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Factors Controlling Myogenic Activity in Smooth Muscle

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Factors controlling myogenic activity in smooth muscle

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The possible roles of Na and Ca in myogenic spontaneous activity are described. Na ions influence the membrane potential by the Na concentration gradient across the membrane, by the relative membrane permeability to Na and by an electrogenic factor involving the Na–K pump. Another role of Na is its contribution to the regulation of the Ca distribution across the membrane, probably through Na–Ca exchange and by affecting Ca permeability. Although these functions of Na are important for the generation of spontaneous activity, the primary role of Ca is emphasized since spontaneous activity can be produced in Na-free, Ca containing solution. It is postulated that the internal surface of the membrane has negative sites, to which Ca can be bound, and that the K permeability is increased when the amount of bound Ca at this site is increased. The K permeability would then be reduced when Ca is extruded by an active Ca pump, and this may be the mechanism underlying the pacemaker potential.

Many smooth muscles exhibit rhythmic spontaneous contractions. These are caused by electrical activity of the muscle fibres. The pattern and the configuration of this spontaneous electrical activity greatly differ in different types of smooth muscles. Accordingly, any generalization is difficult. The origin of the spontaneous activity seems to be myogenic, since various drugs which interfere with the nervous activity or transmission do not abolish the activity.

It seems important, first, to clarify the various components in the electrical activity. In this paper it is assumed that there is one basic underlying component which is metabolically driven and which is responsible for the spontaneous activity in every smooth muscle. The results, obtained mainly from the guinea-pig taenia coli, will be presented and they will be interpreted in connexion with the regulation of the intracellular Ca concentration which, in turn, is probably related to the electrical component underlying the spontaneous activity.

Components of electrical activity

In spontaneously active smooth muscles, two components of electrical activity, slow waves and spikes, are usually classified, although their shape and amplitude are quite different from tissue to tissue. The relative magnitude of the slow wave and spike also varies with the conditions of the preparation and with the methods employed for recording.

Figure 1 shows the electrical activity taken from the guinea-pig oviduct and the effects produced by membrane polarization. The activity was composed of a large slow wave and a spike component. Both depolarization and hyperpolarization had only weak effects on the frequency of spontaneous activity, but the amplitude was clearly modified by polarization. On the rising phase of the slow wave, a notch was often observed, and this became distinctly separated during strong hyperpolarization or following strong depolarization.

Figure 2 shows mechanical and electrical activity recorded from the circular muscle of the guinea-pig stomach. The frequency of the slow waves in this tissue was also only slightly affected by membrane polarization, as in the oviduct. In the stomach also, a notch appeared on the rising phase of the slow wave when conditioning hyperpolarization was applied and, with sufficiently strong hyperpolarization, the second component of the slow wave was abolished, leaving the underlying component unmasked.

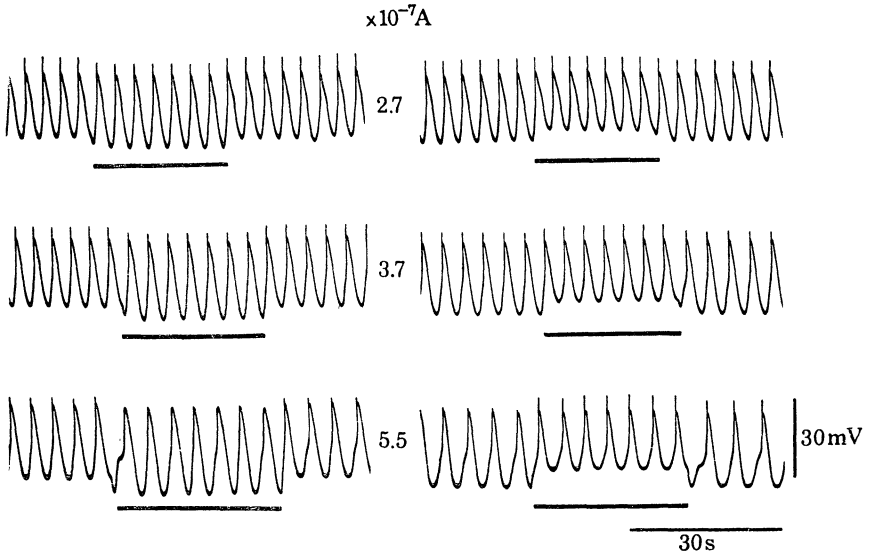

FIGURE 1. Guinea-pig oviduct. Spontaneous electrical activity recorded with the double sucrose-gap method. Effects of hyperpolarizing (left) and depolarizing (right) current application (horizontal bars) with increasing intensities as indicated.

