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MECHANISM OF ACTION OF PENTAGASTRIN AND ACETYLCHOLINE ON THE LONGITUDINAL MUSCLE OF THE CANINE ANTRUM

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SUMMARY

1. Electrical and mechanical activities of the longitudinal muscle of the dog antrum were recorded with the double sucrose-gap technique.

2. The muscle exhibited spontaneous action potentials which consisted of a spike-like potential which, after a brief and partial repolarization, was followed by a negative-going, plateau-type potential. In 97 % of the preparations, no tension changes were produced by spontaneous action potentials.

3. Tetrodotoxin, atropine, alpha- and beta-adrenoceptor antagonists, and H_1 and H_2 receptor blocking agents had no effect on the action potential. It was concluded that the action potential was myogenic in origin.

4. The mean frequency of the action potential at $37 \pm 0{\cdot}5°$ C was $1{\cdot}0/\text{min} \pm 0{\cdot}06$ (S.E. of mean, $n = 92$) and the mean duration $7{\cdot}1 \pm 0{\cdot}2$ sec (S.E. of mean, $n = 11$).

5. Steady depolarizing current increased whereas hyperpolarizing current decreased the frequency of the action potential.

6. Length–tension relations were studied. In twelve strips, the average resting, passive, tension at L_0 was 570 mg. The active force of isometric contraction produced by acetylcholine increased with strip length up to a maximum, then decreased with further increases in length. There were no mechanical responses to pentagastrin.

7. Pentagastrin had two sites of action. On smooth muscle, it increased the frequency of the action potential in a dose dependent fashion. Threshold concentrations ranged from 2×10^{-14} to 10^{-11} M. The ED_{50} was 2×10^{-10} M. The maximum response, 5·4/min, was reached at 10^{-8} M. Pentagastrin also released acetylcholine from intramural cholinergic nerves.

8. Pentagastrin reduced the amplitude and duration of the action potential.

9. Acetylcholine produced phasic contractions which were associated with action potentials. This was its primary action. Initially, however, it produced a transient membrane depolarization, an increase in tension and an increase in the frequency of the action potential. During these initial effects and for as long as acetylcholine was present in the bathing solution, acetylcholine increased the amplitude and duration of the action potential.

10. When acetylcholine and pentagastrin were present together in the bathing solution, phasic contractions occurred at the frequency of the action potential. Under these conditions, an increase in the concentration of acetylcholine increased the force of contraction whereas an increase in the concentration of pentagastrin increased the frequency of contraction but reduced the force of contraction. The reduction was due to a decrease in amplitude and duration of the action potential.

11. Ca^{2+} was important for the genesis of the action potential. Calcium-deficient solution (1·8–0·6 mM-$[Ca^{2+}]_o$) reduced the amplitude and duration of spontaneous action potentials and reduced the muscle response to acetylcholine. In calcium-rich solution (5·0 mM-$[Ca^{2+}]_o$), the frequency of the spontaneous action potential was increased. In 7·5 mM-$[Ca^{2+}]_o$, it was decreased below the control level. In 5·0 mM-$[Ca^{2+}]_o$, the amplitude and duration of the spontaneous action potential were increased and each action potential produced a contraction. The muscle responses to acetylcholine were also increased. In 7·5 mM-$[Ca^{2+}]_o$, either no further increases were observed or they were depressed to near control levels.

12. The results of this study indicate that in the longitudinal muscle of the dog antrum, gastrin increases the occurrence of gastric contraction by releasing acetylcholine from intramural cholinergic nerves and increases the incidence of contraction by acting on smooth muscle to increase and regularize the frequency of the action potential.

INTRODUCTION

Gastrin and pentagastrin (a synthetic polypeptide which contains the active fragment of the whole molecule) modulate gastric emptying in man and dog by varying the strength of phasic contractions of the antrum (Gregory & Tracy, 1964; Bennett, Misiewicz & Waller, 1967; Misiewicz, Holdstock & Waller, 1967; Isenberg & Grossman, 1969; Jacoby & Marshall, 1969; Sugawara, Isaza & Woodward, 1969; Cameron, Phillips & Summerskill, 1970; Kelly, 1970; Sugawara, Isaza, Curt & Woodward, 1970; Kwong, Brown, Whittaker & Duthie, 1972). The electrophysiological mechanism which brings about this effect is not understood. It is known that pentagastrin and gastrin increase contractile activity by acting on

cholinergic nerves in the gastric wall to release acetylcholine (Gregory & Tracy, 1964; Bennett, 1965; Jacoby & Marshall, 1969; Vizi, Bertaccini, Impicciatore & Knoll, 1973). However, the manner in which acetylcholine initiates phasic, rhythmic, motor activity is not known. The purpose of this report is to present and discuss experimental data dealing with the effect of pentagastrin on the electrical and mechanical activity of isolated strips of canine gastric smooth muscle and with the manner in which acetylcholine initiates rhythmic, phasic contractions.

The observations to be described have led to the conclusion that pentagastrin has two effects: it releases acetylcholine from cholinergic neurones and it increases the frequency of occurrence of the myogenic action potential. Acetylcholine produces phasic contraction by acting on the membrane channels which participate in the action potential.

Some of the results have been communicated to the American Gastroenterological Society (Szurszewski, 1974*b*) and to the Satellite Symposium on the Physiology of Smooth Muscles, Kiev, Ukraine, U.S.S.R. (Szurszewski, 1974*c*).

METHODS

One hundred and ten dogs of either sex, ranging in weight from 10 to 22 kg, were used. After each animal was anaesthetized with pentobarbital (Fort Dodge Laboratories, Inc.), the whole stomach was removed and immersed in Krebs solution containing (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; glucose, 11·5; equilibrated with 97 % O_2 + 3 % CO_2, pH 7·3–7·4. To prepare a strip of muscle, a patch of muscle, including circular and longitudinal layers, was removed from the dorsal and ventral surfaces on either side of the greater curvature, placed in Krebs solution and pinned down on to a rubber floor in a dish. With the aid of a dissecting microscope a section of the patch was found in which muscle bundles of the longitudinal layer were parallel to each other. This section was cut from the patch and pinned down in another dish. Under microscopic observation strips were cut which were parallel to the long axis of the longitudinal muscle and were less than 1 mm in width and 3·5 cm in length. Most strips were turned sideways and the circular muscle layer removed. Histological studies indicated that only fragments of circular muscle remained and that the longitudinal strip was devoid of the neuronal plexus which lies between the circular and longitudinal muscle layers. In some strips, the circular layer was not removed. These 'mixed strips' (longitudinal and circular muscle layers) were used in experiments designed to determine if pentagastrin acted on cholinergic motor neurones. All strips were incubated for 1–2 hr in Krebs solution at room temperature.

Double sucrose gap recordings

Strips consisting of longitudinal muscle were used. The double sucrose-gap apparatus has been described in detail (Kuriyama & Tomita, 1970; Szurszewski, 1974*a*). Briefly, regions of the muscle strip were exposed to sucrose, Krebs solution, a second stream of sucrose and isotonic K_2SO_4. The flows of the sucrose and Krebs solution were adjusted so that the width of the strip exposed to Krebs solution (test gap) never exceeded 900 μm. The usual gap width was 700 μm. All solutions

flowed continuously and their rates were kept constant. The K_2SO_4 and sucrose solution were at room temperature whereas the temperature of the Krebs solution was maintained at $37 \pm 0{\cdot}5°$ C.

To measure tension, one end of the tissue was fixed and the other attached by a thread to a semiconductor, isometric force transducer.

Changes in membrane potential were recorded between the isotonic K_2SO_4 and test gap across one of the sucrose gaps with a pair of agar Ag–AgCl electrodes connected to a DC amplifier. After the potential difference was amplified, it was displayed on one beam of dual beam oscilloscope (Tektronix 5100 D12) and on one of the two channels of a Brush Mark 200 oscillograph. The other beam and channel recorded tension changes. Across the other sucrose gap, constant polarizing current was applied, when desired. This was done by using the tissue as a feed-back element in a feed-back loop of an operational amplifier. The input impedance of the operational amplifier was 10^{11} Ω and bias current was 10^{-12} A.

Concentrations of drugs given in the Results are those of the final concentration of the salt reaching the tissue. Drugs used were: *O*-acetylcholine chloride (Sigma), atropine sulphate (Sigma), mepyramine and metiamide (supplied by Dr C. F. Code) ouabain (Sigma), pentagastrin (Sigma), phenoxybenzamine (Smith, Kline and French Laboratories), phentolamine (CIBA), propranolol (Sigma) and tetrodotoxin (Sigma). Pentagastrin was used instead of the whole molecule of gastrin. The synthetic pentapeptide contains the C-terminal tetrapeptide amide Trp-Met-Asp-Phe-NH_2 plus β-alanine attached to tryptophan. Tracy & Gregory (1964) showed that the C-terminal tetrapeptide amide possesses the entire range of physiological activities of the total molecule. A 10^{-3} M stock solution was prepared by dissolving 10 mg pentagastrin in a carrier solution consisting of 0·2 ml. methanol and 0·2 ml. N-NH_4OH, diluted to 10 ml. with 0·9 % NaCl solution. This was divided into 1 ml. aliquots and each kept in the freezer until used.

