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EFFECT OF CALCIUM AND MAGNESIUM ON NEUROMUSCULAR TRANSMISSION IN THE HYPOGASTRIC NERVE–VAS DEFERENS PREPARATION OF THE GUINEA-PIG

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Neuromuscular transmission of excitation in smooth muscle of the vas deferens has been studied by Burnstock & Holman (1961, 1962*a*, *b*). They suggested that the junction potentials evoked by hypogastric nerve stimulation had similar properties to those of end-plate potentials in skeletal muscle (Katz, 1962), and that they might be due to the release of noradrenaline from the nerve terminals. Burnstock & Holman (1961) also observed small spontaneous depolarizations of the smooth muscle-cell membrane resembling the miniature end-plate potentials. The above observations were extended by further investigations (Burnstock, Holman & Kuriyama, 1964; Kuriyama, 1963, 1964), e.g. it was shown that the hypogastric nerve fibres which innervate the vas deferens smooth muscle cells were in part post-ganglionic C-fibres and in part preganglionic fibres, the peripheral ganglia being located at the very end of the hypogastric nerves.

Recently, a transmission process of excitation similar to that at the skeletal muscle end-plate has been observed not only in the vas deferens but also in other types of neuromuscular junction (smooth muscle of the intestine (Gillespie, 1962), the bladder (Ursillo, 1961), the retractor penis (Orlov, 1961) and blood vessels (Speden, 1964); crustacean muscle (Dudel & Orkand, 1960; Dudel & Kuffler, 1961; Grundfest & Reuben, 1961)).

The effect of calcium and magnesium ions on neuromuscular transmission in skeletal muscle has been extensively studied (see reviews by Eccles, 1961, and Katz, 1962). Increasing the calcium ion concentration has been found to increase greatly the amount of acetylcholine released by nerve stimulation. Decreasing the calcium or increasing magnesium concentrations produced the opposite effect. On the other hand, an increase of the calcium concentration reduced the amplitude of the end-plate current produced by acetylcholine and also reduced the total conductance of the end-plate (Takeuchi, 1963).

The purpose of the present experiments was to see if calcium and magnesium ions have a similar effect on the neuromuscular junction in the vas deferens to that observed in skeletal muscle.

METHODS

The isolated guinea-pig vas deferens supplied by the hypogastric nerve was used in all the experiments. About 20 mm of the muscle and the same length of the hypogastric nerve was used. The experimental method and procedures were the same as those previously described by Kuriyama (1963). The preparation, mounted in a constant temperature bath at 35–36° C, was bathed continuously with modified Krebs solution (Bülbring & Kuriyama, 1963*b*). The normal solution used in all experiments contained (mM): Na^+ 137·4; K^+ 5·9; Mg^{2+} 1·2; Ca^{2+} 2·5; Cl^- 134; $H_2PO_4^-$ 1·2; HCO_3^- 15·5; glucose 11·5; and was aerated with 97 % O_2 and 3 % CO_2. The calcium concentration was altered in a range between 0·25 and 25 mM, and the magnesium concentration between 0·12 and 12 mM. The excess calcium and magnesium were added as chloride salts and these solutions were hyperosmotic. Control experiments were carried out with the same hyperosmosis produced by adding solid sucrose to the Krebs solution. Excess chloride solution was prepared by adding Tris-chloride (Bülbring & Kuriyama, 1963*a*). Chloride-deficient solution was prepared by chloride replacement with ethanesulphonate ($C_2H_5SO_3Na$ and $C_2H_5SO_3K$).

RESULTS

Effect of calcium and magnesium ions on the membrane potential

The effect of calcium on the membrane potential, the amplitude and the maximum rates of rise and fall of the spike is shown in Fig. 1.

Excess calcium (5–25 mM) hyperpolarized the membrane and calcium deficiency (lower than 2·5 mM) depolarized it. The changes of the membrane potential were proportional to the logarithm of the calcium concentrations. The maximum slope of the membrane-potential change produced by a tenfold change of the external calcium concentration was 23·5 mV. This observation agrees qualitatively with reports on other smooth muscles as well as skeletal muscle (uterus, Marshall & Csapo, 1961; Kuriyama & Csapo, 1961; Csapo & Kuriyama, 1963; taenia coli, Holman, 1958; Bülbring & Kuriyama, 1963*a*; ureter, Bennett, Burnstock, Holman & Walker, 1962; skeletal muscle, del Castillo & Stark, 1952; Ishiko & Sato, 1960; Jenerick, 1959; Takeuchi, 1963).

In skeletal muscle, excess calcium reduces the sodium conductance in the resting state, but an effect on potassium conductance was less detectable. This means that excess calcium hyperpolarized the membrane towards the potassium equilibrium potential. In the vas deferens, the electrical parameters of the membrane properties have yet to be measured; at the moment we have no adequate techniques applicable to small cells. In spite of this difficulty, it may be possible to explain the hyperpolarization of the membrane produced by excess calcium. The ionic content of the smooth muscle cell (Na, K and Cl ions) was not affected by changes of

external calcium concentrations (R. Casteels, unpublished observation). Therefore the hyperpolarization of the membrane might be due either to a reduction of the sodium conductance or of the chloride conductance. The latter is unlikely, because, when the tissue was bathed in excess calcium in the presence of an anion less permeant than Cl, such as ethanesulphonate, the change of the membrane potential was nearly the same

