews & Scratcherd, 1980). Low-frequency spontaneous discharges have been recorded from abdominal vagal efferents in the ferret (Andrews, Fussey & Scratcherd, 1980) and this may represent tonic activity in the vagal inhibitory fibres. Variations in this tonic activity could contribute to the inter-animal differences in gastric motility previously reported in the anaesthetized ferret (Andrews & Scratcherd, 1980).

In contrast to studies in the cat (Martinson & Muren, 1963; Martinson, 1965), we found no difference in the electrical stimulation threshold between the vagal preganglionic axons responsible for driving the intramural n.a.n.c. neurones and those activating intramural cholinergic neurones. In the cat the vagal inhibitory fibres were reported to have a higher threshold than the excitatory fibres. However, because of differences in (a) the diameters of the vagal trunks in the cat and ferret, (b) the patterns of electrical stimulation, (c) the recording of gastric motility, and (d) the criteria for assessing inhibitory fibre activation (see p. 8), it is not possible to explain the discrepancy. It is of interest that in the cat and pig, VIP is released only in response to activation of the high-threshold vagal fibres alone, and it would be worth measuring VIP release in the ferret to determine the stimulation threshold for its release (Fahrenkrug, Galbo, Holst & Schaffalitzky de Muckadell, 1978).

In addition to the long duration of the n.a.n.c.-mediated corpus relaxation (see below), a second interesting feature of the response is the rebound contraction which occurs on cessation of stimulation. No such rebounds are visible in Martinson's (1965)

records of vagal stimulation in the cat using volume recording of gastric activity. Although a species difference may account for the discrepancy we think it is more likely to be due to methodological differences, as rebound gastric contractions have been recorded in the rat and marmoset (P. L. R. Andrews, unpublished observations) under conditions similar to those used in the present experiment. In addition, *in vitro* studies have demonstrated rebound contractions in the gastric muscle of the pig (Ohga & Taneike, 1977), guinea-pig (Beani, Bianchi & Crema, 1971), kitten (Paton & Vane, 1963) and dog (Schmalz, Morgan & Szurszewski, 1983) in response to activation of the intramural inhibitory nerves. The rebound contraction in the ferret gastric corpus is of particular interest as it is superimposed upon the long-lasting depression in tone produced by activation of the n.a.n.c. nerves. In the corpus the rebound is always a single contraction, in contrast to the multiple contractions which are often observed in the antrum (see Andrews & Scratcherd, 1980). This difference between the corpus and antrum may reflect the differences in the electrophysiological properties of muscle from different regions of the stomach reported by Morgan, Muir & Szurszewski (1981). An extensive discussion of the mechanisms underlying the rebound contraction is beyond the scope of this paper. However, two points are worthy of note. (a) Rebound contractions are only observed when gastric pressure is lowered by activation of n.a.n.c. nerves. Sympathetic stimulation producing similar relaxation of the corpus does not cause rebounds. Therefore, the rebound is a consequence of the way in which the muscle is made to relax rather than the relaxation *per se*. (b) Rebounds only follow electrical stimulation which stops abruptly. N.a.n.c. nerve activation by nicotine or vago-vagal reflexes does not produce rebounds (Andrews & Grundy, 1981; Andrews & Lawes, 1982). Whilst there is no evidence that rebounds occur in the stomach in response to physiological stimuli, a study of the factors affecting their magnitude and frequency may shed light on the mechanism(s) of their production.

The nature of the inhibitory transmitter(s)

The vagal preganglionic fibres appear to activate the intramural inhibitory fibres via a nicotonic cholinergic synapse. The evidence for this is that the gastric relaxation evoked by vagal stimulation in the presence of atropine is blocked by hexamethonium and the intramural inhibitory fibres can be activated by local injections of nicotine in the presence of atropine (Andrews & Scratcherd, 1980; Andrews & Lawes, 1982). This nicotinic cholinergic link between the vagal and intramural n.a.n.c. neurones in the ferret is in agreement with similar studies in the cat (Martinson, 1965), dog (Ohga, Nakazato & Saito, 1970), guinea-pig (Beani, Bianchi & Crema, 1971) and pig (Ohga & Taneike, 1977). The nature of the transmitter(s) released by the intramural inhibitory nerves is still controversial. In the present study we have attempted to identify the type of transmitter by using recognized receptor antagonists and close arterial injections of putative transmitters to mimic the response to vagal stimulation. Three candidate transmitters are discussed below.

