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THE GASTRIC MOTILITY PATTERNS INDUCED BY DIRECT AND REFLEX EXCITATION OF THE VAGUS NERVES IN THE ANAESTHETIZED FERRET

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SUMMARY

1. Under urethane anaesthesia the ferret has a stomach which exhibited spontaneous contractions. The amplitude of the contractions was reduced, but never abolished, by vagotomy, atropine or hexamethonium.

2. Electrical stimulation of the cut peripheral end of the cervical or abdominal vagus nerves activated both excitatory and inhibitory fibres, whereas reflex activation by stomach distension or by cytoglucopenia was predominantly excitatory on gastric motility. The magnitude of the response to electrical stimulation was dependent upon the stimulus frequency, duration and voltage. Stimulation for periods longer than 10 sec at 10 Hz caused an increase in tone and motility which declined almost to control values if it was sufficiently prolonged.

3. Short duration stimulation, 10 sec or less, at 10 Hz produced a single gastric contraction. An identical response could only be produced if a period of rest from 30 to 120 sec separated two such stimuli.

4. The frequency of gastric contractions was independent of the frequency of nerve stimulation.

5. Using a divided stomach, vagal non-cholinergic, non-adrenergic fibres were shown to cause mainly relaxation of the corpus region. Similar fibres supplied the antrum and their activation caused inhibition of spontaneous contractions. The transmitter may be 'purinergic' as rebound contractions were observed.

INTRODUCTION

Direct electrical excitation of the cut peripheral end of the vagus nerves, either in the neck, thorax or abdomen has been frequently used to investigate the role of the extrinsic nerves in the control of gastric motility (Veach, 1925; McSwiney & Wadge, 1928; McSwiney, 1931; Harper, Kidd & Scratcherd, 1959; Martinson & Muren, 1963; Martinson, 1964; Jansson & Martinson, 1965) and gastric emptying (Carr & Brooks, 1978). However, much of the work has been casual, unsystematic and qualitative in nature and the conditions required to produce responses which are consistently reproducible have rarely been studied. The present paper describes conditions under which repeatable responses can be obtained by direct electrical stimulation. The motility patterns induced by direct and reflex vagal stimulation are compared in

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order to examine whether the responses evoked by electrical stimulation mimic those elicited by physiologically activated reflexes.

METHODS

Sixty-five male and female ferrets weighing between 500 and 1650 g were used. They were fed on a standard carnivore diet with free access to water, but were deprived of food for 24 hr before experimentation. Anaesthetization was by a single dose of I.P. urethane (1·5 g kg^{-1}). A glass cannula was inserted into the trachea to allow adequate ventilation and the right external jugular vein cannulated for the administration of drugs. The stomach was intubated via the cervical oesophagus and the tube held in place by a ligature. The abdominal viscera were exposed and a ligature tied just below the pylorus. The abdominal cavity was then closed in layers. Residual stomach contents were washed out with 0·9 % NaCl at 37 °C. The rectal temperature of the animal was maintained between 38 and 40 °C throughout the experiment by radiant heat. Systemic arterial blood pressure, e.c.g., heart rate and respiration were routinely monitored. The above procedures applied to all the animals; below are given the additional operative procedures for each group of experiments.

Divided stomach

The stomach was surgically divided into antral and corpus pouches as previously described (Andrews, Grundy & Scratcherd, 1980). Pressure in the antral pouch was measured via a catheter inserted through the pylorus and that of the corpus pouch through an oesophageal tube.

2-Deoxy-D-glucose (ten animals). Bilateral adrenalectomy was performed in order to prolong the cytoglucopenia. 2-Deoxy-D-glucose itself increases the discharge rate in the sympathetic nerves to the adrenals (Niijima, 1975) and may produce an increase in the production of adrenaline (Hökfelt & Bydgeman, 1961).

Electrical vagal stimulation (fifty animals). The vagi were exposed in the neck, separated from the carotid arteries, cleared of connective tissue for a length of 1 cm, ligated and cut centrally. The peripheral end of the nerve was placed over pipolar silver electrodes to which stimuli were delivered from a Devices isolated stimulator. The pulses were precisely timed by a Digitimer (D4030). Pulses of up to 30 V strength, and of 0·5 msec to 1 msec duration and variable frequency were used. Stimulation time and pattern were varied, and each period of stimulation was followed by a rest period. The electrode was periodically moved caudally to prevent damage to the nerve by prolonged stimulation at one site. In several animals, the abdominal and thoracic vagal trunks were stimulated. The vagal trunks in these animals were approached by resection of the 11th and 12th ribs on the left side, the animal was then artificially ventilated.

Gastric inflation (five animals). Motility was reflexly induced by inflating the stomach with 50 ml. 0·9 % NaCl (37 °C) at a rate of 10 ml./min. This rate and volume are within the physiological range for the ferret (Andrews *et al.* 1980). The vagi remained intact for these experiments.

Recording and analysis

Gastric motility was usually recorded in a constant volume system (20 ml.) by means of a fluid filled pressure transducer (S.E. Labs Ltd) connected to the oesophageal tube and displayed on a chart recorder (Bryans 28000). The following parameters were measured, wave amplitude above the resting level, response duration, wave frequency and integrated response. The rate of rise and the latency of the response were also measured. The integrated reponse was measured by cutting out the shape of the response from the paper chart, weighing it and converting this to a standard unit, the min.cm H_2O. This unit is a compound of pressure and time and may be visualized as the force exerted by the gastric musculature to maintain a pressure of 1 cmH_2O for 1 min. In the Figures 0 cm H_2O refers to atmospheric pressure.

Drugs. All drugs were made up in 0.9 % NaCl. The following drugs were used: atropine sulphate (Koch-light Labs), phentolamine mesylate (Ciba), DL-propranolol (Sigma), acetylcholine chloride (Lematte and Boinott), cimetidine (Tagamet SK & F), neostigmine bromide (Sigma), mepyramine maleate (M & B). All results are expressed as mean ± S.E. (n = number of observations).

RESULTS

Basal gastric motility

Gastric motility was observed in all animals on opening the abdomen, but was apparent in only 60 % of animals (as indicated by intragastric pressure record) after the abdomen was closed on completion of the operative procedures. Spontaneous contractions were present whether or not fluid was in the stomach. The spontaneous gastric contractions were classified on the basis of amplitude into low (< 2 cm H_2O), medium (> 2 < 5 cm H_2O) or high (> 5 cm H_2O) level; 12 % of animals had a high level, 40 % medium and 48 % low level of gastric motility. An example of each type of spontaneous gastric motility is shown in Fig. 1*A*. The waves of contraction often appeared in the form of regularly repeating patterns or bursts of activity and could turn 'on' or 'off' unexpectedly. Both the amplitude and frequency of the gastric contractions varied during the course of an experiment although the frequency of contractions rarely exceeded 10 c/min. The frequency of contractions (including all types of waves) in the vagally intact animals was $7{\cdot}1 \pm 0{\cdot}05$ c/min mean ± S.E. (n = 950 one minute observation periods) and in the vagotomized animals was $6{\cdot}8 \pm 0{\cdot}05$ c/min mean ± S.E. (n = 380 one minute observation periods).