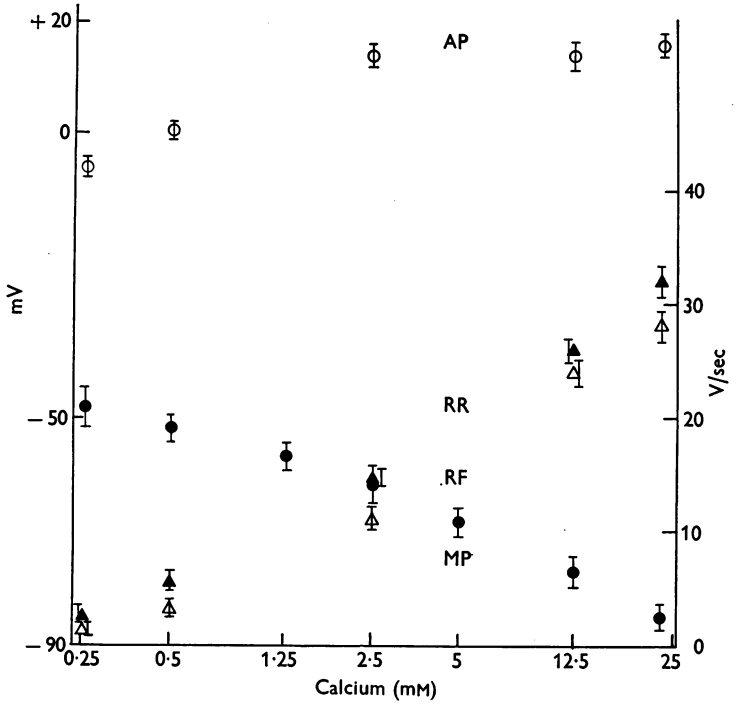

Fig. 1. The relation between the external calcium concentration (abscissa, log scale) and the membrane potential (●), the amplitude (○) and the rates of rise (▲) and fall (△) of the spikes, evoked by hypogastric nerve stimulation.

as that in the presence of chloride. The membrane potential was, in chloride solution, 61·5 mV (S.E. = ±4·4, n = 60) in 2·5 mM-Ca^{2+}, and 85 mV (S.E. = ±5·6, n = 30) in the 25 mM-Ca^{2+}. In ethanesulphonate solution the membrane potential was 59 mV (S.E. = ±5·4, n = 30) in the 2·5 mM-Ca^{2+}, and 80 mV (S.E. = ±5·1, n = 30) in 25 mM-Ca^{2+}.

The effect of magnesium on the membrane potential, the amplitude and the maximum rates of rise and fall of the spikes produced by hypogastric nerve stimulation are illustrated in Fig. 2. Magnesium had a similar, but smaller effect on the membrane compared with Ca. In the range of 0·6–3·6 mM (normal content was 1·2 mM), Mg produced no detectable change

in the membrane potential. The effect produced by a ten-times normal Mg concentration on the membrane potential and spike was comparable to that of three times normal Ca concentration.

Effect of calcium and magnesium on the spontaneous depolarization of the smooth muscle cell membrane

Spontaneous miniature post-synaptic potentials have now been recorded from many muscle fibres in different species (frog skeletal muscle, Fatt & Katz, 1952; insect skeletal muscle, Usherwood, 1963; crustacean muscle,

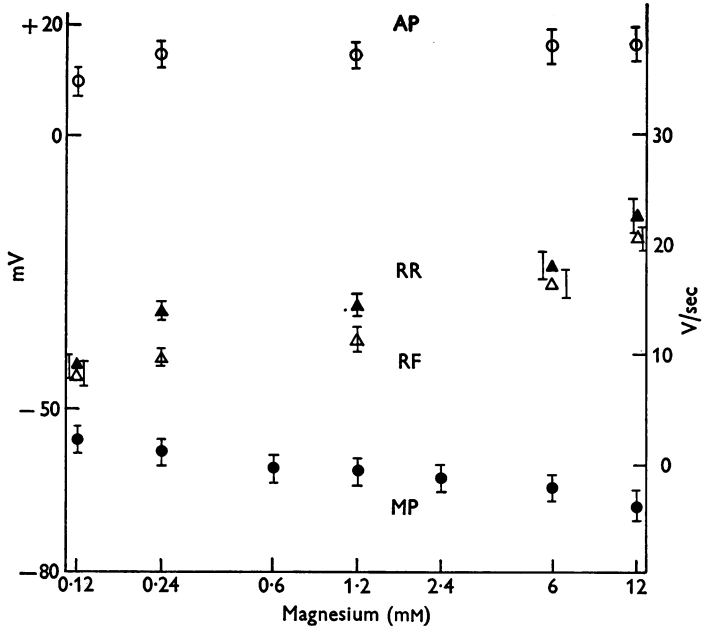

Fig. 2. The relation between the external magnesium concentration (abscissa, log scale) and the membrane potential (●), the amplitude (○), and the rates of rise (▲) and fall (△) of the spikes, evoked by hypogastric nerve stimulation.

Dudel & Orkand, 1960; Dudel & Kuffler, 1961; mammalian smooth muscle, Burnstock & Holman, 1961, Orlov, 1961, as well as in many cells of the central nervous system (see Katz & Miledi, 1963).

Burnstock & Holman (1962*a*) measured the parameters of the spontaneous depolarizations in the vas deferens. The mean amplitude obtained was 3·33 mV (greater than 1·5 mV). The majority of the depolarizations showed maximal rates of rise varying between 0·15 and 0·30 V/sec, and their appearance was random, the mean interval being 3·6 sec. The present experiments generally agreed with the above observations in the normal

solution. The amplitude of the spontaneous depolarizations varied from less than 1 to 17 mV and the frequencies from 0·04/sec to 0·78/sec at 36° C.

Figure 3 shows the effect of excess calcium (12·5 mM) on the spontaneous depolarizations. Initial acceleration was seen after 5 min exposure (*b*) and, after 10 min exposure (*c*), the spontaneous depolarizations were rare, but not abolished. In some experiments, after 30 min exposure to excess calcium (12·5 mM), bursts of spontaneous depolarizations occurred separated by long periods of silence (more than 1 min). In ten-times normal-calcium concentration (25 mM), the spontaneous depolarizations were