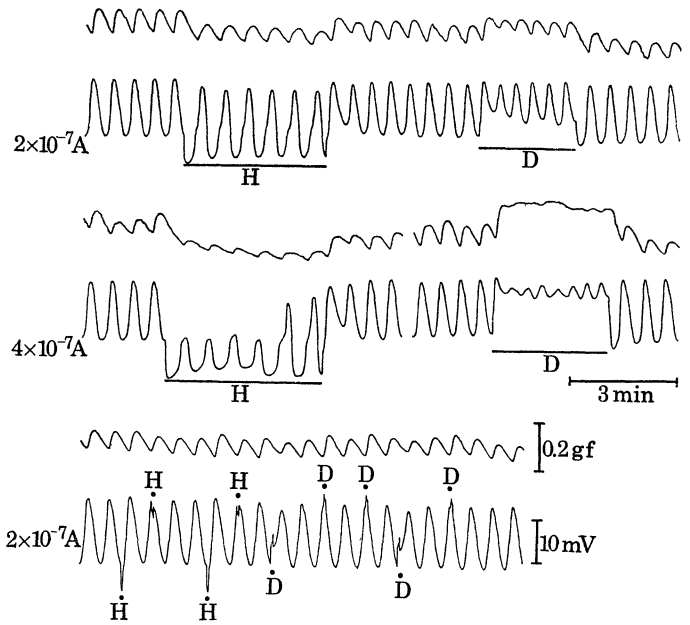

FIGURE 2. Mechanical (upper trace) and electrical (lower trace) activities of the guinea-pig stomach (circular muscle), and effects of hyperpolarizing (H) and depolarizing current (D). (Double sucrose-gap method.) Current intensities are given on the left. For further description see text.

The bottom record of figure 2 shows electrotonic potentials produced by constant current pulses applied between and during the slow waves. Since the electrotonic potentials produced by hyperpolarizing or depolarizing current pulses (1 s) were much reduced near the peak of the slow wave, as reported for the longitudinal muscle of rabbit intestine (Mills & Taylor 1971), the conductance change, probably mainly an increase of Na conductance, may be responsible for the generation of the second components of the slow wave, although convincing evidence is still lacking.

These results suggest that, in these two tissues at least, the slow wave consists of two separate components, and that the underlying basic slow component, the pacemaker potential, is relatively less dependent on the membrane potential, but evokes another slow component which is potential dependent. The second component of the slow wave usually masks the first component and may further trigger the spike component.

The guinea-pig taenia coli has spontaneous electrical activity mostly composed of spikes, although under appropriate conditions the slow wave can be demonstrated. The frequency of spike activity can be easily modulated by depolarization or by hyperpolarization as shown in figure 3. In order to produce the spontaneous spike in the taenia, some underlying slow potential may also be assumed, but in the taenia it is usually rather small in amplitude.

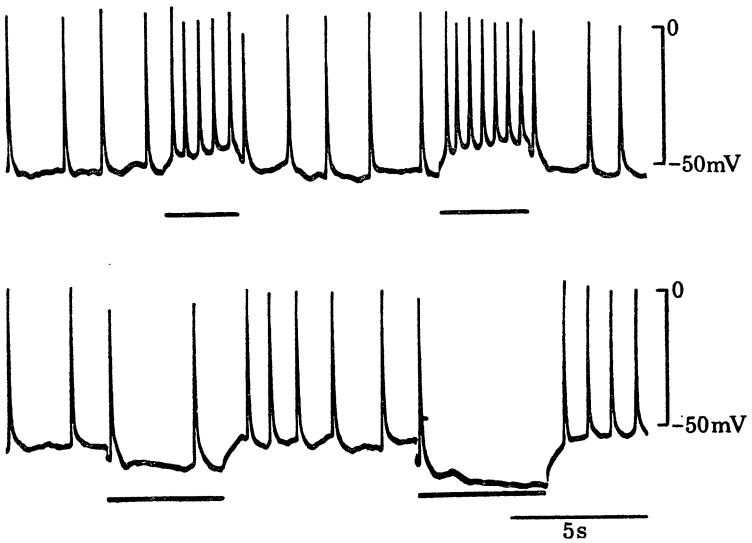

FIGURE 3. Spontaneous spike activity of guinea-pig taenia coli, recorded with intracellular electrode. Frequency modulation by externally applied polarizing current. Depolarization (top) and hyperpolarization (bottom) are shown by horizontal bars. (From Bülbring 1967.)

From the observations of many other types of activity, it may be concluded that in all spontaneously active tissues an underlying basic slow wave exists which is the pacemaker potential. This wave may be metabolically controlled and it is responsible for evoking either the second slow wave or the spikes, or both, to produce the mechanical response. In the taenia, the threshold for spikes is low, and the pacemaker potential is usually very small and difficult to demonstrate.

Effects of temperature on the frequency of activity

Evidence for the possibility of a metabolic control of the pacemaker potential is the very high temperature sensitivity of the spontaneous activity. This is illustrated by the change in frequency of spontaneous spike activity in the taenia (Bülbring & Kuriyama 1963*b*). In a temperature range higher than 23 °C, the Q_{10} of the frequency is about 3, and below 22 °C the frequency sharply decreases, as reported by Loh & Golenhofen (1970). This suggests that the frequency of spontaneous activity may be controlled by some enzymic process, whose activity is sharply reduced below 20 °C, e.g. as that of the Na–K ATPase (Charnock, Doty & Russell 1971; Charnock, Cook & Casey 1971). Not only the spike activity but also the slow waves are temperature sensitive, although the minute-rhythm according to Loh & Golenhofen (1970) in the taenia coli is not sensitive to temperature change. It has been reported that the slow wave in

the cat intestine has a high temperature coefficient (Q_{10} at 30 to 40 °C = 2 and Q_{10} at 10 to 20 °C = 6, Bortoff (1961); Q_{10} at 25 to 35 °C = 2.73, Job (1969)). The Q_{10} of the frequency of peristaltic waves in the guinea-pig stomach is 2.4 (Golenhofen, Loh & Milenov 1970).