Effect of acute vagotomy. Division of the cervical or abdominal vagi produced an immediate fall in the gastric tone that usually recovered within 10 min (Fig. 1*B*). The degree of tone drop varied considerably between animals. In two animals the gastric tone continued rising above control levels after actue vagotomy (cf. Harper *et al.* 1959). Vagotomy also usually produced a permanent decrease in the amplitude of the spontaneous contractions, e.g. in one animal the contractions were reduced from $2{\cdot}0 \pm 0{\cdot}07$ to $0{\cdot}4 \pm 0{\cdot}02$ cm H_2O. Spontaneous motility was never totally abolished by acute vagotomy.

Effect of atropine, hexamethonium and neostigmine. Intravenous doses of atropine in the range 1 to 4 mg/kg did not abolish the spontaneous contractions although the amplitude was reduced, especially in the vagally intact animals. There was either a transient fall in tone lasting two to three minutes or sometimes a permanent fall in the resting tone. This was observed in both the intact and vagotomized animals. In doses of 1–8 mg/kg, hexamethonium produced a fall in gastric tone and in the vagally intact animals a reduction in the amplitude of the contractions. Spontaneous gastric contractions were not abolished by a combination of atropine (1 mg/kg) and hexamethonium (2 mg/kg). In animals with either no or low level spontaneous gastric motility, the gastric tone and contraction amplitude was increased by neostigmine (1 mg/kg). Gastric motility was induced by neostigmine in animals in which it was previously absent. This effect was observed in both vagotomized and intact preparations.

The pattern of pressure changes (motility) induced by efferent vagal stimulation

Frequency of stimulation. Stimulation of the cut peripheral end of either cervical vagus, at a voltage (20 V, 0·5 msec) which was shown to be supramaximal for C-fibre activation induced consistent motility patterns. The latency of the response measured in forty-eight trials in eight animals was $1{\cdot}2 \pm 0{\cdot}05$ sec when a stimulation frequency of 10 Hz was used. The first component of the C-fibre mass potential,

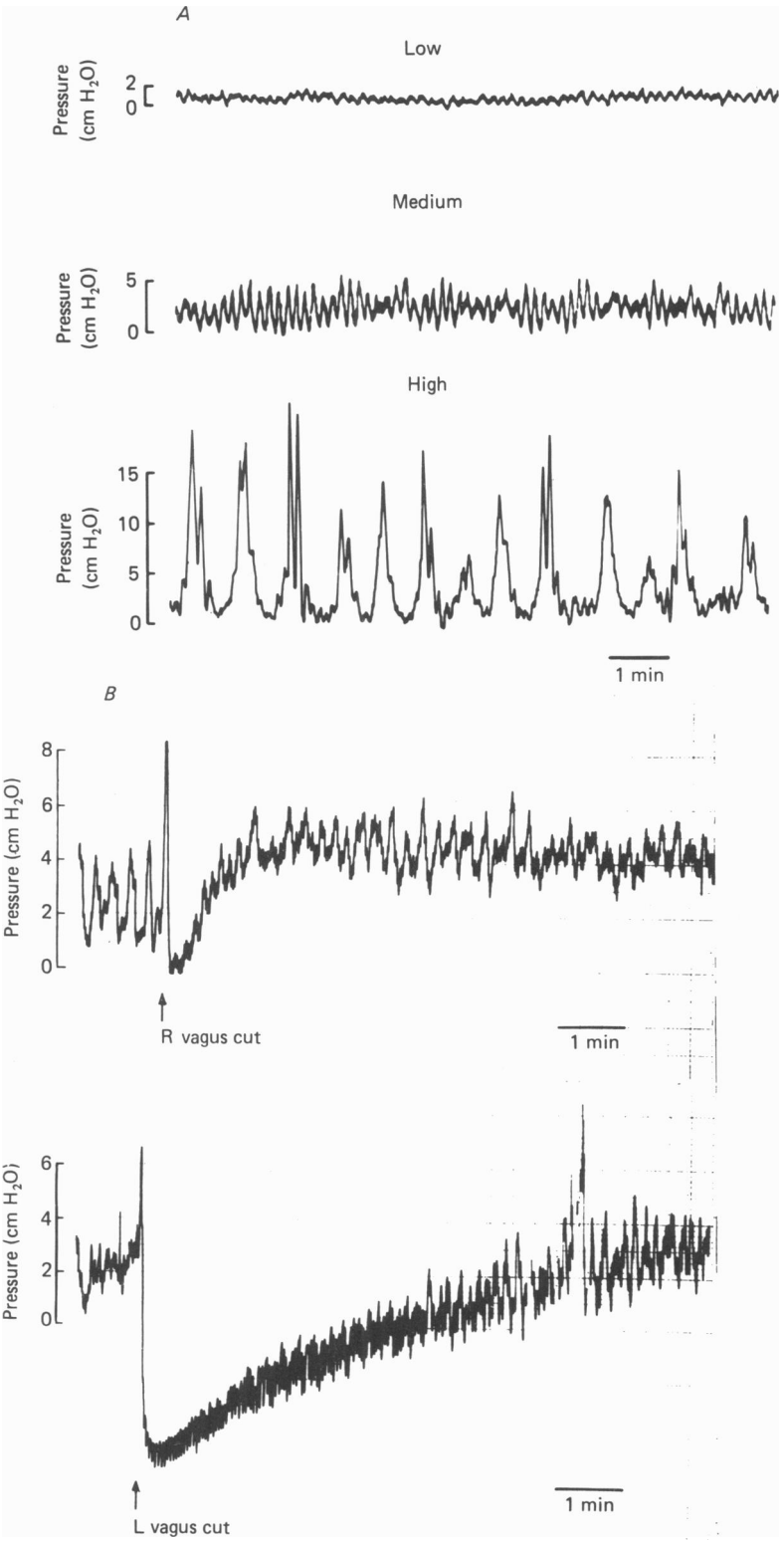

Fig. 1. *A*, illustration of three different levels of spontaneous motility in the anaesthetized, vagotomized ferret. *B*, record of the immediate effect of right cervical vagotomy followed by left cervical vagotomy on the intragastric pressure and spontaneous motility.

