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INTRACELLULAR ELECTRICAL ACTIVITY OF CANINE AND HUMAN GASTRIC SMOOTH MUSCLE

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SUMMARY

1. Intracellular recordings were obtained from circular smooth muscle fibres of the canine fundus, corpus, antrum and pylorus as well as from the human corpus and antrum.

2. In the canine stomach, all regions of the stomach except the fundus exhibited spontaneous action potentials.

3. The spontaneous action potential consisted of an upstroke potential and a plateau potential.

4. There were regional differences in the configuration of the plateau potential. Corporal and antral smooth muscle did not normally spike during the plateau potential whereas terminal antral and pyloric muscle usually showed spikes on top of the plateau potential. Near the intermediate sphincter, there was a zone of transition in which oscillations in potential of variable amplitude were superimposed on the plateau potential.

5. The configuration of the action potential of the human stomach was similar to the configuration of the canine action potential when the same region of the stomach was compared.

6. The ionic dependence of the plateau potential was studied in canine stomach in an area where neither oscillations nor spikes occurred.

7. In calcium-free solution, all spontaneous activity stopped. D600 selectively suppressed the size of the plateau potential.

8. Sodium-deficient solution reduced the size of the plateau potential.

9. These results suggest that both calcium and sodium may be involved as current carriers in the generation of the plateau potential.

INTRODUCTION

In 1922 Alvarez & Mahoney made the first reported extracellular recording of the spontaneous, rhythmic depolarization produced by canine gastric smooth muscle. In the 55 years following that report, most studies on the nature of this electrical activity have been restricted to recordings made with large extracellular electrodes in contact with the serosal surface of the stomach wall or by large suction electrodes introduced into the lumen of the stomach. The sole exception is a report of a few micro-electrode recordings made *in vivo* where impalements could be maintained for

only short periods of time (Daniel, 1965). Typically, the spontaneous rhythmic depolarization when recorded extracellularly originates in the orad corpus at a frequency of 5/min and is propagated to the pyloric ring (Kelly, Code & Elvebach, 1969; Weber & Kohatsu, 1970). Each rhythmic cycle consists of a triphasic oscillation of potential (positive, negative, positive) followed by a small negative deflexion 4–8 sec in duration. When the gastric musculature in the corpus and antrum contract, there is an increase in the size of the prolonged negative deflexion. This negative deflexion has been interpreted to result from fusion of individual spikes (Kelly, 1974). Occasionally, spikes or oscillations in potential are superimposed on the negative deflexion. As far as we can determine from all the published recordings, such activity is generally seen in the terminal 2–4 cm of the canine antrum.

Recently, *in vivo* extracellular recordings have been made from the human stomach at the time of surgery (Kelly, 1974; Hinder & Kelly, 1977). In the antrum, the triphasic oscillations in potential followed by a negative deflexion were observed. In the pyloric ring, the triphasic potential was followed by a burst of spikes.

Little is known regarding the intracellular potential changes in the canine stomach and nothing is known regarding the intracellular potential changes in the human stomach. Quantitative characterization of the intracellular electrical activity of these smooth muscles is a prerequisite for studies aimed at determining the electrophysiological basis of the action of gastric hormones and neurotransmitters thought to regulate gastric motility. Such characterization is also needed before gastric motor dysfunction can be understood. We are unaware of any detailed studies which have characterized the intracellular potentials in the circular muscle of the different functional regions of the stomach of either the dog or human. This lack of information may well be due to the technical difficulties involved in performing micro-electrode studies in contracting smooth muscle. Although this technique is difficult, the use of micro-electrodes to record from single smooth muscle cells *in vitro* has several advantages. First, this method allows greater quantitative accuracy than extracellular recording; secondly, a study at the single unit level simplifies interpretation of the data; and lastly, an *in vitro* study has the advantage of removing the hormonal influences and many of the neural influences which are present *in vivo*.

The aims of this study were threefold. First, to measure the intracellular electrical activity of the circular muscle of the fundus, corpus, antrum and pylorus. Secondly, to determine the differences and similarities in the intracellular potentials between canine and human gastric muscle. Thirdly, to investigate the role of calcium and sodium ions in the transmembrane ionic current underlying the electrical activity of the circular muscle layer of the antrum. Some of this work has been previously communicated (El-Sharkawy & Szurszewski, 1976; El-Sharkawy, Telander & Szurszewski, 1976).

METHODS

Tissue preparations – canine muscle

One hundred and fifteen dogs were used in this study. Dogs of either sex ranging in weight from 10 to 20 kg were anaesthetized with pentobarbitone (Fort Dodge Laboratories, Inc.). The abdomen was cut open and ligatures were placed around the oesophagus 2 cm above the oesophagogastric junction and around the duodenum 3 cm distal to the pylorus. After transecting the oesophagus above the ligature and the duodenum distal to the ligature, the stomach was cut free from its mesenteric attachments and placed in modified Krebs solution equilibrated with 97 %

O_2–3 % CO_2 mixture. Patches of the muscle coat (about 5 × 5 cm) from the fundus region, corpus and antrum of either the ventral or dorsal side were cut free from the underlying mucosa. The patches were pinned to a transparent rubber floor of a dissecting dish filled with Krebs solution. With the aid of a dissecting microscope, an area in each patch was sought in which the muscle bundles of the longitudinal layer ran parallel to each other. When located, 3–4 mm wide strips were cut along the long axis of the longitudinal muscle bundles. The strips were then turned sideways and most of the circular muscle layer was removed. Prepared in this manner, the strips consisted of the serosal layer, the longitudinal muscle layer, the myenteric plexus and the outer part of the circular muscle layer. A piece of each strip, 1 × 2 mm was cut and pinned onto the rubber floor of the recording chamber with the circular muscle layer facing upwards.

To study the pyloric region, a patch of muscle including the terminal antrum, pyloric junction and proximal duodenum was cut free from the mucosa, and pinned down in a dissecting dish. When mildly stretched, the pyloric junction could be determined as the segment most resistant to stretch and therefore was the narrowest region in the preparation. Transverse strips about 2–3 mm wide were cut starting in the terminal antrum, proceeding across the pyloric junction and continuing into the duodenum. The strip was turned sideways and most of the circular muscle removed. The strips, which were 5–7 mm long, were pinned down in the recording chamber as described above.

Tissue preparations – human muscle

Normal human muscle from the corpus, antrum and terminal antrum were used. Such preparations were obtained from patients undergoing surgery for gastric or duodenal ulceration or pyloric stenosis. The tissue used in this study was removed normally during the course of the prescribed surgical procedure, it did not entail excising any additional muscle, or causing additional disadvantage, inconvenience or risk to the patient. Use of such tissue was approved by the Institutional Human Studies Committee. The preparation of strips of human gastric muscle was identical to that described above with the exception that less tissue was available.