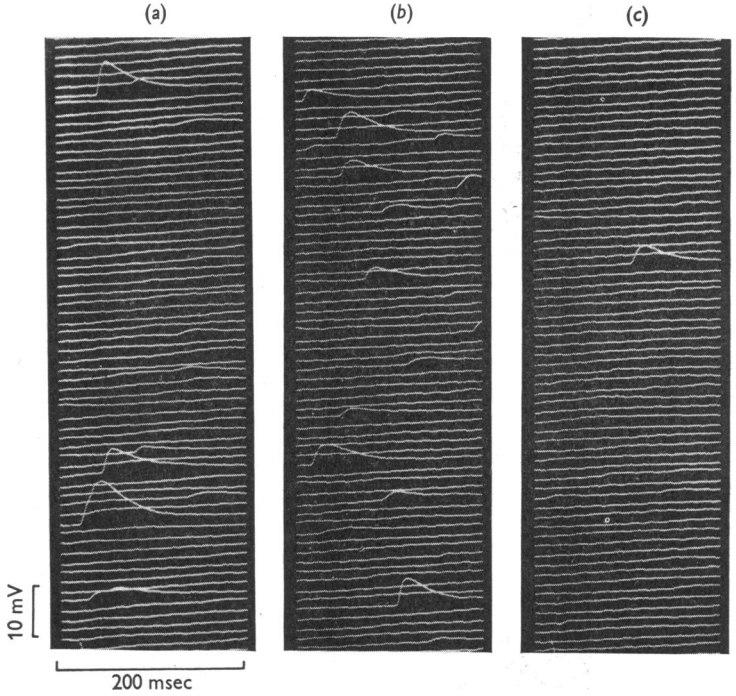

Fig. 3. Effect of excess calcium on the spontaneous depolarizations of the smooth muscle cell membrane. (*a*) control, 2·5 mM-Ca, (*b*) after 5 min in 12·5 mM-Ca, (*c*) after 10 min exposure.

rarely seen after 15 min exposure, but even then they were not completely abolished. Initial acceleration like that shown in Fig. 3 was observed in fourteen out of eighteen experiments. Low calcium (0·25 mM) always lowered the frequency, usually after 10 min exposure.

The histogram of the amplitude distribution of the spontaneous depolarizations (larger than 1 mV) is shown in Fig. 4*A*. The asymmetrical distribution has been described by Burnstock & Holman (1962*a*). This

distribution pattern clearly differs from that of the skeletal neuromuscular junction (Fatt & Katz, 1952), which shows a 'Gaussian' distribution about a mean value. The amplitudes of the spontaneous depolarizations in Fig. 4*A* were recorded from the same cell as those in Fig. 4*B*, showing that excess calcium changed the distribution; but the histogram remained skew.

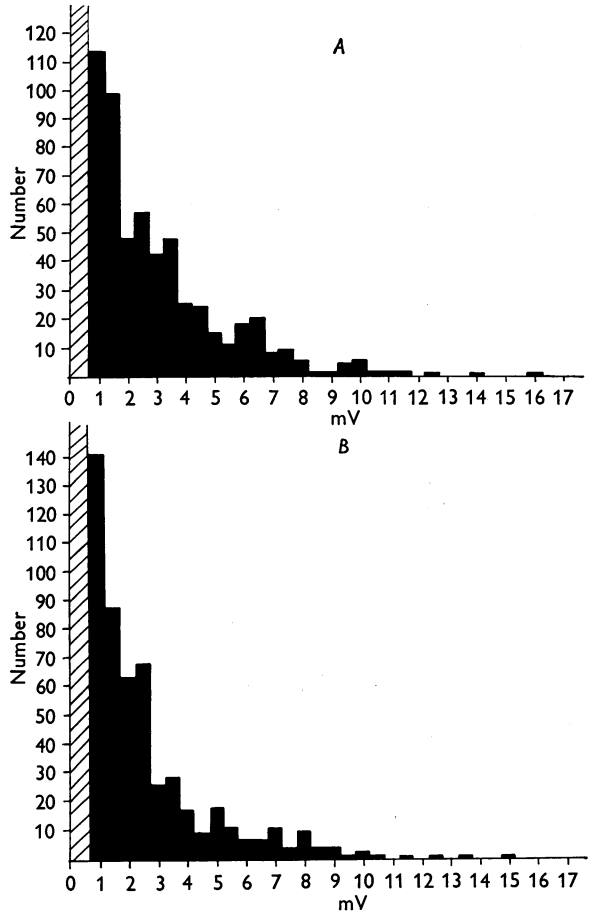

Fig. 4. Histograms of the amplitude distribution of the spontaneous depolarizations of the smooth muscle cell membrane in normal solution containing 2·5 mM-Ca (*A*) and in 12·5 mM-Ca (*B*). The micro-electrode was inserted in the same cell during this experiment.

Excess magnesium (3·6–12 mM) always slowed the frequency of the spontaneous depolarizations but did not abolish them completely. Low magnesium solutions (0·6–0·12 mM) did not always increase the frequency, only in five out of fourteen experiments.

The effect of calcium and magnesium on the junction potentials produced by hypogastric nerve and field stimulation

Effects of frequency, intensity and stimulus duration on the junction potentials. Submaximal stimulation of the hypogastric nerve produced small depolarizations of the post-synaptic smooth muscle cell membrane. These potentials were called 'junction potentials' by Burnstock & Holman (1961). Repetitive stimulation of the hypogastric nerve at more than

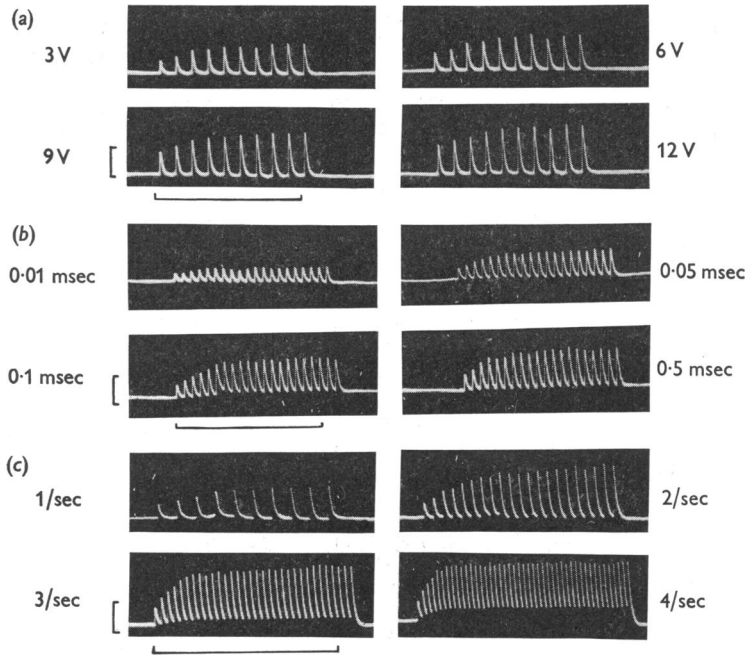

Fig. 5. Effects of varying (*a*) the intensity (duration 0·05 msec, frequency 1/sec), (*b*) the duration (intensity 7·5 V, frequency 1/sec), and (*c*) the frequency of stimulation (duration 0·05 msec, intensity 10 V) on the amplitude and the maximum depolarization after facilitation was complete. Note that the first junction potential became larger when the stimulus intensity and duration was increased. Markers throughout: 10 mV and 10 sec.