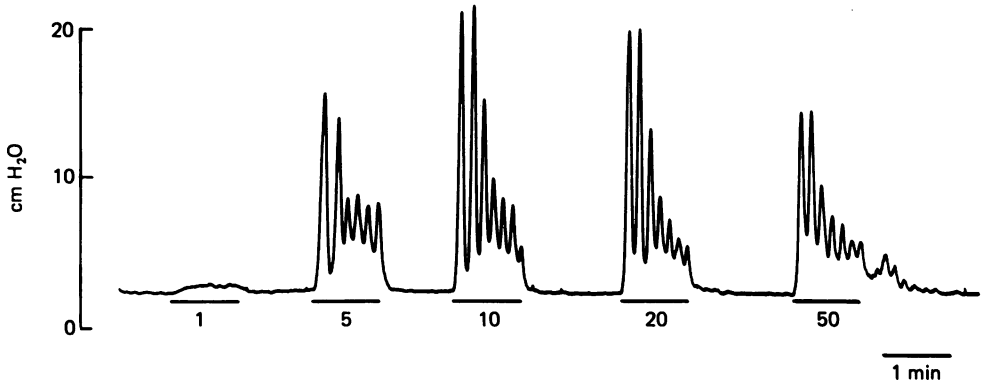

Fig. 2. The effect of peripheral cervical vagal stimulation for 1 min at 1, 5, 10, 20, 50 Hz (20 V, 0·5 msec) on gastric motility. The fall in intragastric pressure usually seen after removal of the stimulus was not present in this animal.

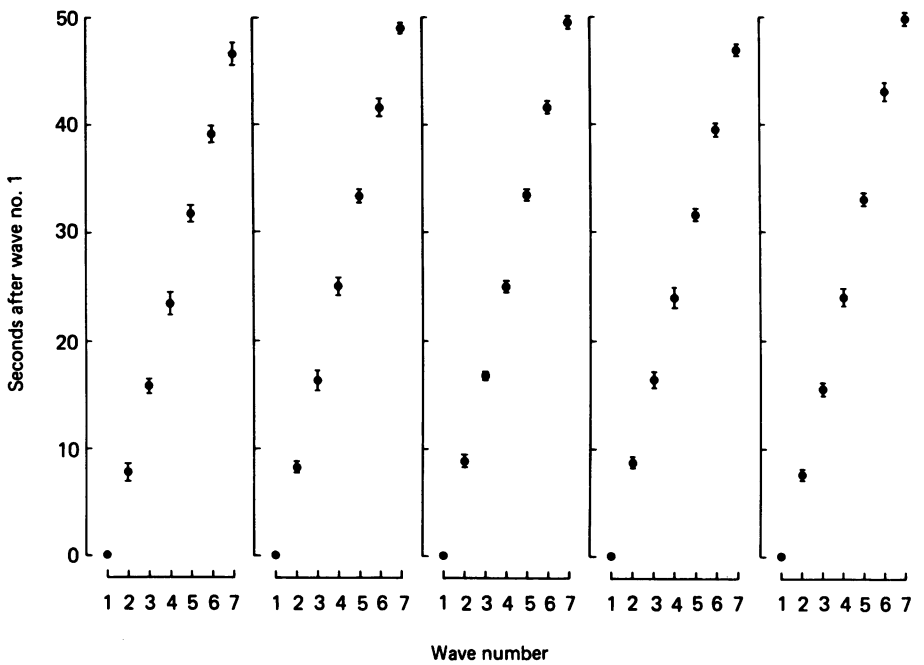

Fig. 3. Graph showing the temporal distribution of gastric contractions in response to 1 min vagal stimulation at 1, 5, 10, 20 and 50 Hz (20 V, 0·5 msec).

recorded at the gastro–oesophageal junction, had a latency of $157 \pm 5{\cdot}2$ msec ($n = 5$), which indicates that the major proportion of the response latency resided in the myenteric plexus and smooth muscle. The rate of rise of the intragastric pressure response was related to the frequency of stimulation. The longest rise to peak pressure was at 1 Hz whereas the maximum rate was observed at 10 Hz when the rate was $3{\cdot}1 \pm 0{\cdot}29$ cm H_2O/sec ($n = 6$).

Regular gastric contractions were evoked when the vagus was stimulated con-

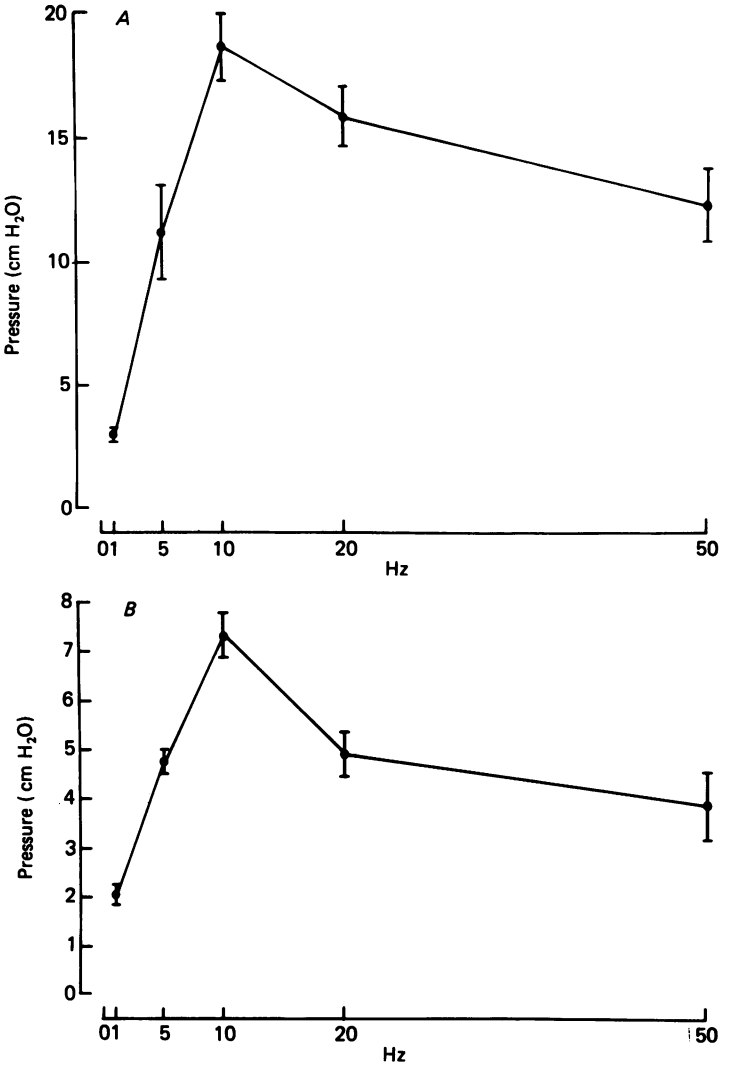

Fig. 4. *A*, the mean amplitude of the gastric contractions in responses to 1 min vagal stimulation at 1, 5, 10, 20, 50 Hz (20 V, 0·5 msec). *B*, the integrated gastric motility response to 1 min vagal stimulation at 1, 5, 10, 20, 50 Hz (20 V, 0·5 msec). In both *A* and *B* the number of stimulations (in parenthesis) at the indicated frequency was – 1 Hz (31), 5 Hz (44), 10 Hz (52), 20 Hz (26), 50 Hz (12).