During an experiment, the change from one Krebs solution to another containing a hormone or drug was made by means of a Y-valve located on the upstream side of a flow control valve. Since the solutions were continuously flowing, it was possible to record the whole sequence of changes associated with the action of pentagastrin or acetylcholine.

Organ-bath tension recordings

Longitudinal muscle strips and 'mixed strips' were used. They were placed in a heated glass chamber containing 30 ml. Krebs solution which was maintained at $37 \pm 0{\cdot}5°$ C and bubbled with 97 % O_2 + 3 % CO_2. To record isometric tension, one end of the strip was securely anchored to a steel hook and the other attached to an isometric strain gauge (Grass FT.03C). Drugs, in a volume 0·5 ml., were added to the chamber. The concentration of drugs given in the Results are those of the final concentrations of the salt reaching the tissue. Drugs were removed from the bathing solution by overflowing the preparation for 30 sec with aerated Krebs solution also maintained at $37 \pm 0{\cdot}5°$ C.

Longitudinal strips. Following 1 hr equilibration period, length–tension curves were constructed. The strip length was changed by adjusting a screw micrometer. Length changes were measured to an accuracy of 0·05 mm. The prime difficulty in smooth-muscle mechanics is obtaining a reliable reference length. For the purposes of this study the following method was adopted. The length of muscle was increased in either 0·5 or 1·0 mm increments until the first increase in passive tension was noted. The length just prior to the development of passive tension was designated L_1, the initial length of zero passive tension. From this point, the length was increased in 0·5 or 1·0 mm increments by adjusting the micrometer. Following each stretch, a 10 min period was allowed for stress relaxation to occur before measurements

were made. In addition to recording the change in length by reading the dial of the micrometer, the length in twelve strips was also obtained by measuring with a *10 × objective* the distance between four nylon markers distributed equidistantly along the length of the strip. In all strips, the imposed stretch was uniform along the length of the strip and presumably so was passive tension. At each length, passive tension and maximum active tension produced by stimulation with acetylcholine and pentagastrin were recorded. The length at which acetylcholine produced maximum active tension was designated L_0.

Mixed strips. Following 1 hr equilibration period, each strip was placed at the optimum point of its length–tension relationship (L_0). In each strip, L_0 was determined by increasing the length until the contraction to supramaximal electrical stimulation of cholinergic motor neurones was maximum. To stimulate the intramural nerve plexus, two rectangular platinum electrode plates were placed parallel to the muscle strip and square wave pulses (8 V, 200 μsec, 15 Hz) were used (Vanhoutte & Leusen, 1969).

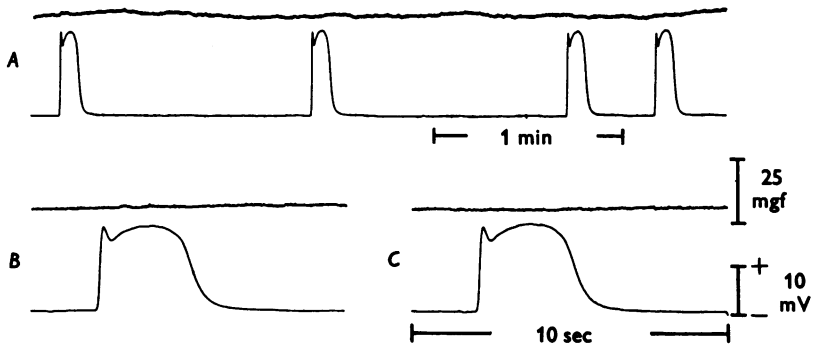

Fig. 1. Double sucrose-gap recordings of spontaneous electrical and mechanical activity in the longitudinal muscle of the dog antrum. Records taken from the same preparation. Note difference in time scale. In this and subsequent figures illustrating electrical and mechanical activity: top tracing, isometric tension; bottom tracing, electrical potential.

RESULTS

General observations

The muscle exhibited spontaneous electrical activity which consisted of two parts: a spike-like potential which after a brief and partial repolarization was followed by a negative-going, plateau-type potential. Fig. 1 shows examples of this activity. The amplitude of the spike-like potential averaged $23 \pm 0{\cdot}4$ mV (S.E. of mean, $n = 50$) whereas the amplitude of the plateau-type potential averaged $17 \pm 0{\cdot}5$ mV (S.E. of mean, $n = 50$). Daniel (1965) has recorded intracellularly from this smooth muscle. The average resting membrane potential calculated from his records was 43 mV and the average amplitude of the spike-like potential and pleateau-type potential were 31 and 24 mV, respectively. In the present studies, the

rate of rise of the spike component averaged $97 \pm 1{\cdot}1$ mV/sec (S.E. of mean, $n = 11$) and the combined duration of the spike and plateau potential averaged $7{\cdot}1 \pm 0{\cdot}2$ sec (S.E. of mean, $n = 11$). In 20 % of the preparations, a slow diastolic depolarization preceded the spike potential. The spontaneous frequency at $37 \pm 0{\cdot}5°$ C averaged $1{\cdot}0 \pm 0{\cdot}06$/min (S.E. of mean, $n = 92$). In all preparations, the period between action potentials was variable.

The spontaneous electrical activity of the stomach has been referred to as 'basic electric rhythm', 'slow wave', 'first potential', 'control potential', 'pace-setter potential' and 'action potential'. Since the rhythmically occurring potential has to be named for easy reference, a choice needs to be made from this list or a new term introduced. In this report, the term 'action potential' will be adopted for regular use when referring to the electrical potential. In the longitudinal muscle of the canine antrum, action potential seems to be an appropriate functional designation for the cyclically occurring potential because it is the electrical event which triggers the contractile process.

In 100 preparations, no tension change occurred either between or during action potentials. In four, weak phasic contractions occurred which ranged from 4 to 60 mg force. In these four preparations the resting passive tension was found to be 600–800 mg greater than the average resting tension in the other 100 strips. The average amplitude of the spike potential of the action potential was 18 mV and of the plateau potential 14 mV.

When tested, 10^{-5} M concentrations of either atropine or tetrodotoxin alone, or in combination, had no effect on either component of the action potential and did not abolish mechanical activity when it occurred. Alpha-adrenoceptor antagonists phentolamine (10^{-6} M) and phenoxybenzamine (10^{-6} M), the β-adrenoceptor antagonist propranolol (10^{-6} M), and H_1 and H_2 receptor blocking agents mepyramine (10^{-6} M) and metiamide (10^{-6} M), respectively, were without effect. These data suggest that the mechanism generating action potentials was a property of the smooth muscle.

In twelve preparations, steady conditioning hyperpolarization decreased or abolished the frequency of the action potentials. Conversely, in these twelve strips, steady conditioning depolarization increased the frequency of the action potentials. The increase was voltage-dependent. For example, in one preparation, 5, 10, 15 and 20 mV depolarization increased the frequency from 0·5 to 2·2, 3·2, 4·1 and 4·1/min, respectively.

The effect of ouabain (10^{-5} M) on the action potential was tested in four preparations. Within 5 min, the membrane depolarized by 4 to 8 mV but the action potentials continued for 25–30 min, during which time their amplitude became smaller. After 30–35 min no spontaneous action potentials occurred and none could be induced with outward current.

Effects of pentagastrin

In all preparations tested (59), pentagastrin increased the frequency of the action potential. The carrier solution for pentagastrin had no effect. The threshold concentration for pentagastrin ranged in different preparations from 2×10^{-14} to 10^{-11} M, the most common being the latter. The effect of four concentrations of pentagastrin on the frequency of the action potential is shown in Fig. 2. Each concentration regularized the occurrence of the action potential. With each tenfold increase in concentration of pentagastrin, the onset of action potentials was sooner, the frequency greater and the recovery to pre-stimulus frequency slower. The increase in frequency lasted for as long as pentagastrin was present. With high concentrations, 10^{-9} M or more, a prepotential sometimes preceded the spike potential of the action potential.

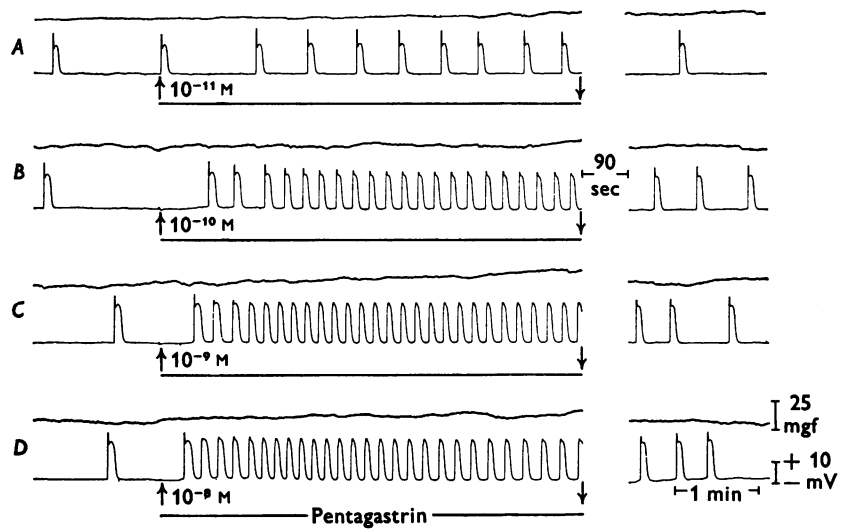

Fig. 2. Effect of four concentrations of pentagastrin on isometric tension and frequency of action potentials. Tracings to the right of gap in records taken 90 sec after wash-out of pentagastrin. In this and several subsequent figures horizontal line indicates period of drug application.