Noradrenaline. In demonstrating inhibition of gastric motility by stimulation of the vagus, the possibility that the inhibitory transmitter is adrenergic must first be excluded, especially as in man and the cat adrenergic fibres are present in the abdominal vagus (Lundberg, Ahlman, Dahlstrom & Kewenter, 1976; Lieberg,

Nielsen, Owman & Sjoberg, 1983). In the cat these adrenergic fibres disappear after removal of the stellate ganglion. The present study and a previous one (Andrews & Scratcherd, 1980) demonstrated that the vagal inhibitory response was unaffected by phentolamine and propranolol. In the guinea-pig vas deferens it has been suggested that the failure of these agents to antagonize an adrenergic contraction is due to the fact that they may not gain access to the receptor site due to the structure of the synapses in this tissue (Furness, 1974). This argument cannot be used in the present study for two reasons: (i) the structure of the neuromuscular junction in gastric smooth muscle is not the same as that in the guinea-pig vas deferens (Daniel, 1977); (ii) the response to n.a.n.c. nerve activation was not antagonized by guanethidine, which displaces noradrenaline from noradrenergic nerve terminals (Cass, Kuntzman & Brodie, 1960; Chang, Costa & Brodie, 1965). The dose of guanethidine used in the present study has been shown to abolish the gastric response to stimulation of the greater splanchnic nerve in the ferret (Andrews & Lawes, 1984) and cat (Jansson & Martinson, 1966). In addition to these observations, noradrenaline does not mimic the inhibitory response to vagal stimulation in detail. The studies with dopamine, histamine, serotonin and enkephalin receptor antagonists and aprotinin indicate that the corresponding neurotransmitters are not involved in the vagal inhibitory response. Although we have used doses of receptor antagonists known to be effective in other species, caution must be exercised in interpreting our results until more data are available on their efficacy in the ferret. Because of these difficulties we undertook a study to mimic the vagal response by bolus arterial injections of putative transmitters.

ATP. Recent studies in the cat (Delbro & Fandriks, 1983) have provided some evidence that ATP may be involved in the vagally driven gastric relaxation in the cat. In the present study we were unable to find any evidence for an involvement of ATP and in view of this apparent species difference it is worth discussing the experiments in detail.

In our experiments ATP, $\alpha\beta$-methylene ATP and $\beta\gamma$-methylene ATP all contracted the corpus in the presence of atropine, guanethidine, vagotomy and greater splanchnic nerve section. It could be argued that the contractions are secondary to the release of prostaglandins, as suggested by Burnstock, Cocks, Paddle & Staszewska-Barczak (1975). However, contractions were still produced in the presence of indomethacin *in vivo* (present study) and *in vitro* (Andrews, Davison & Flatman, 1983). This contrasts with Delbro & Fandriks' (1983) experiments in which the contractions produced by ATP were converted to relaxation by indomethacin. $\alpha\beta$-methylene ATP and $\beta\gamma$-methylene ATP produced relaxations in the cat stomach even in the absence of indomethacin. In addition, Delbro & Fandriks (1984) reported that desensitization of P_2 purinergic receptors by $\alpha\beta$-methylene ATP antagonized the gastric relaxation produced by vagal activation of n.a.n.c. nerves in the anaesthetized cat. The present study, using both submaximal and maximal vagal stimulation, failed to reveal any effect of $\alpha\beta$-methylene ATP on the vagal inhibitory response. The conclusion from these experiments is that we can find no evidence for involvement of ATP in the vagally mediated n.a.n.c. response in the ferret gastric corpus.

Peptides. The gastric corpus responses to the bolus-injected peptides fall into two groups: (i) contraction, and (ii) relaxation. These will be discussed separately.

(i) Bradykinin, CCK-8, substance P, pentagastrin and bombesin all produced increases in intracorpus pressure in the presence of atropine, guanethidine, vagotomy and greater splanchnic nerve section. It would, therefore, appear that their effect is not produced by activation of intramural, atropine-sensitive cholinergic neurones. Because the main aim of the experiments was to identify substances which mimicked the vagally mediated inhibition of the gastric corpus, the final site of action of the above substances was not investigated but work is in progress to compare their effects on the gastric corpus and antrum.

(ii) Neurotensin and VIP both produced rapid-onset falls in intracorpus pressure in the presence of atropine, guanethidine, vagotomy and greater splanchnic nerve section. However, the effect of VIP outlasted that of neurotensin. For each substance a dose of 1 μg/kg I.A. produced a fall in intracorpus pressure comparable to maximal vagal stimulation. The slow recovery of intracorpus pressure from a short period of vagal stimulation (10 s) is one of the striking features of the inhibitory fibres in the ferret and other carnivores (e.g. cat, Jansson & Martinson, 1965). We regard the ability of a single injection of VIP to mimic this phase as an important indicator for the involvement of VIP in the vagal response. In addition to mimicking the over-all shape of the fall in intracorpus pressure, VIP also inhibited the spontaneous corpus contractions. In contrast to VIP, neurotensin matched only the early phase of the vagally driven relaxation of the corpus. The possibility that both VIP and neurotensin contribute to this response cannot be excluded, particularly as the fall in intracorpus pressure can be resolved into an early dynamic phase and a later prolonged phase (see p. 5).

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