Na *contribution to the membrane potential*

Since the main ionic constituent in normal Krebs solution is the Na ion, it would be appropriate first to describe the roles of the Na ion in relation to spontaneous activity. When the external NaCl is reduced by replacing it with sucrose, the spontaneous activity is transiently disturbed, and at about 30 mmol/l Na the activity nearly stops (Holman 1957), but by increasing the external K concentration the spontaneous activity can be produced again, even in a solution containing only one tenth of the normal Na concentration. In low Na solution, repetitive spikes can be evoked by depolarizing the membrane either with current pulses or with excess K (Brading, Bülbring & Tomita 1969*b*). When NaCl is substituted with choline chloride or tris chloride, the spontaneous activity seems to be better maintained than with sucrose (Holman 1958; Bülbring & Kuriyama 1963*a*): in a solution containing only 5 to 20 mmol/l Na, spontaneous activity of normal frequency can be observed, although the amplitude and the rate of rise are both reduced. Therefore, as suggested by Holman (1958), the Na ion does not seem to be essential for the pacemaker potential.

Suppression of the activity in low Na solution is probably mainly due to the hyperpolarization of the membrane, and the contribution of the Na ion to the activity may be partly mediated through its control of the membrane potential. In other words, the membrane potential is maintained near the critical firing level due to the presence of Na ions.

The contribution of Na to the membrane potential is not only through its concentration gradient and the Na permeability of the cell membrane, but also through an electrogenic pump, which actively transports Na outwards across the membrane (taenia: Casteels, Droogman & Hendrickx (1971), Tomita & Yamamoto (1971); uterus: Taylor, Paton & Daniel (1970); portal vein: Kuriyama, Ohshima & Sakamoto (1971)). Thus, modification of the pump activity would lead to a change in the membrane potential. The slow waves of the cat jejunum have been attributed to cyclic changes in the passive influx, causing depolarization, and the electrogenic Na pump, resulting in hyperpolarization (Job 1969; Liu, Prosser & Job 1969). However, more convincing evidence is necessary before one can explain the mechanism of the slow waves according to this hypothesis, as reviewed by Bortoff (1972).

The strongest evidence supporting this idea is the fact that ouabain blocks the spontaneous activity (Daniel 1965; Liu *et al.* 1969; Tomita & Yamamoto 1971). However, since in the presence of ouabain, the membrane is depolarized and the intracellular Na concentration is increased, these changes may produce some secondary effect on the membrane properties. Thus, further analysis seems necessary in order to find out if the suppression of spontaneous activity is simply a direct consequence of blocking the Na-pump.

The block of spontaneous activity which occurs after a period of high spike activity may be related to an activation of the Na-pump. For example, the hyperpolarization observed during recovery from depolarization caused by carbachol has been interpreted in this way (Bolton, this volume, p. 107). However, it is difficult to explain the increase in spike frequency with high temperature as due to a mechanism mediated by the Na-pump, since one would expect that the pump would be more active at higher temperatures and that this would tend to suppress the activity. Thus change of temperature having a high Q_{10} must have another, direct

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stimulating effect on the ionic mechanism responsible for the pacemaker potential, i.e. the higher the temperature, the faster the rate of rise of the pacemaker potential, as has been observed in the giant molluscan neuron (Carpenter 1967) or in cardiac muscle (Coraboeuf & Weidmann 1954).

Possible role of Na *exchanging with* Ca

In many smooth muscles, evidence is being accumulated that the spike is generated mainly, if not entirely, by transmembrane influx of Ca ions. If so, Ca must be effectively pumped out by some mechanisms, otherwise it would be accumulated inside the cells which have high spontaneous activity. Moreover, recent experiments have shown that Na ions are also involved in the regulation of the intracellular Ca concentration (Reuter, Blaustein & Haeusler, this volume, p. 87; Katase & Tomita 1972).

The experiments were as follows. After producing contracture in the K Krebs solution in which Na was completely replaced with K, the K concentration was again reduced to normal by sucrose substitution of KCl, thereby maintaining the absence of Na ions. In this Na-free sucrose solution (with normal K), the tension remained high and the tissue relaxed only when Na ions were added (figure 4). About 5 mmol/l Na was sufficient to cause relaxation, although the rate of relaxation was slower with lower Na concentrations.

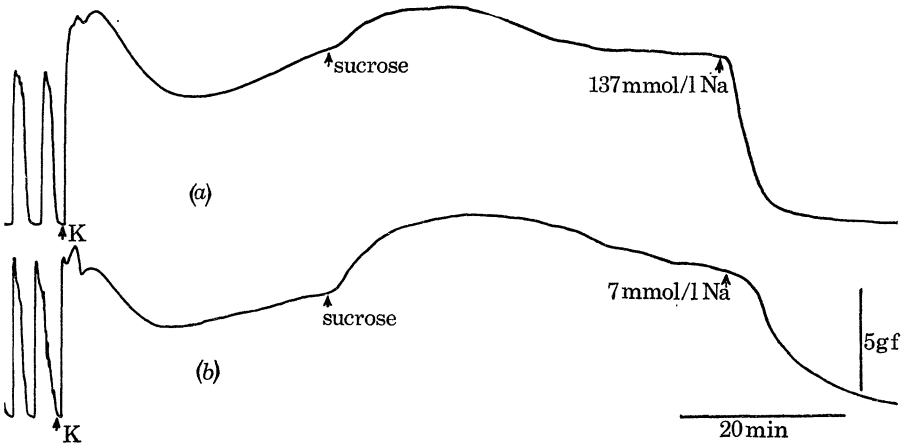

FIGURE 4. Spontaneous mechanical activity of the taenia coli. At arrow, exposure to the K Krebs solution (143 mmol/l K) for 30 min, subsequent exposure to the sucrose Krebs solution (5.9 mmol/l K, 0 Na), and followed with normal Krebs solution (137 mmol/l Na) (upper tracing) or with Krebs solution containing 7 mmol/l Na (lower tracing). (From Katase & Tomita 1972.)