Dose–response relationships for five preparations from five dogs are shown in Fig. 3. In constructing the dose–response relationship for each experiment a series of doses was given, first either in increasing or decreasing order and then in the reverse order. Hence, each point is an average of two results. The time interval between doses on the way up and down the scale of concentrations was never less than 20 min. In these

five preparations, the half-maximum response averaged 2·7 action potentials/min and the corresponding ED_{50} averaged 2×10^{-10} M. The peak effect averaged 5·4/min and was reached at 10^{-8} M. In each of these five experiments, the levelling off of the dose–response relationship was more or less at the same frequency suggesting an upper limit to the action potential frequency. Since the frequency on the ordinate represents the increase in frequency above spontaneous frequency which, in these five preparations, averaged 1·0/min, then the actual frequency during the presence of pentagastrin averaged 6·4/min. This is similar to the maximum frequency (6·0/min) obtained during pentagastrin stimulation (2·5 μg/kg, subcutaneous) in the intact, conscious dog (Kelly, 1970).

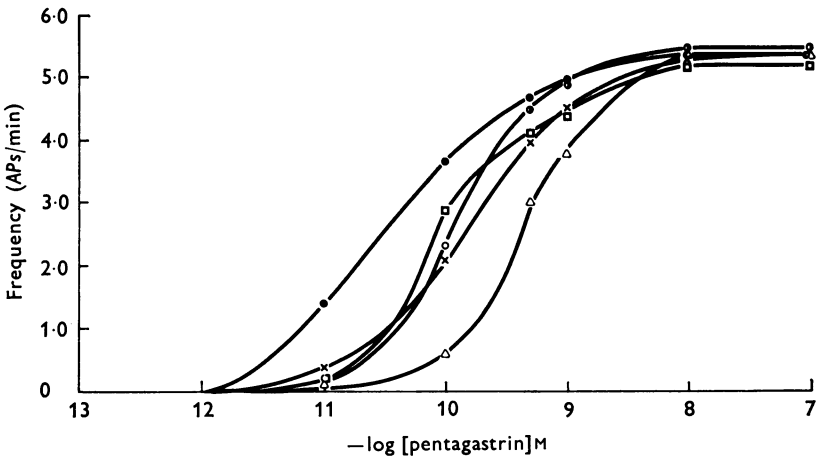

Fig. 3. Dose–response relationship for pentagastrin in five preparations from five dogs. Ordinate, frequency of action potentials produced by pentagastrin; abscissa, −log concentration of pentagastrin. Further explanation, see text.

In fifty-six of fifty-nine preparations, pentagastrin did not produce significant tension development. Sometimes, there appeared to be a slight increase in tone and sometimes a suggestion of weak, phasic contractions (cf. Figs. 2*C*, *D*; 8*D*; 11*A*). However, similar changes in tone were observed without pentagastrin. It should be pointed out that during these experiments, tension recordings were made at high gain. Microphonics from any source could easily account for such changes. For example, at the first arrow in Fig. 2*D*, the turn of the Y-valve to switch from normal Krebs solution to Krebs solution containing pentagastrin produced an artifactual shift in the level of base line tone. To determine whether or not pentagastrin induced tension development, changes in tone were measured in twenty-five strips during stimulation with 10^{-9} and 10^{-8} M

pentagastrin. The mean change in tone was an increase of $1{\cdot}0 \pm 1{\cdot}1$ mg (S.E. of mean). The largest increase was 20 mg; in one, there was a relaxation of 10 mg. In seventeen of twenty-five, there was no change. In the fifty-six strips, there was no consistent relationship between 'bumps' in the tension trace and action potentials.

In three of fifty-nine preparations each spontaneous action potential produced a weak, phasic contraction. In these preparations, pentagastrin increased the frequency of phasic contractions to the same extent as it increased the frequency of the action potential. It did not alter resting tone nor did it potentiate the force of the phasic contractions. In two, there was a reduction in the strength of each phasic contraction.

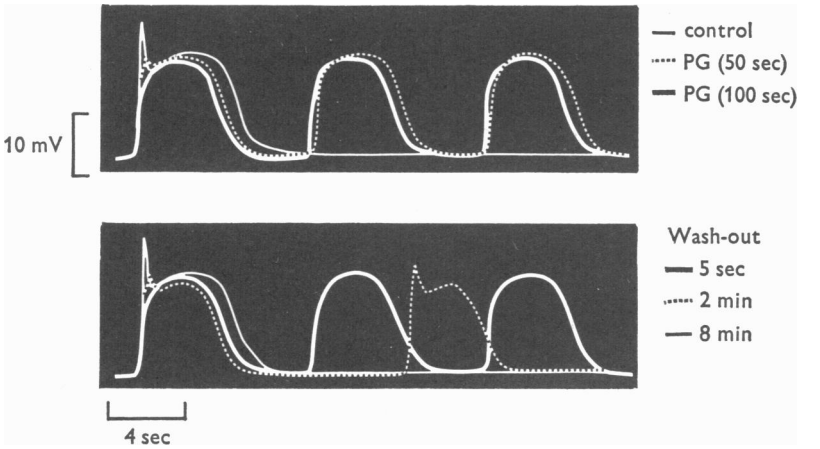

Fig. 4. Effect of pentagastrin (PG, 10^{-8} M) on configuration of action potential. Top panel, action potentials before and during pentagastrin. At 100 sec, note reduction in amplitude of spike and plateau potentials of action potential and shortening of duration. Bottom panel, action potentials after washing out pentagastrin.

Pentagastrin had specific effects on the configuration of the action potential. These effects are shown in Fig. 4. In the upper and lower panels, the thin trace represent an action potential before and 8 min after, the dashed line action potentials 50 sec during and 2 min after, and the thick line action potentials 100 sec during and 5 sec after pentagastrin stimulation, respectively. Pentagastrin produced a prepotential which preceded each action potential, reduced the amplitude of the spike and plateau potentials and shortened the duration of the plateau. In some preparations, pentagastrin had little effect on the amplitude of the spike potential and did not produce a prepotential. In all preparations, however, it reduced the amplitude and shortened the duration of the plateau potential.

In most instances, pentagastrin had no effect on the resting membrane potential. In a few, there was a slight depolarization and this occurred when high concentrations were used (10^{-8} M or more). The greatest depolarization observed was 0·8 mV (Fig. 6).

The responses to pentagastrin were unaffected by atropine (10^{-5} M) or tetrodotoxin (10^{-5} M) present either alone or in combination. α- and β-adrenoceptor antagonists, and H_1 and H_2 antagonists were without effect. Hence, pentagastrin acted directly on the smooth muscle.

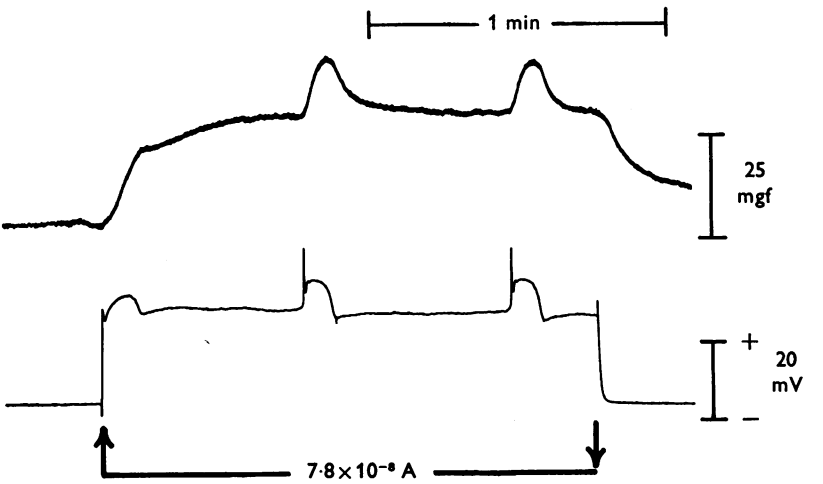

Fig. 5. Effect of conditioning depolarization on membrane potential, action potentials and isometric tension. Before trace, no tension changes accompanied action potential. Further explanation, see text.

Effect of conditioning membrane depolarization during pentagastrin stimulation. The effect of conditioning, depolarizing current was tested on spontaneously occurring action potentials and on action potentials produced by pentagastrin. The effect of depolarizing current on spontaneously occurring action potentials is shown in Fig. 5. Depolarizing current produced an increase in tone and induced action potentials which produced contractions. The contractions were weak, averaging 12 mgf.