Electrophysiological recording set-up

The recording muscle chamber, 5 × 2·3 × 6 cm, was machined out of a 15 mm thick Perspex plate (deck) and filled to one half of its height with a transparent rubber cement. The deck was firmly attached to the top of a thermostatically controlled bath (15 × 6·5 × 5 cm) such that it formed its top wall. Krebs solution flowed by gravity from reservoirs on top of a Faraday cage containing the chamber, through a system of glass tubing in the heated bath, into the chamber and was sucked out by a vacuum system to minimize vibration which might dislodge a microelectrode from a cell. The flow rate was 2–8 ml./min and was constant in any one experiment. The temperature of the bath was adjusted so that the temperature of the Krebs solution near the muscle in the chamber was $37 \pm 0{\cdot}5$ °C.

The transmembrane potential and electrical activity were recorded with 3M-potassium chloride-filled glass micro-electrodes (40–80 MΩ resistance) rigidly mounted on a Perspex electrode holder attached to a micromanipulator. The use of a rigidly held micro-electrode allows recording from undamaged cells in the deeper layers of the circular muscle. A chlorided silver wire conducted the electrical signals to the probe of an electrometer (W-P Instruments, Hamden, Connecticut). The circuit was completed by connecting to the electrometer a chlorided silver wire reference electrode placed at one end of the chamber. The electrometer output was monitored on a storage oscilloscope (Tektronix 5113N) and recorded on an instrumentation tape recorder (Hewlett Packard, Model 3960). The tapes were replayed after the experiments on another oscilloscope (Tektronix 5112) and the desired portions analysed and photographed with a Nihon-Kohden camera. The criteria used for intracellular penetrations were essentially those listed by Kao & Nishiyama (1964).

Solutions and drugs

The standard solution used throughout this study was a modified 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; dextrose, 11·5; it was bubbled with a 97 % O_2–3 % CO_2 gas mixture before and during use in any part of an experiment and the pH was 7·3–7·4. For sodium-deficient solution, all of the sodium chloride

was replaced with an isosmotic equivalent of lithium chloride. Calcium-free solution was prepared by substituting calcium chloride with an isosmotic equivalent of sodium chloride. The drugs D600 5-[(3,4-dimethoxyphenyl) methyl amino]-2-(3,4,5-trimethoxyphenyl-2-isopropyl valeronitrile) and tetraethylammonium (TEA) chloride were dissolved in the Krebs solution.

Anatomy of the canine stomach

Anatomically and functionally, the stomach musculature can be divided into three regions: the fundus, corpus and antrum (Fig. 1). The fundus or cardiac portion is that portion of the stomach cephalad to the oesophagogastric junction. The corpus (or body) is the central portion of the stomach. On the lesser curvature of the stomach there is an angulation approximately

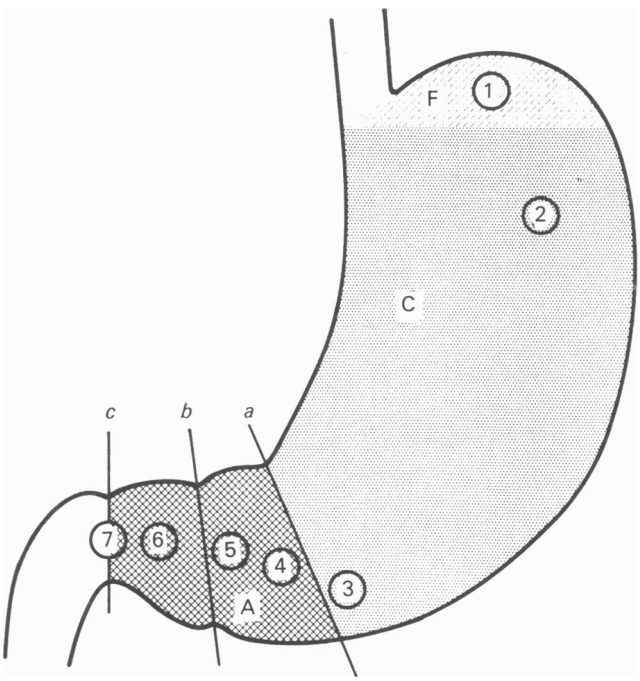

Fig. 1. Schematic diagram of the canine stomach. Areas numbered 1 to 7 indicate regions from which circular muscle was removed for intracellular recording. F, fundus; C, corpus; A, antrum. *a*, incisura angularis; *b*, intermediate sphincter; *c*, pylorus.

7–8 cm from the pylorus which is called the *incisura angularis*. To establish day-to-day boundary conditions, we drew a line parallel to the circular fibres from the *incisura* to a point opposite it on the greater curvature to establish the juncture between the corporal and antral muscle. Hence the antral musculature is defined in this study as the region bounded by this line and the pyloric ring. When the antral musculature is examined with a dissection microscope using transmitted light, a thickening of the circular muscle coat is seen approximately 2–3 cm proximal to the pyloric ring. This is the intermediate sphincter (Torgersen, 1942). The muscle immediately orad and caudad to the intermediate sphincter we will refer to, respectively, as mid-antrum and terminal antrum. The areas studied are numbered in Fig. 1. Fundus muscle was removed from area 1, corpus from area 2 (at a point near the greater curvature and orad to the caudad border of the corpus by 70% of the length of the corpus) and area 3 (near the greater curvature and adjacent to the caudad border of the corpus), antrum from area 4 (orad to the pylorus by 66% of the length of the antrum), and area 5, (orad to the pylorus by 50% of the length of the antrum), terminal antrum from area 6 (orad to the pylorus by 20% of the length of the antrum) and pyloric muscle from area 7.

Analysis of the gastric action potential complex

The following characteristics of the action potential were quantitated: the amplitude of the upstroke potential, A_u (mV); the maximum rate of rise of the upstroke, dV/dt (V/sec); the amplitude of the plateau potential, A_p (mV); and the half-time duration of the action potential complex, $D_{\frac{1}{2}}$ (sec) determined from a line drawn parallel to the resting membrane potential from the half-amplitude of the upstroke potential to the repolarizing phase of the plateau potential (Fig. 2). $D_{\frac{1}{2}}$ was chosen instead of the total duration because of the slow rate of repolarization and the occasional occurrence in some cells of a slower component following the plateau potential. In Fig. 2 and all Figures which follow, the top of the voltage calibration bar denotes zero potential.

