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Effects of Caffeine on Twitch Contraction Elicited in Frog's Skeletal Muscle Fibres Immersed in Osmotically Anomalous Media[1)]

By

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(With 7 Text-figures and 2 Tables)

It has been well known that the caffeine in subcontracture concentrations potentiates the twitch tension of the frog skeletal muscle by prolongation of the active state (Ritchie, 1954; Sandow and Brust, 1966). The effect of the drug in such concentration has been noticed in the study of muscular contraction because it does not significantly alter both the resting and the action potentials of the muscle fibre (Sandow, *et al.*, 1964), while it is suggested that the twitch potentiation may not result from a direct action of the drug on the contractile component but on a process of the excitation-contraction coupling of muscles (Sandow, 1965).

On the other hand, it is discussed widely that the transverse tubular system in the muscle fibre may play an important role as a linking place of excitation with contraction (*e.g.* Hodgkin and Horowicz, 1960; Isaacson and Sandow, 1963; Peachey, 1965). It was also shown that the transverse tubule was swollen when the muscle fibre was exposed in a hypertonic solution which reduced or abolished the twitch tension without impairing the membrane excitation (Freygang, *et al.*, 1964; Hodgkin and Horowicz, 1957).

It is, therefore, of interest to investigate the effects of caffeine on the twitch contraction of the skeletal muscle fibre exposed in osmotically anomalous media for the purpose of elucidating the mechanism of excitation-contraction coupling. The present paper gives the results of such effects using a single or two frog skeletal muscle fibres and shows the potentiation by caffeine is greater or the same in the hypertonic solution and less in the hypotonic than in the isosmotic one. The resting and the action potentials of fibres under the above conditions are also given using intracellular micro-electrodes.

Materials and methods: Single fibres or bundles of two fibres isolated from the semitendinosus or iliofibularis muscles of the frogs (*Rana japonica* or *R. chensinensis*) were used for measuring twitch and tetanus tensions; and bundles of several fibres were used to measure the resting and action potentials because it was very difficult to record the

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action potentials using a single fibre with repeated penetrations of a micro-electrode under serial conditions.

The Ringer's solution contained NaCl, 110 mM; KCl, 2.5 mM; $CaCl_2$, 1.8 mM; and $NaHCO_3$, 2 mM, with which the solution was adjusted to pH 7.2. In order to prepare the osmotically anomalous solutions, the amount of sodium chloride in the Ringer's solution was varied and the hypertonic solution used contained 220 mM NaCl (2× Na-Ringer's solution) and the hypotonic, 55 mM (1/2 × Na-Ringer's solution). The other compositions in both fluids were the same as those in the normal one. The concentration of caffeine contained in each of the solutions was, unless otherwise stated, 1mM which was in subthreshold concentration to induce contracture in the skeletal muscle (Sandow, *et al.*, 1964). All solutions used, contained 6×10^{-6} g/ml d-tubocurarine-chloride.

The fibres were mounted horizontally in the experimental chamber, the volume of which was 0.7 ml. In order to exchange the medium, a test solution of 7 ml was flushed into the chamber with a pipette while sucking at the other end of the chamber. This procedure finished within several seconds. Using a dye, the remainder of the fluid was estimated, by a spectrophotometer, at about one fiftieth of the total volume, which was negligible for the experiments.

Repeating replacements of the media by such a procedure a series of measurements were made on one preparation under the respective conditions.

Stimuli were square shocks of 0.5 msec duration. Tetani consisted of a 0.3 sec train of these shocks at a frequency of 80/ sec, which were applied to the fibre transversely by a pair of Ag-AgCl electrodes.

Tension output was displayed on a cathode-ray oscilloscope by the following procedure: at one tendon, the fibre was connected with a glass rod with a length of 4.5 cm extended from the anode pin of a 5734A mechano-electronic transducer tube (Toshiba) and the tension was recorded isometrically stretching the fibre just tautly by moving the tube with a micromanipulator.

For the measurement of the membrane potentials, a micro-electrode amplifier (Nihon Kohden MZ-3B) with compensated input capacity connected to an oscilloscope, was used. The intracellular glass micro-electrodes used were filled with 2 M KCl and had the resistance of 10–30 MΩ.

