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EFFECTS OF CALCIUM DEFICIENCY ON STRIATED MUSCLE OF THE FROG

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The behaviour of striated muscle in a solution deficient in calcium shows certain similarities with the normal behaviour of the smooth muscle of the intestine. It exhibits spontaneous rhythmic activity (see Mines, 1908); it responds, like a sensory organ, to pressure and to stretch by an increased discharge of impulses (Adrian & Gelfan, 1933) and it is hypersensitive to chemical stimuli (Kuffler, 1945), for example it can be stimulated by histamine (Bülbring, 1955*b*). The present investigation was undertaken in order to find out whether the spontaneous rhythmic activity was accompanied, or possibly preceded, by a fall in membrane potential, and whether a similar correlation could be established between tension and membrane potential and the frequency of electrical activity as has been described recently for normal smooth muscle (Bülbring, 1955*a*).

METHODS

Isolated striated muscle preparations of the frog (*Rana temporaria*) were used, mostly m. iliofibularis and m. sartorius, occasionally m. rectus abdominis and m. tibialis posterior. All muscles showed rhythmic contractions when immersed in calcium-deficient solutions. The 'normal' medium was Krebs's solution in which the sodium bicarbonate was reduced to half; of this solution 1 part was diluted to 1·35 with distilled water. The composition was as follows: NaCl 127 mM; KCl 4·5 mM; $CaCl_2$ 2·5 mM; $NaHCO_3$ 11·5 mM; NaH_2PO_4 1·1 mM; glucose 11 mM; equilibrated with 95% O_2 + 5% CO_2 at room temperature; pH 6·8. The calcium-deficient medium used for most experiments contained only 1/20 of the normal amount of $CaCl_2$ and 1/20 of the normal amount of KCl. This solution was preferred to 0·6% NaCl, or to a solution which contained no $CaCl_2$, or to a medium in which the calcium was reduced by addition of oxalate or citrate, because the muscles continued to be rhythmically active over periods of many hours. All preparations were fully curarized by adding to the bathing solution tubocurarine in a concentration of 5×10^{-6} or 10^{-5}. The solution flowed at a constant rate of 1–2 ml./min. All experiments were carried out at room temperature.

Tension was recorded isometrically with a mechano-electronic transducer valve (RCA 5734) (Talbot, Lilienthal, Beser & Reynolds, 1951).

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Extracellular electrical recording. The muscle lay on a convex support and was superfused from a small capillary, the fluid making continuous contact with the centre of the preparation which was at earth potential. The solution flowed down to both ends of the muscle, where two small cotton-wick recording electrodes made contact. These were connected to an a.c. amplifier by Ringer–Agar bridges and Ag/AgCl electrodes.

Intracellular electrical recording. The muscle was immersed in the bathing solution, and glass capillary electrodes, filled with 3 M-KCl, were used. The outer tip diameter was less than 0·5 μ, the resistance varied from 12 to 21 MΩ. A conventional cathode follower input stage was used (input valve ME 1400), the silver chlorided silver wire making direct contact with the valve grid cap. The input capacity was about 2–3 pF.

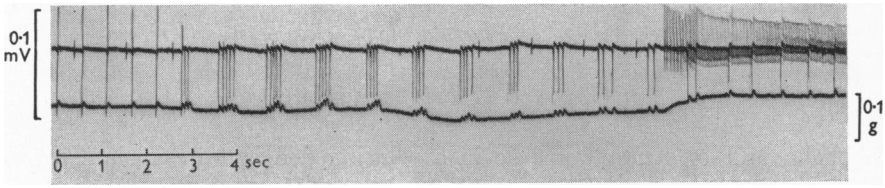


Fig. 1. Frog, m. iliofibularis. $\frac{1}{20}$ Ca^{2+} + $\frac{1}{20}$ K^{+}. Top, external electrical recording; bottom, mechanical recording of spontaneous activity.

RESULTS

Spontaneous rhythmic activity

The skeletal muscle of the frog became spontaneously active when the normal solution was changed to the solution containing only one-twentieth of the normal amount of $CaCl_2$ and of KCl. Observing the muscle microscopically, it was noticed that not all fibres were active at the same time, and that they did not contract synchronously. Activity usually began in small groups of fibres and, after some minutes, spread to those in the neighbourhood. Sometimes the activity took the well-known form of regular twitches like those recorded by Mines (1908), which have often been compared with the heart beat. Sometimes the pattern of the rhythmic activity underwent frequent changes. On other occasions the activity was quite irregular. Periods of inactivity occurred from time to time in all preparations; but most muscles continued to be active, with varying regularity and varying frequency, for 4 hr or longer. An example of rapid changes in the rhythmic pattern is shown in Fig. 1. In this record contractions can be seen, passing from single twitches to groups of twitches, then to a tetanus on which were superimposed the slower single twitches. The surface records of the electrical activity accompanying these tension changes also ranged from single action potentials to groups of action potentials, occurring rhythmically, which gave way to a burst at high frequency corresponding to the tetanus. Examples of more regular rhythms are shown in Figs. 10 and 12. In other records (e.g. Figs. 11 and 13) the activity was quite irregular.