tinuously at 1–50 Hz for 1 min (Fig. 2). The waves were precisely timed and independent of the frequency of stimulation. The intervals between each contraction in response to various frequencies of stimulation were $7{\cdot}8 \pm 0{\cdot}12$ sec (1 Hz, $n = 8$); $8{\cdot}2 \pm 0{\cdot}2$ sec (5 Hz, $n = 18$); $8{\cdot}3 \pm 0{\cdot}12$ sec (10 Hz, $n = 23$); $7{\cdot}8 \pm 0{\cdot}2$ sec (20 Hz, $n = 18$); $7{\cdot}8 \pm 0{\cdot}3$ sec (50 Hz, $n = 13$). The frequency of vagal stimulation had little effect on the frequency of the evoked gastric contractions as can also be seen from the plot of the relationship between the first contraction and the subsequent six contractions in the response to 1 min stimulation at various frequencies (Fig. 3).

The frequency of evoked gastric contractions was 7·2–7·7 contractions/min. Not

only were the evoked waves precisely located in time but in the same animal the amplitude of the individual waves was remarkably consistent. Separate waves of contraction of a similar nature could be obtained by intra-arterial infusions of acetylcholine. When the stimulation frequency was 1 Hz, the response normally rose to a plateau with small superimposed waves and remained at that level until the stimulus was removed. With stimulation frequencies greater than 1 Hz, the response rose rapidly to a peak with the first or second wave and thereafter declined

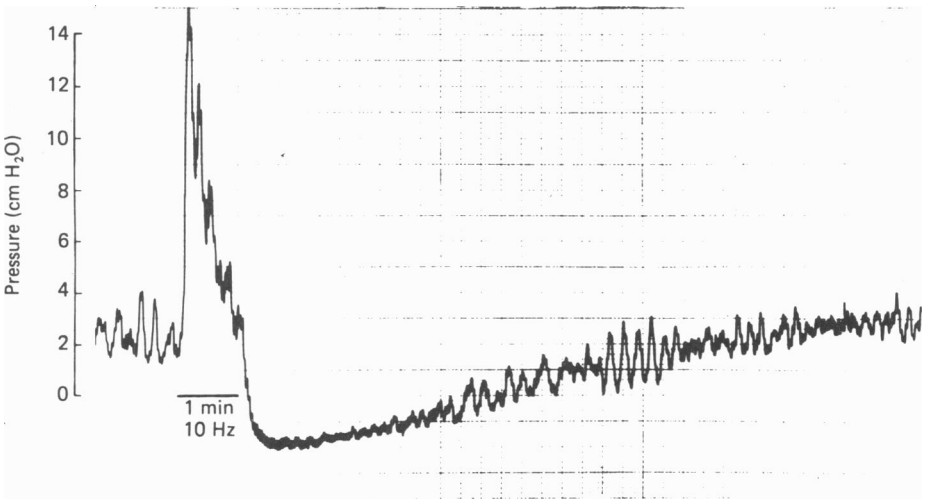

Fig. 5. Record of the effect of 1 min stimulation of the peripheral cervical vagus on gastric motility (10 Hz, 20 V, 0·5 msec). Note the fall in tone and inhibition of motility after removal of the stimulus.

even though the stimulus was continued (Fig. 2). The amplitude of the contractions and the integrated response to variations in frequency of the applied stimuli are plotted in Fig. 4*A*, *B*.

Typically, on removal of the stimulus, there was a decrease in tone and an inhibition of motility which lasted several minutes (Fig. 5). After stimulation for 60 sec at 10 Hz, the time taken for the relaxation to reach its lowest value was $37{\cdot}2 \pm 5{\cdot}6$ sec ($n = 10$) and the time for the tone to recover to the pre-stimulation level was 259 ± 34 sec ($n = 10$). With only 10 sec stimulation at 10 Hz the corresponding values were $20{\cdot}8 \pm 2{\cdot}1$ sec ($n = 4$) and $187{\cdot}4 \pm 22{\cdot}0$ sec ($n = 4$). The spontaneous motility always returned before the tone had regained its pre-stimulation level. This inhibitory/relaxatory response was unaffected by either an adrenergic receptor blockade, with phentolamine (2 mg/kg) and propranolol (2 mg/kg), or by H_1 or H_2 histamine receptor blockade with mepyramine (2 mg/kg) and cimetidine (4 mg/kg).

Duration of stimulation. Stimulation of the cervical vagus for 10 sec at a frequency of 10 Hz produced a single gastric contraction of maximal amplitude (Fig. 6*A*). Increasing the duration of the stimulation (> 10 sec) resulted in multiple waves of contraction which became decremented with increasing duration of the applied stimulus. The contractions often fell in amplitude until they became no larger than