0·1/sec enhanced the amplitude of the junction potentials, i.e. facilitation occurred. When a junction potential reached the firing level, it triggered a spike.

Like the end-plate potential in skeletal muscle the amplitude of the junction potential was increased when the frequency of stimulation was increased (Fig. 5*c*). In addition, however, the amplitude was also increased

if the intensity or the duration of the stimuli was increased at a constant frequency (Fig. 5*a*, *b*).

Figure 6 shows the effect of changing the stimulus duration (0·01–5 msec) on the junction potential in the presence of 12·5 mM-calcium. These junction potentials were evoked by field stimulation (10 V, 1/sec). Since excess calcium increased the threshold for triggering the spike, the relation between the amplitude and the duration of the falling phase of the junction potential could be observed over a wider range than in normal solution.

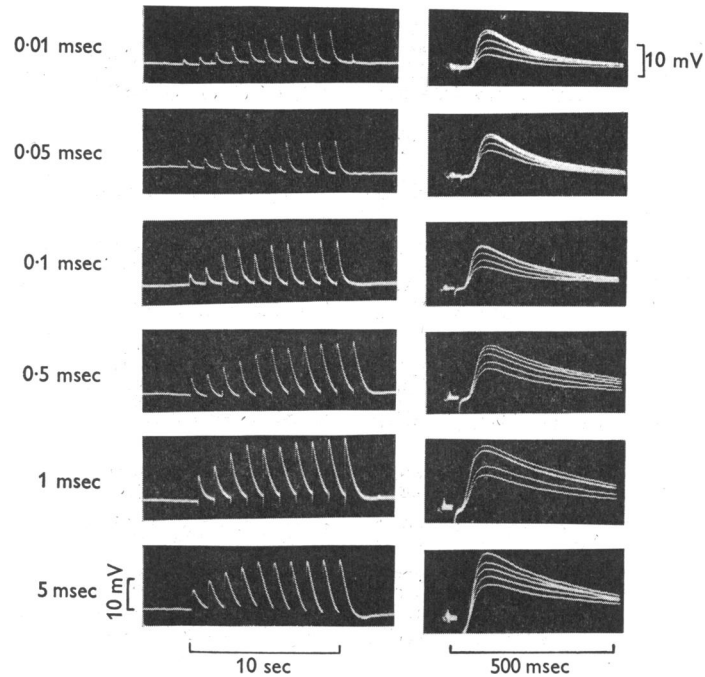

Fig. 6. Effect of increasing the stimulus duration (0·01–5·0 msec, 10 V, 1/sec) on the junction potentials produced by field stimulation in the presence of 12·5 mM-Ca. Left side; junction potentials produced by ten successive stimuli. Right side; superimposed junction potentials produced by five successive stimuli.

The amplitude of the junction potential was not further increased by prolonging the pulse duration beyond 1 msec, but the repolarization time continued to increase up to 5 msec pulse duration.

Interaction between the junction potentials and the spikes. Figure 7 shows various patterns of the spike generation superimposed on the junction potentials. When the amplitude of the junction potential reached a certain level, the local response further depolarized the membrane to trigger the spike. The spike was followed by a brief increase of the membrane potential

which 'wiped out' the junction potential (*a*). The junction potential then continued at a diminished amplitude (*a*, *b*). When the spikes were triggered by the propagation of excitation from neighbouring cells, the spikes were superimposed on the falling phase of the junction potential, and these did not 'wipe out' the junction potential (*c*). Figure 7*d* shows two further observations. First, the threshold to trigger the spike, became lower when

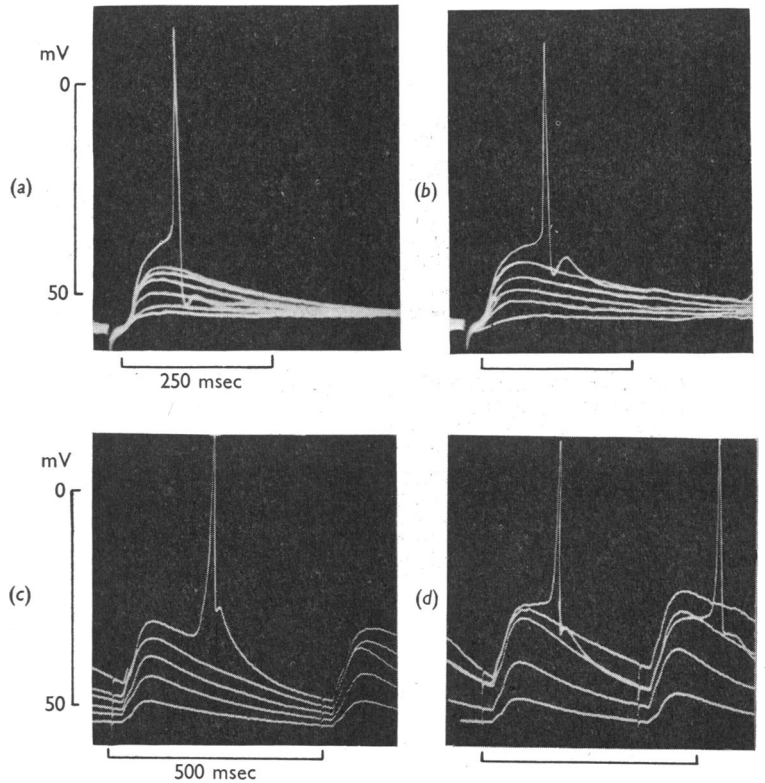

Fig. 7. Interactions between the spikes and the junction potentials produced by hypogastric nerve stimulation. The spikes which were triggered locally partially 'wiped out' the junction potential (*a*, *b*, *d*) but not the propagated spike (*c*). For further details, see text.

a successive spike was generated. Secondly, repetitive triggering of spikes suppressed the facilitation process. The first eight junction potentials increased progressively in size; the ninth triggered a spike and reduced the size of the tenth junction potential, which nevertheless triggered a second spike.

