J. Physiol. (1975), **251**, *pp.* 317–331
With 10 *text-figures*
Printed in Great Britain

SOME DIFFERENCES IN CONTRACTILE RESPONSES OF ISOLATED LONGITUDINAL AND CIRCULAR MUSCLE FROM THE GUINEA-PIG STOMACH

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(*Received 2 December 1974*)

SUMMARY

The mechanical and electrical properties of the longitudinal (fundus and corpus) and circular (antrum) muscle fibres of the guinea-pig stomach were investigated.

1. In the longitudinal but not in the circular muscle isotonic K Krebs and Na-free (sucrose) Krebs solutions produced a contracture with a tonic component. The different mechanical responses were not accompanied by different membrane responses. Verapamil abolished both phasic and tonic components of K-induced contracture.

2. During the tonic response of the K-induced contracture, repolarization of the membrane by current pulses relaxed the tissue; after cessation of the current pulse, rebound contracture occurred. In the circular muscle, the Q_{10}^{*} value for the rate of relaxation induced by inward current pulse was 3·1 and for the development of rebound contracture was 2·4.

3. After the tissue had been immersed in Ca-free isotonic K Krebs solution, application of Ca produced a large contracture in the longitudinal muscle, but contracture in the circular muscle was small or absent. However, the amplitude of subsequent carbachol-induced contracture in the above solution was enlarged in proportion to the durations of Ca treatment in both tissues.

4. Direct tetanic electrical stimulation could produce tension in both tissues. With low frequency of stimulation (0·1 Hz) a positive staircase was observed in the circular but not in the longitudinal muscle.

5. It is concluded from these results that topical differences of the motility in the stomach may be due not only to the activity of nervous elements, but also to differences in the properties of the muscle fibres themselves.

INTRODUCTION

Investigations of the mechanism of excitation–contraction coupling in visceral smooth muscles are complex because not only are there organ and species differences, but even the longitudinal and circular muscles within one organ show marked differences in electrical and mechanical responses. For example, in guinea-pig stomach muscle, the antrum region generates slow potential changes with superimposed spikes, but such potential changes are not observed in the fundus and cardia regions (Osa & Kuriyama, 1970). It was also reported in the cat intestine that longitudinal but not circular muscle showed spontaneous electrical activity (Prosser, 1974).

The different electrical and mechanical responses of the various regions of the guinea-pig alimentary canal (namely stomach, ileum, jejunum, caecum, and rectum) to caffeine, a potent Ca releaser from the sarcoplasmic reticulum in the striated muscle, are thought to be caused mainly by different requirements for Ca which in turn reflect differences in stores (Ito, Osa & Kuriyama, 1973).

It appears that all visceral smooth muscles require Ca ion for contraction and it can be assumed that the amplitude of the contraction depends on the amount of free Ca in the cells as in striated muscle (Ebashi & Endo, 1968; Somlyo & Somlyo, 1968; Bülbring, Brading, Jones & Tomita, 1971; Bülbring & Needham, 1973).

The present experiments are intended to investigate further the mechanical properties of the longitudinal and circular muscles of the stomach, and the results obtained are discussed in relation to the roles of Ca ion in the generation of different responses in both tissues.

METHODS

Guinea-pigs, weighing 250–300 g, were stunned and bled. The stomach was excised and dissected starting along the greater curvature. The muscle layers were separated from the mucous layer. Longitudinal muscle was prepared from the fundus and upper region of the corpus along the greater curvature, and circular muscle from the pyloric region (antral region).

To measure the mechanical activity induced by electrical stimulation, preparations 10–12 mm in length and 1–2 mm in width were put into a chamber of small capacity (0·5 c.c.). To measure the spontaneous mechanical activity, preparations 15 mm in length and 2–3 mm in width of longitudinal muscle and circular muscle were perfused together in one chamber (2·5 c.c. in capacity).

The micro-electrode method was used to record the electrical activity alone (Osa & Kuriyama, 1970), and the double sucrose-gap method was used to measure the electrical and mechanical activities (Kuriyama & Tomita, 1970). For current clamp in the double sucrose-gap experiments, a strip 20 mm in length and 0·3 mm in width was dissected. The space between the two isosmolar sucrose solutions was kept at

0·2 mm by controlling the perfusion velocity. The apparatus for the voltage-clamp method was almost the same as that described by Anderson (1969). For voltage clamp experiments, the preparation was dissected to 0·15–0·2 mm in diameter and 10–15 mm in length, and was placed in the double sucrose-gap apparatus with an artificial node width of 0·1–0·15 mm for measurements of voltage and current.