All experiments were carried out at room temperature 22–26°C.

Results

I Mechanical responses of muscle fibres to single or tetanic stimuli

As a preliminary test, the effect of caffeine on the twitch and tetanus tensions and its recovery in the normal Ringer's solution were examined. Fig. 1 illustrates a typical example of such an effect. The fibre is stimulated at intervals of about 30 seconds or a minute. The twitch tensions developed were potentiated with the lapse of time and attained to a plateau about one minute and a half after addition of the drug. This plateau value varies from fibre to fibre and it amounts to 150 to 250 percent of the control twitch tension. Recovery times from the tension potentiation are much longer and the present results show them about 4 minutes. It was reported that the similar phenomena of potentiation and recovery were found both in the sartorius muscle (Sandow and Brust, 1966) and in a single or two fibre preparation (Matsumura, 1967), the latter of which was stimulated at a frequency

of 1/sec. Although Matsumura's preparation was just the same as the present one, his result showed more rapid potentiation and recovery. The cause of the discrepancy cannot be realized for the present. Ten minutes after the recovery, re-administration of caffeine causes the same effect as the first, and the time course of its recovery is also the same. Contraction time (time to peak tension) is also prolonged by caffeine reversibly. Tetanic tensions are almost the same whether caffeine is present or not. These enable the following experiments which examine the drug effect under the osmotically anomalous conditions.

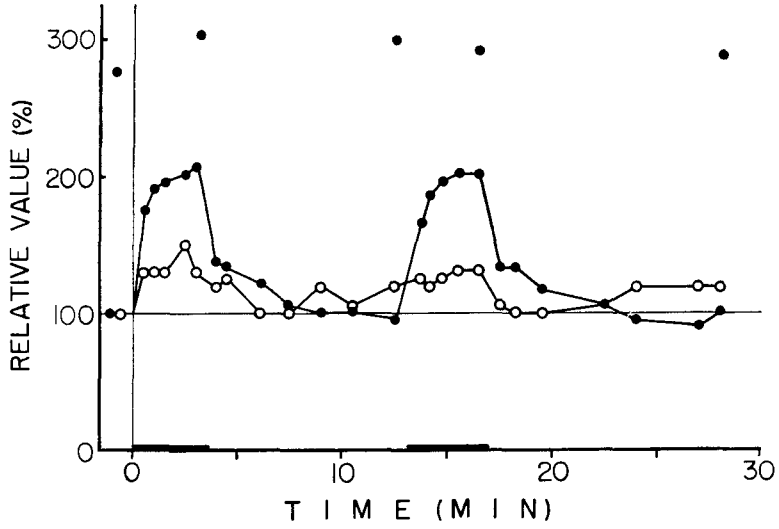

Fig. 1. Serial measurement of mechanical responses of a single fibre in the isosmotic Ringer's solution with or without 1 mM caffeine to a single or tetanic stimulation. Ordinate, relative values of tension and contraction time plotted as percentage of the twitch values obtained in the control solution: abscissa, time after the first applciation of caffeine: thick line on abscissa, duration of caffeine application: filled circles, twitch and tetanic tensions, the latters are in the upper position and unconnected with line: open circles, contraction time. June 27, 1967, 23°C.

a) *Twitch potentiation by caffeine in 2 × Na-Ringer's solution* Fig. 3 illustrates the tension output and contraction time of a fibre as function of time in this experiment. After examination of the twitch potentiation by caffeine and its recovery in the normal medium, the latter was replaced by 2 × Na-Ringer's solution. The twitch tension in this hypertonic medium was reduced to about 30 percent and the contraction time increased by 50 percent. By addition of caffeine, to this hypertonic medium, the twitch tension was increased either nearly to the potentiated level or by almost the same amount as that developed in the normal fluid. The contraction time was prolonged and it amounted to 300–400 percent of that of the normal twitch. The relaxation time was much more prolonged and the minimal

and measurable value was several times greater than the normal one, but in other cases it was so prolonged that it could not be counted from the frame photographs. These twitch potentiation in 2 × Na-Ringer's solution disappeared with removal of the drug and the suppressed twitch was observed again. Finally, the normal Ringer's solution was substituted for the hypertonic solution and the mechanical responses recovered to the initial state. If 0.5 mM caffeine was added instead of 1mM, the potentiating effect was similar to that of the foregoing result. Fig. 2 presents the records of the twitch and tetanic contractions in the normal and 2 × Na-Ringer's solutions, with or without 0.5 mM caffeine. It is noticed in Fig. 2 that the tetanic tension developed in the hypertonic solution are less and the rising and falling times are prolonged.