The resting potential

In order to determine any changes in resting potential which might lead to spontaneous activity, many observations were made in which the normal solution was replaced by a solution deficient in calcium and potassium, while a microelectrode was inserted inside a muscle fibre. It was found that in some fibres the potential fell, but in others it rose. For example, in one fibre the resting potential was 90 mV at the time when the solution was changed. During the first 5 min in the solution deficient in Ca and K the resting potential rose to 95 mV, in the next 5 min to 106 mV, and in the following 4 min to 112 mV. At this time other fibres at the edge of the preparation were already spontaneously active. It was sometimes possible to keep the fibres successfully impaled and to observe that the resting potential was either maintained at a high level of about 115 mV for 10–20 min (no fibre was observed for a longer period), or that it began to fall. Such fibres remained inactive until the resting potential fell below 90 mV, or until 'slow waves' developed which will be described below. Activity continued until the resting potential had fallen to about 50 mV. Below this level the fibre became inactive though the resting potential continued to fall. It was evident that the values for resting potentials of muscle fibres in calcium-deficient solution varied over a wide range. When m. iliofibularis was used, it was, moreover, not always possible to distinguish between 'slow' and 'quick' fibres. The m. sartorius was therefore used to obtain results which could be treated statistically. In several experiments the following procedure was adopted. About 100 resting potentials were determined in normal solution, which was then replaced by the test solution. Measurements were continued in this solution for $2\frac{1}{2}$ hr. Fig. 2 shows the results of all experiments presented as a scatter diagram. Section (*a*) shows the resting potentials in normal solutions; (*b*) in a solution deficient both in calcium and in potassium; and (*c*) when only one of the two ions was reduced. The mean resting potential of all observations in normal solutions was 84·6 mV, S.E. of mean $\pm$ 0·3 (586 observations.) (The potassium concentration was 4·5 mM instead of the usual 2·5 mM; Fenn, 1936). A great increase in the spread of the values occurred when both calcium and potassium were reduced. The mean resting potential was at first not diminished but it fell with time, the important change being the greater variation. The mean value of all fibres measured during the first hour was 101·05 mV, S.E. of mean $\pm$ 1·26 (233 observations), and the mean of the measurements during the next 90 min was 91·9 mV, S.E. of mean $\pm$ 1·19 (270 observations). The ratio of the number of fibres with resting potentials above 85 mV to those below was in the first 30 min 5·4 : 1, and fell during successive 30 min periods to 4·8, 3·9, 2·1 and 1·1 in the fifth period. The regression line was calculated as $y = 109{\cdot}14$ mV $- 0{\cdot}181\,x$. (y = mV, x = time in min). When K^+ only was reduced to one-twentieth of the normal

amount, the mean resting potential rose to 123 mV, S.E. of mean ±0·2 (166 observations). The spread was not increased and there was no spontaneous activity. When Ca^{2+} only was reduced to one-twentieth of the normal amount the resting potential fell gradually and the spread was greater. The mean of the membrane potentials measured during the first hour was 71·62 mV, S.E. of mean ±1·54 (69 observations); during the next 30 min it was 63·46 mV, S.E. ±2·59 (49 observations). The regression line was calculated as $y = 75{\cdot}32\ \text{mV} - 0{\cdot}147x$. Spontaneous activity started immediately after the solution was changed but it was not well maintained. Most of the fibres near the surface ceased to contract after 30 min.

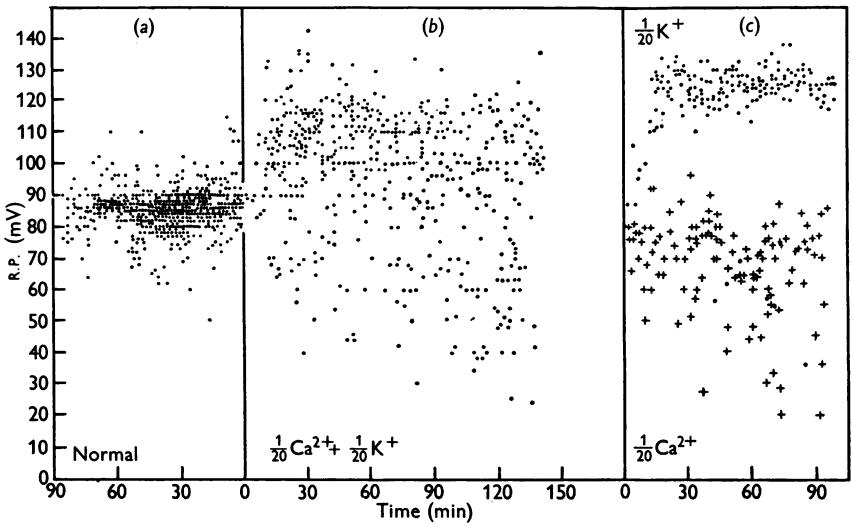


Fig. 2. Frog, m. sartorius. Scatter diagram of resting potentials (ordinate) measured in (*a*) normal solution, (*b*) deficient in Ca^{2+} and in K^+, (*c*) deficient in K^+ (dots), deficient in Ca^{2+} (crosses): plotted against time (abscissa).

