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# EFFECT OF STIMULATION OF THE VAGUS NERVE IN BURSTS ON GASTRIC ACID SECRETION AND MOTILITY IN THE ANAESTHETIZED FERRET

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SUMMARY

1. The effect of electrical stimulation of the vagus nerve with different patterns of impulses (the total number of stimuli remaining constant) on gastric acid secretion and gastric motility were investigated in the anaesthetized ferret. Three stimulus patterns were used: continuous, bursts at ten times the continuous frequency but for a tenth of the time, and a natural burst pattern obtained from a recording of vagal efferent fibre discharge.

2. The natural burst pattern gave rise to gastric contractions of larger amplitude than either the artificial burst or continuous stimulation, while continuous stimulation gave rise to larger changes in tonus. Acid secretion, however, was reduced by the natural pattern of stimulation as compared to the output during continuous stimulation, but to a lesser extent than that due to artificial burst stimulation.

3. Burst stimulation at 10 impulses/sec resulted in a larger output of acid and greater amplitude of gastric contraction than the equivalent continuous stimulation. This potentiation was lost at stimulation frequencies above 30 impulses/sec.

4. At burst frequencies of 60 and 120 impulses/sec there was a marked reduction in acid output and amplitude of contraction as compared with continuous stimulation at 6 and 12 impulses/sec respectively.

5. These results are discussed in relation to the functional significance of the different patterns of vagal discharge seen in the anaesthetized ferret.

## INTRODUCTION

A proportion of efferent fibres in the vagus nerve of the anaesthetized ferret show a discharge which is modulated in phase with gastric motility (Grundy, Salih & Scratcherd, 1981). This modulated efferent discharge is a consequence of reflex activation by vagal afferent fibres whose endings lie in the antral region of the stomach (Andrews, Grundy & Scratcherd, 1980*a*). Since the vagus nerve is considered only to influence the amplitude of gastric contractions, the basic electric rhythm being responsible for the frequency and wave form of the contractions (Szurszewski, 1977), the functional significance of the modulated efferent discharge is unknown.

Recent papers have suggested that the submaxillary glands of cats and the parotid

gland of lambs give bigger responses and respond over a greater frequency range when the parasympathetic supply is stimulated electrically with high frequency bursts as opposed to the same number of impulses applied at a constant lower frequency, and imply that the normal pattern of nerve discharge may be of this type (Andersson & Edwards, 1982; Andersson, Bloom, Edwards & Järhult, 1982). The purpose of the present investigation was to determine whether a similar situation occurred in the ferret stomach. In addition, access to tape recordings of activity from vagal efferent fibres allowed us to use the discharge from one of these to trigger the stimulator in order to compare the gastric motility and secretory responses to both natural and artificial patterns of electrical stimulation of the vagus nerve. In this way it was intended to gain insight into the functional role of the different patterns of vagal efferent fibre discharge seen in the anaesthetized ferret.

## METHODS

### *Animals and general surgery*

The experiments were performed on male and female ferrets anaesthetized with a single dose of urethane (1·5 g/kg, I.P.) following an overnight fast. A glass cannula was inserted into the trachea, and the right external jugular vein was cannulated for administration of further anaesthetic if required. The stomach was intubated via the cervical oesophagus and also via the pylorus. Intragastric pressure was measured by connecting the fluid-filled pyloric tube to a pressure transducer (S. E. Labs Ltd). The oesophageal tube was used for the introduction and removal of fluid into the stomach for the estimation of gastric acid secretion. Bilateral vagotomy was performed in the neck. The thoracic vagi were exposed via a left thoracotomy with resection of the lower ribs; the branch of the left vagus nerve which joined the dorsal trunk (crossing branch) was placed over a pair of platinum electrodes and covered with cotton wool swabs soaked in liquid paraffin. After thoracotomy, the animals were ventilated artificially with room air and exhaled against a water resistance of 3 cm $H_2O$. End-tidal $CO_2$ was monitored continuously (EL 100 $CO_2$ analyser) and maintained at approximately 6%. Body temperature was maintained at 38 °C by means of a Palmer Homeothermic blanket.