Intracellular measurement showed that the membrane potential was nearly normal and that the spike could be evoked in the Na-free sucrose Krebs solution after treatment with the K Krebs solution. Addition of Na caused only a small further hyperpolarization and reduced the excitability. Therefore, the inability to relax in the sucrose Krebs solution is not due to inability to repolarize, showing that relaxation is not greatly dependent on the membrane potential.

Similar experiments to that shown in figure 4 were carried out with LiCl and tris chloride, instead of NaCl. Li caused relaxation, although less effectively than Na, but tris was ineffective. Since tris chloride does not relax the tissue, the Cl contribution is negligible. Furthermore, Na salts of large anions, such as benzenesulphonate, are as effective as NaCl. Therefore, Na ions are responsible for the relaxation.

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Active transport of Na does not seem to be directly involved in the relaxation caused by the addition of NaCl because treatment with ouabain had only a small slowing effect on relaxation. Lowering the temperature to 20 °C also had only a weak effect, and the Q_{10} of the rate relaxation by Na was about 1.4 (Katase & Tomita 1972). A similar low Q_{10} has been obtained for the loss of ^{45}Ca in the normal Krebs solution from the taenia coli (Goodford 1965).

If the mechanical response is taken to be an indication of the intracellular Ca concentration, then the results described above are generally in accordance with those reported for squid giant axon (Baker, Blaustein, Hodgkin & Steinhardt 1969; Blaustein & Hodgkin 1969), and for cardiac muscle (Reuter & Seitz 1968). The concentration gradient of Na across the membrane or the downhill movement of Na would drive the outward movement of Ca and reduce the intracellular Ca concentration. The energy for this Na–Ca exchange would be supplied by the electrochemical gradient of Na across the membrane.

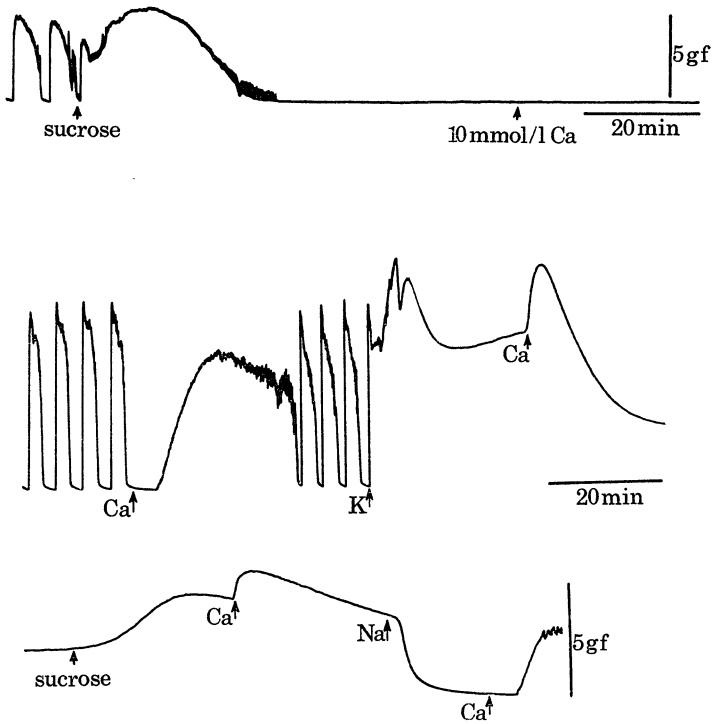

FIGURE 5. Top: mechanical response of the taenia coli produced by exposure to sucrose Krebs solution. When activity stopped, Ca (10 mmol/l) was added to the bathing solution. Middle and bottom: continuous record from another preparation, showing the effects of adding 20 mmol/l Ca to the normal Krebs solution, to the K Krebs solution, to the sucrose Krebs solution, and again to the normal Krebs solution. Ca was injected into the organ bath, so that the initial concentration of 20 mmol/l was transient, and Ca was washed out within a few minutes. (Modified from Katase & Tomita 1972.)

In the taenia, the Ca distribution seems to be controlled not only by a Na–Ca exchange mechanism, but also by some other mechanism, such as an active Ca-pump as found in the erythrocyte (Schatzmann & Vincenzi 1969), since the simple removal of Na from the Krebs solution, without pretreatment with excess K, does not cause an increase in tension, as shown in the top record in figure 5. Similar results have been reported for the rat portal vein (Biamino & Johansson 1970). Furthermore, treatment with ouabain caused only transient excitation and contraction followed by complete relaxation (Casteels 1966; Tomita & Yamamoto 1971). If the Na gradient is the main factor in keeping the Ca concentration inside the cells low, then

contracture would be expected in response to a reduction of the external Na concentration or to an increase in the intracellular Na concentration by ouabain. The proportions of the contribution of the Na–Ca exchange and the active Ca-pump to the regulation of the intracellular Ca concentration seem to vary greatly from tissue to tissue, and they also depend on the condition of the tissue.