Potential changes during spontaneous activity

(*a*) *Slow waves* of depolarization lasting about 100 msec were frequently recorded. They occurred with high as well as low membrane potential. They occurred both as discrete events (Fig. 3*a*) or they were oscillatory (Fig. 3*b*). Usually the slow waves became progressively greater until, at the peak of the depolarization, one or several (Fig. 3*c*) spike potentials arose. This did not, however, occur in fibres with a resting potential above 100 mV. The degree of depolarization during a slow wave at which a spike potential was set up was generally constant, within 5 mV, for an individual fibre, but differed from other fibres in the same preparation. Fig. 4 (upper part) shows the record of a fibre with 83 mV resting potential. Spike potentials arose when the slow wave depolarization exceeded 10 mV, but not if it was less. The lower record

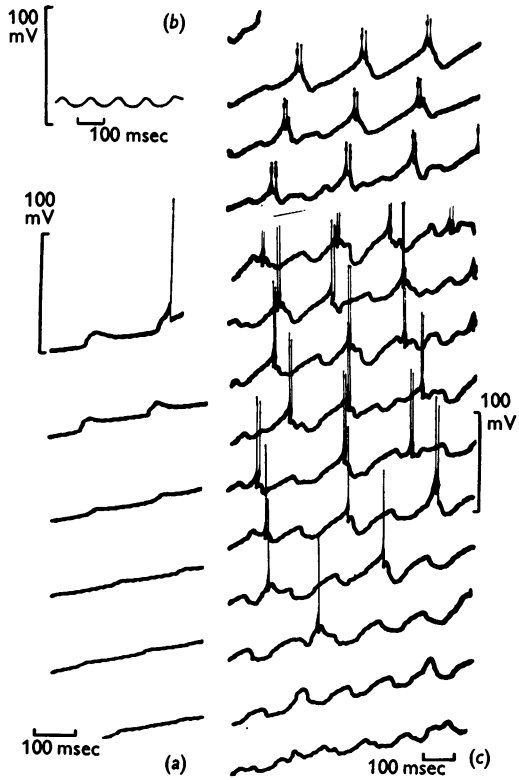


Fig. 3. Intracellular recording. Slow waves (*a*), oscillatory (*b*), or gradually increasing in size (*a* and *c*) until they gave rise to one or to several action potentials. Read from below upwards.

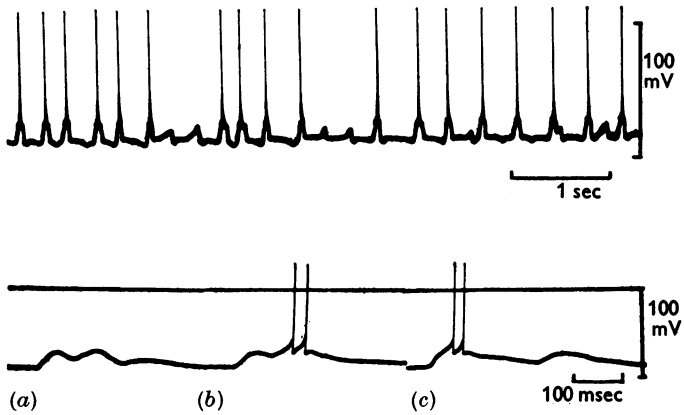


Fig. 4. Intracellular recording. Slow waves and spike potentials arising at a critical height of the slow depolarization.

was taken from another experiment using a faster time-base. The resting potential was 85 mV. In (*a*) there were two slow waves, but no spike potentials; in (*b*) the second wave gave rise to two spike potentials; in (*c*) the spike potentials arose from the first, but not from the second wave. Slow waves, without spike potentials, sometimes continued for several minutes with no change in the mean resting potential. They often appeared to coincide with the contractions of neighbouring fibres.

(*b*) *Spike potentials* without any preceding slow potential changes were only recorded from fibres with high resting potentials of about 90 mV (Fig. 5*a*). The rate of such a discharge was slow (1–10 per sec) and often irregular. As the resting potential fell the slow depolarization preceding the spike potential became more and more pronounced (Fig. 5*b*, *c*). This led to fluctuations, examples of which are shown in Fig. 6. As a rule, the frequency of discharge became faster as the resting potential declined. This is shown in Fig. 7, in which additional spike potentials were initiated by slow waves which had gradually appeared in the intervals between spikes. However, when the potential fell to the critical level below 60 mV the spike discharge often became slower before it ceased altogether. It must be emphasized that the resting potential of any one fibre did not necessarily decline progressively but often fluctuated. If the fibre was active, depolarization was accompanied by a higher frequency of spike discharge, while the rate of spike discharge became slower during repolarization (see Fig. 8). Fibres with resting potentials between 85 and 65 mV showed spontaneous activity of all frequencies, with and without slow waves. At frequencies below 20/sec the activity was often very regular over many minutes. Higher frequencies (20–100/sec), which were usually coincident with low resting potentials, occurred in shorter bursts. The spike potentials arising from high resting potentials were not different from normal action potentials (see Fig. 5*a*), showing an overshoot of 25 mV. As the resting potentials became lower the action potentials became smaller and finally reached only partial depolarization (e.g. Fig. 7). Not only the height of the action potentials but also the rate of rise was variable. A very common finding was an initial slow depolarization from which the spike started; this became more marked as the resting potential fell (note Fig. 8*d*). Action potentials arising at low membrane potentials showed some after-positivity which increased as the resting potential fell and was most pronounced when the action potential occurred at the peak of a slow wave. This is shown in Fig. 9 in which the action potentials produced by electrical stimulation (upper record) were compared with a spontaneous discharge (lower record) occurring in this preparation. The 'prepotential' and the after-positivity, characteristic for the spontaneous discharge, were both absent when the muscle was stimulated electrically.