A modified Krebs solution (hereafter referred to as Krebs) of the following composition was used (mM): Na^+, 137·4; K^+, 5·9; Mg^{2+}, 1·2; Ca^{2+}, 2·5; Cl^-, 134·0; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2 and glucose, 11·5; equilibrated with 97% O_2–3% CO_2. Isotonic K Krebs solution was prepared by replacing NaCl and $NaHCO_3$ with isosmolar KCl and $KHCO_3$ respectively and Na-free Krebs solution by replacing NaCl and $NaHCO_3$ with isosmolar sucrose and 5·6 mM $KHCO_3$, with KCl omitted. The pH of the solution was adjusted by Tris to 7·2.

All experiments, except as quoted in the text, were carried out at $35 \pm 0{\cdot}2°$ C. The drugs used in the experiments were carbachol (Ishizu Pharmaceutical Co. Ltd), verapamil (Knoll A. G.) and caffeine (Ishizu Pharmaceutical Co. Ltd) and the concentrations are expressed in g/ml.

RESULTS

Some factors controlling the development of the mechanical response

The mean membrane potential, with S.D. of observation, recorded from the longitudinal muscle fibres was $-59{\cdot}5 \pm 2{\cdot}8$ mV ($n = 30$) and from the circular muscle fibres $-60{\cdot}5 \pm 2{\cdot}1$ mV ($n = 30$).

With the micro-electrode method, the threshold for contraction induced by excess $[K]_o$ in the longitudinal muscle was -42 ± 3 mV ($n = 20$) and in the circular muscle -46 ± 4 mV ($n = 20$).

Fig. 1 shows the effects of 2 sec potential steps on tension in the longitudinal and circular muscle of the stomach obtained with the voltage-clamp method. Depolarization of more than 20 mV produced contraction without generation of a transient inward current in longitudinal muscle (*a*) but inward current was generated in the circular muscle (*b*). When inward current was generated by a step depolarization, the amplitude of contraction and the rate of upstroke of contraction were greater (*b*). The general features of the relationship between the size of the step depolarization and contraction are the same as those described for the rat myometrium by Mironneau (1973).

In circular muscle, isotonic K Krebs solution evoked a contracture which was composed of phasic and tonic responses. The former developed rapidly then declined to a level close to the resting tension level. The residual tension represents a persisting tonic response. On the other hand, in the longitudinal muscle, the tonic response developed gradually and its amplitude often exceeded the peak tension of the initial phasic response (Fig. 2*a*). When the circular muscle was perfused with Na-free solution, spontaneous contractions were transiently enhanced (for several min), before a slight contracture after which the muscle relaxed to the resting

tension level. However, in the longitudinal muscle, a large contracture was evoked and the amplitude of this contracture often exceeded the peak tension of the K-induced contracture (Fig. 2*b*). The minimum Na ion concentration to suppress the generation of contracture was 54 mM, i.e. two-thirds of the normal Na concentration.

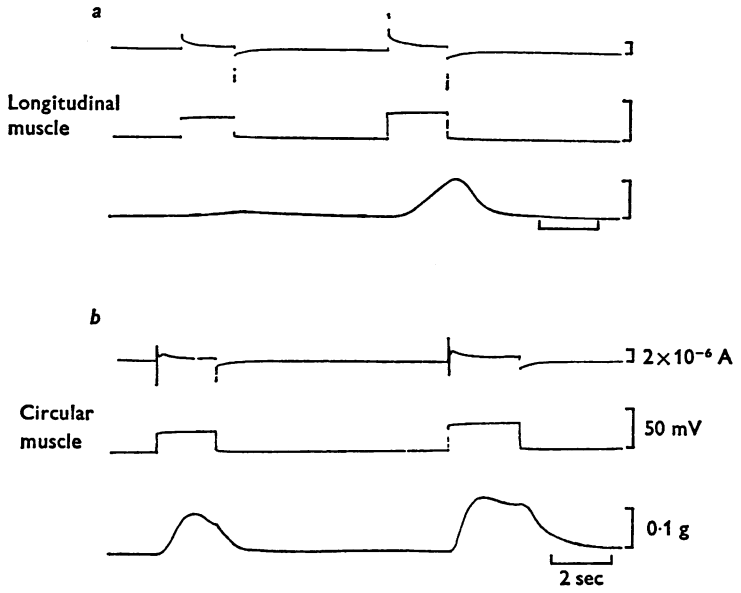

Fig. 1. The effects of step depolarizations on the membrane currents and tension recorded from longitudinal (*a*) and circular (*b*) muscle preparations of guinea-pig stomach. Depolarizations were applied with the double sucrose-gap voltage-clamp technique. Top trace, current recording; middle, voltage recording; bottom, tension recording. A longitudinal muscle strip of fundus and a circular muscle strip of pylorus were used.