In some tissues, contracture has been observed on simply removing Na or by treating with ouabain (mouse uterus: Osa (1971); guinea-pig ileum: Judah & Willoughby (1964); dog trachea: R. F. Coburn, personal communication). In the taenia, it is possible that the Ca permeability of the membrane is so low that, following removal of external Na or treatment with ouabain, the intracellular Ca concentration does not increase sufficiently within the experimental period of 1 h to result in a contracture, or that a metabolically driven Ca pump is enough to compensate for the increased Ca influx under these conditions.

Ca *permeability and contracture*

During the contracture produced in excess K solution or maintained in the sucrose Krebs solution following excess K treatment, the tension depended very much on the external Ca concentration (figure 5), suggesting a high Ca permeability of the membrane. However, in Na-free solution without pretreatment with excess K, the tissue stayed relaxed when excess Ca was added, and in normal Krebs solution excess Ca suppressed the activity. Therefore, in order to demonstrate the Na-dependent Ca movement in the taenia, the Ca permeability of the membrane must first be increased. The Ca permeability is increased by treatment with excess K and it remains high without Na ions. It is possible that addition of Na reduces the Ca permeability by competing at the outer surface of the membrane, and also increases the outward movement of Ca by the Na–Ca exchange process, thereby causing relaxation.

If the active Ca-pump is working, then a reduction of Ca influx by suppressing the membrane permeability to Ca to such an extent that the influx becomes less than the outflux, should relax the tissue. The results shown in figure 6 can probably be explained in this way. Mn, Mg and La ions, all of which are considered to reduce the Ca permeability in many tissues (e.g. barnacle muscle: Hagiwara & Takahashi (1967); guinea-pig ileum: Weiss & Goodman (1969)), terminated the contracture in the sucrose-Krebs solution. It was also found that Ca in a concentration exceeding 30 mmol/l reduced the tension, although up to 30 mmol/l Ca increased the tension as described above. It is likely that concentrations of Ca in excess of 30 mmol/l suppress the Ca permeability.

Summarizing the results, it may be concluded that Na ions have several roles. One is that they modify the membrane potential by their concentration gradient across the membrane, their relative permeability, and probably partly through the electrogenic factor involving the Na–K pump. The second role is a contribution to the regulation of the Ca distribution across the membrane, probably through Na–Ca exchange and by reducing the Ca permeability.

Spontaneous activity in Na-*free solution*

In normal conditions, the various functions of Na may be very important for the generation of the spontaneous activity. However, under some conditions, the spontaneous activity can be produced in the complete absence of Na ions. For example, spontaneous contractions can be observed in Ca Krebs solution, containing 5.9 mmol/l HCO_3 and in which Na is isosmotically replaced with Ca (the bottom record in figure 6). Therefore, Na ions may not be essential

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for the spontaneous activity, although their importance should be stressed under normal conditions.

As shown in figure 7, the spontaneous activity stopped for about 50 min when the taenia was exposed to the Ca Krebs solution, but then the activity started again. At the beginning of exposure to the Ca Krebs solution, the excitability of the tissue was very low but the responses to the same intensity of current pulse gradually increased until spontaneous activity appeared.

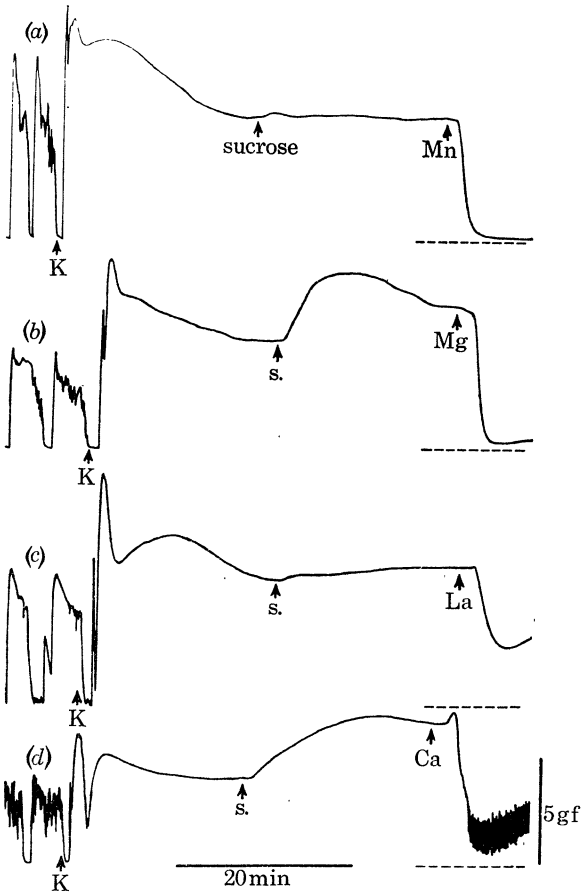

FIGURE 6. Effects of polyvalent cations: Mn (91 mmol/l), Mg (91 mmol/l), La (68 mmol/l) and Ca (91 mmol/l) on contracture after treatment with the K Krebs solution and sustained in the sucrose Krebs solution. The sucrose was isosmotically replaced with polyvalent cations. Dashed lines show zero tension level.