### *Vagal stimulation*

The crossing branch of the thoracic vagus nerve was stimulated with square-wave pulses (500 $\mu$sec duration) at supramaximal voltage for the middle 5 min of a 7 min collection period, using different patterns of stimulation. In any one experiment the number of impulses applied during the 5 min stimulation period was the same; however the pattern of stimulation varied from a continuous low-frequency train to a burst of impulses triggered from a Digitimer D4030 applied at a frequency ten times that of the continuous frequency but for only a tenth of the time. A 10 sec cycle was chosen, first because the natural rhythm of gastric contractions in the ferret is approximately 6/min (Andrews & Scratcherd, 1980), and secondly to allow a comparison with similar experiments on salivary glands (Andersson *et al.* 1982). A third pattern of impulses was obtained from a 5 min recording of a vagal efferent fibre whose discharge increased during antral motility (Fig. 1; see also Fig. 3 in Grundy, *et al.* 1981). This delivered a total of 1820 impulses in the 5 min period and was therefore compared with 6 impulses/sec continuously and 60 impulses/sec for 1 sec every 10 sec. Other comparisons were made between 1, 3 and 12 impulses/sec continuous and 10, 30 and 120 impulses/sec in bursts.

### *Analysis*

Gastric acid secretion was estimated using a wash-out technique. 20 ml. isotonic glycine was placed in the stomach and drained passively every 7 min. Aliquots of 5 ml. were titrated against 0·05 M-NaOH to pH 5·9 using a Radiometer TTT 81 Digital titrator. The acid output was corrected for the volume of wash-out and expressed as $\mu$mole/7 min. There was no correlation between acid

secretion and body weight and therefore no attempt has been made to normalize results on the basis of body weight. Acid outputs following vagal stimulation were calculated as shown in Fig. 2*A* from the increase in acid above basal in successive 7 min periods until basal conditions were reached again. When comparing the different patterns of nerve stimulation, periods of continuous stimulation bracketed the burst stimulation and the mean of these two periods of continuous stimulation used as the control for the burst. No more than nine periods of stimulation were performed on any one animal, allowing two periods of natural and digitimer bursts to be bracketed by periods of continuous stimulation. The acid output in response to the first and last period of continuous stimulation was not significantly different and was therefore not a determinant of experimental duration. Results are presented either as absolute values of acid output (mean ± S.E.) or as the difference in acid output during continuous and burst stimulation.

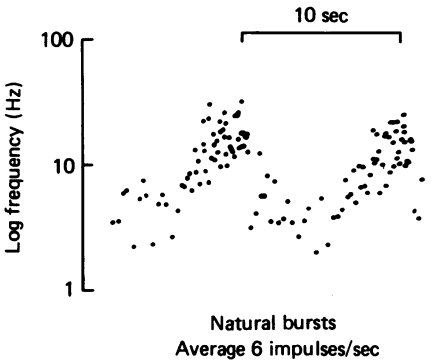


Fig. 1. Log display showing the natural pattern of vagal stimulation used. Each dot represents a stimulus interval whose reciprocal is plotted on a log scale. Note that the natural burst has a frequency which never falls below 1 impulse/sec and which has a maximum of approximately 34 impulses/sec.

Analysis of variance was carried out on the basal acid secretion in the groups of animals receiving different frequencies of stimulation, and because these were not significantly different at the 5% level individual responses were compared by Student's *t* test. In the text *n* refers to number of animals unless otherwise stated.

Gastric motility evoked by vagal stimulation was analysed in several ways. The amplitude of the first contraction and the mean amplitude of contraction during different 5 min stimulation periods were compared by again bracketing a burst stimulation with a continuous stimulation. An additional parameter measured was the ratio (*R*) of the pen excursion (i.e. the total length of the ink line drawn on the chart recorder) during stimulation and that of an equivalent period when no stimulation occurred. Two additional experiments were performed on the contractions evoked by short periods of stimulation and the effect on these of different intervals between stimulation.