To find out whether the different mechanical responses of the two layers were accompanied by different membrane responses, the effects of the above solutions on the membrane potential were observed. As shown in Fig. 3, no detectable difference in the responses of the membrane potential to either isotonic K or Na-free Krebs solutions were observed in the two muscle tissues. Depolarization induced in the latter was small.

When the electrical activity of the circular muscle in Na-free Krebs solution was observed, the membrane was transiently depolarized within a few minutes and slow potential changes ceased (Fig. 4*a*). However, within 10 min slow potential changes of small amplitude reappeared (*b*). although after 20 min the slow potential changes had again ceased (*c*), When the tissue was washed with Krebs solution, the membrane hyper-

polarized and slow potential changes of larger amplitude reappeared (*c* and *d*).

Some factors controlling the relaxation of muscle tone

As shown in Fig. 5, the contracture induced by excess K Krebs solution could be relaxed by repolarization of the membrane by strong inward current. The experiments were carried out by the current-clamp method. Isotonic K Krebs solution depolarized the membrane to 8–5 mV in both muscle tissues. When strong intensity current pulses were applied, the

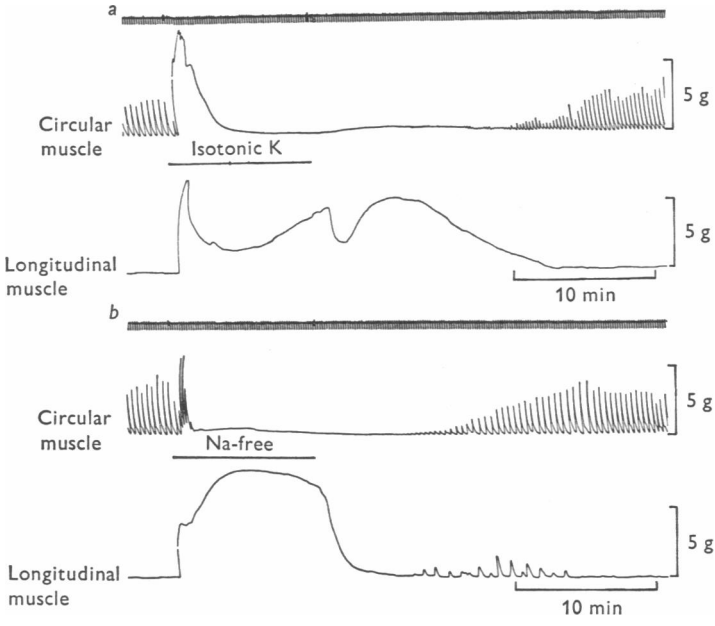

Fig. 2. The effects of isotonic K (*a*) and Na-free (sucrose) Krebs solutions (*b*) on the tension developed by the circular and longitudinal muscle of guinea-pig stomach. Bars indicate application of the test solutions. Uppermost traces in each set of records are time marks (10 sec).

depolarized membrane was repolarized, and the K-induced contracture was relaxed. Cessation of the current pulse produed a mechanical response (rebound contracture) which exceeded the level before application of the inward current. The amplitude of the relaxation and of the rebound contracture was roughly in proportion to the applied current intensity. In the circular muscle the rebound contraction rapidly declined to a level close to the level of the tonic response (*a*) but in the longitudinal muscle it gradually relaxed and even after 2 min the tension had not reached the level before application of the current (*b*). Therefore successive application

of inward current pulses produced summation of the tonic response more easily in the longitudinal muscle than in the circular muscle (*b*).

The relationship between the relaxation and rebound contraction induced by various intensities and durations of the inward current pulse during the generation of the K-induced contracture was examined (Fig. 6). The membrane potential in circular muscle preparations measured by the double sucrose-gap method was −43 mV, and in 118 mM-K Krebs solution it was

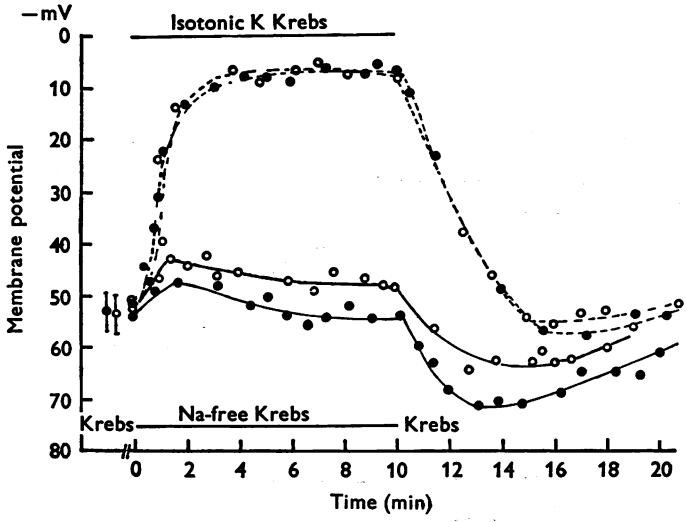

Fig. 3. The effects of isotonic K and Na-free (sucrose) Krebs solutions on the membrane potential measured from the circular and longitudinal muscle tissue with the micro-electrode method. Ordinate, membrane potential (−mV); abscissa, the time after exposure to the test solution (min). The mean ± 2 S.D. of an observation in normal Krebs solution are shown. O—O, Na-free longitudinal muscle; ●—●, Na-free circular muscle; O- - -O, isotonic K longitudinal muscle; ●- - -●, isotonic K circular muscle.