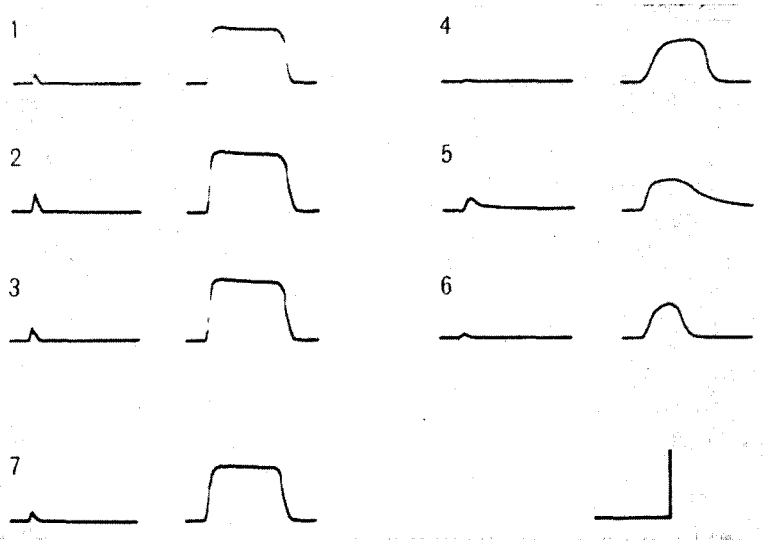

Fig. 2. Effects of 0.5 mM caffeine on twitch and tetanic tensions elicited in a single fibre immersed in isosmotic and 2 × Na-Ringer's solution. Left and right records represent the twitch and the tetanic tensions, respectively. 1, the control. 2, after 3 min. and a half after addition of caffeine to the control medium. 3, 4 min. after removal of the drug. 4, twitch: 3 min. after exchange by 2 × Na-Ringer's solution; tetanus: 4 min. after. 5, 3 min. after addition of caffeine to 2 × Na-Ringer's solution. 6, twitch: 3min. after removal of the drug from the hypertonic medium. 7, 2 min. after return to the normal Ringer's solution. Calibration: vertical line, 200 mg, horizontal line, 300 msec. June 12, 1967, No. 3, 24°C.

In some experiments made in this hypertonic medium a contracture was induced by 1 mM caffeine, that coincided with the result of Caputo (1966) but on some of these fibres the successive experiment could not be performed, for the fibres failed to respond to a stimulus during the experiment. The fibre was damaged by caffeine in the medium of higher osmotic strength which contained 275 mM NaCl.

b) *Twitch potentiation by caffeine in 1/2 × Na-Ringer's solution* The same

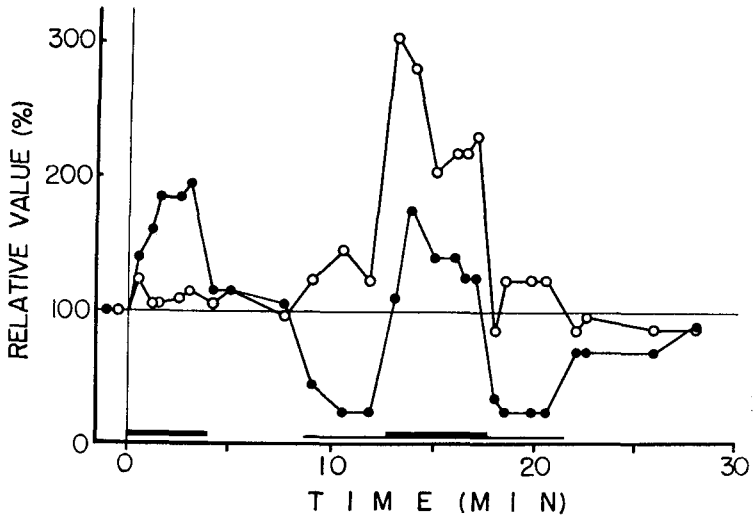

Fig. 3. Serial measurement of twitch tension and contraction time in isosmotic and 2 × Na-Ringer's solution. Thin line on the abscissa indicates the treatment by the hypertonic medium. Other characteristics should be referred to the explanation of Fig. 1. June 28, 1967, 2 fibres, 24°C.