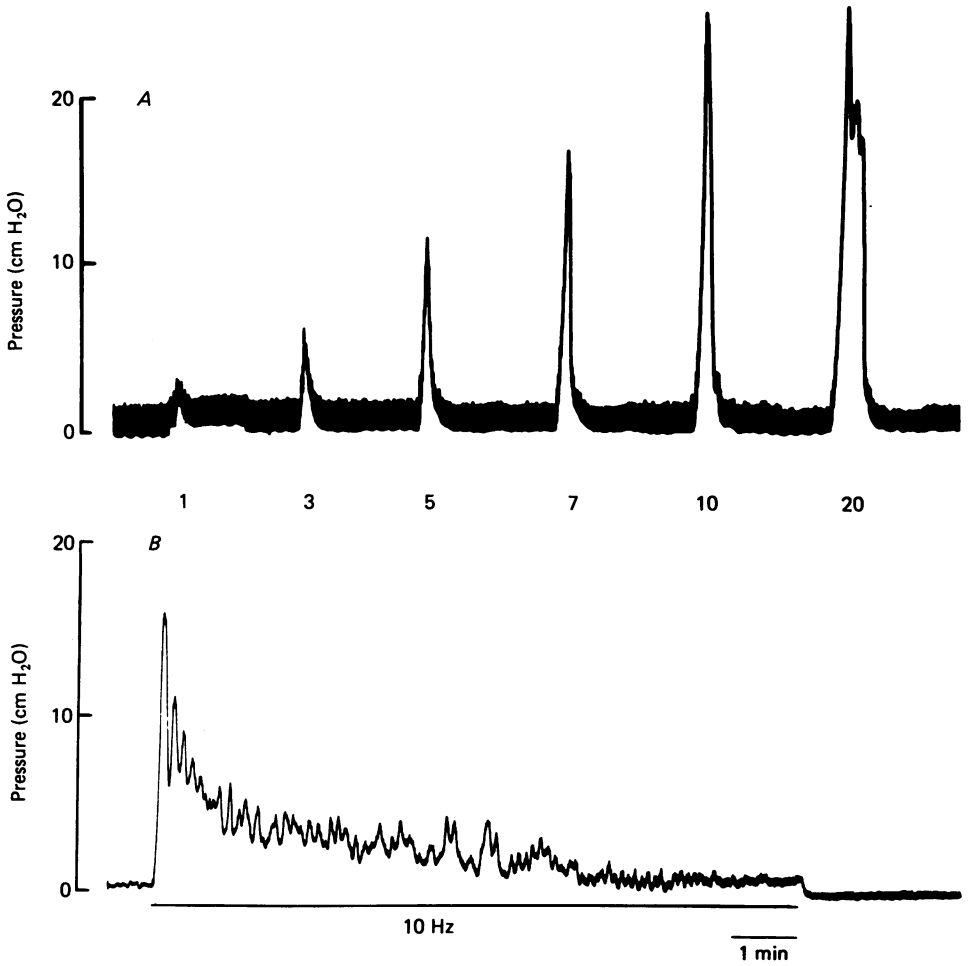

Fig. 6. *A*, the effect on gastric motility of changing the duration of vagal stimulation between 1 and 20 sec at a frequency of 10 Hz (20 V, 0·5 msec). *B*, the effect of continuous cervical vagal stimulation for 10 min at 10 Hz (20 V, 0·5 msec) on the gastric motility.

the spontaneous contractions existing before stimulation (Fig. 6*B*). However, the gastric tone remained elevated above the pre-stimulation value until the stimulus was removed. The cause of this decline in contraction amplitude was further investigated. The injection of atropine (1 mg/kg) or hexamethonium (1 mg/kg) during the phase when there were no contractions caused a fall in tone below the base line level. This indicates that the vagus still exerted an influence on the tone even in the absence of evoked contractions. Stimulation of the contralateral vagus was ineffective during the phase when there were no evoked contractions produced by stimulation of the ipsilateral nerve, whereas the intra-arterial injection of acetylcholine produced a large gastric contraction. These observations implicate the myenteric plexus as the site of some 'fatigue' phenomenon.

The influence of a period of rest on the response to vagal stimulation. When either the

right or left cervical vagus nerves were stimulated for 10 sec at 10 Hz, a maximal amplitude gastric contraction occurred. This response was quite characteristic and consistently repeatable. Two such stimulation periods were separated by an interval of rest which varied from 2·5 to 120 sec. When the interval between the periods of stimulation was short, the second stimulus always produced a contractile response which was less than that produced by the preceding stimulus (Fig. 7). As the interval between the periods of stimulation was increased the amplitude of the response to the second stimulus also increased until the two responses became equal (Fig. 7). The time taken for this to be achieved varied from animal to animal. It could be as

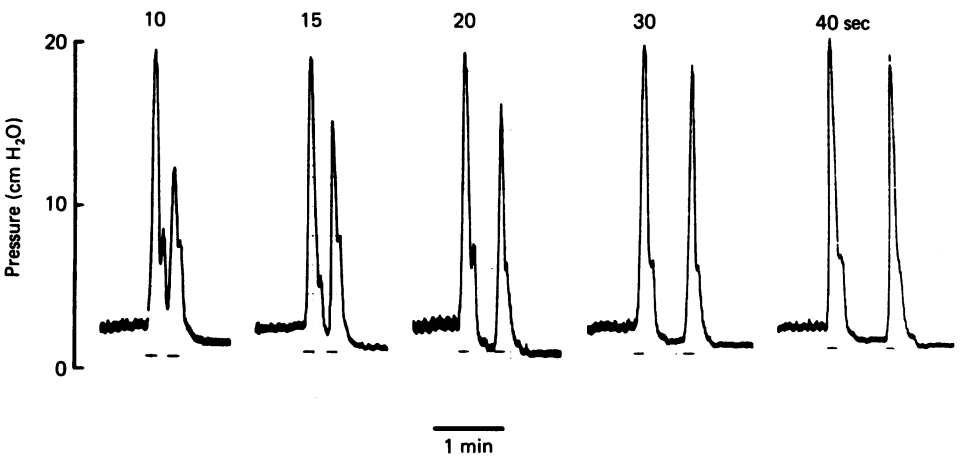

Fig. 7. The effect of increasing the period of rest between stimulations from 10 to 40 sec on the gastric response to 10 sec (10 Hz, 20 V, 0·5 msec) periods of stimulation.

little as 30 sec but was usually much longer. However, both responses were identical after a period of rest of 120 sec. The results were similar if the second stimulus was applied to the ipsilateral nerve or to the contralateral nerve.

Effect of stimulus voltage. Gastric pressure changes were elicited when the stimulus voltage was sufficient to evoke a C-fibre mass potential (conduction velocity 1·33 m/sec) in the vagi at the level of the gastro–oesophageal junction. The threshold of the vagal gastric motility fibres was always higher than those responsible for causing bradycardia. A voltage–response curve was constructed by stimulating either cervical vagus at 10 Hz for 10 sec with varying voltages in a random fashion. The amplitude of the response increased rapidly for only small changes in voltage, indicating that a fairly homogeneous population of vagal fibres was involved in the activation of motility. The decline in amplitude at high stimulus strengths described by Martinson & Muren (1963) was not observed. The amplitude and the rate of rise in intragastric pressure increased with increasing voltage. This indicates that the rate of rise of the response is dependent upon the number of activated pre-ganglionic nerve fibres as well as on the frequency of stimulation.