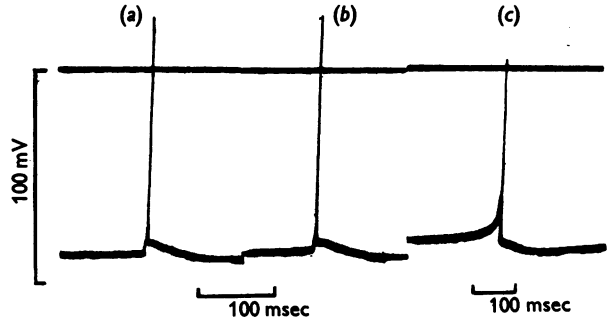


Fig. 5. Three intracellular records from the same fibre. (*a*) no prepotential; (*b*) first indication of prepotential; (*c*) prepotential and after-positivity (slower time base), reduced overshoot.

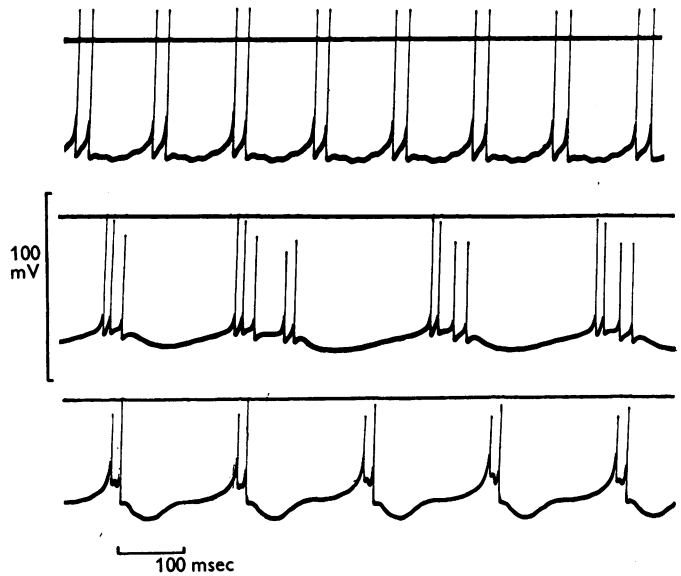


Fig. 6. Intracellular recording from different fibres. Examples of slow depolarization before and hyperpolarization after the spike potentials.

The effect of stretch

It is well known that, in calcium-deficient skeletal muscle, stretch and other mechanical stimuli increase the rate of spontaneous activity, and we were always able to observe this effect microscopically. Fig. 10 shows an example of a muscle with a very regular rhythm of twitches occurring at a rate of 48/min. This rate was increased in response to stretch which raised the tension by 0·1 g. Several experiments were carried out to see whether the rate of activity was proportional to the tension. In some experiments tension only

was recorded, in others electrical records were also obtained using surface electrodes. Stretch was applied in steps every 2 min. Within a small range, when the total increase in length was not more than 2 mm, a graded increase in the rate of impulse discharge was observed in response to a graded increase

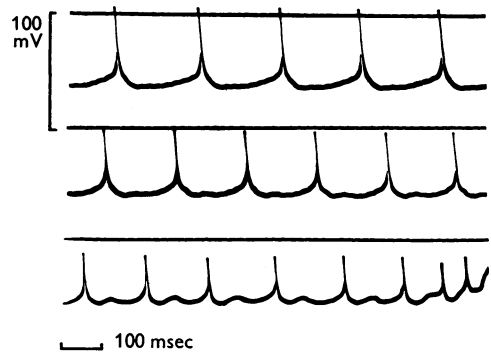


Fig. 7. Intracellular recording; gradually appearing slow waves which in third record give rise to spike before dislodgement of electrode. Note that spikes produced only partial depolarization when membrane potential declined.