## RESULTS

### *Acid secretion*

The mean basal acid secretion in these ferrets was 18·8 ± 10 μmole/7 min (mean ± S.D., $n = 10$). Following vagal stimulation the output of acid increased for several periods, the extent of which depended upon the frequency and pattern of stimulation (Fig. 2*A*). When the same number of impulses (mean of 6 impulses/sec) was applied in the different pattern described in the methods the acid output was greatest with

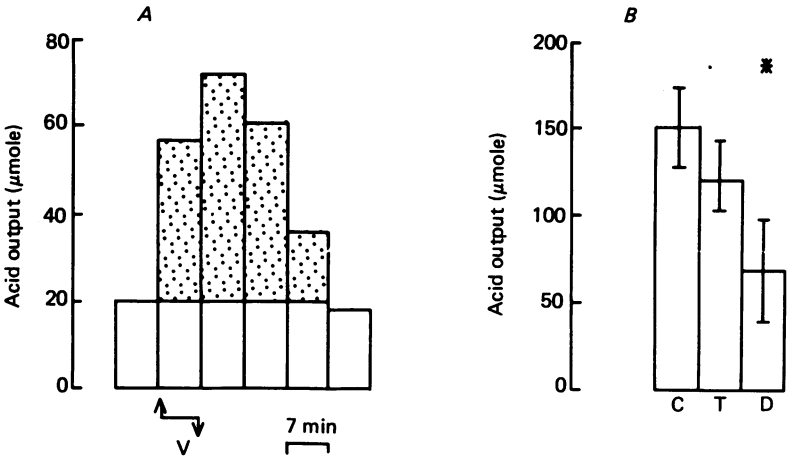


Fig. 2. *A*, acid output in consecutive 7 min periods in one experiment before, during and after a 5 min period of continuous vagal stimulation at 6 impulses/sec. The stippled area represents the acid output as a consequence of the vagal stimulation and is the parameter used in subsequent Figures. *B*, acid output following vagal stimulation with three different patterns of stimulation. Mean ± S.E. C, continuous; T, tape bursts (i.e. the natural bursts) and D = bursts programmed from the Digitimer. * $P < 0{\cdot}01$ (paired $t$ test).

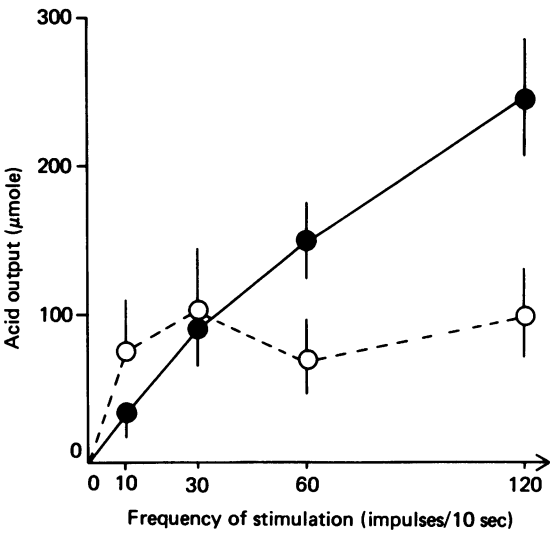


Fig. 3. Acid output following continuous stimulation (●) and Digitimer bursts (○) over the frequency range 10–120 impulses/10 sec.

continuous (C) and significantly less with the bursts programmed from the digitimer (D) ($P < 0{\cdot}01$, $n = 4$) (Fig. 2*B*). The tape bursts (T) produced a slight reduction in acid output but this failed to reach the 5% level.

The relationship between acid output and frequency of stimulation when applied either continuously or in bursts in shown in Fig. 3. With continuous stimulation the output of acid increased with increasing frequency over the range 1–12 impulses/sec.

When the same number of stimuli were delivered in bursts of 1 sec every 10 sec a maximum response occurred with bursts at 30 impulses/sec. Bursts at higher frequencies failed to produce a further increase in acid production.

When the acid output during individual burst stimulation was subtracted from that during continuous stimulation for each of the four different frequencies tested (i.e. subtracting the two curves in Fig. 3) it can be seen that stimulating in bursts of 60 and 120 impulses/sec significantly reduced the output of gastric acid while bursts of 10 impulses/sec gave a significant increase in acid output (Fig. 4).