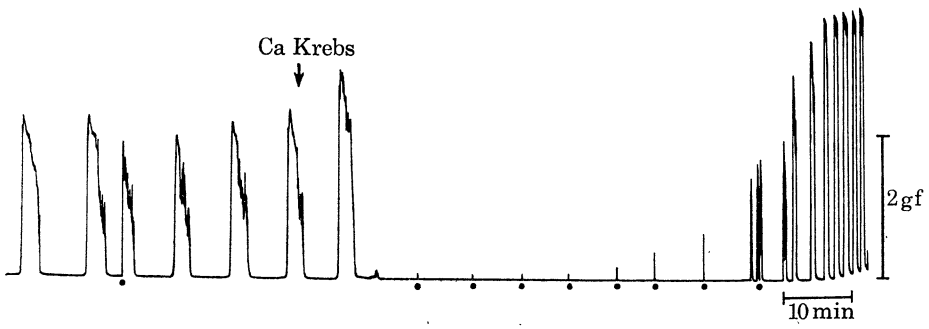

FIGURE 7. Spontaneous and evoked (dot, 10 ms, 10 V) mechanical activity of the guinea-pig taenia coli. At arrow normal Krebs solution was replaced by the Ca Krebs solution in which NaCl (130 mmol/l) was completely replaced by $CaCl_2$ (87 mmol/l) and HCO_3 was reduced to 5.9 mmol/l.

The relaxed state in the Ca Krebs solution is probably due to a very low Ca permeability of the cell membrane and an adequate extrusion of Ca by the active Ca-pump.

Figure 8 shows simultaneous recording of mechanical and electrical activity. (Electrical recording by external electrodes placed on the tissue in the organ bath.) Otherwise, the experimental conditions were the same as those shown in figure 7. After 45 min in the Ca Krebs solution, the electrical and mechanical activity started and was quickly terminated when normal Krebs solution was introduced. After 10 min the normal pattern of activity recovered.

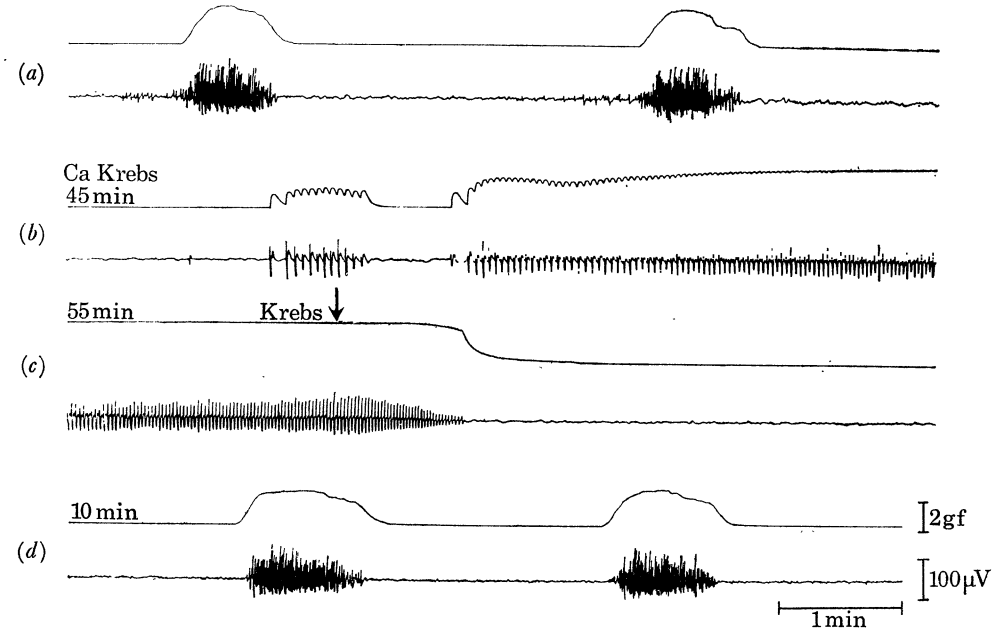

FIGURE 8. Mechanical (upper trace) and electrical (lower trace) activities in the taenia coli. Electrical activity was recorded with two external electrodes placed 5 mm apart along the preparation: (*a*) in normal Krebs solution; (*b*) after 45 min in the Ca Krebs solution; (*c*) 10 min later. At arrow, change to normal Krebs solution, and (*d*) after 10 min in normal Krebs solution.