The effect of depolarizing current on action potentials produced by pentagastrin is shown in Fig. 6. The traces are continuous. Pentagastrin was added at the arrow at the beginning of Fig. 6*A* and removed at the arrow in *D*. At each smaller arrow, the strength of the depolarizing current was increased by the amount indicated. The dashed line represents the original resting potential. A current 9×10^{-9} A had no effect on either tone or phasic activity but it increased the frequency of the action

potential; $1{\cdot}8 \times 10^{-8}$ A produced a slight increase in tone and each action potential produced a weak contraction; $2{\cdot}7 \times 10^{-8}$ A produced a further increase in tone and phasic contractions were more evident. The strength of each contraction averaged 14 mgf. Although each additional increment in current produced a further increase in tone, there was no further increase or decrease in the force of contraction. When the conditioning depolarizing current was turned off and pentagastrin removed in *D*, the membrane repolarized and tension returned to the pre-stimuli level. The

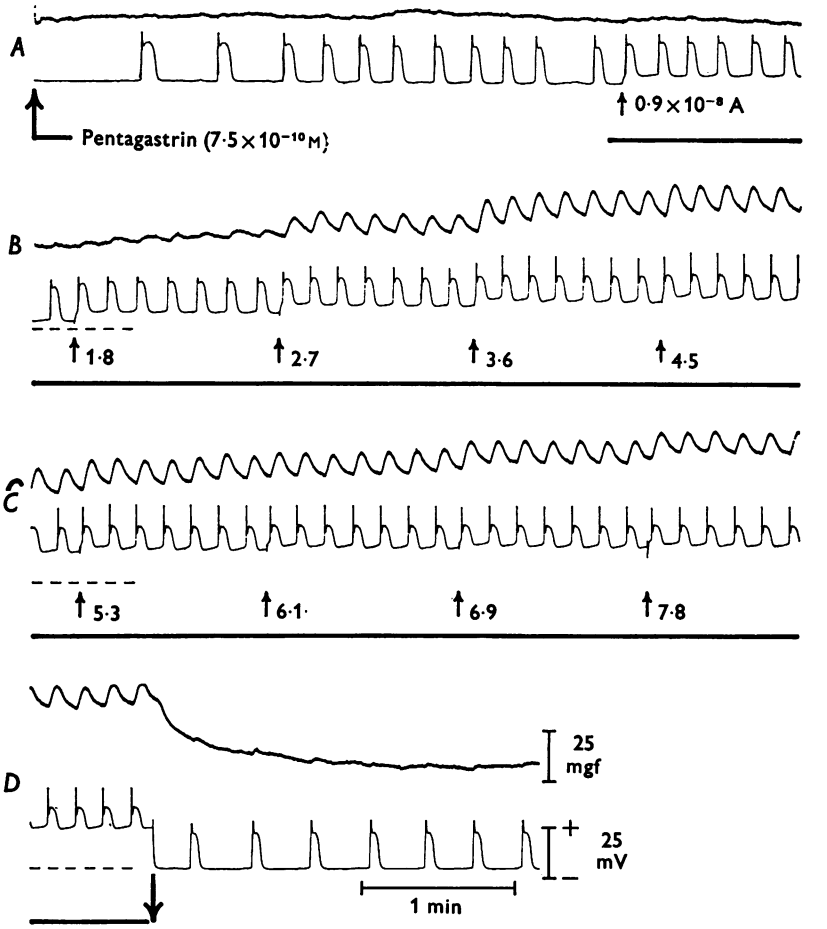

Fig. 6. Electrical and mechanical responses to pentagastrin during conditioning depolarizing current. Traces continuous. At arrow in *A*, pentagastrin added. At each smaller arrow, *A–C*, strength of depolarizing current increased by amount indicated. At large arrow in *D*, pentagastrin removed and current turned off. Further explanation, see text.

first action potential after the arrow triggered a phasic contraction which generated 1 mgf. This occurred when the tension had fallen to the level present in *B* when the conditioning current was $3{\cdot}6 \times 10^{-9}$ A. If the occurrence of phasic contractions with each action potential was related to tone, then the force of this contraction would have been similar to those in *B*. These data suggest that phasic contractions observed during conditioning depolarizing current were not directly related to changes in tone.

Effect of stretch on response to pentagastrin using the double sucrose-gap method. In the experiments described above, the muscle strips were held at a constant over-all length and constant passive tension. In three experiments, the effect of pentagastrin was examined after the muscle was actively stretched. In each, the muscle response to pentagastrin (10^{-9} M) was tested at 50, 100, 300, 600, 900, 1200 and 1500 mg passive tension. At each level of passive tension in each strip, pentagastrin did not initiate active tension, either tonic or phasic. However, it still increased the frequency of the action potential. In the three experiments, the mean frequency before pentagastrin stimulation was 0·6/min whereas during pentagastrin it was 3·7/min at each increased level of passive tension up to 600 mg. At each level beyond 600 mg the frequency during pentagastrin averaged 2·9/min.

Effect of strip length on active tension development using the organ-bath method. The evidence to the point indicates that pentagastrin increases the frequency of the action potential but does not produce either tonic or phasic tension. It is possible that with the double sucrose-gap method, active tension developed by the muscle in the test node might pull on portions of muscle in the sucrose gaps and lengthen the muscle in the sucrose gaps. Hence, tension development in the active portion of the muscle might be damped by the inactive portions and further might lead to a significant displacement along the length–tension relationship. The stretch experiments in the double sucrose gap described above indirectly argue against this. To deal more directly with the possibility, the length–tension relationship was determined for twelve strips taken from twelve animals. Tension records were made from strips placed in the organ bath. The object of these experiments was not to examine the mechanics of the longitudinal muscle of the canine antrum but rather to determine if pentagastrin failed to produce active tension in the double sucrose-gap apparatus because the muscle strips were on an unfavourable part of the length–tension relationship.

Length–tension curves are shown in Fig. 7. In Fig. 7*A*, *B* and *C*, tension (gf) is plotted on the *y*-axis and the ratio L/L_1 on the *x*-axis. L is the actual length and L_1 the length just before the development of

passive tension. The length–tension curves from the strip in which the ratio L/L_i was the smallest are shown in Fig. 7*A*, the largest in *B* and the more typical in *C*. The mean value for L/L_i in the twelve strips was $1{\cdot}7 \pm 0{\cdot}1$ (S.E. of mean). The length at which maximum active tension was developed was designated as L_0. In twelve strips at L_0 there was an

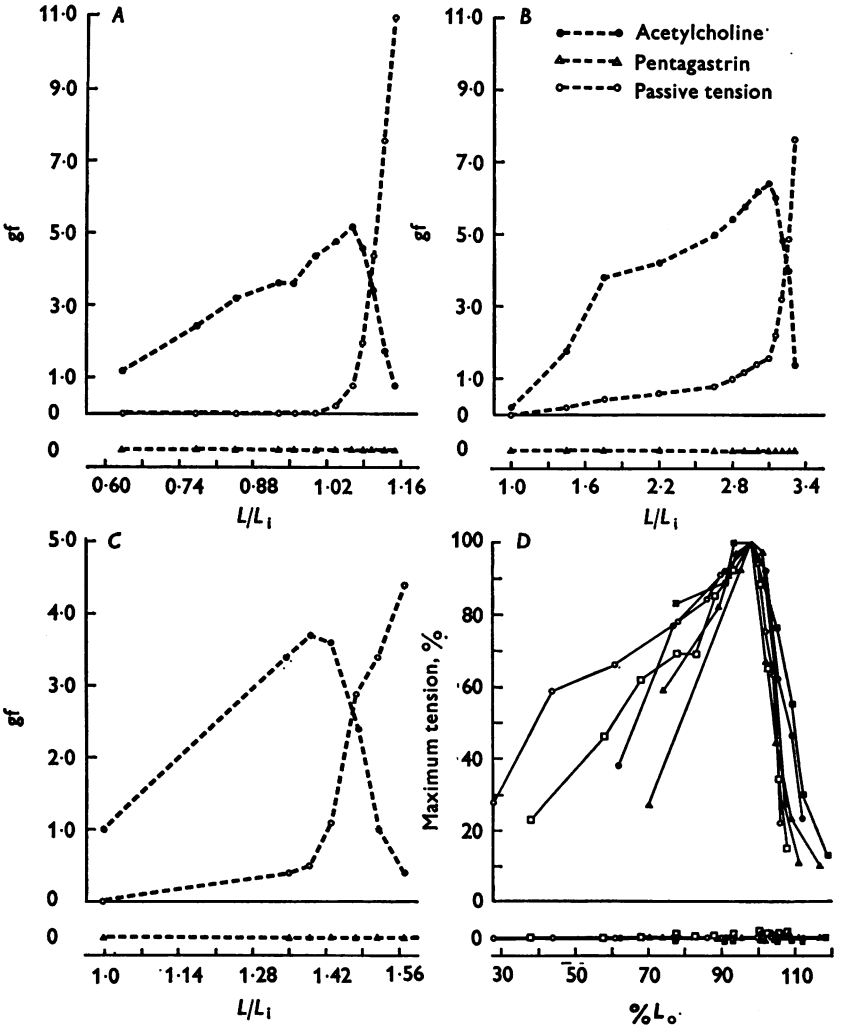

Fig. 7. Tension–length curves. In *A*, *B* and *C*: ○, passive tension; ●, active tension produced by acetylcholine; △, tension produced by pentagastrin. In *D*, active tension expressed as percentage of maximum, length expressed as percentage of L_0. Top six curves, active tension produced by acetylcholine in six strips; bottom line, active tension produced by pentagastrin in same six strips. Further explanation, see text.

appreciable resting tension which averaged 570 ± 70 mg (S.E. of mean). At each length, passive tension due to stretch and active tension produced by acetylcholine ($5{\cdot}5 \times 10^{-5}$ M) and pentagastrin (10^{-9} M) were recorded. The curves show that active tension produced by acetylcholine increased with stretch up to a maximum and then decreased with further increases in length. In contrast to acetylcholine, pentagastrin did not produce active tension, either phasic or tonic, at any point along the length–tension relationship. This was found in all twelve strips.