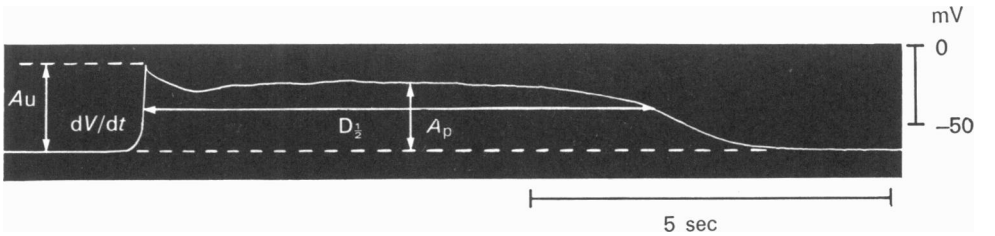

Fig. 2. Electrical characteristics of the intracellularly recorded gastric action potential (area 4, Fig. 1) as analysed in this report. A_u, amplitude of upstroke potential; dV/dt, its maximum rate of rise; A_p amplitude of plateau potential; $D_{\frac{1}{2}}$, half-time duration of action potential complex.

RESULTS

The values for each characteristic of the action potential in the circular muscle of each gastric region are shown in Table 1.

Electrophysiological recordings from the canine stomach

Fundus. The circular muscle cells of fundus muscle did not exhibit spontaneous electrical activity during the time periods through which impalements could be maintained (up to 42 min). These cells had a mean resting potential of $-57{\cdot}5 \pm 1{\cdot}4$ mV (S.E. of mean, $n = 40$).

Corpus. Recordings from the rest of the stomach showed spontaneous electrical activity. In area 2 the gastric action potential complex consisted of an upstroke potential followed by one to two oscillations which were followed by a plateau-type potential (Fig. 3*A*, *B*). In 89% of the preparations a diastolic depolarization preceded the upstroke potential. Area 3, unlike area 2, showed no small oscillations between the upstroke potential and the plateau potential (Fig. 3*C*, *D*), and a diastolic depolarization preceded the upstroke potential in only 8% of the impalements. The frequency, maximum rate of rise of the upstroke potential and amplitudes of the upstroke and plateau potentials were greater than those observed in area 2 of the corpus (Table 1).

Antrum. The spontaneously occurring action potential complex recorded from proximal antral circular muscle fibres (area 4, Fig. 1) was similar to that seen in area 3 of the corpus but there was seldom (1–2% of cells) a diastolic depolarization before the action potential. The potentials were recorded from the outer layer of circular muscle attached to the longitudinal muscle coat. Similar results were obtained from the inner half of the circular layer after removing the longitudinal coat, the myenteric

plexus and outer half of the circular muscle layer (three strips, three dogs, twenty-two impalements). Intracellular recordings were also made from longitudinal muscle fibres after dissecting away all of the circular muscle layer. Recording was difficult, probably because of the thinness of the preparation and the toughness of the surrounding connective tissue but the spontaneous action potentials had the same configuration and characteristics as those in circular muscle (five strips, five dogs, sixteen impalements).

TABLE 1. Comparison of resting membrane potential and characteristics of spontaneous action potential in circular muscle in different regions of the canine and human stomach. R.m.p., resting membrane potential; F, frequency. A_u, A_p, dV/dt and $D_{\frac{1}{2}}$ defined in Fig. 2. Results are expressed as mean ± S.E. of mean. Number of observations indicated in parentheses in left hand column. For canine and human stomach, n indicates number of impalements. Corporal antral and terminal antral muscle obtained from 1, 10 and 1 patients respectively

		Action potential complex				
Area of stomach	R.m.p. (mV)	F (c/min)	A_u (mV)	A_p (mV)	dV/dt (V/sec)	$D_{\frac{1}{2}}$ (sec)
		Canine stomach				
Fundus (n = 40)	57·5 ± 1·4	—	—	—	—	—
Corpus Area 2 (n = 32)	68·6 ± 0·5	3·7 ± 0·1	28·5 ± 1·5	20·8 ± 1·2	0·54 ± 0·04	5·9 ± 0·2
Area 3 (n = 11)	68·8 ± 1·0	3·5 ± 0·1	42·4 ± 0·51	38·7 ± 0·81	1·09 ± 0·1	6·9 ± 0·4
Antrum Area 4 (n = 57)	68·1 ± 0·7	1·4 ± 0·4	45·8 ± 0·7	34·2 ± 0·8	1·4 ± 0·05	7·5 ± 0·2
Area 5 (n = 7)	68·6 ± 0·9	1·4 ± 0·4	55·9 ± 1·8	42 ± 0·9	1·6 ± 0·9	10·4 ± 1·1
Terminal antrum (n = 7)	71·4 ± 1·4	0·66 ± 0·1	61·4 ± 2·9	44·7 ± 1·5	2·18 ± 0·1	11·3 ± 0·2
Pyloric ring (n = 6)	73·8 ± 1·9	0·15 ± 0·03	71·3 ± 1·7	31·2 ± 0·9	2·15 ± 0·1	—
		Human stomach				
Corpus (n = 3)	67·7 ± 1·4	6·17 ± 0·2	38·6 ± 2·2	31·3 ± 2·3	0·6 ± 0·1	3·6 ± 0·2
Antrum (n = 31)	70·5 ± 0·9	4·22 ± 0·2	36·8 ± 1·6	30·6 ± 1·1	0·60 ± 0·7	4·15 ± 0·1
Terminal antrum (n = 2)	67 ± 2·8	5·5 ± 0·2	26·4 ± 2·2	27 ± 1·4	0·14 ± 0·04	6·6 ± 0·1

Smooth plateaus recorded in the corpus and antrum could be converted into plateaus with spikes by adding TEA (5 mM) to the bathing solution (Fig. 4). These data indicate that spike potentials can either be induced or unmasked by TEA.

Fig. 5 illustrates the spontaneous action potentials recorded in the circular muscle of the distal antrum (area 5, Fig. 1) in normal Krebs solution. On average, this area

was only 2 cm distal to area 4. The oscillations on top of the plateau potential were a distinguishing feature. The amplitude of these oscillations ranged from 2 to 14 mV, and the largest occurred near the end of the plateau potential.