Electrical stimulation of the thoracic and abdominal vagi

In three animals the gastric motor response to cervical, thoracic and abdominal vagal stimulation to the same parameters (60 sec, 1–50 Hz) were compared. The responses were similar in terms of number of waves, overall shape and after relaxation. The observation that a decline in the response to one minute stimulation of 10 Hz (see Fig. 2) was observed in responses evoked by stimulation of the cervical, thoracic and abdominal vagi indicates that the decline is not due to the dramatic cardiovascular effects produced by stimulation of the cervical vagus. A similar decline in the response was also observed by Paton & Vane (1963) in the isolated guinea-pig stomach, in which there can be no cardiovascular effects. The threshold of activation was similar in all three sites as were the actions of atropine, phentolamine, propranolol, mepyramine and cimetidine on the vagally evoked responses. Quantitative differences were observed in the amplitude of the contractions evoked by stimulation at each vagal site. This has been investigated in detail by Andrews, Bower & Lawes (1978).

The vagal influence on corpus and antral motility. Experiments were performed on a surgically divided stomach in order to compare the effect of the vagus on the two major functionally different regions. After division of the stomach, there was frequently an increase in the amplitude of spontaneous motility of both corpus and antral pouches. The frequency of the corpus contractions always exceeded those of the antrum and this is consistent with Daniel's (1975) relaxation oscillator model of gastric motility. Continuous vagal stimulation affected both pouches to different degrees. In the case of the corpus after an initial contraction and an increase in tone, a progressive fall in tone occurred with superimposed small contractions which waxed and waned. The frequency of these contractions was $8{\cdot}0 \pm 0{\cdot}44$ ($n = 15$) per minute (Fig. 8).

In contrast, after an initial large contraction, the antral tone also increased and remained elevated with large waves of contractions superimposed. The frequency of the evoked contractions was $6{\cdot}9 \pm 0{\cdot}36$ ($n = 15$) per minute, about 1 contraction per minute slower than the corpus. A stimulation frequency response curve was constructed for the divided stomach in the same way as that for the intact organ. Its shape was similar for both corpus and antrum and both showed a peak at 10 Hz. However, the magnitude of the response was much greater in the antrum. On cessation of stimulation, the corpus and the antrum also exhibited different responses. In the corpus there was a marked decrease in tone which fell below prestimulation values and this was accompanied by a loss of spontaneous contractile activity (Fig. 8). The tone then gradually increased and spontaneous activity returned with a time course similar to that observed in the intact stomach (Fig. 5). In the antrum, there was a fall in tone which was only just perceptible but there was no loss of spontaneous contractile activity (Fig. 8). Vagal stimulation after atropinization (1 mg/kg) of the animal caused a fall in intragastric pressure after latency of $1{\cdot}3 \pm 0{\cdot}6$ sec ($n = 5$) in the intact stomach. This effect was studied in detail in the divided stomach. The fall in tone was almost entirely confined to the corpus and fundus (Fig. 9) and here again the recovery of tone and spontaneous contractions was similar to that observed in the intact stomach.

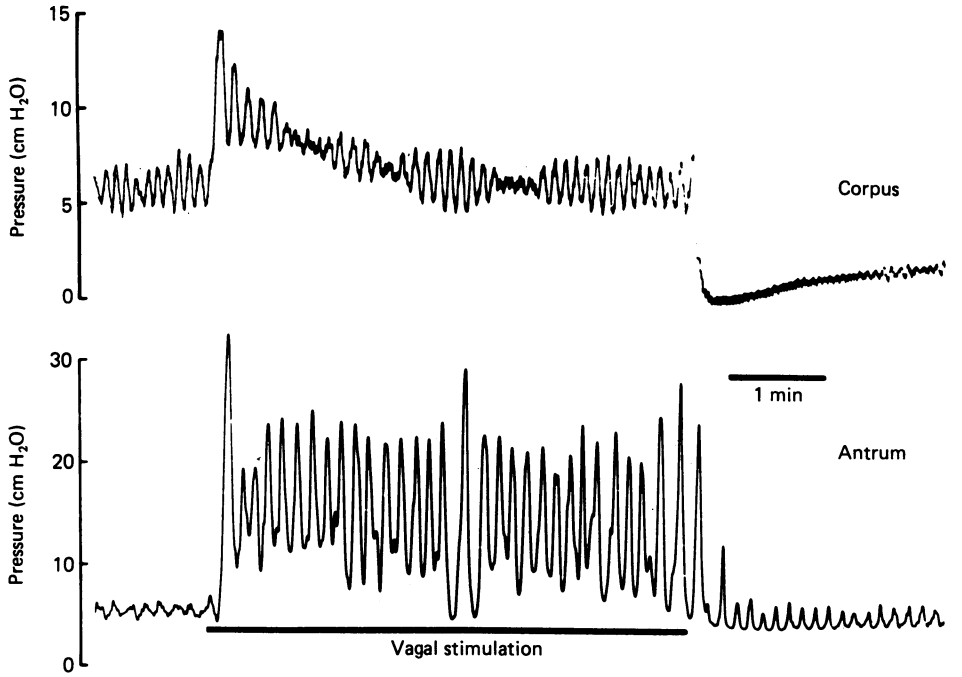

Fig. 8. The simultaneous response of a corpus and antral pouch to 5 min vagal stimulation (20 V, 10 Hz, 0·5 msec). Note the difference in the frequency of the spontaneous contractions and the over-all nature of the response in the two regions.

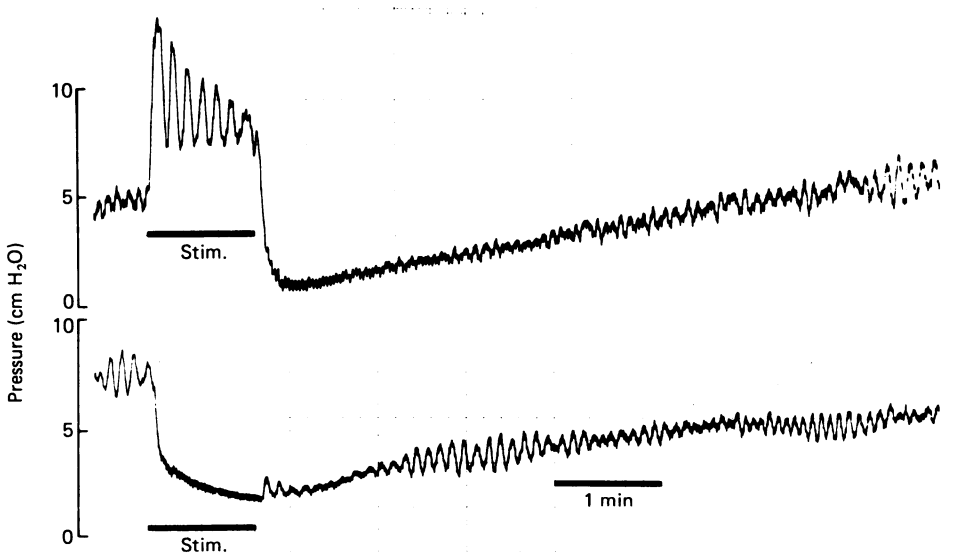

Fig. 9. Records from the corpus region of a divided stomach in response to vagal stimulation (20 V, 0·5 msec, 10 Hz). Note the similarity between the time course of the pressure return in the control (upper) and atropinized (lower) preparation.