The fact that the depolarization following the 'wiping out' is always less than the level expected during the repolarization phase of the original

junction potential can be explained in two ways. It might be due either to the reduction of the transmitter concentration in the receptor fields during the active state of the membrane, or to the electrical shunt between the receptor membrane and the non-receptor membrane caused by the suddenly increased conductance of the entire smooth muscle cell membrane.

Effects of calcium and magnesium on the junction potential. Figure 8 shows the effect of excess calcium (12·5 mM) on the junction potentials produced by hypogastric nerve stimulation (0·5/sec, 0·1 msec, 10 V).

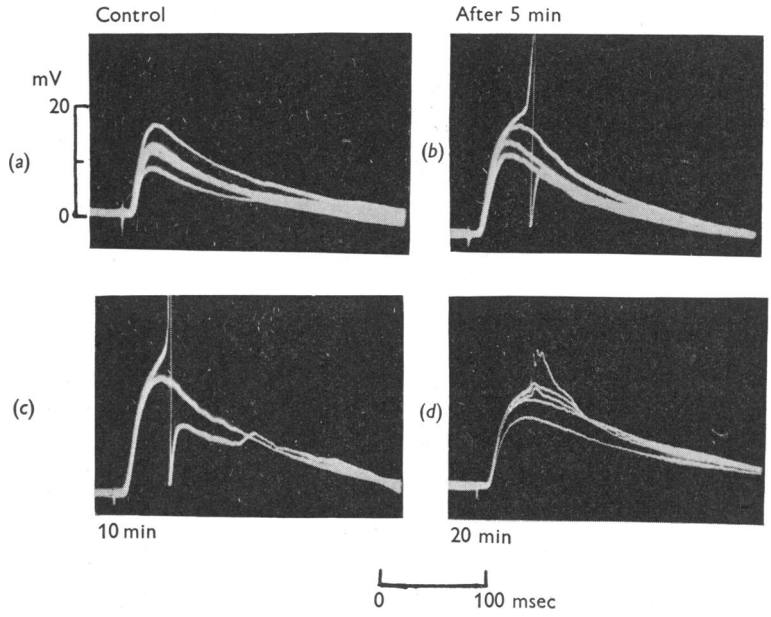

Fig. 8. Effect of excess calcium (12·5 mM) on the junction potentials produced by hypogastric nerve stimulation (0·1 msec, 10 V, 0·5 c/s). (*a*) control. (*b*)–(*d*) after exposure to excess calcium as indicated. Note 'wipe out' phenomenon in (*b*) and (*c*) and in (*d*) the abortive spike.

Excess calcium enhanced the amplitude of the junction potentials produced by the first stimulus, and repetitive stimulation enhanced it further. However, the effect changed during the time of exposure. Whereas, during the first 10 min of exposure the amplitude of the first junction potential continued to increase (*b*, *c*) it declined again during the following 10 min (*d*). The threshold for triggering the spike was greatly raised so that even when the membrane was depolarized by more than 20 mV no spike was triggered or only an abortive spike was seen. Excess calcium prolonged the falling phase of the junction potential, while calcium deficiency reduced it. No junction potentials could be produced at concentrations

below 0·5 mM-Ca. In excess calcium solution the spikes were also followed by the 'wipe out' phenomenon similar to that in normal solution (*b*, *c*).

Figure 9*a* shows that the amplitude and falling phase of the junction potentials produced by field stimuli (0·05 msec, 5 V) are a function of the external calcium concentrations (0·25–25 mM). However, the amplitude and the falling phase of the junction potential had a negative correlation with the external magnesium concentration in the range of 2·4–12 mM

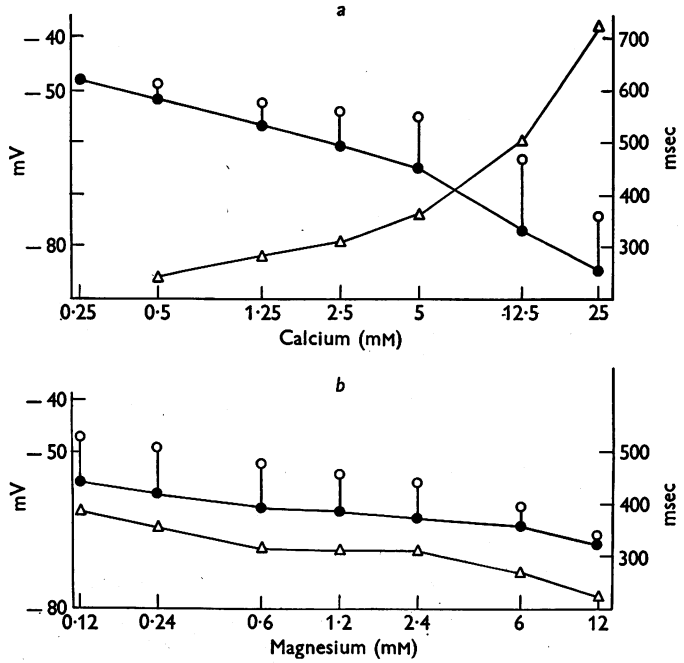

Fig. 9. The relation between the external calcium (*a*) and magnesium (*b*) concentration (abscissa, log scale) and the amplitude (open circles), the falling phase of the junction potentials produced by field stimulation (open triangles), and the membrane potential (filled circles).

(Fig. 9*b*). Excess magnesium (2·4–12 mM) reduced the amplitude and shortened the falling phase of the junction potentials without any remarkable change of the membrane potential. Low magnesium (less than 1·2 mM) did not, as expected, enhance the amplitude of the junction potentials; sometimes the amplitude was reduced, sometimes it became more irregular than in normal Krebs solution.