The peak acid output often appeared in the 7 min period following vagal stimulation and remained elevated for up to three additional periods (see Fig. 2*A*). In order to determine whether release of antral gastrin was responsible for the duration of this response, two experiments were performed with the stomach divided along the incisura to separate the antrum from the corpus (Andrews, Grundy & Scratcherd, 1980*b*) so that the pH in the antrum could be reduced to 1·5 and would thereby inhibit gastrin release (Pe Thein & Schofield, 1959). In these experiments the acid output and the time course of this output following vagal stimulation showed no significant difference whether the antrum was buffered to pH 5·9 or whether the pH was 1·5. We conclude from these observations that vagal release of antral gastrin did not contribute significantly to the acid output in these experiments.

*Motility*

The effect of the different patterns of stimulation on gastric motility were more difficult to quantify. At all frequencies tested burst stimulation produced a greater mean amplitude of contraction and a larger excursion of the pen than the equivalent continuous period of stimulation (Fig. 5) but only reached significance for stimulations at 10 and 120 impulses/10 sec. The natural burst stimulation also gave rise to a significantly bigger response ($P < 0{\cdot}05$, $n = 4$) which was also bigger than that in response to the equivalent artificial burst but which failed to reach the 5% significance level (Fig. 6). During burst stimulation the interval between the burst (either from the tape or the digitimer) allowed the intragastric pressure to fall to near pre-stimulation levels before the next burst of impulses arrived, each burst of impulses giving rise to a discrete contraction, the amplitude of which gradually fell during the 5 min stimulation period (Fig. 7). During the periods of continuous stimulation the mean amplitude of individual contractions was less than during burst stimulation but these were superimposed on a marked change in tone; as the stimulus continued the contractions attenuated until only the change in tone persisted (Fig. 6 and 7). These differences in response to continuous and burst stimulation gave rise to the differences in pen excursions illustrated in Fig. 5. This difference was greatest during stimulation in bursts at 10 impulses/sec and decreased as the frequency was increased. This was because, as the frequency of stimulation increased, the response to continuous stimulation also increased but the response to burst stimulation decreased. This is adequately demonstrated by the change in amplitude of the first contraction following either continuous or burst stimulation. Fig. 8 shows the percentage change in amplitude of the first contraction when changing from continuous to burst stimulation. At 10 impulses/10 sec the first contraction was bigger during burst stimulation than during continuous stimulation but as the

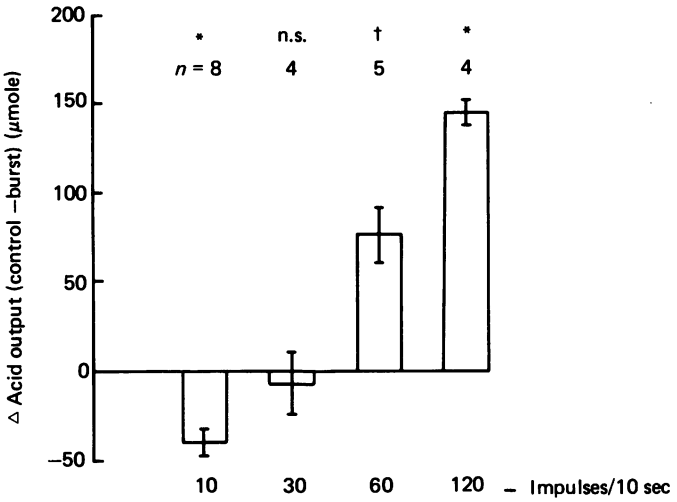


Fig. 4. Difference in acid output between continuous and Digitimer bursts (C-D). Positive difference represents a reduction in acid output during burst stimulation. * $P < 0{\cdot}001$, † $P < 0{\cdot}01$ (paired $t$ test). n.s. = not significantly different. ($n$ represents the number of observations).