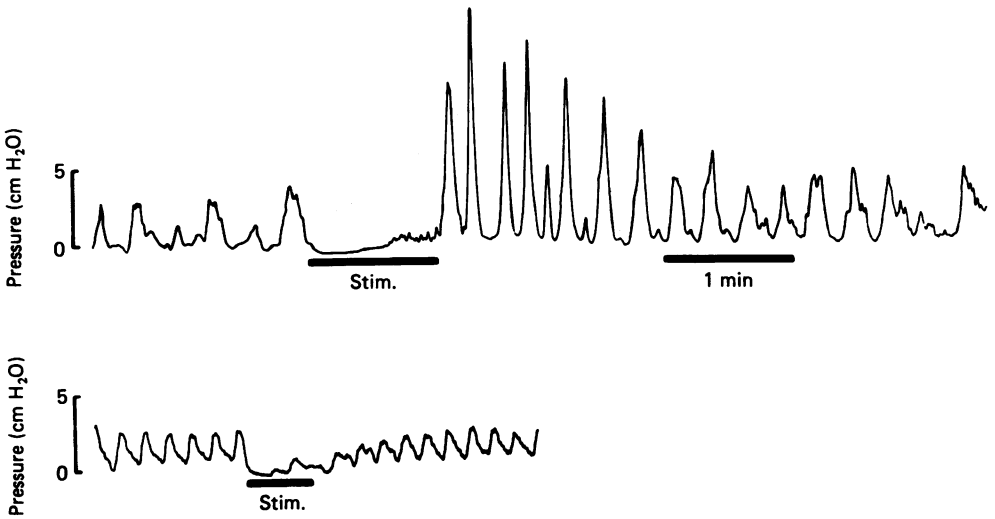

Fig. 10. Records from the antral region of a divided stomach in two atropinized animals. Note that the major effect of vagal stimulation is an inhibition of the spontaneous rhythmic activity.

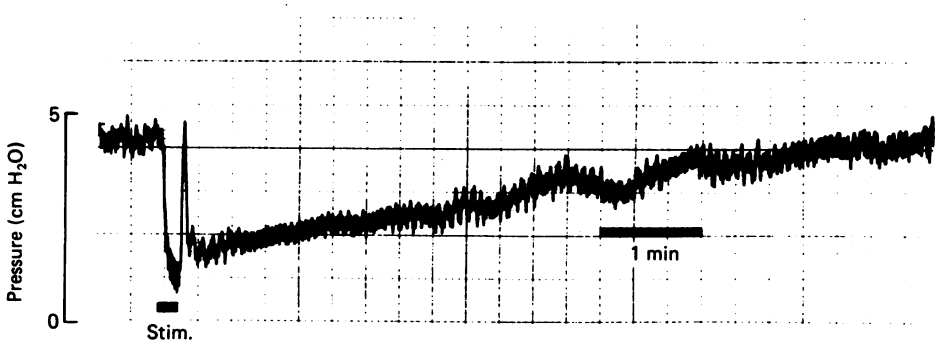

Fig. 11. Record from the corpus region of the divided stomach of an atropinized animal. Note that *immediately* on removal of the vagal stimulus there is a rebound contraction and that many minutes are taken for the pressure to return to control levels.

In the antrum, the major effect was inhibition of spontaneous motility during the period of stimulation with either no fall in tone or just a perceptible fall (Fig. 10) demonstrating the presence of inhibitory fibres to this part of the stomach also. When the stimulus was discontinued to the antrum, spontaneous activity returned immediately often more vigorously than before for a short period (Fig. 10).

A phenomenon observed on a number of occasions was a rebound contraction (Fig. 11) in the corpus on cessation of the stimulus. This has been used as an indicator of a purinergic response (Burnstock, Cocks, Paddle & Staszewska-Barczak, 1975). All vagally induced effects were blocked by hexamethonium, but not the spontaneously occurring contractions.

Gastric motility induced by reflex vagal excitation

The inflation of the stomach with 0·9 % NaCl at a rate of 10 ml./min to a volume of 50 ml. reflexly induces an increase in antral motility. The receptors are tension receptors in the corpus and both afferent and efferent pathways lie in the vagus nerves (Andrews *et al.* 1980). This rate and volume have been shown to be physiological and the increased motility persists for as long as the saline remains in the stomach. The frequency of the contractions was 7·0 ± 0·13 (n = 17) contractions per

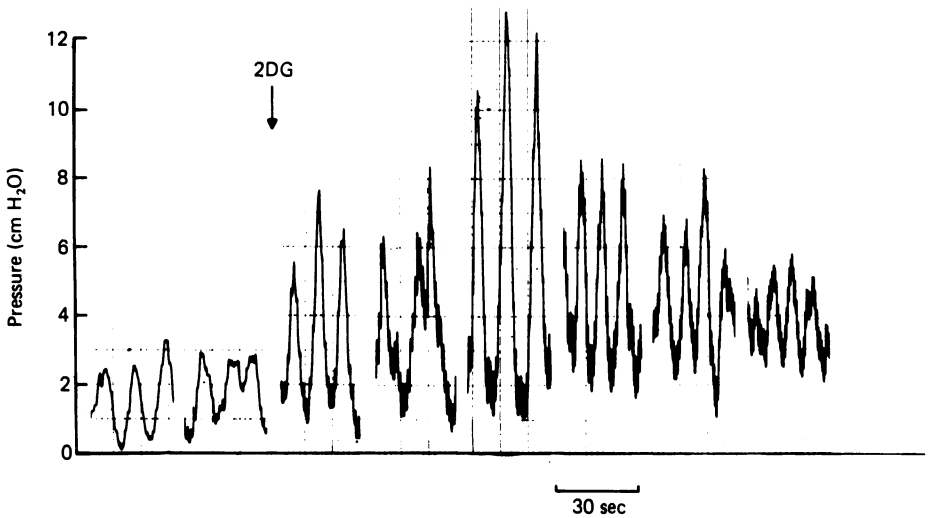

Fig. 12. Gastric motility changes in response to 2-deoxy-D-glucose (DG, 135 mg/kg). A 30 sec record from each 15 min period of the response is illustrated.

minute and the amplitude of the contractions showed little decrement with time in contrast to that observed as a consequence of electrical stimulation.