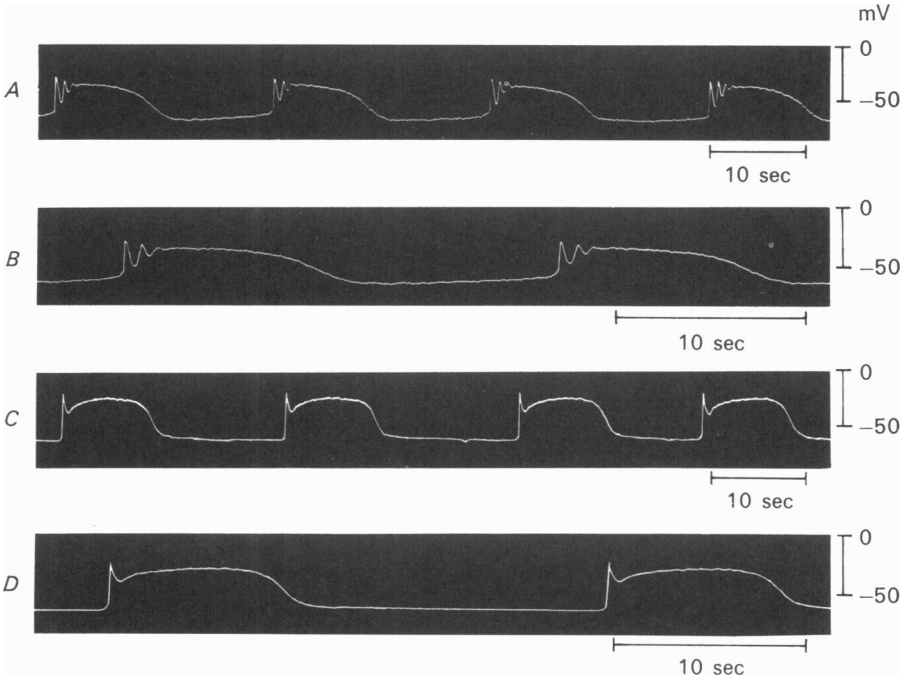

Fig. 3. Intracellular recordings from circular muscle of the corpus from area 2 (*A* and *B*), and from area 3 (*C* and *D*). Note prominent diastolic depolarization preceding each action potential in area 2.

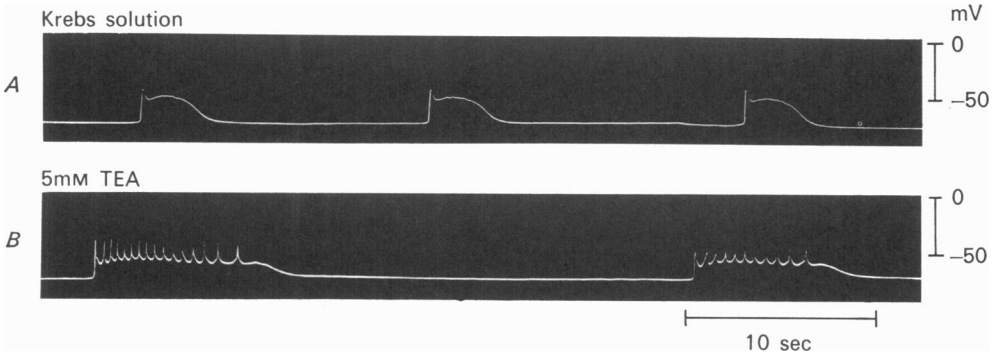

Fig. 4. Effects of 5 mM-TEA on the spontaneous action potential recorded intracellularly from area 4 (Fig. 1). *A*, Krebs solution; *B*, 15 min after adding TEA. Note burst of spikes on top of plateau and prolongation of action potential.

Terminal antrum. Since extracellular recording techniques record oscillations and spike-like potentials more frequently in the most distal part of the antrum, we studied the intracellular electrical activity of circular muscle at decreasing intervals between the intermediate sphincter and the pyloric ring. Fig. 6 illustrates a typical action potential recorded from the terminal antrum which in this dog was located

2·8 cm from the pyloric ring. Superimposed on the plateau potential were oscillations; each usually gave rise to a spike. Typically, an oscillation which occurred during the later half of the plateau potential led to a spike.

Pylorus. Spontaneous action potential complexes in circular pyloric ring muscle

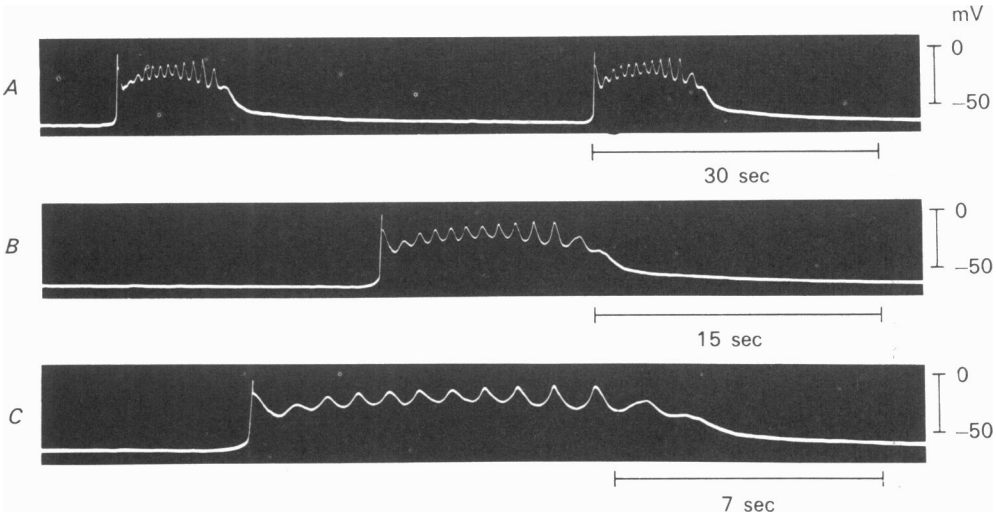

Fig. 5. Intracellular recording from circular muscle located in area 5 (Fig. 1). Recordings in this region characterized by occurrence of oscillations on top of the plateau potential.

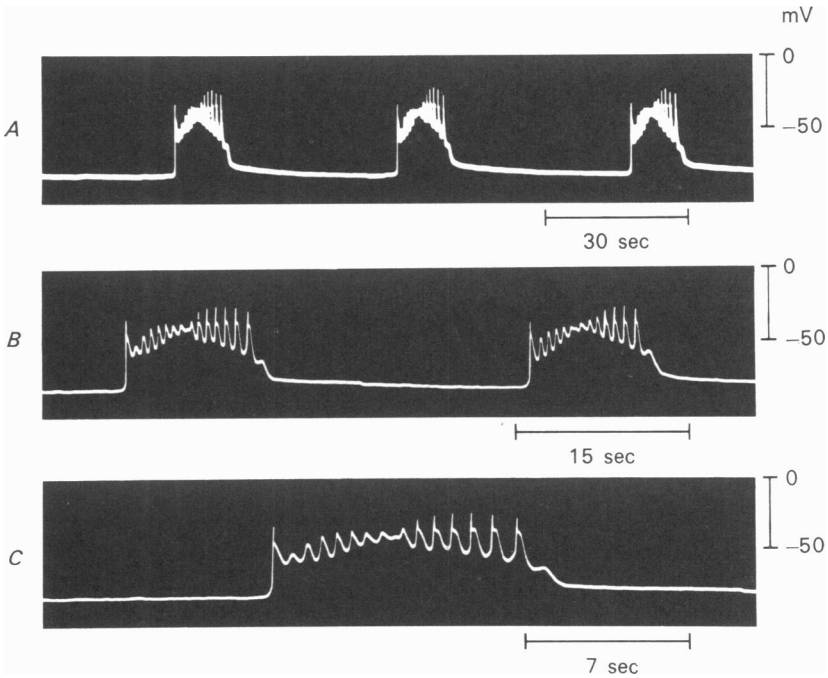

Fig. 6. Intracellular recordings from circular muscle of terminal antrum. Note throughout the plateau potential oscillations occurred which led to a spike during the later half of the plateau potential.

occurred infrequently, usually 1 every 6 min. The most characteristic feature was the superposition of spikes on a plateau potential of long duration (Fig. 7). The upstroke potential either was close to or exceeded zero potential and many of the spikes exceeded zero potential. The pyloric $D_{\frac{1}{2}}$ of the action potential is not given in Table 1 because the amplitude of the plateau potential was less than the half-amplitude of the upstroke potential. However, the total duration of the plateau potential ranged from 21 to 26 sec. Impalements of cells from this region were most difficult to maintain during the action potential because of the associated strong contractions. For this reason, the n values are small.