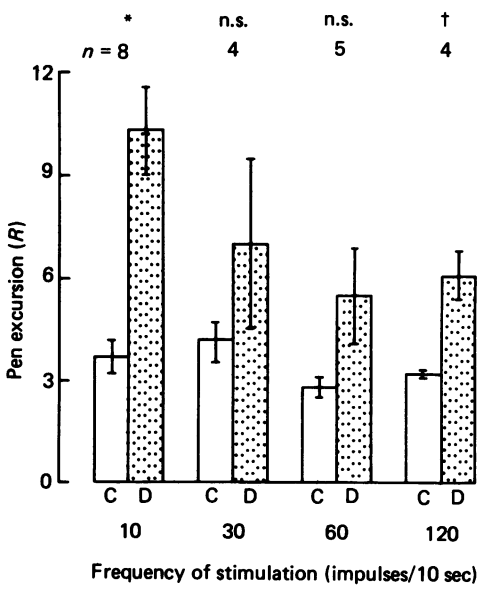


Fig. 5. Ratio of the pen excursion ($R$) for continuous (C) and Digitimer bursts (D) (stippled bars) over the frequency range 10–120 impulses/10 sec. * $P < 0{\cdot}001$; † $P < 0{\cdot}05$. ($n$ refers to number of observations). Note that the bursts give rise to bigger responses than continuous and that the biggest difference occurs between 1 impulse/sec continuous and 10 impulses/sec given in bursts.

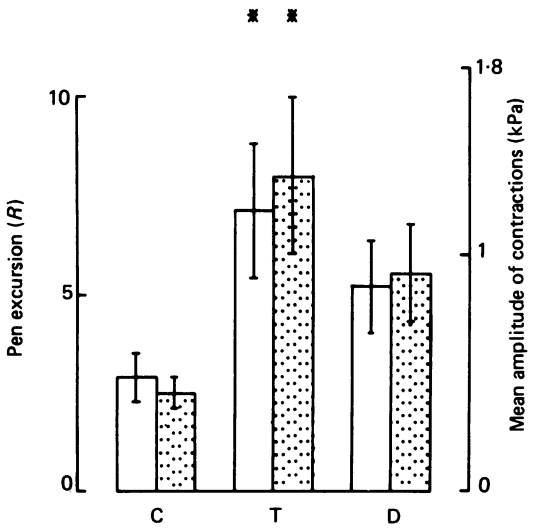


Fig. 6. Ratio of pen excursion ($R$) and mean amplitude of contractions (stippled bars) during the three different patterns of vagal stimulation (total no. of impulses remaining constant). * $P < 0{\cdot}05$. Note that both are increased by burst stimulation but only the taped bursts (T) are significantly different from the continuous control (C).

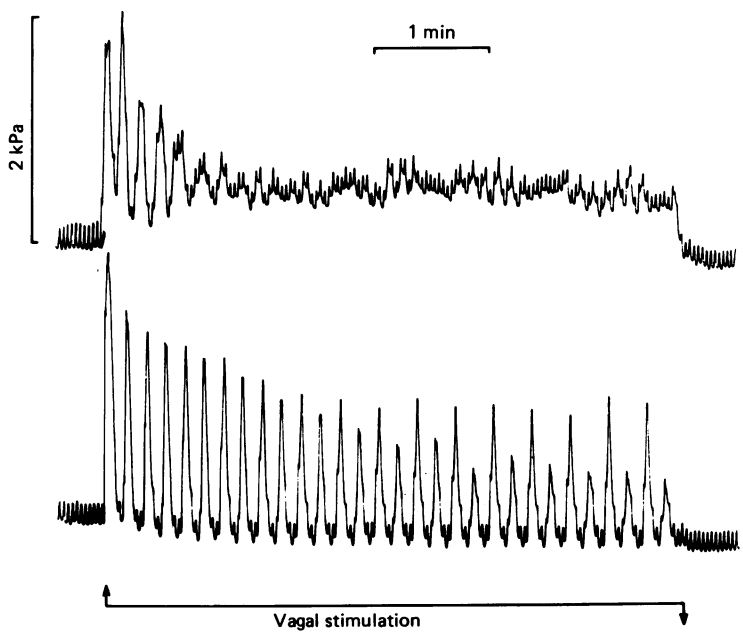


Fig. 7. Actual record of changes in intragastric pressure during continuous stimulation (upper record) at 1 impulse/sec and Digitimer bursts (lower record) at 10 impulses/sec every 10 sec.

frequency increased the amplitude of the first contraction during burst stimulation was reduced. The natural pattern of stimulation had a greater effect than the artificial pattern of stimulation in bursts on the mean amplitude of contraction and pen excursion and produced significant increases over the response to continuous stimulation ($P < 0{\cdot}05, n = 4$). The first contraction following natural burst stimulation was not reduced compared with that following continuous stimulation while the response to the equivalent artificial burst was significantly reduced ($P < 0{\cdot}05, n = 4$).