The results illustrated in Figs. 9*a* and *b* suggested that excess calcium and magnesium had an antagonistic action on the amplitude and the falling phase of the junction potentials in spite of their similar effect on the membrane potential. This was confirmed by the observation illustrated

in Fig. 10. The depression of the amplitude and shortening of the falling phase of the junction potential produced by excess magnesium (3·6 mM) was not seen when excess calcium (7·5 mM) was also present. The antagonism was, however, not complete, since the effect of low calcium (less than 1·25 mM) on the junction potential was not prevented by lowering the

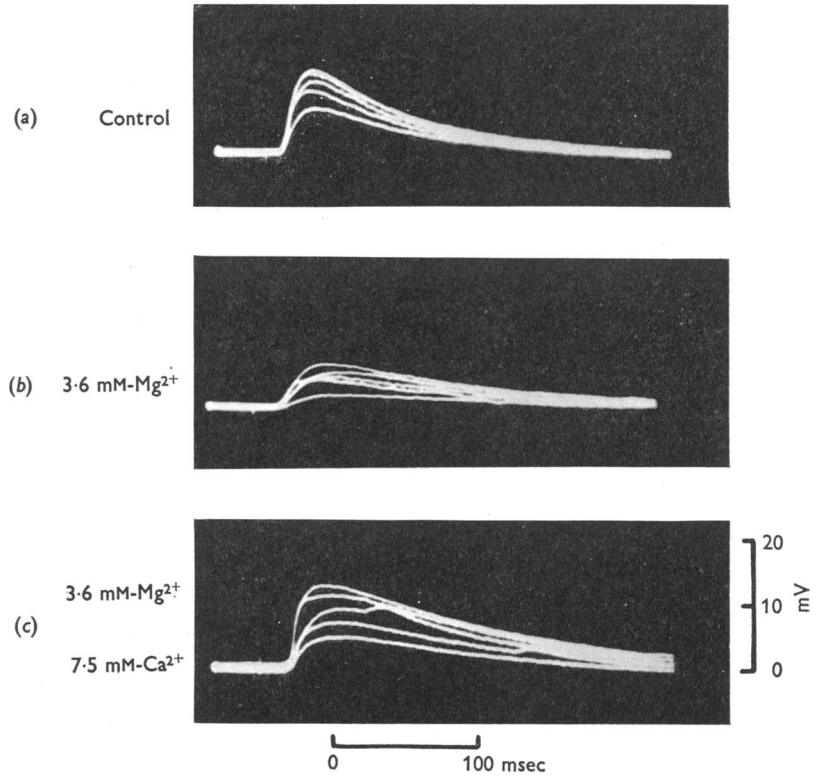

Fig. 10. Antagonistic action of excess magnesium (3·6 mM) and excess calcium (7·5 mM) on the amplitude and duration of the junction potentials produced by hypogastric nerve stimulation (0·05 msec, 5 V, 0·5 c/s). (*a*) Control. (*b*) Effect of excess magnesium. (*c*) Effect of excess magnesium and excess calcium. Note that the effect of excess magnesium was reversed by adding excess calcium, and that in excess magnesium the shape of the junction potential fluctuated.

magnesium concentration. Furthermore, in low calcium (0·5 mM) with high magnesium (7·5 mM) no junction potentials could be evoked by field or hypogastric nerve stimulation.

Figure 11 shows the effect of excess calcium (12·5 mM) in normal magnesium (*b*, *c*) and in low magnesium (0·12 mM) solutions, on the facilitation of the junction potentials produced by repetitive stimulation

of the hypogastric nerve (0·05 msec, 5 V, 1/sec). This figure shows the highest amplitude of the junction potential which has been observed in these experiments. In normal solution (*a*), the maximum depolarization appeared after nine successive stimuli, representing an increase from 1 to 11·5 mV. The enhancement ratio: (ninth amplitude/first amplitude) was therefore = 11·5. After 5 min exposure to excess calcium (*b*) the amplitude increased from 1·7 to 22 mV, the enhancement ratio being = 12·9. After 10 min exposure to excess calcium (*c*), the amplitude of the junction

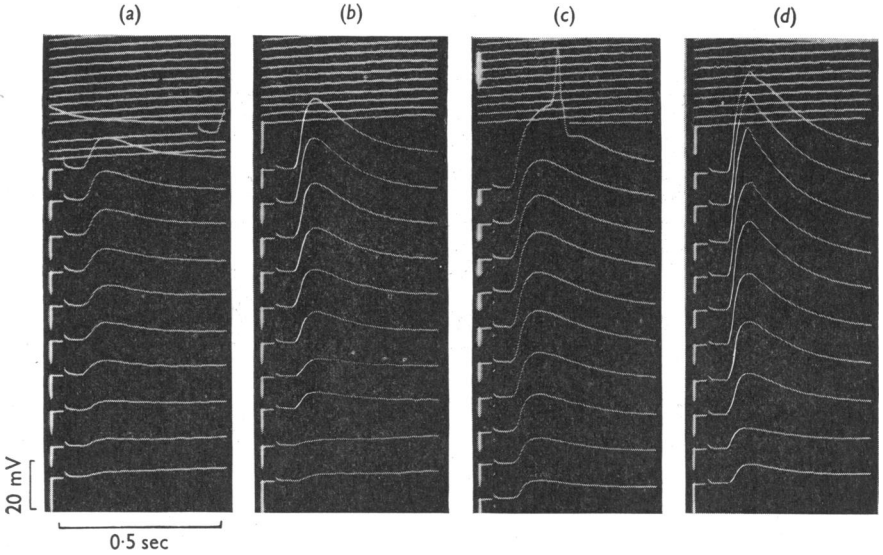

Fig. 11. Effect of excess calcium (12·5 mM), and excess calcium (12·5 mM) with low magnesium (0·12 mM) on the facilitation of the junction potentials produced by hypogastric nerve stimulation (0·05 msec, 5 V, 1 c/s). (*a*), control (2·5 mM-Ca); (*b*), 12·5 mM-Ca after 5 min; (*c*), 12·5 mM-Ca after 10 min; (*d*), 12·5 mM-Ca and 0·12 mM-Mg after 10 min.

potential increased from 6·3 to 42 mV, but the enhancement ratio was decreased to 6·6. The tenth junction potential generated an abortive spike, no overshoot potentials were observed at this time. (The amplitude of the spike from the resting membrane potential was 69 mV, the resting membrane potential being 72 mV.) After 10 min exposure to excess calcium with low magnesium (*d*), the amplitude of the junction potential was increased from 6·8 to 53 mV and the enhancement ratio was 7·8. The inability to trigger a spike is evident from the shapes of the junction potentials.