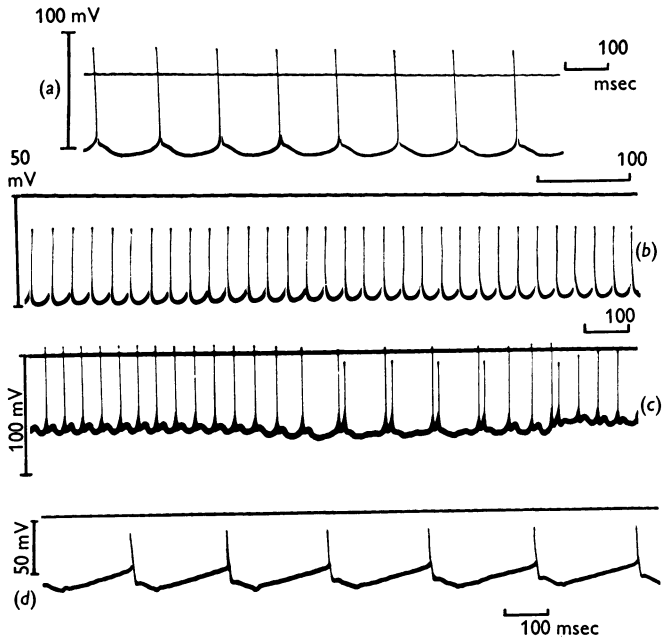


Fig. 8. Intracellular recording. Examples of slow discharge (*a*) at high membrane potential and fast rate (*b*) at low potential. Fluctuation in (*c*). Gradual depolarization preceding each spike (*d*); record obtained after 20 μg histamine.

in tension. The results of seven experiments are summarized in Table 1. It was found that excessive stretch had the opposite effect, causing slowing (Expt. 7) or cessation of activity which would then start again when the pull was released. It will be noticed that in Expt. 5 the rate of activity was only increased in response to the first stretch, while it was slowed the second and third time. The record shown in Fig. 11 is taken from this experiment and illustrates the observation that sometimes the response to stretch consisted in a greater synchronization. The irregular state of activity in Fig. 11*a*, *b* was changed to

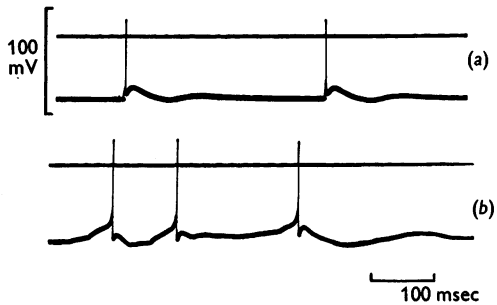
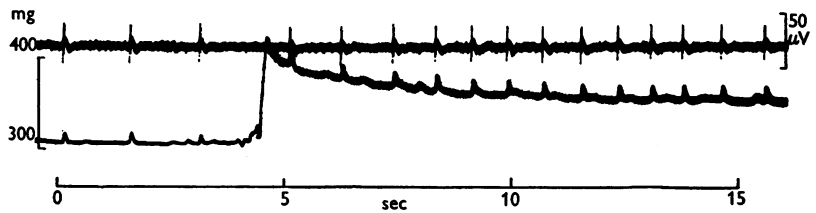


Fig. 9. Intracellular recording: (*a*) response to electrical stimuli; (*b*) spontaneous discharge.



10. Records as in Fig. 1. Response to stretch.

a rhythm of synchronized contractions of greater strength in Fig. 11*c*, *d*. These occurred at a frequency which was similar to that of the dominant fibres participating in the earlier twitching. The synchronization could easily be observed with the microscope: whereas at first only some fibres showed a regular rhythm, after the stretch a larger number joined in. A third but only momentary effect of stretch which was also seen consisted in an immediate burst of activity, often resulting in a tetanus, following which no measurable change of activity was detectable.

Since it was not possible to keep the same fibre impaled while the muscle was stretched, only a few records were obtained with intracellular electrodes. Two experiments in which different fibres were sampled, before and after stretch, indicated that there was a fall in resting potential. However, in view of the possibility that the resting potential of the fibres may gradually fall, and of the great variation which was encountered without disturbing the muscle, no

conclusive evidence could be obtained. Intracellular records taken immediately after the muscle had reached its new length showed the same type of activity as occurred spontaneously before, but it was always increased.

TABLE 1. Effect of tension on activity

Expt.	Tubocurarine concn.	Tension (g)	Twitches per min
1. Sartorius 0·7% NaCl	10^{-5}	0·1	9
		0·4	13
		0·8	15
		0·3	4
2. Sartorius 0·7% NaCl	10^{-5}	0·1	8
		0·35	11
		0·7	15
		0·1	3
3. Iliofibularis 0·7% NaCl	5×10^{-6}	0·25	23
		0·35	38
		0·60	54
4. Iliofibularis $\frac{1}{20}$ Ca + $\frac{1}{20}$ K	Not curarized	0·05	34
		0·25	50
		0·45	119
		0·60	200
5. Iliofibularis $\frac{1}{20}$ Ca + $\frac{1}{20}$ K	5×10^{-6}	0·03	44
		0·07	53
		0·115	30 (large)
		0·25	27 (larger)
6. Iliofibularis $\frac{1}{20}$ Ca + $\frac{1}{20}$ K	5×10^{-6}	0·09	27
		0·26	47
		0·37	58
		0·54	72
		0·66	75
		0·80	120
7. Iliofibularis $\frac{1}{20}$ Ca + $\frac{1}{20}$ K	5×10^{-6}	0·14	20
		0·57	49
		0·97	74
		1·3	89
		1·75	25

The effect of histamine

Histamine is a well-known stimulant of many types of smooth muscle but it has no action on normal striated muscle. Histamine was found to stimulate frog's striated muscle when it had been rendered more excitable by calcium deficiency. If the muscle was quiescent histamine provoked twitching; if the muscle was already spontaneously active histamine increased its activity.