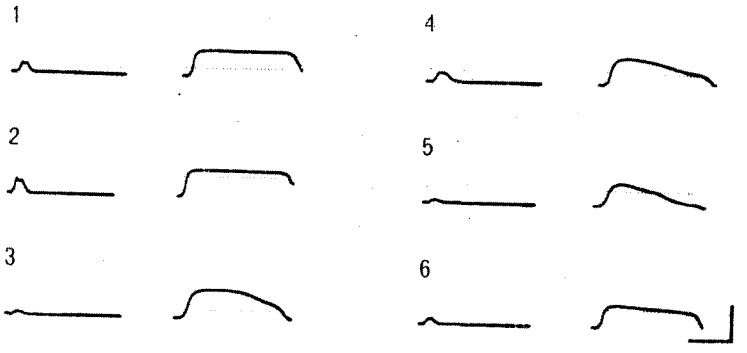

Fig. 4. Effect of 1 mM caffeine on the twitch and the tetanic tensions elicited in a single fibre immersed in 1/2 × Na-Ringer's solution. Left and right records represent the twitch and the tetanic tension, respectively. The irregular form of the twitch tension was caused by a fraction of a fibre attached. 1, the control. 2, 3 min. after addition of caffeine to the control medium. 3, 3 min. after treatment with 1/2 × Na-Ringer's solution following examination of twitch and tetanus in the normal medium. 4, 5 min. after addition of caffeine to the hypotonic medium. 5, 3 min. and a half after removal of the drug. 6, 4 min. and a half after return to the normal medium. Calibration: vertical line, 300 mg, horizontal line, 150 msec. September 1, 1967, No. 3, 24°C.

procedure as for the hypertonic solution was followed in the experiment. Fig. 4 illustrates the records of twitch and tetanus tensions developed in the experiment. As will be mentioned below, the twitch tension is decreased in the hypotonic solution, while the tetanus tension attains to about the same level as in the control, though it declines during the repetitive stimulation. This implies that contractility of the fibre may not be impaired by the hypotonicity. Fig. 5 presents the tensions and the contraction times of the twitches elicited in a single fibre as function of time. When the external solution was replaced by 1/2 × Na-Ringer's solution, the fibre was elongated and swollen so that the tension was recorded after the fibre was

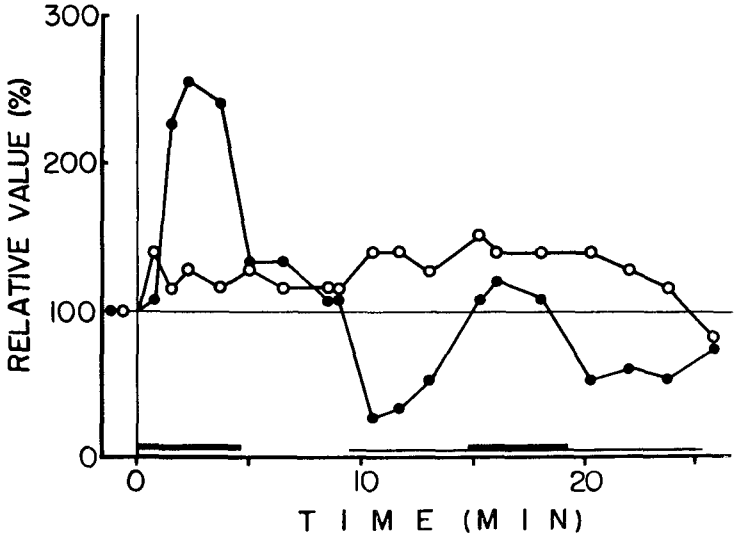

Fig. 5. Serial measurement of the twtich tension and contraction time elicited in a single fibre immersed in the isosmotic and 1/2 × Na-Ringer's solution. Thin line on the abscissa indicates the treatment with the hypotonic solution. Other characteristics should be referred to the explanation of Fig. 1. August 31, 1967, No. 2, 24°C.