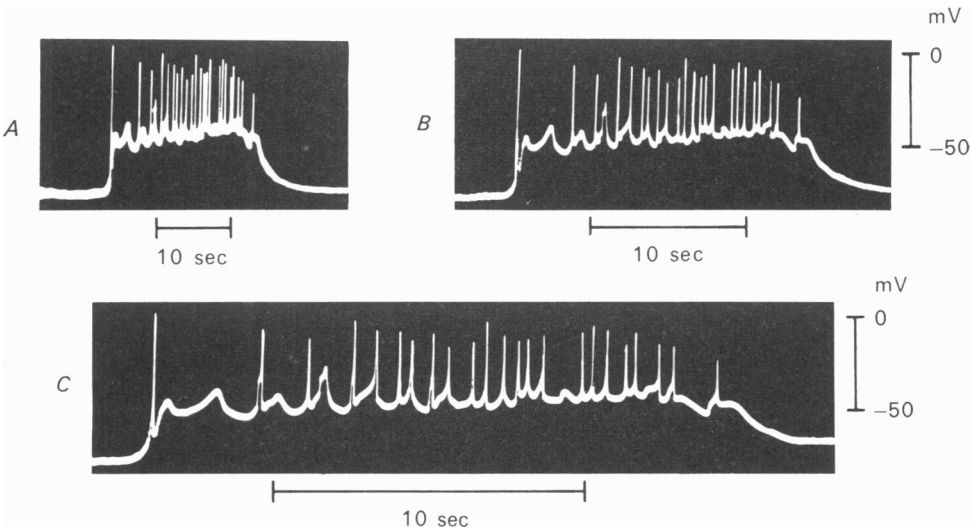

Fig. 7. Intracellular recordings from circular muscle located in pyloric ring. Upstroke potential and some spike potentials exceeded zero potential.

The above results indicate that circular muscle between the intermediate sphincter and pyloric ring had a greater tendency to produce spike potentials than did antral circular muscle. The closer the muscle was to the pyloric ring, the greater the incidence of spike activity. The plateau potential was generally smooth in the antrum but sometimes included oscillations of 2–14 mV amplitude immediately orad to the intermediate sphincter. Caudad to the intermediate sphincter these oscillations gave way to spikes. Occasionally the zone of transition from a smooth plateau potential to a plateau potential with spikes occurred much higher in the antrum. For example, in one dog the action potential complex of the circular muscle 6 cm orad to the pyloric ring had spike potentials. The action potential of the corpus of this dog had a smooth plateau potential. Thus, the exact location of the transition zone varied from dog to dog but was generally near the intermediate sphincter.

To ensure that the regional differences described above were not due to experimental variation, on two occasions we removed strips of muscle from areas 1, 2, 4, 5 and 7 (Fig. 1) from the same dog, placed each strip in the same recording chamber and made intracellular recordings from circular muscle fibres in each strip using the same micro-electrode. We found the same regional differences described above for strips removed from different dogs.

Electrophysiological recordings from the human stomach

To date, we have recorded from normal human gastric muscle obtained from twelve patients. All muscle was judged to be normal based on histologic examination using haematoxylin–eosin and Masson's Trichrome staining techniques.

Corporal and antral muscle. Three recordings were obtained from one strip of corporal muscle. The rhythmically occurring action potential complex was similar to that in the same region of the canine stomach (area 3). The action potential in human antral circular muscle (Fig. 8*A*) from the region equivalent to area 4 (Fig. 1) of the dog stomach, shows an upstroke potential and a plateau potential.

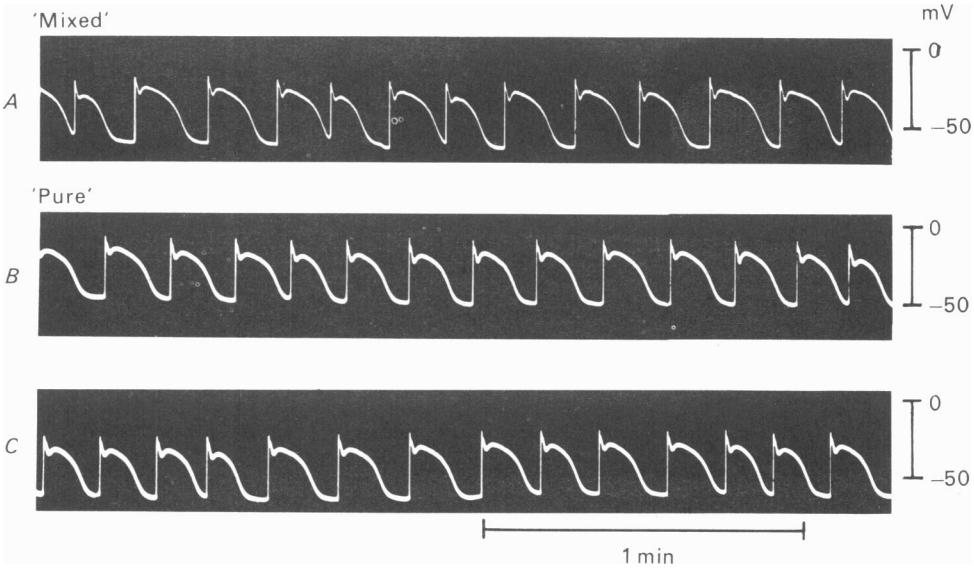

Fig. 8. Intracellular recordings from normal human antral circular muscle. *A*, recording from an outer circular muscle fibre with longitudinal muscle layer attached. *B* and *C*, different patients, recordings from an inner circular muscle fibre after removing longitudinal and outer circular muscle.

In three experiments, we recorded from the inner half of antral circular muscle dissected free from the longitudinal muscle layer, the myenteric plexus and the outer half of the circular muscle coat. Action potentials were recorded in every cell impaled, with a frequency and configuration the same as those recorded from circular muscle attached to the longitudinal muscle (Fig. 8*B*, *C*).