Histamine hydrochloride was used; the solution was neutralized with isotonic $NaHCO_3$ and doses from 15 to 50 μg were added to the perfusion fluid. In the bath of 2–3 ml. this would produce a concentration of 5×10^{-6} to $2{\cdot}5 \times 10^{-5}$. It is possible that lower concentrations were also effective, but only with the higher concentrations was the histamine effect convincing. It was important to test the action of histamine early in the experiment while activity was fairly regular. Later in the experiment sudden changes in rhythmic pattern, such as shown in Fig. 1, might well occur spontaneously.

The response to 15 μg histamine is shown in Fig. 12. The muscle was regularly active (*a*) at a rate of 72/min; 10 sec after histamine (*b*) the action potentials appeared in pairs and the twitches were accordingly greater. After 1 min 40 sec the peak effect was reached and the first tetanus occurred (*c*), and was repeated several times; then activity decreased. Three minutes after the

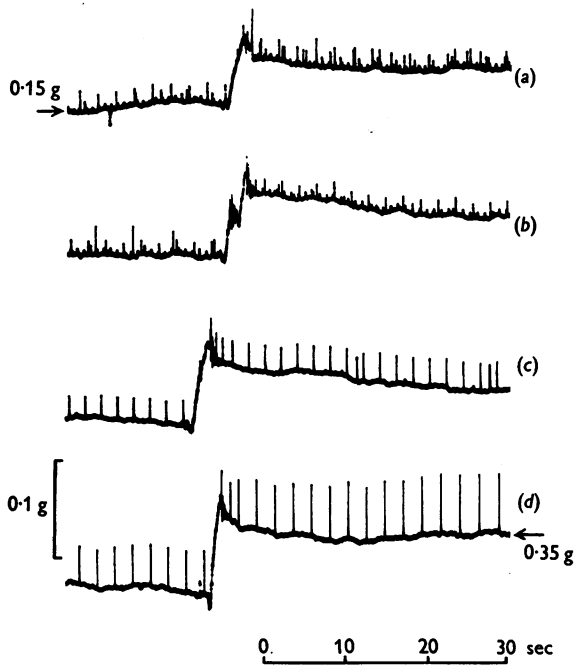


Fig. 11. Mechanical record. Response to stretch. Note synchronization with dominant fibre.

histamine (*d*) the muscles twitched at a rate of 36/min but the greater force of contraction indicated greater synchronization. 15 min later the synchronization had disappeared and single twitches occurred regularly again as in (*a*) at a rate of 78/min. Fig. 13 shows the effect of stretch (top record), of 20 μg (middle record) and of 40 μg histamine (bottom record). Between top and middle record there was an interval of 9 min; between the middle and lower record 22 min. It is interesting to see that within the period of 43 min between the beginning of the top record and the beginning of the bottom record the degree of spontaneous activity returned to a comparable level after each disturbance. Stretch produced irregular bursts of activity; both doses of histamine produced not only an increased rate, but also a greater synchronization. This is most evident from the electrical record after 20 μg, and from the tension record after 40 μg histamine.

Intracellular records showed no qualitative difference between the electrical activity after histamine and that which occurred spontaneously. There was no doubt that the rate of activity was always greatly increased, but records obtained from the whole muscle showing the summed increase in activity of all fibres were, of course, more conclusive.

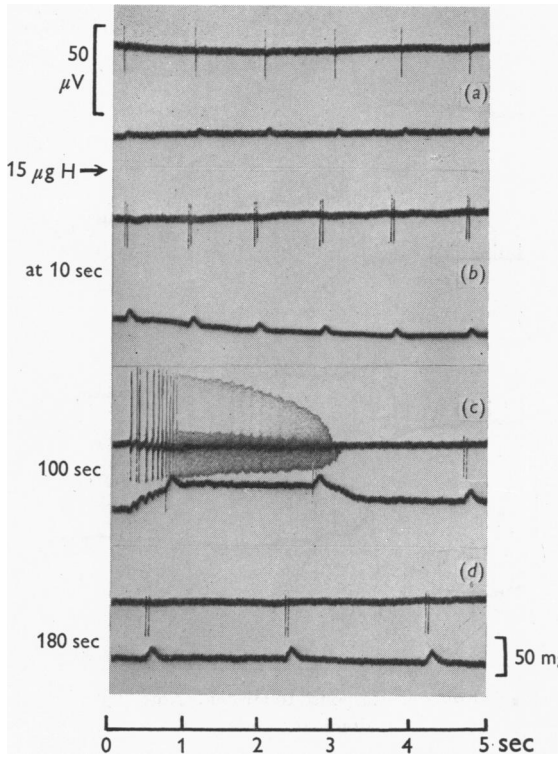


Fig. 12. Records as in Fig. 1. Response to 15 μg histamine.