stretched again just tautly. The twitch tensions in the hypotonic solution are also attenuated and their value are about 40 percent of the control twitch. The contraction times are prolonged a little. Application of caffeine to the medium causes potentiation in the twitch tensions and in the seven cases, the six show their potentiation from 0.5 to 0.7 times of that under the normal condition. The remaining one shows just the same potentiation.

The contraction time is increased much more or stays constant under this condition. These potentiation by caffeine disappears by removal of the drug. The mode of twitch tension development under the following conditions is similar to the case of 2 × Na-Ringer's solution.

II Resting and action potentials of muscle fibres

In order to examine the relation between the mechanical and the electrical responses elicited in the experimental solutions, the action potentials as well as

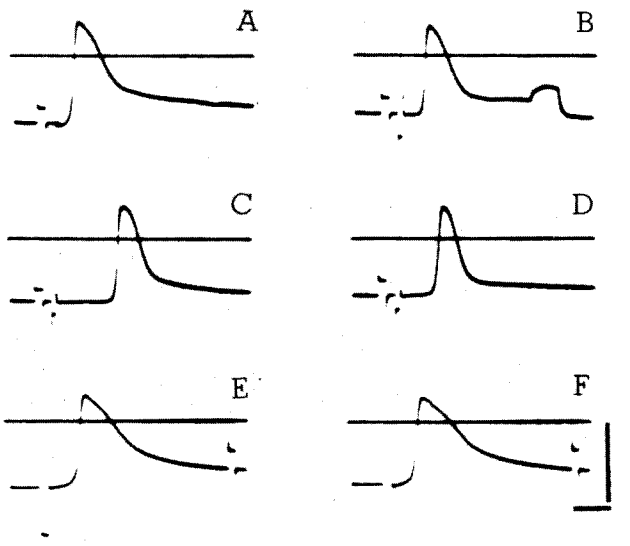

Fig. 6. Action potentials elicited in the experimental solutions. A and B, in the normal Ringer's solution, C and D, in 2 × Na-Ringer's solution; E and F, in 1/2 × Na-Ringer's solution. 1 mM caffeine was contained in B, D and F. Ineffective monitoring pulses to compensate input capacity are shown near the starts in A-D and near the ends in E and F. In B the distortion is caused by fibre movement. Calibration: vertical line, 100 mV; horizontal line, 2 msec. A-D, January 24, 1968, 25°C. E and F, December 23, 1967, 22°C.

Table 1. Resting potential and action potential in 2 ×

Resting potential (mV)

Media**	A	B	C	D	E
Mean	81	84	82	83	83
Standard error of the mean	±2.3	±2.5	±1.6	±1.8	±2.9
Significant difference (P<.05) between A and other columns		–	–	–	–
Significant difference (P<.05) between D and E					–
Number of observations	11	11	11	9	9

* Durations of action potentials are given at half value of the spike height.

** A, the control; B, the normal medium containing 1 mM caffeine; C, the

the resting potentials were measured by means of the intracellular micro-electrodes. In one bundle of several fibres, one or two fibres were used to measure both potentials under the respective conditions.

a) Measurements in 2× Na-Ringer's solutions Table 1 gives the results of the resting and action potentials obtained in the experiments performed on the fibres in ten bundles in 2 × Na-Ringer's solution, comparing with the results in the normal medium. A large number of measurements were finished from 2 to 5 minutes after exchanging the solution, but a few were done much later at about 8 or 9 minutes after. The discrepancy between the values measured at the different times was not observed. As is shown in Table 1, in the magnitudes of the resting potential and the action potential, statistical tests reveal no significant differences ($P<.05$) between the control value and those in the test solutions. But in the duration of the action potential, the difference between the control value and that in the isosmotic medium containing 1 mM caffeine is signfiicant at the 10–percent level but not at the 5-percent level. In another measurement, the significant difference ($P<.05$) between them is obtained, as is illustrated in Table 2. It may be, therefore, considered that the duration of the action potential has a tendency to be shortened by 1 mM caffeine, that is contrary to the result of Sandow, *et al.* (1964). The difference between the duration of the action potential elicited in the control medium and that in 2 × Na-Ringer's solution, is highly significant ($t=3.6$), that is the duration of the action potential is clearly shortened in 2 × Na-Ringer's solution. But in every characteristic in the hypertonic solution, the significant difference was not obtained between the value with caffeine and that without the drug. It is, therefore, concluded that caffeine does not affect the action potential in the 2 × Na-Ringer's solution. The records of the action potentials obtained in the experiment are illustrated in Fig. 6.