A surprising observation in human antral muscle was the rapid frequency of spontaneous action potentials. The frequency is 3/min *in vivo*, but in our *in vitro* experiments, it was 4·2/min (Table 1). Since the potassium concentration in our modified Krebs solution is higher than that in human plasma, we lowered the potassium concentration to 4·0 mM, a normal concentration found in human plasma. This had no effect on the frequency or shape of the action potentials.

Terminal antrum. As in the canine antrum, oscillations and spike potentials occurred on the plateau of the action potential complex recorded caudad to the intermediate sphincter (Fig. 9).

Effect of atropine and tetrodotoxin. Although the effects of atropine (10^{-6} g/ml.) and tetrodotoxin (10^{-6} g/ml.) on the gastric action potential were not systematically studied, the effects of both agents were studied on the action potential on at least three separate occasions in each of areas 2 to 6 (Fig. 1) of the canine stomach. Neither agent alone or in combination altered any component of the action potential. These drugs were also tested on human antral muscle ($n = 3$) and were again without effect. These results indicate that the gastric action potential complex in canine and human stomach is totally myogenic in origin and not dependent on neurogenic influences.

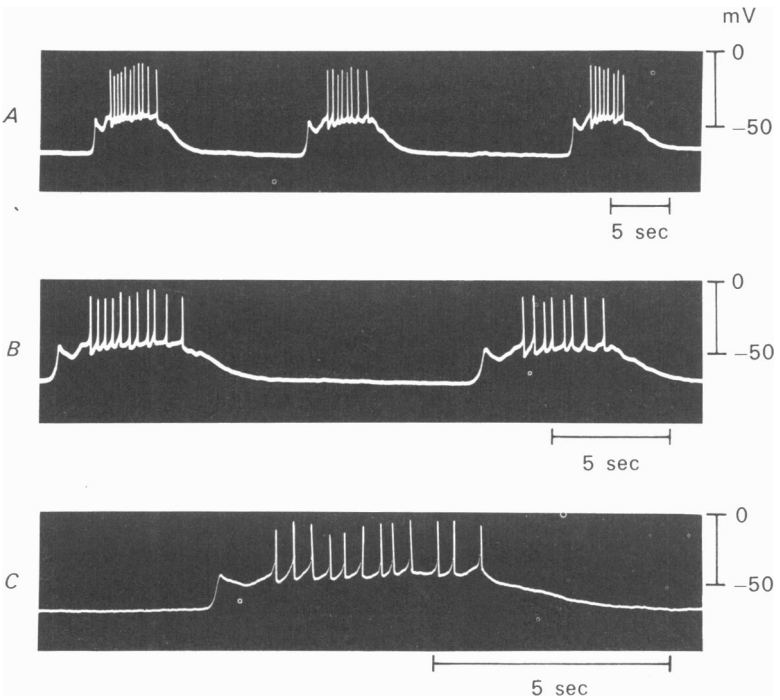

Fig. 9. Intracellular recording from human circular muscle fibre in the terminal antrum. Spike potentials on top of the plateau potential approach zero potential.

Resumé

In addition to regional differences in the configuration of the canine action potential complex, the data in Table 1 show several trends. First, there was a gradient in the resting membrane potential, increasing from a mean of 57 mV in the fundus to approximately 74 mV in the pyloric ring muscle. Secondly, there was a gradient in the frequency of spontaneous action potential decreasing from 3·7/min in the orad corpus, to 0·15/min in the pyloric ring. Associated with the decrease in frequency was an increased duration. Thirdly, the rate of rise of the upstroke potential and the amplitudes of the upstroke and plateau potentials increased from corpus to the terminal antrum. In human stomach, the configurations of the action potentials resembled those in similar regions of the dog stomach. At the present time, there are too few data to determine if regional differences exist in the electrical characteristics of the human gastric action potential.

Ionic dependence of the canine antral action potential

It has previously been shown using the double sucrose gap that calcium is important for the genesis of the action potential complex in the longitudinal muscle of the canine antrum (area 4) (Szurszewski, 1975). Calcium-deficient solution reduces the amplitude and duration of the plateau potential whereas calcium-rich solution has

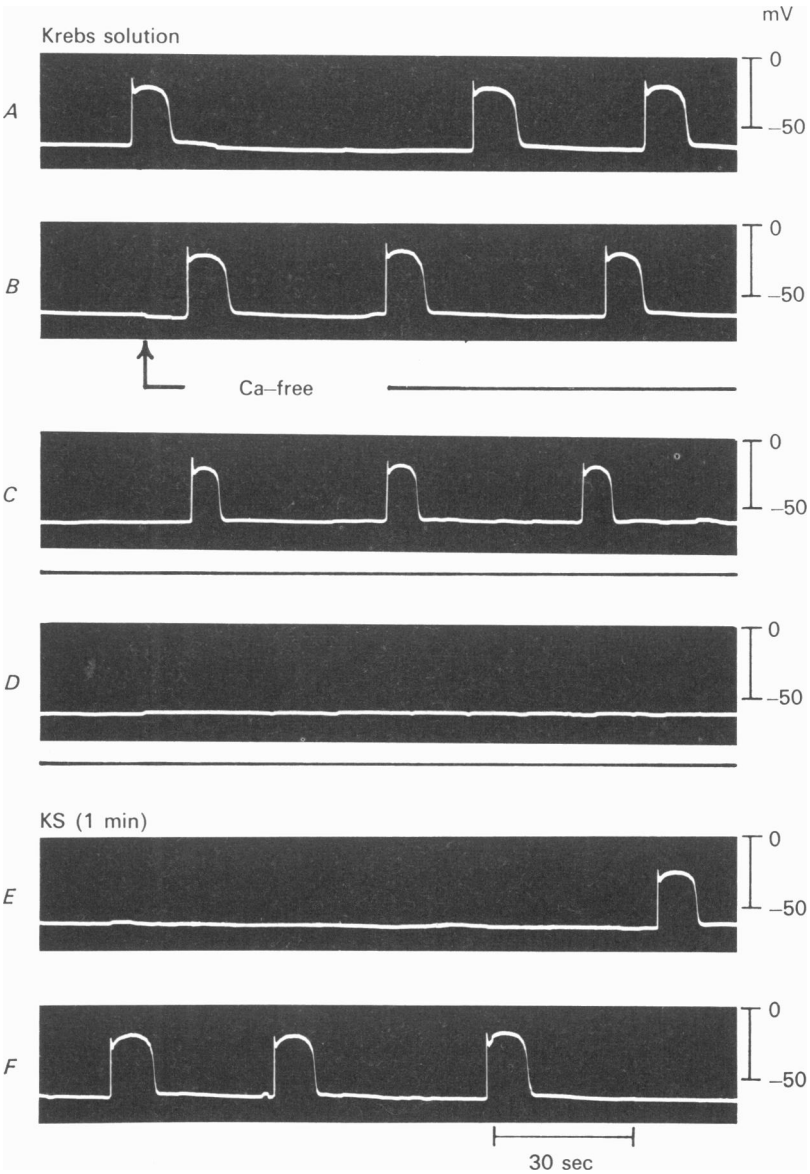

Fig. 10. Effects of calcium-free solution on spontaneous action potential in canine stomach recorded from area 4 (Fig. 1). *A*, Krebs solution; *B*, calcium-free solution introduced at beginning of trace and maintained throughout *C* and *D*. At beginning of *E*, normal Krebs solution re-admitted and maintained to end of *F*. Recordings continuous from *A* to *E*.

the opposite effect. We here report studies on the circular muscle of the proximal antrum (area 4, Fig. 1) using alteration of the calcium concentration and the calcium blocker D600.