depolarized to −5 mV. During generation of the tonic responses, the membrane was repolarized by inward current pulses to −32, −52 and −68 mV respectively, and the amplitudes of the relaxation and the rebound contraction were measured. The minimum duration to produce rebound contraction was 0·2 sec with repolarization of the membrane to −68 mV. Weaker intensities of current required longer duration to generate relaxation and rebound contraction. The degree of relaxation induced by application of inward current pulses varied not only with the current intensity but also with the amplitude of the original tonic response, so that when inward current pulses were applied, the relaxation was larger in the longitudinal muscle than in the circular muscle.

When the effects of temperature were measured, both the latency of the relaxation and the rate of relaxation were more sensitive to changes in temperature than were the corresponding parameters of the rebound contracture. Fig. 7 shows the effects of temperature on the mechanical response induced by inward current pulses during the K-induced contracture in circular muscle. Below 25° C inward current pulse did not relax the tonic response even when the membrane was repolarized to the control

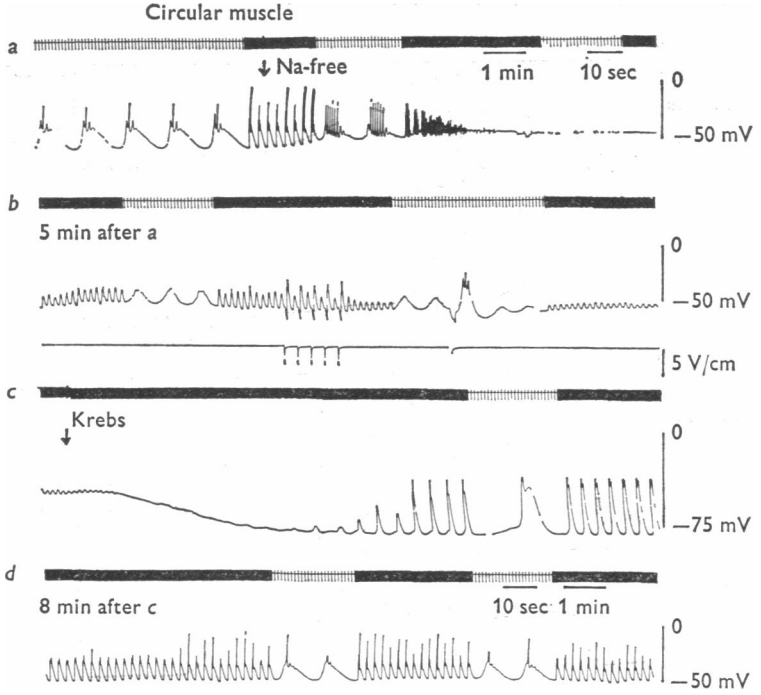

Fig. 4. Effects of Na-free (sucrose) Krebs solution on the membrane potential of the guinea-pig circular muscle studied with the micro-electrode method. The upper trace is a time marker and indicates the two different speeds of the continuous records. Application and removal of Na-free solution are indicated by arrows. Downward deflexions in *b* indicate the application of inward current pulses (2 sec).

value, but rebound contracture still occurred. The Q_{10} value for the rate of upstroke of the rebound contracture was 2·4 ($n = 5$) and for the rate of the relaxation was 3·1 ($n = 5$). These results might indicate that reabsorption of Ca ion from the myoplasm requires more energy than the release of Ca ion into the myoplasm.

It has been reported by Magaribuchi, Ito & Kuriyama (1973) that rapid cooling of the visceral muscle to below 15° C produces contracture. This

phenomena was confirmed in the present experiments. As shown in Fig. 8, when the temperature of Krebs solution was lowered to 14° C, a small tonic contracture was evoked in both muscle tissues (*a*). However, when the temperature was lowered at 13° C during treatment with Na-free Krebs solution, contracture occurred in the circular muscle but in the longitudinal muscle the contracture was relaxed (*b*). A similar effect was observed in isotonic K solution (*c*), i.e. lowering the temperature caused contracture in the circular muscle and relaxation in the longitudinal muscle.