Effects of hyperosmotic solution and excess chloride. Hyperosmotic solutions were used for comparison with the excess calcium and magnesium solutions which were prepared as hyperosmotic solutions as described in

the method. Three different hyperosmotic solutions were prepared: (1·25 times, 1·5 times and 2 times) by adding solid sucrose to the normal solution. Ten-times normal calcium solution (25 mM) corresponded to 1·25 times hyperosmotic solution.

A 1·25-times hyperosmotic solution increased the membrane potential from 59 to 63 mV in the three experiments. Twice hyperosmotic solution decreased it from 59 to 56 mV. Both hyperosmotic solutions enhanced the amplitude and prolonged the falling phase of the junction potentials produced by hypogastric nerve stimulation. The amplitude of the junction

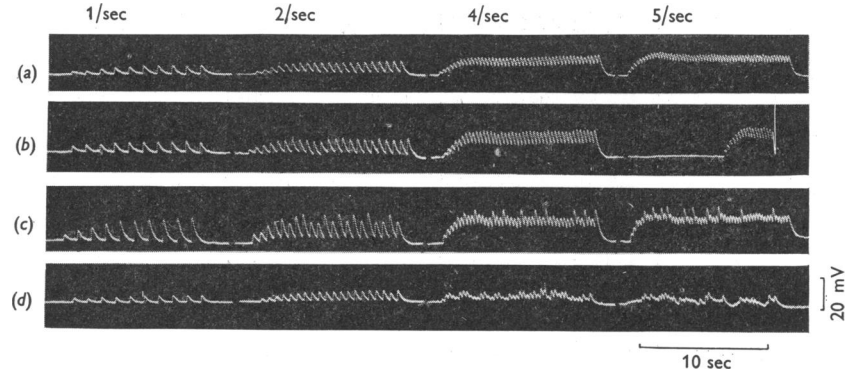

Fig. 12. Effect of hyperosmotic solution on the junction potentials produced by hypogastric nerve stimulation at different frequencies of stimulation (0·01 msec, 5 V) applied for 10 sec. (*a*) normal solution, (*b*) 1·5 times normal tonicity by adding solid sucrose. (*c*) excess calcium (7·5 mM) in hyperosmotic solution 1·25 times. (*d*) low calcium (0·5 mM) in hyperosmotic solution 1·5 times. For details see text.

potential produced by single stimuli (0·05 msec, 5 V) was increased after 15 min exposure from 5 mV to 6, 7·2 and 8 mV respectively, in the 1·25, 1·5 and 2-times hyperosmotic solutions. The falling phase of the junction potential was prolonged from 290 to 310, 340, 380 msec respectively in the three different solutions.

Figure 12 shows the facilitation produced by increasing the frequency of nerve stimulation (0·01 msec, 5 V) in normal solution (*a*), in hyperosmotic solution (1·5 times) (*b*), in excess calcium (7·5 mM) (*c*) and in low calcium (0·5 mM) in the same hyperosmotic solution (*d*). The hyperosmotic solution (1·5 times) increased the amplitude of the junction potentials and the maximum depolarization and hence triggered the spike (*b*). Excess calcium in the hyperosmotic solution further increased the amplitudes of the junction potentials, but the amplitude fluctuated at higher frequencies (> 2/sec) in a stepwise fashion. The threshold to trigger the spike, however, was increased (*c*). Reduced calcium in the hyperosmotic

solution lowered the amplitude of the junction potentials, and fluctuations of the amplitude were also observed at the higher frequencies of stimulation (> 2/sec). The occasional failure of the generation of junction potentials brought the facilitation back to the start (*d*).

The hyperosmotic solutions (1·5–2 times) did not increase the frequency of the spontaneous depolarizations of the membrane.

The reason for investigating the effect of excess chloride solution (45 mM) was that the excess calcium solution (25 mM) not only increased the osmoticity but also increased the chloride concentration. However, no detectable change of the membrane potential nor of the amplitude of the junction potentials was observed.

Effect of calcium and magnesium on the spikes

The amplitude and the maximum rates of rise and fall were measured from spikes triggered by field and hypogastric-nerve stimulation. The intensity, pulse duration and frequency of the stimulus were increased until they triggered spikes in different calcium concentrations. The effect of calcium and magnesium on the amplitude and rates of rise and fall of the spike is shown in Figs. 1 and 2. In excess calcium (12·5 mM) the amplitude of the action potential increased from 76·5 to 87·5 mV (mean overshoot potential 15·5 mV, S.E. = $\pm$ 2·6, $n = 30$). The most significant effect of calcium appeared on the maximum rates of rise and fall of the spikes. It is generally believed that the maximum rate of rise indicates the velocity of the sodium influx (nerve fibre, Hodgkin & Huxley, 1952; cardiac muscle, Weidmann, 1956; skeletal muscle, Ishiko & Sato, 1960). In the skeletal muscle (Ishiko & Sato, 1960), a ten-times increase in Ca concentration reduced the overshoot potential and the maximum rate of rise of the spike. However, in the vas deferens, the overshoot potential was increased from 14·6 mV (S.E. = $\pm$ 2·1, $n = 65$) to 18 mV (S.E. = $\pm$ 2·8, $n = 30$) and the maximum rates of rise of the spike from 14·5 V/sec (S.E. = 1·8, $n = 30$) to 32·2 V/sec (S.E. = $\pm$ 2·2, $n = 30$), when the external calcium concentration was raised to 25 mM. This difference between smooth and skeletal muscle might be due to the difference in membrane potential which in the normal solution is 61·5 mV $\pm$ 4·4 in the vas deferens and 96 mV $\pm$ 1·07 in frog muscle.

DISCUSSION

Spontaneous depolarizations

The spontaneous depolarizations of the smooth-muscle cell membrane are likely to be due to the release of chemical transmitter from the presynaptic site. Supporting evidence comes from the observations by

Burnstock & Holman (1962*b*) who found that partial denervation by cutting the hypogastric nerve, or chronic reserpine treatment, reduced the frequency of these potentials, and that the amplitude histograms for individual cells from partially denervated preparations differed in shape. Furthermore, in the present investigation it was found that excess calcium and magnesium (10 times normal) which raised the threshold of excitation by nerve stimulation, did not abolish the spontaneous depolarizations. The following questions remain unsolved: whether the chemical transmitter is released in 'quanta', and whether the chemical transmitter is released from nerve terminals or from an 'autonomic ground plexus' (Hillarp, 1959), i.e. a fine mesh of non-myelinated nerve fibres, in part enveloped by a Schwann syncytium, but in part incompletely covered by Schwann cells and in these regions packed with vesicles and mitochondria (Richardson, 1958, 1962; Burnstock & Holman, 1963).