Effect of calcium-free solution. Experiments were done in six strips from six dogs. Replacement of the calcium chloride with sodium chloride initially reduced the amplitude and duration of the action potential plateau with no significant effect on

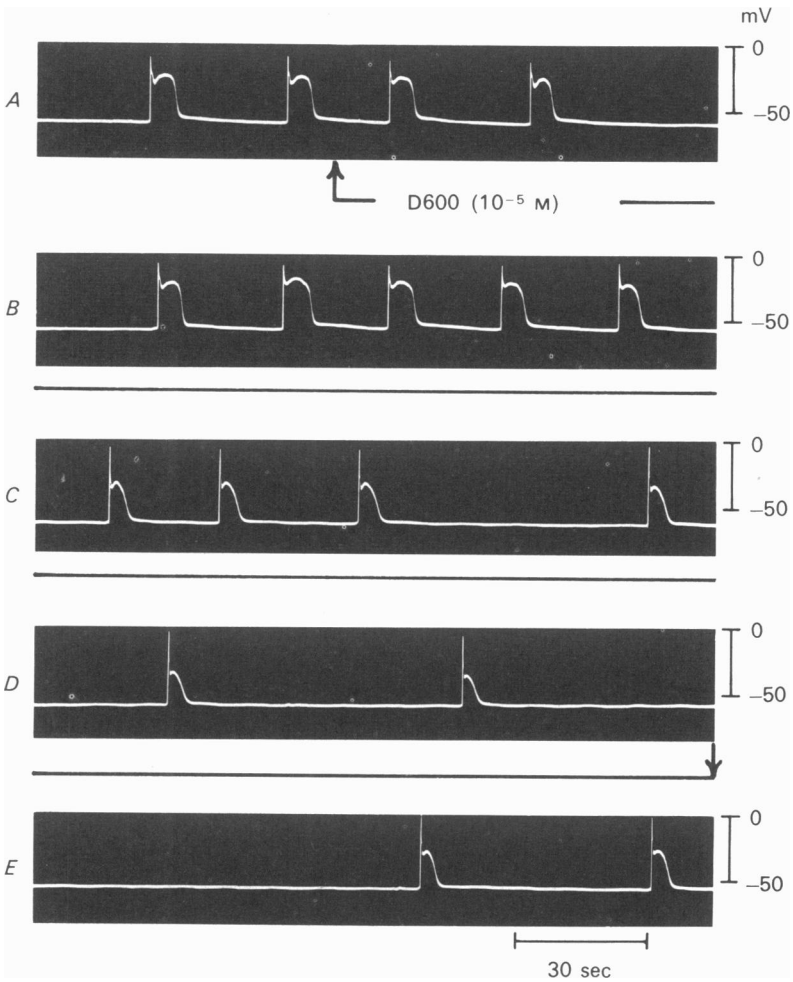

Fig. 11. Effects of D600 (10^{-5} M), on the spontaneous action potential recorded from canine antral circular muscle in area 4 (Fig. 1). D600 introduced at arrow in *A* and withdrawn at arrow in *D*. All traces continuous. Note selective suppression of plateau potential.

the upstroke potential (Fig. 10*B*). In some cells these effects were accompanied by a slight decrease in the resting membrane potential. After 3 min in calcium-free medium the upstroke potential became smaller and after 5 min the spontaneous activity completely ceased (Fig. 10*D*). After re-admission of calcium the activity re-appeared (Fig. 10*E*).

Effect of D600. This compound causes a relatively selective blockade of transmembrane calcium movements in both cardiac (Tritthart, Volkmann, Weiss & Fleckenstein, 1973) and smooth muscle (Fleckenstein, Griin, Tritthart & Byon, 1971; Golenhofen & Lammel, 1972; Mayer, van Breemen & Casteels, 1972). Fig. 11 shows the effect of compound D600 on the antral action potential. In five of five cells, resting membrane potential was unaffected but, D600 significantly depressed the amplitude