DISCUSSION

The behaviour of striated muscle deprived of calcium, which has been described in detail by Adrian & Gelfan (1933), was studied once more because it has recently been found that smooth muscle from the intestine shows a similar behaviour under normal conditions. The average membrane potential of the smooth muscle is lower than that of normal skeletal muscle and depends on the degree of stretch and the production of tension thereby evoked. In addition, this smooth muscle is spontaneously rhythmically active and the frequency of its activity is related to membrane potential and tension.

Adrian & Gelfan (1933) came to the conclusion that the general state of polarization could not be responsible for the tendency to activity because the

injury potential rose in NaCl but fell in sodium citrate solution. When they used citrate to reduce the calcium they found that, at the point of application, each spike potential was preceded by a slow depolarization. They also produced local depolarization in calcium-deficient muscle by pressure. Kuffler (1944) observed that the muscle portion which was immersed in the calcium-deficient solution became negative relative to the normal part of the muscle; the depolarization developed slowly and reached a maximum after 20 min.

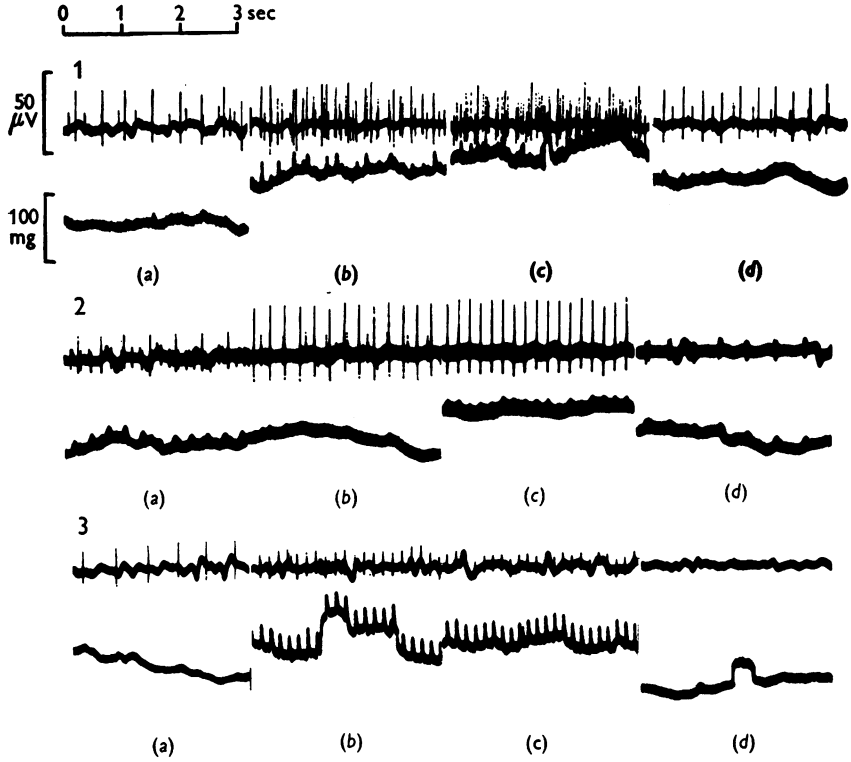


Fig. 13. Records as in Fig. 1. Top: response to stretch (*a*) before, (*b*) 1 min, (*c*) 3 min, (*d*) 6 min after stretching. Middle: response to 20 μg histamine. Bottom: response to 40 μg histamine. Time intervals the same in all three records.

We have used a solution deficient both in calcium and in potassium to produce rhythmic activity in frog's skeletal muscle. We found that the activity was more regular and continued for a longer time than when Ca only was reduced. Using intracellular electrodes we found no fall of the average resting potential at a time when rhythmic activity was in full progress. On the contrary, a high degree of spontaneous activity existed while the membrane potential was raised, i.e. the tendency for auto-rhythmicity was not dependent on the absolute value of the membrane potential. The important change was the much greater variation of resting potentials than that found in normal

fibres as is shown by the scatter diagram. When calcium only was reduced a similar increase in scatter was seen and the resting potential fell slowly. As the rate of decline varied greatly from fibre to fibre and as the time factor was involved, the mean value obtained in the course of one hour is, of course, not strictly valid. The reduction of both Ca and K subjected the muscle fibres to two opposing forces, one to reduce and one to increase the resting potential. The depolarization was thus delayed. Nevertheless, spontaneous discharge of spike potentials started already in some fibres at a membrane potential of the order of 90 mV, showing that the depolarization was not in itself the cause of rhythmic activity.

The resting potential became unstable. Fluctuations and concomitant changes in the frequency of spike potentials were observed, depolarization being accompanied by quickening and repolarization by slowing of the rate of activity. This observation is reminiscent of the finding in decalcified nerves (Brink, Bronk & Larrabee, 1946) that the frequency of spontaneous firing was increased by cathodal and decreased by anodal polarization. We found that generally the lower the resting potential the greater the frequency of spike discharge. As the resting potential fell the action potentials became smaller; first the overshoot disappeared and gradually each spike caused only partial depolarization.