b) *Measurements in 1/2 × Na-Ringer's solutions* Measurements of the resting potentials and the action potentials of the muscle fibre in 1/2 × Na-Ringer's solution were made by the similar procedure to those in the hypertonic solution.

Na-Ringer's solutions with or without caffeine (22–25°C)

Action potential (mV)					Duration of action potential (msec)*				
A	B	C	D	E	A	B	C	D	E
127 ±2.3	128 ±2.9	126 ±3.3	130 ±2.9	126 ±4.8	1.7 ±.14	1.4 ±.06	1.6 ±.16	1.1 ±.05	1.0 ±.06
	−	−	−	−		−	−	+	+
				−					−
11	11	11	9	9	11	10	10	9	9

normal medium; D, 2 × Na-Ringer's solution; E, 2 × Na-Ringer's solution containing 1 mM caffeine.

Table 2. Resting potential and action potential in 1/2 × Resting potential (mV)

Media**	A	B	C	D	E
Mean	83	86	85	82	81
Standard error of the mean	±2.7	±2.5	±2.2	±1.7	±2.5
Significant difference (P<.05) between A and other columns		–	–	–	–
Significant difference (P<.05) between D and E					–
Number of observations	9	9	9	11	11

* Durations of action potentials are given at half value of the spike height.
** A, the control; B, the normal medium containing 1 mM caffeine; C, the

Table 2 illustrates the results of the measurement made on the fibres in 9 bundles. In the resting potential no significant differences were obtained between the control magnitude and those in the other experimental solutions.

It was observed that the shape of action potential elicited in the hypotonic solution was changed: that is, the rising phase and in particular the falling phase were prolonged (Fig. 6), and the potential magnitude seemed to be decreased. But the statistical tests show no significant differences (P<.05) between the normal amplitude and that under the hypotonic condition (between A and D or E in Table 2). From Table 2 and Fig. 6, it is evident that the application of caffeine to the hypotonic solution does not induce any change in the shape of the action potential as well as in the resting potential.

III Volume changes in the muscle fibres immersed in the experimental solutions

The volume changes in the muscle fibres exposed in the osmotically anomalous media were indicated by the change in fibre diameter as illustrated by Reuben *et al.* (1963). Under a microscope at the magnification of 10×7 the fibre diameter was measured by an eyepiece micrometer on a single or two fibres. The procedure for exchanging the solution was the same as mentioned previously. After measuring the diameter in the normal fluid, the diameter of the same position was measured again 2 to 4 minutes after replacement of the fluid by the anomalous media. It is illustrated in Fig. 7 that the volume is increased by about 20 percent in 1/2 × Na-Ringer's solution and decreased by about 25 percent in 2 × Na-solution. It is reported that the resembling result is obtained on a single muscle fibre exposed in the osmotically different media containing higher potassium concentration (12.5 mM) (Reuben *et al.*, 1963).

Discussion

In the foregoing results, it was shown that the twitch tension was potentiated by caffeine in subcontracture concentration not only under the isosmotic condition

Na-Ringer's solutions with or without caffeine (22–25°C)

Action potential (mV)					Duration of action potential (msec)*				
A	B	C	D	E	A	B	C	D	E
128 ±4.3	129 ±4.9	128 ±3.1	121 ±3.6	117 ±4.0	2.1 ±.16	1.7 ±.10	1.9 ±.16	2.3 ±.15	2.2 ±.14
	−	−	−	−		+	−	−	−
				−					−
9	9	9	11	11	9	9	9	11	10

normal medium; D, 1/2 × Na-Ringer's solution; E, 1/2 × Na-Ringers solution containing 1 mM caffeine.

but also under the hypertonic or the hypotonic condition. Before discussing the effect of the drug on a process of the excitation-contraction coupling, it must be considered if the mechanical response is altered by the change in membrane excitation and the change in the internal condition which are brought about by the external media.