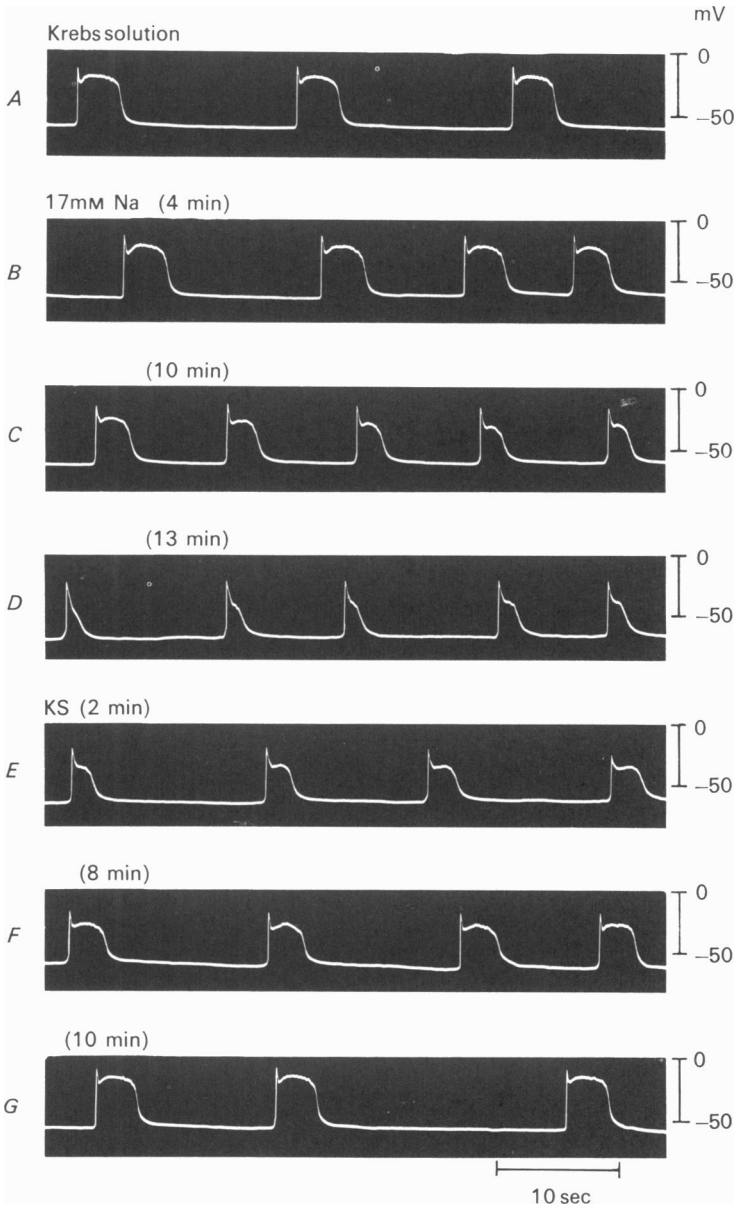

Fig. 12. Effect of sodium-deficient solution (17 mM-sodium, lithium substitution) on spontaneous action potential recorded from area 4 (Fig. 1). *A*, Krebs solution; *B*, *C* and *D*, 4, 10 and 13 min respectively after change to sodium-deficient solution; *E*, *F* and *G*, 2, 8 and 10 min respectively after returning to normal Krebs solution.

and duration of the action potential plateau (Fig. 11*C*, *D*). Whereas the upstroke in four out of five cells was unaffected by D600, it was augmented in one. These effects were not reversed after at least 40 min of washing out the calcium antagonist (Fig. 11*E*).

Effect of sodium-deficient (lithium-substituted) Krebs solution. Substitution of lithium chloride for the sodium chloride, leaving approximately 17 mM-sodium in the buffers, depolarized the smooth muscle cell membrane by 6–8 mV and slightly enhanced the action potential frequency (Fig. 12*C*). The upstroke potential was unaffected during the 20 min period of exposure, but the amplitude and duration of the plateau potential were reduced (Fig. 12*D*). These effects were readily reversed after replacing the normal Krebs solution (Fig. 12*E–G*).

DISCUSSION

This work gives the first quantitative description of the intracellular electrical activity of isolated circular smooth muscle and the way it varies in different regions of the canine and human stomach. Canine fundus circular muscle exhibited no spontaneous, myogenic electrical activity; but, in contrast, the region from the orad corpus to the pyloric ring did show spontaneous activity. Previous studies using extracellular electrodes *in vivo* have pointed out such differences (Kelly *et al.* 1969; Weber & Kohatsu, 1970; Kelly & Code, 1971). The intact canine corpus and antrum *in vivo* show spontaneous electrical activity of approximately 5 c/min. The *in vitro* frequencies we recorded depended upon the location from which the strips were removed and corresponded well with the intrinsic frequencies recorded *in vivo* from the transected stomach (Sugawara, 1964; Kelly & Code, 1971). In such experiments, the frequency proximal to the transection was the same as before the transection (5/min) whereas distally the frequency fell.

The data indicate that autorhythmicity is a property of gastric smooth muscle from the corpus to the pyloric ring muscle and is not restricted to the 'pace-maker region' thought to reside somewhere in the corpus (Kelly & Code, 1971; Weber & Kohatsu, 1970). However, as seen in the Table, there is a decrease in the intrinsic frequencies from the corpus to the pyloric ring. It is clear from other studies (Kelly & Code, 1971) that although circular muscle distal to the corpus has an endogenous frequency of about 1 c/min, this oscillatory frequency can be driven up to 5 c/min by the pace-maker located in the orad corpus.

Papasova, Nagai & Prosser (1968) reported that the circular muscle layer of the cat small intestine is not spontaneously active and that the slow-wave (action potential) recorded in the circular layer is propagated from the longitudinal layer. Isolated circular and isolated longitudinal antral muscle are both spontaneously active in the dog, as is isolated human circular antral muscle.

When peristaltic contractions occur *in vivo*, they begin in the corpus and propagate to the pyloric antrum in phase with the extracellularly recorded action potential (Carlson, Code & Nelson, 1966; Kelly *et al.* 1969). The triphasic component of the extracellularly recorded action potential in the corpus and antrum is followed by a slow negative dip in the electrical tracing, whereas in the terminal 2–3 cm of the antrum small oscillations or spikes are recorded (Daniel & Irwin, 1968). From these

regional differences, in the presence and absence of spikes, the concept was developed that peristaltic contractions in the electrically active regions of the stomach are initiated by spike bursts throughout the stomach but for some reason in the corpus and antrum the spike discharge becomes fused under the experimental recording conditions (Kelly, 1974). Our data, however support the concept that a gradient of spike activity exists in the stomach circular muscle. The greatest incidence of spike activity occurred in the terminal antrum and pyloric ring muscle, and the least in the corpus and antrum. In between is a transition zone, usually 3–4 cm from the pyloric ring, with oscillations of variable amplitude superimposed on the plateau potential. This transition zone occurred within a limited segment of the stomach and it is probable that its location is variable between dogs. We did not record such a zone of transition in human stomach probably because of insufficient tissue; one probably exists since circular muscle from the proximal antrum exhibited a smooth plateau whereas circular muscle from the terminal antrum exhibited spikes.

Spikes did not normally occur in canine corporal and antral muscle, but could be caused by TEA. This suggests that there are regional differences in the control of spike activity.

The dependence of the contractile events in antral smooth muscle cells (area 4, Fig. 1) on the size of the plateau potential (Szurszewski, 1975) raises the question of ionic fluxes underlying this component of the gastric action potential. The contractile machinery in smooth muscle is probably activated by an increase in intracellular free calcium, either released from intracellular stores and/or moving inward across the cell membrane. The inhibition of the plateau potential in a calcium-free solution or in the presence of the calcium transport-blocking agent D600 suggests that calcium is a major component of the plateau potential (but not of the upstroke potential which is not inhibited). Both conditions inhibit contractions of canine antral smooth muscle (unpublished observations). The incomplete suppression of the plateau component may be due to an incomplete effect or because another ionic current may be involved; the effects of lithium-substituted Krebs solution indicate that this might be sodium. The action potential complex disappeared in calcium-free solution which might be attributable to the non-specific effects of a calcium-free solution or might indicate that calcium is important for regulating the ionic current of another channel which causes the generation of the upstroke potential.

The intracellular electrical events in human and canine antral and terminal antral smooth muscle are similar so that canine gastric muscle can be used as a model for the human stomach. The concepts developed for the canine model may help explain motor dysfunctions in human gastric disease as we have recently reported (Telander, Kelly, Morgan, Kreulen, Schmalz & Szurszewski, 1977).

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