The results from six strips are shown normalized in Fig. 7*D*. This is done because the comparison between strips is best assessed by plotting the active tension expressed as a percentage of the maximum against the length expressed as a percentage of $L_{\hat{0}}$. In each strip, active tension produced by acetylcholine falls off rapidly at lengths greater than $L_{\hat{0}}$ (top curves). At lengths shorter than $L_{\hat{0}}$ there was a fairly wide scatter. Pentagastrin did not produce active tension (bottom line).

In twenty other strips, each placed at $L_{\hat{0}}$, pentagastrin had no mechanical effect. In one of these strips, spontaneous contractions occurred at a frequency of 1·3/min. A complete length–tension relationship was done to determine the effect of stretch on the force of spontaneous contractions. Acetylcholine ($5{\cdot}5 \times 10^{-5}$ M) was used to determine $L_{\hat{0}}$. The force of spontaneous contraction fell off on either side of $L_{\hat{0}}$. At each point of the length–tension relationship pentagastrin increased the frequency of contraction to 3·3/min but it did not produce tone and it did not potentiate the force of phasic contraction.

Effects of acetylcholine

The effects produced by acetylcholine differed from those produced by pentagastrin. For each preparation tested a concentration of acetylcholine could be found which produced phasic contractions at the frequency of the action potential. Threshold concentration ranged, in sixty experiments, from 2×10^{-8} to 6×10^{-8} M. The response of the same preparation to three concentrations of acetylcholine and to a high concentration of pentagastrin is shown in Fig. 8. There was no response to 10^{-8} M acetylcholine (Fig. 8*A*). Acetylcholine, 3×10^{-8} M, produced a transient depolarization which was associated with a transient increase in tone and in frequency of the action potential (Fig. 8*B*). There was an increase in the amplitude and duration of the action potential and each action potential produced a contraction. In addition, oscillations of the membrane potential followed each action potential. As the membrane repolarized, the frequency of oscillation was reduced. When conditioning outward current was used to depolarize the membrane to the same extent as that which occurred during stimulation by acetylcholine, there was a similar increase in action potential

frequency. This suggests that the increase in frequency of action potentials during acetylcholine may be related to the membrane depolarization. The frequency of oscillation depended on the concentration of acetylcholine and this was so because larger concentrations of acetylcholine produce a greater depolarization of the membrane potential (Fig. 8*C*). Sometimes, the oscillations produced contractions. In 5×10^{-8} M acetylcholine (Fig. 8*C*), all responses were potentiated. Although resting tone was restored by the

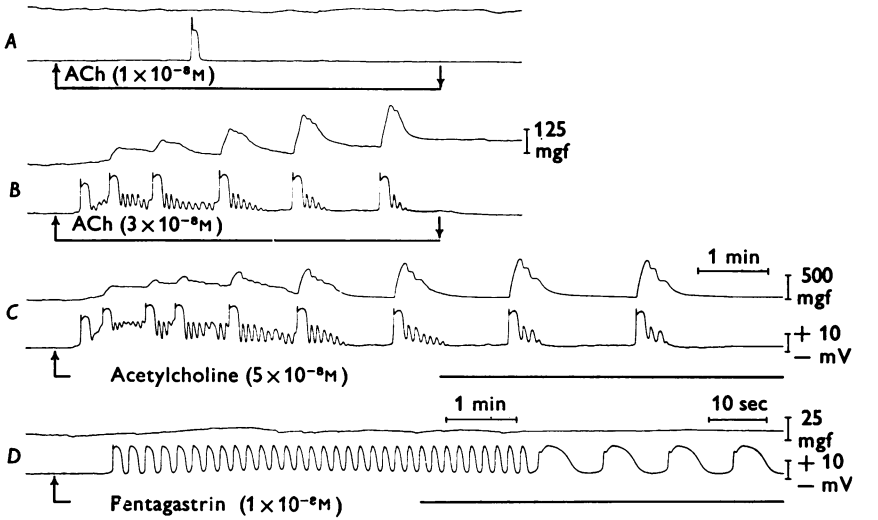

Fig. 8. Effect of three concentrations of acetylcholine (ACh) and a high concentration of pentagastrin on electrical and mechanical activity. Recordings taken from same tissue. In *A*, no response to acetylcholine (1×10^{-8} M). In *B* and *C*, above-threshold concentrations of acetylcholine depolarized the membrane, increased tone, increased amplitude of action potential and produced phasic contractions. In *D*, pentagastrin (10^{-8} M) increased frequency of action potential but had no motor effect. Note, gain of tension record higher in *D* than in *A–C*. Depolarization due to pentagastrin largest ever observed. Further explanation, see text.

seventh action potential, each subsequent action potential produced a stronger phasic contraction. The force of these contractions was stronger than the ones which occurred during the transient change in tone. In Fig. 8*C* acetylcholine was not removed from the bathing solution at the 5 min mark but rather at 35 min (not shown). Throughout this period, each action potential produced a phasic contraction. In the same tissue, pentagastrin increased the frequency of the action potential but did not produce motor activity (Fig. 8*D*; note increase in sensitivity of tension recording). Although the increase in frequency produced by acetylcholine was transient, the increase by pentagastrin was maintained throughout.

Some of the responses to acetylcholine were time-dependent (Fig. 8 *B*, *C*). This was more easily studied with higher concentrations of acetylcholine. Thus, in Fig. 9, the initial depolarization, increase in tone, increase in frequency of the action potential and of oscillation passed off and there was a dispersion between the electrical and mechanical changes. Although the increase in the amplitude and duration of the action potential reached steady state by the fifth action potential, the force of each phasic contraction continued to increase thereafter until steady-state conditions were reached. This phenomenon may be similar to the one seen in cardiac muscle during stimulation by noradrenaline (Reuter, 1974).

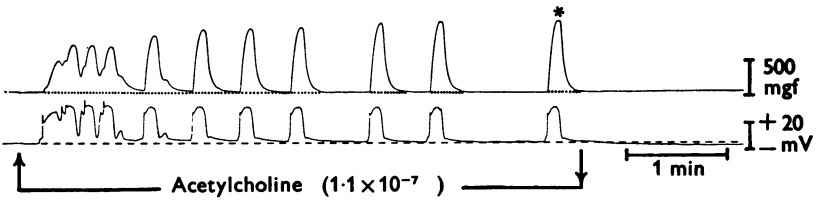

Fig. 9. Effect of higher concentration of acetylcholine on electrical and mechanical activity. Initial depolarization and increase in tone return to control levels. Action potentials, however, still produced contractions which increased in force even though tone had returned to pre-stimulus level. Dashed line, level of resting potential before acetylcholine; dotted line, level of tone before acetylcholine. *, contraction off-scale.

The increase in the amplitude of both the spike and plateau potentials and the increase in the duration of the plateau potential were dose-dependent. Fig. 10 shows the results from one of eleven experiments. The results in the other ten were similar. The traces in the top panel are tension changes whereas those in the bottom panel are action potentials. To construct this Figure, action potentials were aligned by superimposing the first detectable depolarization which occurred at the foot of the spike of each action potential. In both panels, trace *C* is the control and traces 1–7 were obtained 10 min after exposure to seven progressively increasing concentrations of acetylcholine. In each concentration at the 10 min mark, the membrane potential had completely repolarized and the resting tension completely returned to the control level. Acetylcholine, 5×10^{-9} and 1×10^{-8} M, increased the amplitude of the spike and plateau potentials and prolonged the duration of the action potential. There were no changes in tension. An increase to $1{\cdot}5 \times 10^{-8}$ M produced a further increase in amplitude and duration of the spike and plateau potentials and these changes produced contractions. Each additional increase in the concentration of acetylcholine increased the amplitude of both the plateau and spike potentials. However, the changes in the plateau potential were

greater and more consistent than those in the spike potential suggesting that phasic tension development in this smooth muscle may be more dependent on changes in the plateau potential.

All of the effects produced by acetylcholine were unaffected by tetrodotoxin (10^{-5} M) but were completely blocked by atropine (10^{-8} M). Hence, muscarinic receptors mediated these effects.

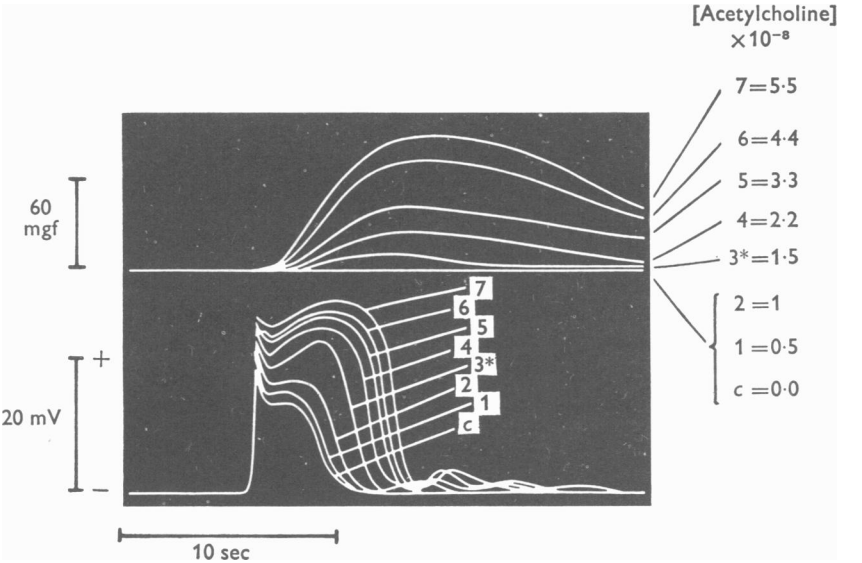

Fig. 10. Relationship between change in configuration of action potential produced by seven concentrations of acetylcholine and force of phasic contraction. *c*, control traces. Trace 3* indicates threshold response to acetylcholine. Progressively increasing concentrations of acetylcholine increased amplitude and duration of action potential and force of contraction. Changes in plateau potential greater than those in spike potential.