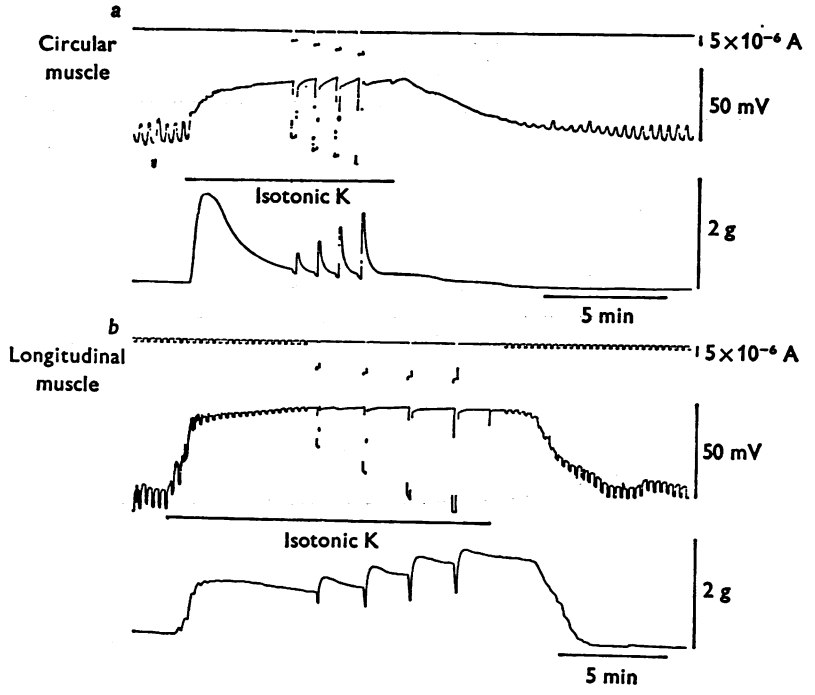

Fig. 5. Effects of repolarization of the membrane during K-induced contracture. The current-clamp method was used. Top trace, current monitor; middle, membrane potential; bottom, mechanical response.

On treatment with verapamil (10^{-5} g/ml.), the phasic and tonic responses of the K-induced contracture were suppressed in both muscle tissues.

Release of bound Ca in the muscle fibres by carbachol

Carbachol produces a further contracture during the tonic phase of a K-induced contracture (Evans, Schild & Thesleff, 1958; Durbin & Jenkinson, 1961). If the carbachol-induced contracture in Ca-free isotonic K Krebs solution is due to release of sequestered Ca in the muscle fibre, the amplitude of the contracture should indicate the amount of the sequestered Ca.

In either isotonic K or Na-free Krebs solutions, carbachol (10^{-5} g/ml.) produced contracture. However, in tissues pre-treated with Ca-free Mg (2·5 mM) Krebs solution, Ca-free isotonic K and Ca–Na-free Krebs solutions produced only small contractures in both tissues. Moreover, carbachol (10^{-5} g/ml.) also produced only a small contracture. After the muscles had lost the capability to generate a carbachol-induced contracture in Ca-free isotonic K Krebs solution, the tissues were perfused with a Ca containing isotonic K Krebs solution for different durations. As shown in

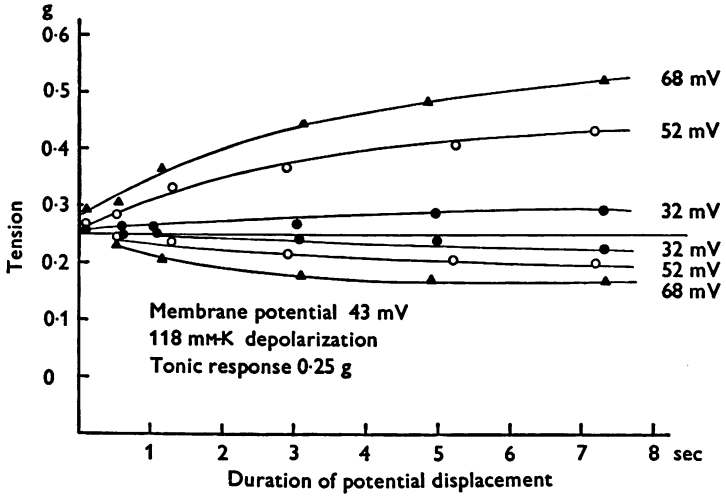

Fig. 6. Effects of various intensities and durations of the inward current pulse on the relaxation and rebound contracture of the circular muscle fibres during the K-induced contracture. Ordinate, mechanical response expressed as g; abscissa, duration of the inward current pulse. The membrane potential was displaced from −5 to −32 (●), −52 (○) and −68 mV (▲) in isotonic K Krebs solution (resting membrane potential was −43 mV). The inward current pulses were applied during the tonic response of K-contracture which remained steady at 0·25 g. The records were taken with the double sucrose-gap current-clamp method.