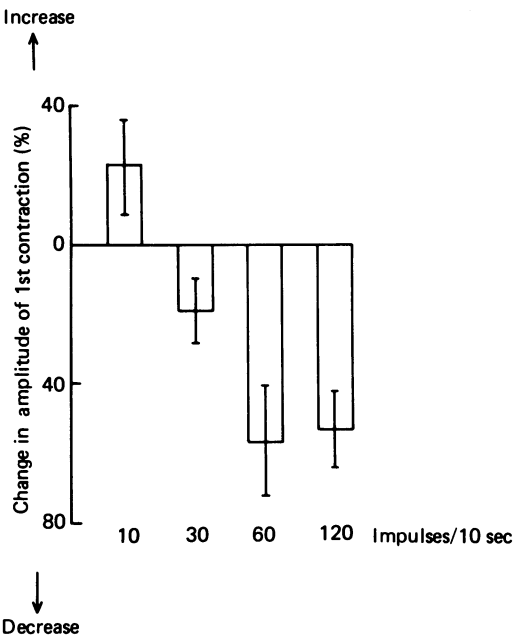


Fig. 8. Percentage change in amplitude of the first contraction to occur during vagal stimulation when changing from continuous to Digitimer bursts over the frequency range 10–120 impulses/10 sec. A negative change represents a decrease in contraction amplitude during burst-type stimulation.

To investigate the underlying mechanisms behind these observations, two further experiments were conducted in which short bursts of impulses at varying frequencies were applied to the thoracic vagus. In these experiments it was seen that there was a relationship between frequency of stimulation and amplitude of contraction, the total number of impulses remaining constant (Fig. 9). The amplitude of contractions increased with increasing frequencies up to a maximum at about 12 impulses/sec; at frequencies above 30 impulses/sec the amplitude of contractions fell off rapidly. This observation adequately explains the reduced amplitude of contractions seen during burst stimulation frequencies above 30 impulses/sec.

The importance of the interval between bursts was also investigated in these experiments. The gastric contractions could be driven at frequencies up to 12/min (1 sec stimulation/4 sec off), but at intervals less than this the contractions summated, giving rise to a change in tonus similar to that seen during continuous stimulation. The period between bursts was therefore important in allowing individual contractions to occur in the absence of a change in tonus.

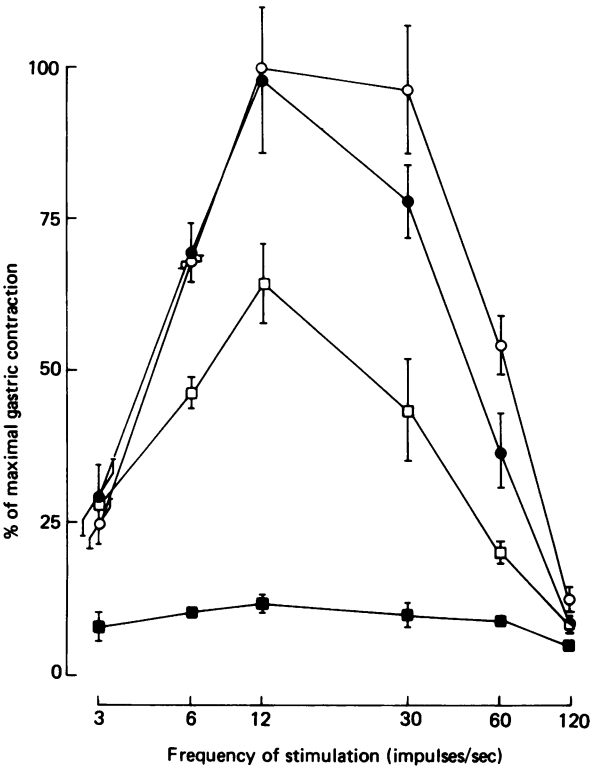


Fig. 9. Graph showing the contraction amplitude as a percentage of the maximal contraction (12 impulses/sec for 10 sec) which occurred as a consequence of vagal stimulation over the frequency range 3–120 impulses/sec using four different total numbers of pulses. ■, 15 impulses; □, 30 impulses; ○, 60 impulses; ●, 120 impulses. For example, 15 impulses were delivered at 120 impulses/sec for 0·125 sec while 120 impulses were delivered at 120 impulses/sec for 1 sec.