Effects of combining acetylcholine and pentagastrin

To determine the muscle response when both acetylcholine and pentagastrin are present, two series of experiments were conducted. In the first, acetylcholine was added to a preparation being stimulated by pentagastrin. Fig. 11 shows the results of one of five experiments. Similar results were obtained in all five. The tracings in Fig. 11 are continuous. At the arrow in Fig. 11*A*, pentagastrin was added and maintained at 10^{-11} M throughout *B* and *C*. In *B*, acetylcholine was added and after a brief delay each action potential produced a contraction which increased in strength as a function of time. Increasing the concentration of acetylcholine by 50% in *C* led to a further increase in the force of each phasic contraction.

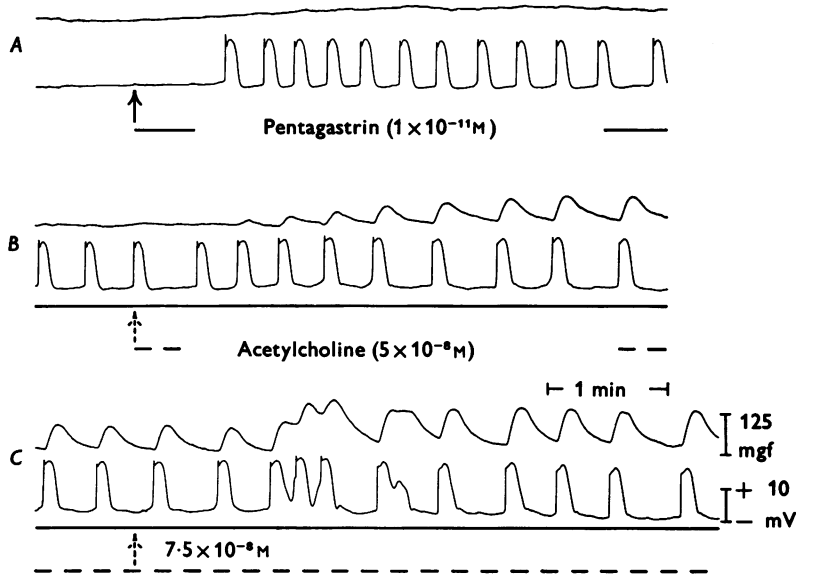

Fig. 11. Effect of adding acetylcholine during pentagastrin stimulation. Traces are continuous. At arrow in *A*, pentagastrin added and maintained throughout. At arrow in *B*, acetylcholine added and at arrow in *C*, concentration increased by 50 %. Note acetylcholine produced contractions in *B* and potentiated them in *C*.

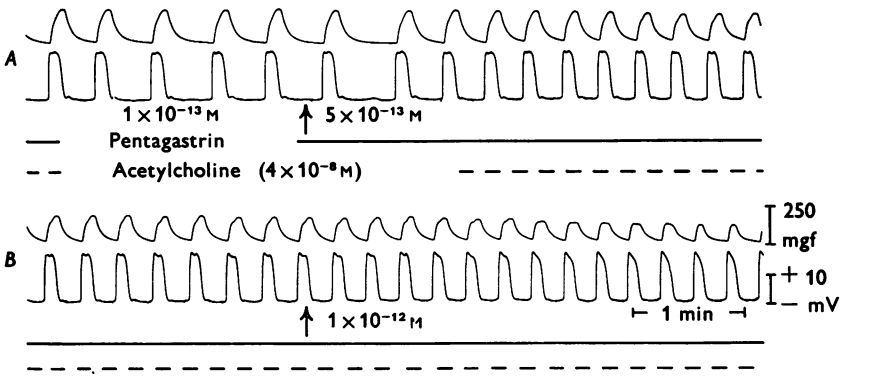

Fig. 12. Effect of increasing concentrations of pentagastrin on frequency and amplitude of electrical and mechanical activity. *A* and *B* continuous. Acetylcholine (4×10^{-8} M) and pentagastrin (10^{-13} M) present at beginning of *A*. Concentration of acetylcholine maintained constant throughout *A* and *B* but concentration of pentagastrin increased at arrow in *A* to 5×10^{-13} M and at arrow in *B* to 10^{-12} M. Each increment increased frequency of action potential and contraction but reduced amplitude of contraction. Reduction in amplitude and duration of plateau potential after increasing concentration of pentagastrin to 10^{-12} M led to weaker contractions.

In the second series of experiments, pentagastrin was added against a constant background of acetylcholine. Fig. 12 shows one of six experiments. Similar results were obtained in each experiment. In Fig. 12, *A* and *B* are continuous and acetylcholine (4×10^{-8} M) was present throughout. At the beginning of *A*, phasic contractions occurred at the frequency of the action potential. An increase in the concentration of pentagastrin from 1 to 5×10^{-13} M increased the frequency of action potentials and phasic contractions occurred at the frequency of the action potential. An increase in the concentration of pentagastrin from 1 to 5×10^{-13} M increased the frequency of action potentials and phasic contractions but decreased the force of each phasic contraction. A doubling

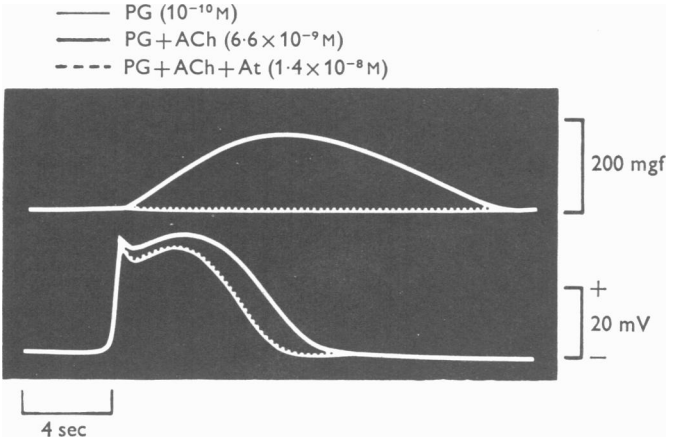

Fig. 13. Effect of actylcholine and atropine (At) on action potential produced by pentagastrin. Increase in tension and increase in amplitude and duration of action potential blocked by atropine.

of the concentration of pentagastrin in *B* further increased the frequency of both action potentials and contractions and further reduced the force of each contraction. The reduction in force was associated with a reduction in amplitude and duration of the plateau potential of the action potential. These data are consistent with the data illustrated in Fig. 4 where it was shown that pentagastrin reduced the amplitude and duration of the plateau potential.

The effect of acetylcholine and atropine on the configuration of pentagastrin initiated action potentials is shown in Fig. 13. Traces in the top panel are tension changes whereas those in the bottom are action potentials. Thin lines represent activity 15 min after adding pentagastrin, thick line 10 min later after adding acetylcholine and the dashed lines 2 min later after adding atropine. The action potential in the presence of acetyl choline and pentagastrin was larger in amplitude and longer in duration

than one which occurred during pentagastrin alone. When the stimulant action of acetylcholine was blocked by atropine, the action potential was similar to the one which occurred when only pentagastrin was present. Clearly, differences can be discerned between action potentials occurring in pentagastrin alone and in pentagastrin plus acetylcholine. These electrical differences may play an important role in excitation–contraction coupling.

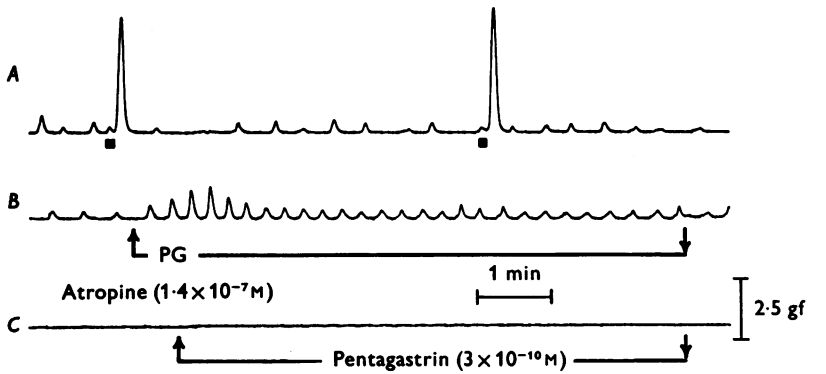

Fig. 14. Mechanical response of whole strip of mixed preparation to: *A*, nerve stimulation at ■ (9 V, 200 μsec, 15 Hz); *B*, to pentagastrin alone; *C*, to pentagastrin in the presence of atropine. Atropine-blocked mechanical response to pentagastrin.