Fig. 9, in the circular muscle, the amplitude of the mechanical response evoked during the application of Ca was small, however, when carbachol (10^{-5} g/ml.) was applied to the tissue 2 min after the tissue was rinsed with Ca-free isotonic K Krebs solution contracture was evoked. The amplitude of the carbachol-induced contracture increased in proportion to the duration of perfusion with Ca containing solution (*b–e*). On the other hand, in the longitudinal muscle, application of Ca produced a mechanical response and the amplitude was proportionally increased with the duration of Ca application (*b–e*). Carbachol also produced a contracture although the amplitude was smaller than the Ca-induced contracture (*c*).

A single electrical stimulus produced a twitch contraction while repetitive electrical stimulation produced a tetanic contraction in both muscle tissues. The falling phase of the twitch and the tetanus were much shorter in the circular muscle than in the longitudinal muscle. Moreover, in the circular muscle, the amplitudes of the twitch and tetanus were larger than the K-induced contracture, but in the longitudinal muscle the reverse was observed. Another difference in mechanical properties between the tissues was seen on repetitive electrical stimulation (0·1 Hz, 30 msec): a positive

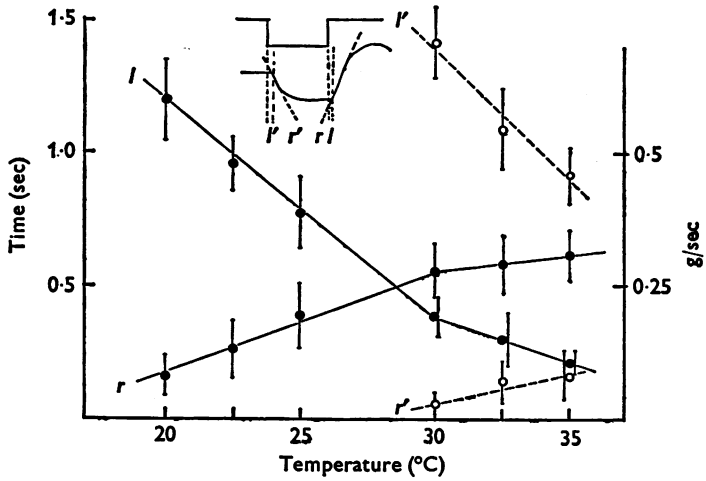

Fig. 7. Effects of temperature on the latency and rate of relaxation and of rebound contracture induced by inward current pulses during K-induced contracture in circular muscle. l' and l; latencies of onset of relaxation and of onset of rebound contraction respectively after application and cessation of the inward current pulse respectively (sec). r' and r, rates of relaxation and of rebound contracture respectively (g/sec). Continuous lines, rebound contracture; dashed lines, relaxation; bars indicate 2 × S.D. of an observation, $n = 5$.

staircase phenomenon was observed in the circular muscle but not in the longitudinal muscle. As shown in Fig. 10, the amplitude of the first twitch produced after various quiescent periods was inversely proportional to the silent period up to a certain duration, and successively applied electrical stimuli gradually enlarged the twitch tension to a steady level. It has already been reported by Sakamoto & Kuriyama (1970) that repetitively applied outward current pulses (1 Hz) did not modify the amplitude of the spike in circular stomach muscle. Therefore the positive staircase phenomenon might be due to increased mobilization of Ca from sequestration sites.