In those regions which are not covered by Schwann cells the space between the nerve membrane and the muscle membrane is 200–300 Å (Burnstock & Holman, 1963). Since the duration of the rising phase of the spontaneous depolarizations varies widely (5–35 msec), they may not only be due to the release of chemical transmitter from adjacent nerve fibres, but also to diffusion of chemical transmitter from more distant places, and to passive electrotonic spread of excitation from neighbouring cells. This is unlikely since three successful impalements with two microelectrodes which were less than 50μ apart, did not indicate any causal relation between the generation of the spontaneous depolarization of adjacent cells (unpublished observations).

The asymmetrical amplitude distribution in the histogram of the spontaneous depolarizations resembles that of the slow muscle fibres of the frog (Burke, 1957), neurone soma of the frog (Katz & Miledi, 1963), insect muscle (Usherwood, 1963) and crustacean striated muscle fibres (Dudel & Kuffler, 1961). This is in striking contrast to frog skeletal muscle (Fatt & Katz, 1952), and indicates that the release is not from the same focus, as has been discussed by Burnstock & Holman (1962). Moreover, the amplitude of the spontaneous depolarizations is often higher than that of the junction potentials and it is likely that the chemical transmitter is released in many places.

An explanation for the large variation in amplitude (less than 1 mV to more than 15 mV) might be derived from the membrane properties of the muscle cell. The amplitude of the miniature end-plate potential in skeletal muscle depends on the 'input' resistance of the muscle cell membrane (Katz & Thesleff, 1957). It might be assumed that a long, thin, spindle-shaped smooth muscle cell (100 × 5μ) has a higher 'input' resistance than a skeletal muscle cell (mammalian skeletal muscle 1–2 MΩ (Axelsson &

Thesleff, 1959); frog skeletal muscle 0·2–10 MΩ according to the fibre diameter from 140–10μ (Katz & Thesleff, 1957); neurone soma 1–2 MΩ (Rall, 1959); cat intestinal circular muscle 70 MΩ (Nagai & Prosser, 1963)). In addition, Nagai & Prosser (1963) have found the space constant in cat intestinal muscle to be 1 mm. The receptors may be distributed over the whole cell surface and these parts may have an even higher resistance than the non-receptor regions, as postulated for skeletal muscle (Axelsson & Thesleff, 1959). Recently, Katz (1962) emphasized that in skeletal muscle the frequency of the miniature end-plate potentials is controlled entirely by the conditions of the presynaptic membrane, while their amplitude is controlled by the properties of the post-synaptic membrane. Therefore it is possible that the widely scattered values of the amplitude are the consequence of the non-homogeneous electrical properties of the smooth muscle cell membrane.

Junction potentials

The rising and falling phases of the junction potential were much longer than those of the end-plate potential, but they resembled the prolonged end-plate potential in the presence of a cholinesterase inhibitor, prostigmine (Fatt & Katz, 1951), lasting for more than 200 msec. Brown & Gillespie (1957) showed that noradrenaline, released during sympathetic stimulation of cat spleen, is destroyed in about 100 msec, and in cat intestine the destruction of transmitter might be even slower (Brown, Davies & Gillespie, 1958).

The effect of calcium and magnesium on the junction potential appeared to be the same as that on the end-plate potential (del Castillo & Engbaek, 1954; del Castillo & Katz, 1954), i.e. excess calcium enhanced the amplitude and prolonged the falling phase of the junction potential, and excess magnesium had the opposite action. Moreover, the effect of excess magnesium on the junction potential was reversed by adding excess calcium. Therefore, it might be assumed that calcium increases the transmitter output during nerve stimulation and magnesium interferes with the release of the transmitter, as postulated for the neuromuscular transmission in skeletal muscle (Katz, 1962).

del Castillo & Katz (1954) observed that when the skeletal muscle was soaked in high magnesium and low calcium, the end-plate potential could be reduced to that of the miniature end-plate potential. In the vas deferens, low calcium and high magnesium also decreased the amplitude of the junction potential and produced stepwise fluctuations in size. The interpretation of these observations may be that the release takes place in 'quanta'. However, since the amplitudes of both the spontaneous depolarizations and the junction potential varied over a wide range and

since the junction potentials were evoked by submaximal stimulation, activating only a part of the multiple nerve fibres which innervate a muscle cell, no clear causal relation between the spontaneous depolarizations and the junction potential could be established.

SUMMARY

1. The effect of calcium (0·25–25 mM) and magnesium (0·12–12 mM) on the mechanism of neuromuscular transmission of excitation was studied in the isolated hypogastric nerve–vas deferens preparation of the guinea-pig.

2. *Membrane potential.* Excess calcium hyperpolarized and low calcium depolarized the membrane. The maximum potential change produced by a tenfold change of the external calcium concentration was 23·5 mV. Magnesium had a similar but smaller effect.

3. *Spontaneous depolarizations.* Excess calcium initially increased the frequency in fourteen out of eighteen experiments and then decreased it. The spontaneous depolarizations were, however, never abolished completely. Low calcium reduced the frequency. The frequency was reduced both by excess and by low magnesium. At low magnesium concentrations this was preceded by acceleration.

4. *Junction potential.* Increasing the intensity and duration of the nerve stimulus enhanced the amplitude and the duration of the junction potentials. Increasing the frequency of stimulation (> 1/sec) only enhanced the amplitude. Excess calcium enhanced the amplitude and prolonged the falling phase of the junction potentials. Excess magnesium had the opposite action. The effect of excess magnesium was reversed by adding excess calcium.

5. *Action potential.* Excess calcium enhanced the amplitude and the rates of rise and fall of the spikes. Low calcium reduced them. The effects of magnesium were similar but smaller.

6. The view is discussed that the neuromuscular-transmission mechanism and the effects of calcium and magnesium on the release of the chemical transmitter in this smooth muscle may be fundamentally the same as in skeletal muscle, and that differences may be brought about by the presence of multiple receptor regions diffusely distributed over the whole cell membrane.

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