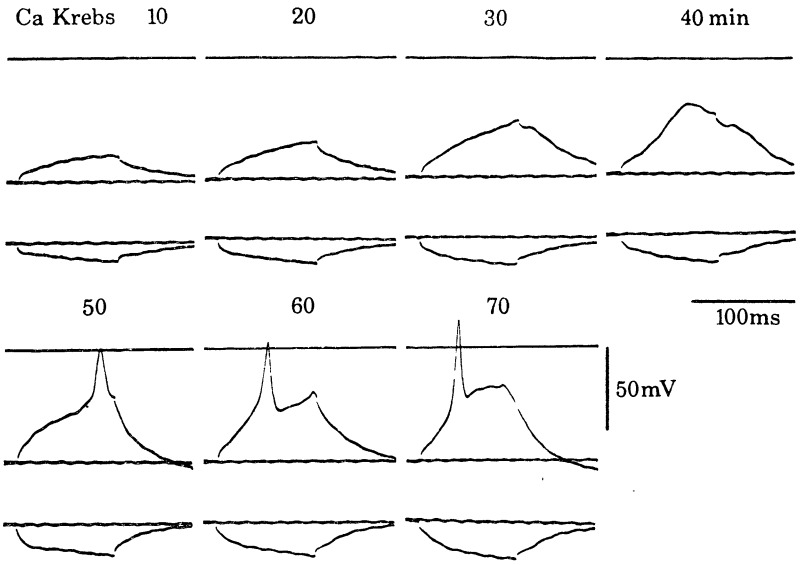

FIGURE 9. Taenia coli. Intracellular records of electrical response to current pulses of constant intensity (100 ms duration). Each pair (depolarization: top and hyperpolarization: bottom) of records was taken at 10 min interval in the Ca Krebs solution.

When the intracellular recording technique was used, a gradual increase in the electrotonic potential and a gradual reduction in the membrane potential were observed in the Ca Krebs solution (figure 9). Thus, it became easier to evoke a spike with time of exposure to the Ca Krebs solution. Action potentials of the taenia in Na-free Ca solution have previously been demonstrated by Sakamoto (1971). The increase in the size of the electrotonic potentials and the depolarization in the Ca Krebs solution suggests a decrease in the K conductance of the membrane.

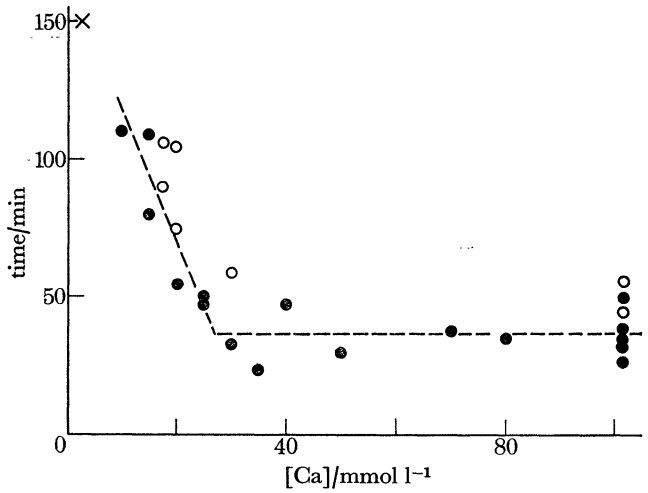

FIGURE 10. Relation between external Ca concentration and the time required for reappearance of spontaneous contractions in Na-free solution. Osmolarity was maintained by sucrose. There is a tendency for the time to be shorter with lower HCO_3 concentration. ○, 5.9 mmol/l HCO_3; ●, 2.5 mmol/l HCO_3.

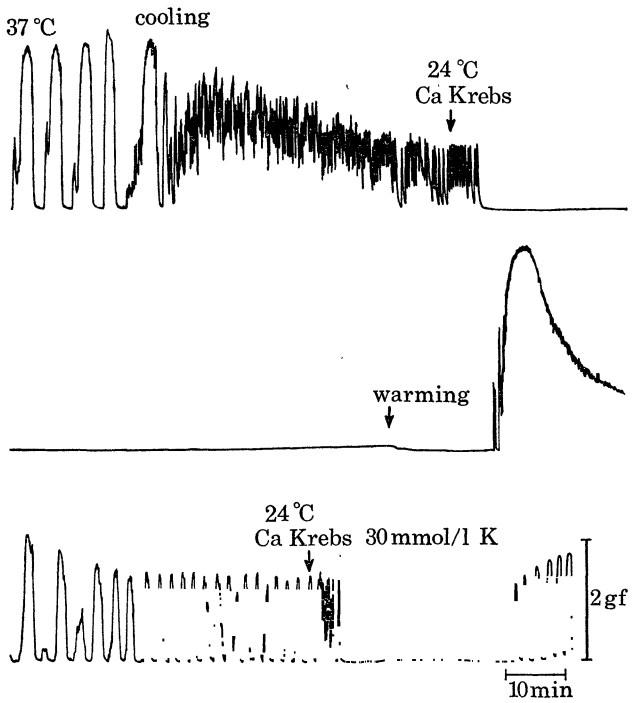

FIGURE 11. Effects of temperature on the spontaneous contractions in the Ca Krebs solution. Top and middle records are continuous (for description see text). Bottom record from another preparation, when the normal Krebs solution was replaced with the Ca Krebs solution (67 mmol/l Ca) containing 30 mmol/l K (see text).

Figure 10 shows the relation between the external Ca concentration and the time which elapsed until spontaneous activity appeared after exposure to Na-free solution containing various concentrations of Ca. At high concentrations of more than 30 mmol/l Ca, the spontaneous activity started after about 40 min, but at lower concentrations a longer time was necessary. At normal Ca concentration, 2.5 mmol/l, no spontaneous activity appeared without Na ions. As described above, a high external Ca concentration probably suppresses the Ca permeability in the resting state but, on the other hand, provides a large concentration gradient (the driving force) for the generation of the Ca-spike.