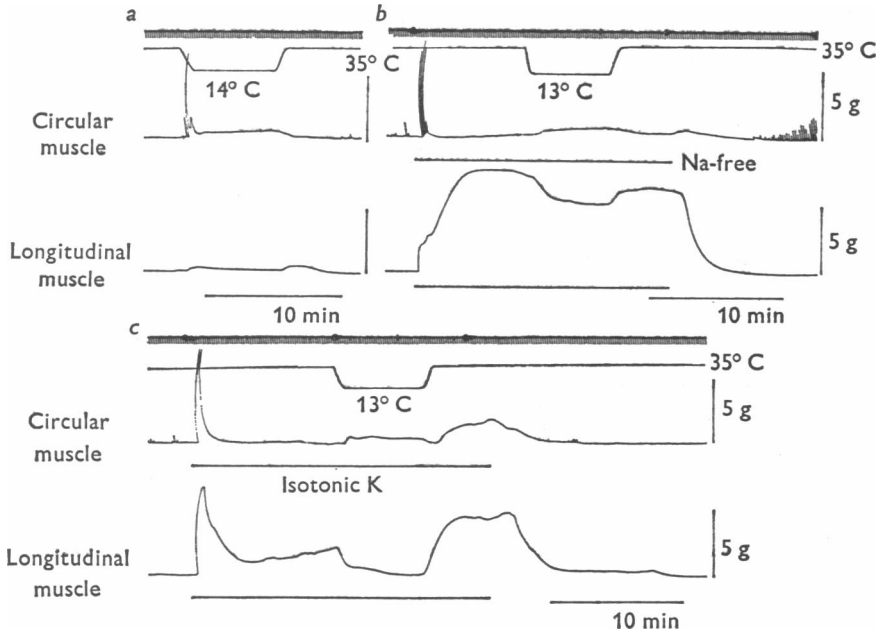

Fig. 8. Effects of rapid cooling on the mechanical activity of circular and longitudinal muscle in Krebs solution (*a*), Na-free (sucrose) (*b*) and isotonic K Krebs solutions (*c*). Temperature is lowered from 35 to 14° C in *a* and to 13° C in *b* and *c*.

DISCUSSION

The results obtained in the present experiments show that there are marked differences in the contractile responses of the longitudinal and circular muscle tissues of the stomach. It is postulated that these differences are caused by differences in Ca sequestration in the muscle fibres and also by differences in the mechanism of extrusion of Ca from the myoplasm. In the longitudinal muscle fibres, increased influx of Ca ion due to K-depolarization of the membrane might directly increase the amount of $[Ca]_i$ and reduction of free Ca from the myoplasm may require $[Na]_o$. On the other hand, in the circular muscle fibres, Ca that enters due to K-depolarization might mainly be sequestered in the store sites and $[Ca]_i$ may not increase.

In some excitable tissues, namely cardiac muscle and nerve, Ca influx and efflux are partially dependent on $[Na]_i$ and $[Na]_o$ respectively and therefore the possible existence of Na–Ca exchange system in these cells must be considered (Reuter & Seitz, 1968; Baker, Blaustein, Hodgkin & Steinhardt, 1969; Blaustein & Hodgkin, 1969; Glitsch, Reuter & Scholz,

1970). A similar mechanism to maintain a low concentration of Ca in the myoplasm of visceral smooth muscles is also postulated (Sitrin & Bohr, 1971; Katase & Tomita, 1972; Reuter, Blaustein & Haeusler, 1973; Tomita & Watanabe, 1973), although it has also been suggested that in smooth muscle the large transmembrane Ca gradient depends on cellular ATP rather than on the Na gradient. ATP depletion abolished the Ca gradient by increasing Ca influx (van Breemen, Farinas, Casteels, Gerba,

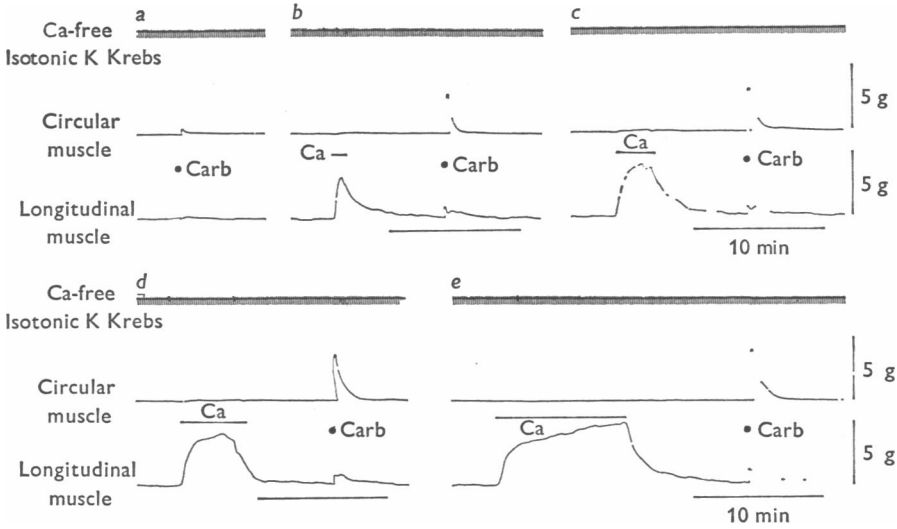

Fig. 9. Effects of pre-treatment with Ca on the carbachol-induced contraction in Ca-free isotonic K Krebs solution. (*a*) control, carbachol (10^{-5} g/ml.) was applied 20 min after immersion in Ca-free isotonic K Krebs solution; (*b–e*) application of Ca (2·5 mM) for 1, 2, 5 and 10 min. Bars indicate application of Ca containing isotonic K Krebs solution and dots indicate application of carbachol (Carb).