## DISCUSSION

Electrical stimulation of the vagus nerve increases gastric acid secretion and stimulates gastric motility. However, the magnitude of these responses depends upon the pattern of stimulation, the total number of stimuli remaining constant, in so much as there is a frequency of vagal stimulation above which there is a decrease in both acid output and amplitude of contractions. This frequency above which a reduction in response was observed was approximately 30 impulses/sec. Thus there was a graded increase in the output of acid and motility in response to continuous stimulation over the frequency range 1–12 impulses/sec, similar to that seen by Sjödin (1975) and McSwiney & Wadge (1928), while the same number of impulses delivered in 1 sec bursts at 10–120 impulses/sec respectively produced responses which were maximal at 30 impulses/sec and which were progressively reduced at higher frequencies. This response maximum to high frequency bursts may be limited by failure of either the target cell, ganglionic transmission or pre-ganglionic activation to translate impulses above a given frequency. The latter seems to be the most likely explanation since the frequency maximum is about the same level as the maximal

discharge frequency recorded in efferent vagal fibres of rat, dog and ferret (Davison & Grundy, 1978; Miolan & Roman, 1974; 1978; Grundy, *et al.* 1981) and also the maximal following frequency of vagal afferent fibres recorded in nodose ganglion (Stansfeld & Wallis, 1982).

Stimulation in bursts at frequencies lower than this maximum did give rise to a bigger acid output and a larger amplitude of gastric contractions than did continuous stimulation in a similar way to that seen in the cat sub-maxillary gland (Andersson *et al.* 1982). This is probably due to temporal summation within the enteric plexi whereby successive post-synaptic events are superimposed, resulting in an increase in acetylcholine output during stimulation in bursts, as has been described in cat superior cervical ganglion (Birks, 1977). This effect being lost in these experiments, due to the inability of the vagal C fibres to follow above about 30 impulses/sec. The greater range of response in the cat sub-maxillary gland may possibly be due to these small myelinated fibres being able to follow at higher frequencies.

In the anaesthetized ferret, the vagal efferent fibres discharge observed during reflex activation by gastric distension is of two basic types: a continuous type of discharge with a relatively low peak discharge frequency and a type which is modulated in phase with antral motility (Grundy, *et al.* 1981). Stimulating the vagus nerve in a way which mimics this latter type of discharge would seem to serve little advantage to the parietal cell. However, there may well be an advantage in this type of innervation of the smooth muscle.

It is generally believed that in monogastric animals the gastric pace-setter potential sets the frequency of contraction, while the level of vagal discharge influences only the amplitude of contractions (Szureszewski, 1977), i.e. the frequency of contractions is independent of the vagal innervation. This may well be true of the ferret stomach in which the spontaneous contraction frequency is the same as reflexly activated motility (Andrews & Scratcherd, 1980). However, as these results indicate, a modulated vagal stimulation may allow the full expression of individual contractions to occur.

One of the main differences between reflexly activated gastric motility and that elicited by continuous vagal stimulation is that the latter rapidly attenuates (Andrews & Scratcherd, 1980). This may be due to electrical vagal stimulation activating both the excitatory cholinergic pathway and the non-adrenergic, non-cholinergic inhibitory pathway to the gastric smooth muscle (Andrews & Grundy, 1981), the latter causing the fall-off in response. Another possibility, however, is that continuous stimulation results in saturation of either the ganglion or target cell with transmitter, which results in a type of depolarization block which may be overcome by stimulating in bursts. This would be particularly relevant to the smooth muscle of the stomach as opposed to the parietal cell as the latter responds with a long latency and long duration. This may be borne out by the observation of Andersson & Järhult (1981) that the long-latency, long-duration atropine-resistant contractions of the cat colon were readily elicited by continuous stimulation of the pelvic nerve but not by the equivalent period of burst stimulation.

In conclusion, since vagal efferent fibre discharge in the anaesthetized ferret can be either a continuous low-frequency irregular discharge or a discharge which is temporarily related to gastric motility (Grundy *et al.* 1981), and since the response

of the smooth muscle is modified significantly by periodic stimulation, it seems likely that the fibres that fire intermittently innervate the smooth muscle and those that fire continuously innervate the parietal cells.

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