Mechanical responses to pentagastrin in mixed preparations using the organ bath method. These preparations were spontaneously active and transmural stimulation (filled squares, 9 V, 200 μsec, 15 Hz) produced a contraction (Fig. 14*A*). Pentagastrin produced contractions at a frequency of 3·5/min (Fig. 14*B*). Atropine blocked the response to both transmural stimulation (not shown) and to pentagastrin (Fig. 14*C*), indicating that in mixed preparations which included neurones of Auerbach's plexus, pentagastrin released acetylcholine from cholinergic neurones.

Effect of altering the external calcium concentration

In calcium-deficient solution, part or all of the $CaCl_2$ was replaced with NaCl. Calcium-rich solutions were prepared by replacing part of the NaCl with $CaCl_2$.

Calcium-deficient. The effect of calcium-deficient Krebs solution on spontaneous and acetylcholine induced action potential and contractions is shown in Fig. 15. With each progressive decrease in extracellular calcium there was a reduction in the amplitude of the spike and plateau potentials

of the spontaneous action potentials and in 1·2 mM-$[Ca^{2+}]_o$, a significant shortening of the action potential. In 0·6 mM-$[Ca^{2+}]_o$, spontaneous action potentials were abolished. Similar changes were observed in acetylcholine induced action potentials (right side, Fig. 15). Each reduction in extracellular calcium reduced the amplitude and duration of the action potential and the force of contraction.

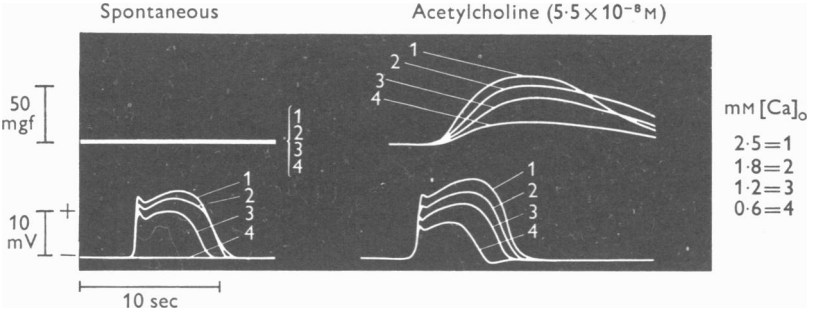

Fig. 15. Effect of calcium-deficient solutions on spontaneous (left side) and acetylcholine induced action potentials and contractions (right side). Reduction in extracellular calcium progressively reduced amplitude of action potentials. In 0·6 mM $[Ca^{2+}]_o$, spontaneous action potentials abolished but acetylcholine able to produce action potential. Further explanation, see text.

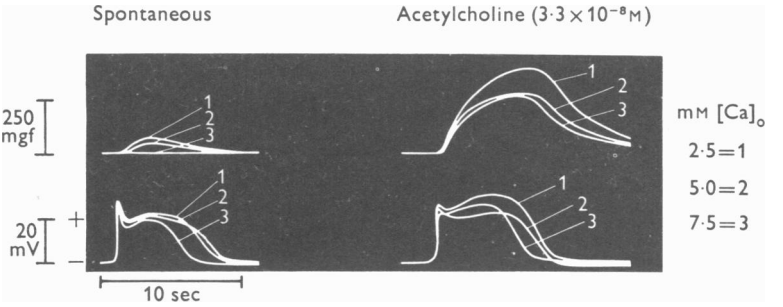

Fig. 16. Effect of calcium-rich solution on spontaneous action potential (left side) and acetylcholine induced action potential and contractions (right side). Further explanation, see text.

Calcium-rich. In five experiments, the frequency of spontaneous action potentials increased from an average of 1·9/min in normal Krebs solution to an average of 2·6/min in 5·0 mM-$[Ca^{2+}]_o$ Krebs solution. However, in 7·5 mM-$[Ca^{2+}]_o$ Krebs solution, the frequency was reduced to 0·3/min. The effect of calcium-rich solutions on spontaneous and acetylcholine induced action potentials and contractions is shown in Fig. 16. Increasing the calcium concentration to 5·0 mM produced an increase in the amplitude

and duration of the plateau potential of spontaneous action potentials. Each spontaneous action potential produced a contraction. When acetylcholine was present, the amplitude and duration of the plateau potential and amplitude of contraction were greater than those observed in normal Krebs solution. In 7·5 mM-$[Ca^{2+}]_o$, spontaneous action potentials and the amplitude of contractions were greater than those in normal Krebs solution but were the same or slightly less than those observed in 5·0 mM-$[Ca^{2+}]_o$ (Fig. 16). The effects of acetylcholine in 7·5 mM-$[Ca^{2+}]_o$ were depressed to near control levels.

DISCUSSION

The pattern and configuration of the action potential of the longitudinal muscle of the dog antrum recorded in this study with the double sucrose-gap method were the same as those recorded with intracellular electrodes (Daniel, 1965) and those obtained by integration of the differentially recorded extracellular potential (Bozler, 1945). In each of these studies and in the present one, the occurrence of the plateau was the most prominent feature of the action potential. The amplitude of the action potential recorded with the double gap was less than that recorded with an intracellular electrode (Daniel, 1965) but this is to be expected because the short circuiting factor in practice never equals unity. The primary mechanism underlying the action potential was not voltage-insensitive because hyperpolarization reduced the frequency whereas depolarization increased the frequency of the action potential or triggered action potentials when none occurred spontaneously.

The average action potential frequency in the antrum of an intact stomach of a conscious dog is 5·0/min (Kelly, 1970), whereas the average frequency in this study was 1·0/min. However, the frequency of the action potential in the antrum of a stomach in which the pace-maker in the orad corpus is removed is approximately 1·0/min (Kelly, 1970). It is likely then that the frequency obtained in the present study is the natural frequency of the action potential in the longitudinal muscle of the antrum when it is not driven by the pace-maker in the corpus.

In 97% of the preparations spontaneous action potentials did not produce contractions. Hence, action potentials do not normally reach threshold for contraction. It is possible in these experiments that the level of the resting membrane potential might have been significantly hyperpolarized from the true resting membrane potential because of loop currents arising from the liquid junction potential between the sucrose solution and Krebs solution (Blaustein & Goldman, 1966). Thus, under more physiological conditions, action potentials might reach threshold

for contraction. However, in thirty-one of thirty-two experiments, spontaneous contractions were not seen when whole strips of muscle were bathed in Krebs solution in the organ bath even though such strips were at L_0. This further suggests that the spontaneously occurring electrical potential does not normally reach threshold for contraction.

In three preparations using the double sucrose-gap method, weak spontaneous contractions occurred with each action potential. Stretch alone could not have been the reason because when quiescent strips were actively stretched or shortened in the length–tension experiments, no spontaneous contractions were observed. There is no immediately apparent explanation for the occurrence of contractions in these preparations.

Contractions associated with action potentials were always seen during stimulation by acetylcholine or when the membrane was depolarized by outward current. These data indicate that both voltage and acetylcholine altered the action potential or some component of it coupling membrane excitation with contraction.

This raises the question as to whether or not there exists a voltage threshold which must be crossed by the action potential for tension development. It is possible using the double gap method to detect the presence of a voltage threshold for contraction and to make measurements relative to this value and the resting level. The effect of conditioning depolarizing current and acetylcholine suggests that relative to the average resting membrane potential of the muscle cells in the test node, there is a threshold or 'trigger' potential for contraction. The double gap method cannot define the absolute magnitude of this voltage level relative to the resting membrane potential because neither were absolutely determined. The absolute magnitude of the electrical parameters in question might be settled with intracellular recordings.

In the length–tension experiments, it was found that in this smooth muscle appreciable resting tension was present at L_0. In this respect, this muscle resembles intestinal (Aberg & Axelsson, 1965), uterine (Csapo & Goodall, 1954) and vascular (Speeden, 1960) smooth muscle. The force of isometric contractions induced by acetylcholine depended on initial ength. In contrast, pentagastrin did not induce tonic or phasic active tension regardless of the muscle length. It seems reasonable then to conclude that pentagastrin does not induce tension development. However, the similarities between the isometric condition *in vitro* and the physiological condition *in vivo* may be limited. Under *in vivo* conditions antral smooth muscle probably contracts neither isometrically nor isotonically.

Although pentagastrin had no effect on tension, it increased the frequency of the action potential. More often than not, this effect was not associated with a depolarization in the resting membrane potential

indicating that pentagastrin did not increase the frequency of the action potential through a voltage change in the membrane potential. In addition to this action on smooth muscle, pentagastrin also acted on the cholinergic nerve endings in the intramural plexus. Here, it caused the release of acetylcholine because in mixed muscle strips, consisting of circular and longitudinal muscle and intramural plexus, atropine blocked the motor response to pentagastrin. This confirms the observations of others who have shown that gastric motor responses induced by pentagastrin in the dog are reduced or abolished by atropine (Gregory & Tracy, 1964; Bennett, 1965; Jacoby & Marshall, 1969). In the guinea-pig ileum-pentagastrin doubles the output of acetylcholine from Auerbach's plexus (Vizi *et al.* 1973).