Gastric motility during vagal stimulation induced by cytoglucopenia

Cytoglucopenia induced by the intravenous injection of 2-deoxy-D-glucose (135 mg/kg) caused activation of cells in the hindbrain and hypothalamus which in turn activate vagal motoneurones (Kadekaro, Timo-Iaria & Vicentini, 1977). The amplitude of gastric contractions increased rapidly after the injection of the drug, with a latency of 246 ± 15 sec (n = 10). The response reached a peak at about 45 min (Fig. 12) and usually returned to baseline levels by 90 min from the time of injection. No consistent change in contraction frequency was observed. The range of mean frequencies in ten animals during the response was 6·4–8·3 contractions per minute and the maximal contraction rate in any individual experiment never exceeded that observed for the spontaneous activity. The gastric tone was always elevated and often remained so even when the gastric contractions had returned to pre-stimulation levels. No decrement occurred as was observed with electrical stimulation. Vagal section or the administration of atropine abolished the response.

DISCUSSION

The urethane anaesthetized ferret has an active gastrointestinal tract which is exemplified by the presence of motility patterns in the empty stomach. These gastric contractions are described as spontaneous, as they occur in the absence of any obvious stimuli. Vagotomy, atropine and hexamethonium all reduce the amplitude of this spontaneous activity but do not abolish it. The results described in this paper indicate that the contraction amplitude is regulated by a cholinergic excitatory mechanism acting via the vagus and myenteric plexus but a non-cholinergic pathway is also involved. The frequency of contractions appears to be controlled mainly by intramural mechanisms and this view is supported by the observation that they are largely independent of the frequency of electrical vagal stimulation. Also the contractions evoked by reflex activation of the vagi have a similar frequency. The smooth muscle itself appears to make a major contribution to the determination of the frequency as infusions of acetylcholine (probably acting mainly on the cholinergic receptors on the smooth muscle) evoked contractions of a similar frequency to those produced by vagal stimulation.

In contrast to the frequency, the amplitude of contractions increased with stimulation frequencies up to 10 Hz and thereafter declined. The frequency producing the largest effect is similar for other gastrointestinal functions influenced by the vagus, e.g. acid secretion, 8 Hz (Sjödin, 1975); lower oesophageal sphincter tone, 10 Hz (Matarazzo, Snape, Ryan & Cohen, 1976); pancreatic juice volume and amylase secretion, 10–20 Hz (Hickson, 1970*a*, *b*); pancreatic blood flow, 10 Hz (Greenwell & Scratcherd, 1974), jejunal motility, 6–8 Hz (Kewenter, 1965); VIP release 8 Hz (Fahrenkrug, Galbo, Holst & Schaffalitzky de Muckadell, 1978). The amplitude of the contractions is also dependent upon the number of active vagal fibres as indicated by the stimulus strength–response curve but the decline in amplitude at high stimulus strengths observed by Martinson & Muren (1963) was not confirmed. At a fixed frequency and voltage of stimulation the amplitude is also influenced by the duration of stimulation up to 10 sec. The effect of the vagal preganglionic fibres on motility is mediated by the action of nicotinic cholinoreceptors presumably located on the intramural ganglion cells and by the action of muscarinic cholinoreceptors probably located on the smooth muscle cells. The amplitude of the contractions evoked by systemic acetylcholine is dependent on the dose of drug which indicates that the effects of frequency and voltage of vagal stimulation on the motility are mediated by increasing the amount of acetylcholine released on to the smooth muscle. Paton (1963) demonstrated that the release of acetylcholine in unit time, from post-ganglionic cholinergic fibres increased with the rate of discharge.

Stimulation of the vagus for periods greater than 10 sec evoked separate waves of contraction superimposed upon a declining mean level of pressure (see Fig. 6*B*). This gradual decline appears to be due to activation of vagal relaxatory fibres as evidenced by the large fall in the intragastric pressure below resting levels on removal of the stimulus. When the cholinergic excitatory effects of the vagus are blocked by atropine, vagal stimulation evokes a decrease in intragastric pressure with a similar time course to that observed in the unatropinized preparation. The action of the transmitter responsible for mediating the relaxation has a long half-life

as the intragastric pressure takes many minutes to return to control levels even after a short period of stimulation. Experiments with the divided stomach show that this relaxation is confined to the corpus-cum-fundus region. Therefore, during electrical vagal stimulation, both excitatory and relaxatory fibres to the corpus are activated and the recorded intragastric pressure is the resultant of their actions, which makes interpretation of results from electrical excitation of the nerve difficult. The corpus excitatory and relaxatory efferent fibres are simultaneously activated by electrical stimulation of the central end of the abdominal vagus (Ohga, Nakazato & Saito, 1970) and this casts doubt on the usefulness of this type of study for investigating the control of gastric emptying (see Carr & Brooks, 1978).

The present investigation has also demonstrated that the vagus supplies fibres to the antrum which are capable of inhibiting the 'spontaneous' antral contractions in an atropinized divided stomach. Specific activation of these nerves may be responsible for the initial 'turn off' of gastric contractile activity sometimes observed at the start of a meal (Wolf, 1965). They may also be implicated in the waxing and waning of gastric contractions observed during prolonged vagal stimulation. The time of rest between periods of stimulation was also shown to be important in determining the magnitude of the response. A period of between 30 and 120 sec was needed before a reproducible response was obtained. Because the response of one vagus could be influenced by prior stimulation of the contralateral nerve, the structures responsible for the effect are distal to the preganglionic fibres. Whether this phenomenon is another expression of the action of a long lasting effect of an inhibitory transmitter is not known.

The gastric motor responses to vagal reflex activation are in marked contrast to those evoked by direct electrical vagal stimulation. The reflex responses do not show a gradual decline in the mean pressure towards baseline levels even with prolonged periods of stimulation, nor is there a marked waxing and waning of the superimposed gastric contractions. After removal of the driving stimulus (particularly distension) there is not a period of decreased intragastric pressure and absence of spontaneous contractions. These differences in the responses obtained by the two methods of vagal efferent activation, indicate that the reflexes activate specifically the cholinergic excitatory fibres to the stomach with little stimulation of the non-adrenergic, non-cholinergic inhibitory fibres, whereas electrical stimulation activates both groups of fibres and the response observed is the resultant of the two effects.

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