Wuytack & Deth, 1973). It is difficult to decide between the two alternative hypotheses concerning the Ca extrusion mechanism on the basis of the present experiments, since the observations on stomach muscle fit both mechanisms. In the circular muscle a Ca pump may be the mechanism reducing the Ca ion in the myoplasm, and in the longitudinal muscle a Na–Ca exchange mechanism may be more likely, because in isotonic K and Na-free solutions the developed tension relaxed in the circular muscle but not in the longitudinal muscle.

It is known that the sarcoplasmic reticulum in visceral smooth muscles is less developed than in skeletal muscle. However, the vesicles and sarcoplasmic reticulum are relatively more developed in circular muscle of the

stomach than in longitudinal muscle (Yuko Nishio, personal communication). Therefore the Ca pump mechanism may be developed predominantly in the circular muscle associated with the sarcoplasmic reticulum and the Na–Ca exchange mechanism in the longitudinal tissue. The present results also showed that reduction of the amplitude of the tonic response during K-induced contracture was not due to reduction of influx of Ca ion but due to accumulation of Ca ion in the store sites, since transient hyperpolarization of the membrane produced larger rebound contractions in the

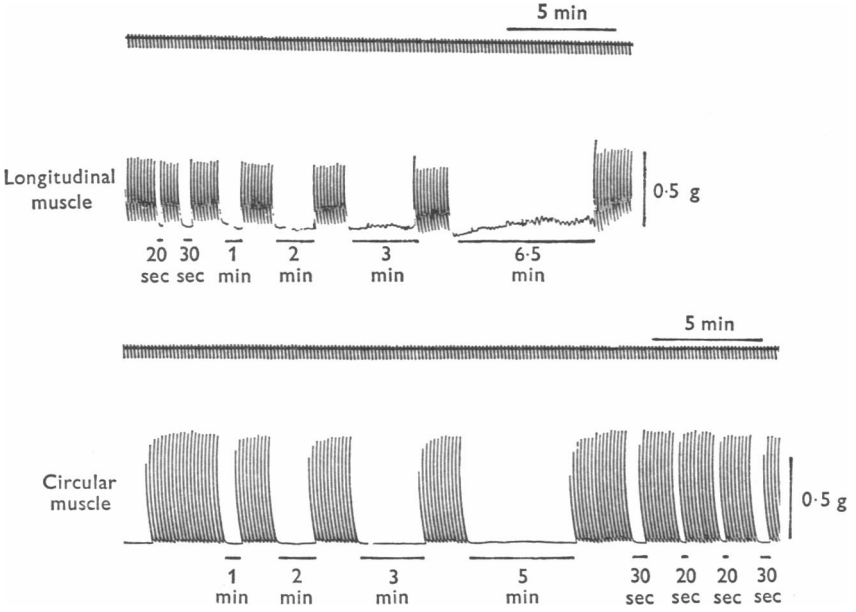

Fig. 10. Effects of electrical stimulation on the longitudinal and circular muscles applied at various intervals. Electrical stimulation (30 msec pulse duration and 0·1 Hz) was applied. Bars in the figure indicate the intervals between the electrical stimulation.

circular than the longitudinal muscle. Moreover, repetitive electrical stimulation produced a positive staircase phenomenon in the circular but not in the longitudinal muscle. In general, the Na–Ca exchange mechanism is more complex and depends not only on $[Na]_o$ and $[Ca]_i$ but also on $[Na]_i$ which is modified by $[K]_o$ and by the Na–K pump mechanism. Moreover $[Ca]_i$ controls the K-conductance in the taenia coli (Brading, Bülbring & Tomita, 1969; Tomita & Yamamoto, 1971; Brading, 1973; Casteels, Droogmans & Hendrickx, 1973).

It has been reported that the stomach muscle is composed of electrically excitable muscle fibres and less-excitable muscle fibres. In normal

conditions, depolarization of the membrane only produces an abortive spike or does not generate an active response in the latter. However, after treatment with tetraethyl ammonium all the cells produced spikes with overshoot potential. The latter are mainly found in the longitudinal muscle of the cardia and fundus regions and the former mainly in the circular and longitudinal muscles of antral region (Osa & Kuriyama, 1970).

It is generally thought that the stomach has functional regions which correspond to its anatomical divisions (Code & Carlson, 1968). The fundus and an upper region of the corpus both act as reservoirs. The major functions of the antrum are propelling, retropelling and triturating the contents. The longitudinal muscle fibres in the upper region may only be activated by extension of the stomach wall through increased contents. It may not be necessary for this muscle to possess the properties required by the antral region. Hence, it is postulated that the activity of the stomach might be controlled not only by the activity of the nervous elements, but also by topical differences in the properties of the smooth muscle tissues themselves.

The authors wish to express their appreciations to Professor E. Bülbring and Dr T. Bolton for their critical comments and their help in the preparation of this manuscript.

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