In the Ca Krebs solution, spontaneous activity always started within 50 min at 37 °C, but when the temperature was lowered to 24 °C no activity was observed even after 80 min exposure to the Ca Krebs solution. Activity started quickly on rewarming (figure 11). The lack of activity was not due to the inability of the tissue to contract, because the activity was observed at 24 °C in the Ca Krebs solution containing excess K (30 mmol/l). Thus, the activity in the Ca Krebs solution seems to be evoked when the depolarization of the membrane reaches the critical firing level, and the rate of depolarization is probably very much reduced at low temperature, as the pacemaker potential observed in other tissues.

Ca *pump and pacemaker potential*

There is evidence that the sarcoplasmic reticulum and mitochondria can accumulate Ca. Thus it is likely that these structures also contribute to the regulation of the intracellular Ca concentration. Furthermore, under some conditions, enzymic activity of mitochondria produces slow oscillations (Gooch & Packer 1971). It is, therefore, tempting to correlate these biochemical oscillations with the spontaneous activity of the muscle fibres. However, cyanide and oligomycin, which strongly interfere with the mitochondrial metabolism and its ATPase, have a rather weak suppressing effect on spontaneous activity in the taenia (unpublished observations), and do not significantly change the frequency of the slow wave in the small intestine of the cat (Job 1971). Therefore, it is likely that the Ca transport across the plasma membrane is more important for the spontaneous activity in at least some smooth muscles.

Since Ca must cross the plasma membrane to the interior of the cell during spike activity, translocation of Ca within the cell is not enough to maintain a constant Ca content inside the cell, and Ca must be pumped out of the cell to maintain equilibrium in physiological conditions. The simplest idea for this mechanism would be a similar Ca pump to that observed in the erythrocyte since this pump can operate in the absence of Na and in the presence of ouabain, the Q_{10} of Ca transport being 3.5 (Schatzmann & Vincenzi 1969).

In the taenia coli, an increase in the external Ca concentration or a reduction of external Na decreases the electrotonic potential. This can be attributed to an increase in the intracellular Ca concentration (Bülbring & Tomita 1969; Brading, Bülbring & Tomita 1969*a*). The decrease of membrane resistance by excess Ca becomes very pronounced when the Ca exchange is first increased, for example by ouabain treatment (unpublished observations).

It is known that an increase in the intracellular Ca concentration reduces the membrane resistance in the squid giant axon (Tasaki, Watanabe & Lerman 1967), and in the cat motoneuron (Feltz, Krnjevic & Lisiewicz 1972). Also in the erythrocyte the K efflux is increased by increasing the intracellular Ca concentration (Romero & Whittam 1971; Lew 1971). Therefore, it may be postulated that the internal surface of the membrane has negative sites, as assumed for the squid giant axon (Hodgkin & Chandler 1965; Chandler, Hodgkin & Meves 1965), to

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which the Ca can be bound, and that the K permeability is increased when the amount of bound Ca at this site is increased (figure 12). The K permeability would then be reduced by the active Ca pump transporting Ca bound at the inner surface to the outside of the membrane.

It is difficult to explain why the binding of Ca at the inner surface of the membrane should increase the K permeability. One possible explanation would be a reduction of negative charges at the internal surface of the membrane. When Ca is bound at the outer surface of the membrane, the potential field across the membrane is increased (Frankenhaeuser & Hodgkin 1957). This produces effects similar to hyperpolarization of the membrane reducing the ionic permeability. On the other hand, when Ca is bound at the inner surface of the membrane, the transmembrane potential gradient would be reduced (dashed line in figure 12). This corresponds to depolarization of the membrane increasing the ionic permeability, possibly mainly to K because the highest concentration of ions inside the cell is that of K ions.

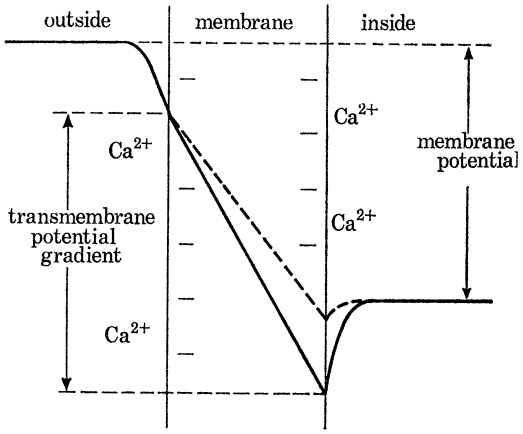

FIGURE 12. Hypothetical membrane, having negative charge at both the external and internal surface, and its potential field. Membrane potential is dependent on ionic concentration gradient and on relative permeability of ions distributed across the membrane. The permeability is controlled by the transmembrane potential gradient which can be modified by negative charges at both surfaces. Dotted line indicates a reduction of transmembrane potential gradient caused by neutralizing internal negative sites by Ca ions.

Then, the pacemaker potential, or metabolically driven slow wave, can be visualized as a decrease in the K conductance of the membrane produced by the Ca pump which reduces the amount of bound Ca at the inner surface of the membrane. It is difficult to demonstrate the conductance change responsible for the spontaneous activity in the taenia under normal conditions. However, it may be supposed that the gradual change of the K conductance observed in the Ca Krebs solution is fundamentally analogous to the pacemaker potential in the normal Krebs solution, and it is hoped that further experiments along this line of approach may throw some light on the basic mechanism of the spontaneous activity in various smooth muscles.

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