The instability of the membrane potential was also evident from the slow waves which were regularly seen. Since they frequently coincided with contractions of neighbouring fibres, and as similar waves were also observed in normal fibres when neighbouring cells were activated with weak electrical shocks, it seemed likely at first that they were movement artifacts. However, the facts (*a*) that as a rule the slow waves gradually increased in size until they gave rise to a spike potential, (*b*) that the spike potential always started from the peak of the slow wave, (*c*) that spike potentials arose only if the slow depolarization exceeded a fairly constant level for any particular fibre, provide evidence that the slow waves represent true phases of depolarization. The observation that the resting potential of a fibre remained constant after a great number of slow waves makes it unlikely that they were due to dislodgement of the electrode tip. Further, we were unable to produce similar waves by moving the electrode within the fibre.

There are two possible explanations for our constant observation that the spikes always arise from the crests of the slow waves: (1) slow waves and spikes are initiated only at the electrode, (2) slow waves are synchronous along the whole length of the fibre; they initiate a spike at some distant point or at the electrode and, as the conduction time is short compared with the duration of the slow waves, the spike always appears at the crest. We believe that our observations support the second possibility.

Several observations make it unlikely that spikes which are preceded by

a prepotential are always set up at the point of insertion of the microelectrode and that those without prepotential arise elsewhere in the fibre. First, prepotentials were recorded by Adrian & Gelfan (1933) and by us using surface electrodes (e.g. Fig. 1). Secondly, spikes without prepotential were only seen in fibres with high resting potential and never when the membrane potential was low. It is unlikely that in fibres with high resting potential the spike should always arise at a distant point and in those with low potential always at the electrode.

The slow waves are probably the events preceding synchronization by a mechanism postulated by Adrian & Gelfan (1933). 'The essential conditions seem to be (*a*) an intense and fairly uniform activity in several fibres, and (*b*) the existence of damaged or permeable regions at neighbouring points in them. These would bring the interiors of the fibres into free electrical communication and give a chance for a group to behave as a single unit. An active region developing in one fibre close to the injury would tend to activate not only the neighbouring points in the same fibre but also those in the fibres next to it, and thus rhythmic discharge in one fibre might come to dominate the rhythm of its neighbours.' This is exactly what was repeatedly observed if external stimuli were applied, mechanical, such as stretch, or chemical, such as histamine. In this connexion it may be mentioned that during the measurements of resting potentials we often found a group of fibres having similar values, whereas the resting potentials of another group farther away were all by 10–20 mV different.

In the normal smooth muscle of the intestine the stimulus of stretch and also the administration of histamine lower the membrane potential and increase the frequency of spontaneous discharge of impulses (Bülbring, 1954, 1955*a*). Recently Born & Bülbring (1955, 1956) have shown that the rate of loss of radioactive potassium from the smooth muscle is increased by the mechanical as well as by the chemical stimulus. It is possible that the response of calcium-deficient skeletal muscle to stretch and to histamine is due to a similar mechanism.

The slow 'prepotential' described for nerve by Arvanitaki (1939*a*, *b*) leading up to the rapid spike potential was a common finding. It was not limited to one specific area of the fibre. It was seen to develop gradually as the resting potential declined. Its appearance, and that of the action potentials arising from the peak of the slow wave, were very similar to the records obtained by Eyzaguirre & Kuffler (1955*a*, *b*) from crustacean sensory nerve cells (stretch receptors) and to records obtained in intestinal smooth muscle (Bülbring, unpublished) which has properties of a stretch receptor. Thus the shape of the action potential in skeletal muscle undergoes changes when, in the extreme conditions of calcium deficiency, it becomes spontaneously active and behaves like a stretch receptor.

SUMMARY

1. The behaviour of isolated frog's skeletal muscle in calcium-deficient medium has been studied.

2. Lowering the Ca^{2+} to one-twentieth of the normal amount led to a fall in membrane potential and a greater scatter of the values measured. The muscle became rhythmically active.

3. Lowering of both Ca^{2+} and K^{+} to one-twentieth of the normal amount caused a greater scatter both above and below the normal values. Spontaneous rhythmic activity began at a time when the mean resting potential was still normal. Later the membrane potential slowly declined.

4. As a result of calcium deficiency the membrane potential became unstable and fluctuated. Slow waves appeared which grew in size until they gave rise to action potentials.

5. The appearance of the action potential was normal, or, as the resting potential fell, it was preceded by a slow depolarization ('prepotential') which suddenly deflected into the rapid rising phase of the spike potential.

6. The frequency of discharge of spike potentials was related to the resting potential; at a high membrane potential the rate was slow, at a low potential the rate was fast.

7. The size of the action potentials varied from the normal size with reversal of the membrane potential, to small spikes which reached only a partial depolarization.

8. Within a small range of stretch a graded increase in tension was accompanied by a graded increase in the rate of spike discharge and muscle twitches.

9. The rate of activity of calcium-deficient striated muscle was increased by histamine.

10. External stimuli not only increased the rate of activity but often produced synchronization.

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