The characteristic effect of acetylcholine was initiation of active tension. Initially acetylcholine produced an increase in tone. But this was transient leaving phasic contractions at the frequency of the action potential. Acetylcholine produced this phasic inotropic effect by increasing the amplitude and duration of the action potential suggesting that the neurotransmitter acted on the potential dependent slow membrane current which generated the action potential. In this respect, the effect of acetylcholine may be similar to its action in the longitudinal muscle of the guinea-pig ileum (Bolton, 1971). In addition, Shuba (1974) has recently observed that noradrenaline and adrenaline increase the amplitude and duration of the plateau potential of the action potential in the guinea-pig ureter and suggested the amines do so by increasing the slow inward sodium current.

When high concentrations of acetylcholine were used (cf. Fig 6) there was a larger depolarization and increase in tone. Hence, in addition to its action on the potential dependent membrane conductance of the action potential, acetylcholine also activated ionic current through voltage, independent channels. This latter effect most likely conforms to the standard model for transmitter action (Ginsborg, 1967). Interestingly, there appears to be a selective desensitization to prolonged exposure to acetylcholine. Whereas the membrane potential repolarizes and tone returns to the resting level the effect on the action potential and phasic contractions continue to occur with each action potential.

Often when acetylcholine was present, action potentials were followed by oscillations in membrane potential. It is not certain whether such oscillations occur *in vivo* in this smooth muscle during cholinergic, nervous activity. Judging from published records obtained in the intact unanaesthetized dog, they do not (Kelly, 1970). The occurrence of oscillations in this study may in part be due to the sudden increase in concentration of acetylcholine from zero to a level approaching the measured

concentration. Because of the lack of specialized close neuromuscular junctions (J. H. Szurszewski, unpublished observations), the concentration of acetylcholine *in vivo* in the vicinity of the smooth muscle cells may increase more gradually and not reach the concentration at which oscillations occur.

Under *in vivo* conditions after ingestion of a meal, it is likely that gastrin is released in response to the meal and that both it and acetylcholine simultaneously effect gastric antral motility. The results obtained in this study indicate that the nature of the motor pattern in the longitudinal muscle layer may depend upon the relative concentration of gastrin and acetylcholine. It was found that an increase in the concentration of acetylcholine increased the amplitude and duration of the action potentials which lead to stronger contractions. On the other hand, an increase in the concentration of pentagastrin increased the frequency of contraction, but it reduced the amplitude and duration of the plateau potential which led to weaker contractions. Thus, although gastrin produces contractions by stimulating release of acetylcholine from cholinergic neurones, it may regulate the force of contraction by modulating in the smooth muscle membrane, the electrical event which triggers the contractile process.

There remains to be discussed the ionic requirements for the action potential. Although the primary mechanism responsible for the action potential was not studied in detail, a few preliminary comments can be made. The negative effect of tetrodotoxin on the action potential should not be taken as unequivocal evidence for a calcium mechanism. Although the action potential in muscles of the *Taricha* newt and *Tetrodon* fish is sensitive to changes in the external sodium concentration, it is resistant to tetrodotoxin (Kao, 1966). In four experiments, ouabain (10^{-5} M) did not abolish the action potential in the first 30 min but did produce a 4–8 mV depolarization. The disappearance of action potentials after 30 min might be attributed to a non-specific effect because of the high concentration of ouabain used in these experiments. Calcium was important for the occurrence of the action potential. Calcium may contribute to the development of the action potential either by a calcium conductance change or by an oscillatory calcium pump. Or, calcium may be important in regulating conductance channels for other ions or oscillatory pumps for other ions. Quantitative studies to analyse the current flow during the action potential are needed before anything more conclusive can be said about the precise role of calcium.

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REFERENCES

ABERG, A. K. G. & AXELSSON, J. (1965). Some mechanical aspects of an intestinal smooth muscle. *J. Physiol.* **64**, 15–27.

BENNETT, A. (1965). Effect of gastrin on isolated smooth muscle preparations. *Nature, London* **208**, 170–173.

BENNETT, A., MISIEWICZ, J. J. & WALLER, S. L. (1967). Analysis of the motor effects of gastrin and pentagastrin on the human alimentary tract *in vitro*. *Gut* **8**, 470–474.

BLAUSTEIN, M. P. & GOLDMAN, D. E. (1966). Origin of axon membrane hyperpolarization under sucrose-gap. *Biophys. J.* **6**, 453–470.

BOLTON, T. B. (1971). On the nature of the oscillations of the membrane potential (slow waves) produced by acetylcholine or carbachol in intestinal smooth muscle. *J. Physiol.* **216**, 403–418.

BOZLER, E. (1945). The action potentials of the stomach. *Am. J. Physiol.* **144**, 693–700.

CAMERON, A. J., PHILLIPS, S. F. & SUMMERSKILL, W. H. J. (1970). Comparison of effects of gastrin, cholecystokinin-pancreozymin, secretin, and glucagon on human stomach muscle in vitro. *Gastroenterology* **59**, 539–545.

CSAPO, A. & GOODALL, M. (1954). Excitability, length tension relation and kinetics of uterine muscle contraction in relation to hormonal status. *J. Physiol.* **126**, 384–395.

DANIEL, E. E. (1965). The electrical and contractile activity of the pyloric region in dogs and the effects of drugs. *Gastroenterology* **49**, 403–418.

GINSBORG, B. L. (1967). Ion movement in junctional transmission. *Pharmac. Rev.* **19**, 289–316.

GREGORY, R. A. & TRACY, H. J. (1964). The constitution and properties of two gastrins extracted from hog antral mucosa. I: The isolation of two gastrins from hog antral mucosa. II: The properties of two gastrins isolated from hog antral musoca. *Gut* **5**, 103–117.

ISENBERG, J. I. & GROSSMAN, M. I. (1969). Effects of gastrin and SC 15396 on gastric motility in dogs. *Gastroenterology* **56**, 450–455.

JACOBY, H. I. & MARSHALL, C. H. (1969). Gastric motor-stimulating activity of gastrin tetrapeptide in dogs. *Gastroenterology* **56**, 80–87.

KAO, C. Y. (1966). Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. *Pharmac. Rev.* **18**, 997–1049.

KELLY, K. A. (1970). Effect of gastrin on gastric myo-electric activity. *Am. J. dig. Dis.* **15**, 399–405.

KURIYAMA, H. & TOMITA, T. (1970). The action potential in the smooth muscle of the guinea pig taenia coli and ureter studied by the double sucrose-gap method. *J. gen. Physiol.* **55**, 147–162.

KWONG, N. K., BROWN, B. H., WHITTAKER, G. E. & DUTHIE, H. L. (1972). Effects of Gastrin I, secretin and cholecystokinin-pancreozymin on the electrical activity, motor activity and acid output of the stomach in man. *Scand. J. Gastroenterol.* **7**, 161–170.

MISIEWICZ, J. J., HOLDSTOCK, D. J. & WALLER, S. L. (1967). Motor responses of the human alimentary tract to near-maximal infusions of pentagastrin. *Gut* **8**, 463–469.

REUTER, H. (1974). Localization of beta-adrenergic receptors and effects of noradrenaline and cyclic nucleotides on action potentials, ionic currents and tension in mammalian cardiac muscle. *J. Physiol.* **242**, 429–451.

SHUBA, M. (1974). Mechanism of action of catecholamines and histamine on smooth muscle cells of guinea-pig ureter. *J. Physiol.* **245**, 88–89*P*.

SPEEDEN, R. N. (1960). The effect of initial strip length on the noradrenaline-induced isometric contraction of arterial strips. *J. Physiol.* **154**, 15–25.

SUGAWARA, K., ISAZA, J., CURT, J. & WOODWARD, E. R. (1970). The effect of pentagastrin on gastric motility following vagotomy. *J. surg. Res.* **10**, 73–80.

SUGAWARA, K., ISAZA, J. & WOODWARD, E. R. (1969). Effect of gastrin on gastric motor activity. *Gastroenterology* **57**, 649–658.

SZURSZEWSKI, J. H. (1974*a*). Recording of electrical activity of smooth muscle by means of the sucrose gap. In *Fourth International Symposium on Gastrointestinal Motility*, ed. E. E. DANIEL, pp. 409–425. Vancouver, B.C.: Mitchell Press.

SZURSZEWSKI, J. H. (1974*b*). Effects of pentagastrin on transmembrane potential changes and phasic contractions of isolated longitudinal muscle of the canine antrum. *Gastroenterology* **66**, 867.

SZURSZEWSKI, J. H. (1974*c*). Effect of pentagastrin and acetylcholine on the electrical and mechanical activity of isolated longitudinal muscle of the canine antrum. In *Symposium of Physiology of Smooth Muscles* (Kiev), ed. BÜLBRING, E. New York: Raven Press (in the Press).

TRACY, H. J. & GREGORY, R. A. (1964). Physiological properties of a series of synthetic peptides structurally related to Gastrin I. *Nature, Lond.* **204**, 935–938.

VANHOUTTE, P. M. & LEUSEN, I. (1969). The reactivity of isolated venous preparations to electrical stimulation. *Pflügers Arch. ges. Physiol.* **306**, 341–353.

VIZI, S. E., BERTACCINI, G., IMPICCIATORE, M. & KNOLL, J. (1973). Evidence that acetylcholine released by gastrin and related polypeptides contributes to their effect on gastrointestinal motility. *Gastroenterology* **64**, 268–277.