First, it was shown that the duration of the action potential measured at the half value of the spike potential in the normal fluid had a tendency to be shortened by caffeine, whereas the twitch tension was potentiated by the drug. In general, as muscle contraction is initiated by membrane depolarization, it is plausible that

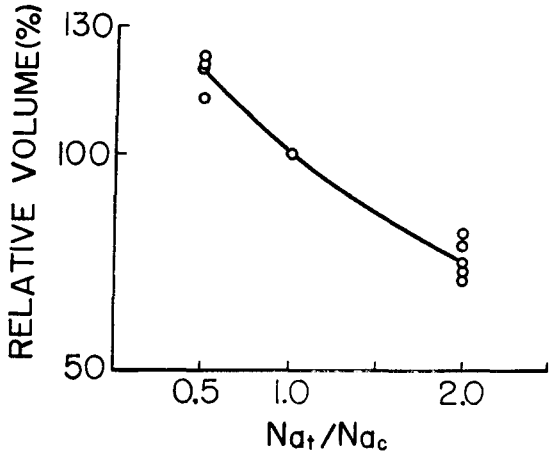

Fig. 7. Relation between the external osmotic strength and the fibre volume. Ordinate, the relative volume calculated from the fibre diameter. Abscissa, the osmotic strength indicated by the ratio of NaCl concentration contained in the test solution to that in the control solution.

the prolongation of the duration of the action potential as well as enlargement of the negative after potential, in other words, prolongation of the depolarization, will cause the twitch potentiation. In fact, such evidence is presented by some workers (Lubin, 1957; Mashima and Matsumura, 1962; Sandow, *et al.*, 1964). It cannot be, therefore, expected that the action potential itself with its shorter duration elicited in the presence of caffeine, may potentiate the twitch tension.

The differential action of the hypertonic solution on electrical and mechanical responses of the muscle fibre has been well known (Hodgkin and Horowicz, 1957) and the present result also shows the action. But the careful measurement shows the shortening of the duration of the action potential (Table 1).

It may be, therefore, suggested that the attenuation of the twitch tension in the hypertonic solution may result from (1) the shorter duration of the action potential, or shorter time of depolarization which may be responsible for contraction, (2) increase in internal viscosity that may be caused by dehydration by hypertonicity (Howarth, 1958), and (3) the blocking effect on a linking process of excitation with contraction. If we make further explanations on the latter two causes, the following explanations will be given: For (2), besides the result of Howarth (1958), it is shown in Fig. 2 and Fig. 3 that contraction time is prolonged, and the rising phase and the falling phase of the tetanic tension are prolonged in the hypertonic solution. These phenomena seem to be attributed to the internal resistivity to mechanical response caused by dehydration. Such dehydration may also suppress the tension output. For (3), blocking effect of hypertonicity on the link is concluded from the work of Fujino *et al.* (1961) that the twitch contraction recovers during the dehydrated state and the work of Fujino and Fujino (1964) that the inhibitory action of hypertonicity on the twitch contraction in sartorius muscle is weakened temporarily after treatment with potassium. Furthermore, it is reasonable to consider the blocking effect of hypertonicity on the excitation-contraction link, from the following discussion on the caffeine effect.

Addition of 1 mM caffeine to the hypertonic solution did not cause any change in the shape of action potential, and the change in tonicity due to the applied drug is negligible. If the diminution of the twitch tesnion were attributed mainly to the viscosity change of the fibre, the potentiation by caffeine could not be obtained. It is, therefore, concluded that the potentiation effect of caffeine in the hypertonic solution might be ascribed to the action of the drug on a process of the excitation-contraction coupling. As was previously shown, the twitch potentiation observed in 2 × Na-Ringer's solution is the same or greater than that induced in the normal fluid, and particularly, the contraction and the relaxation times are prolonged. Considering the suppressing effect of the internal enhanced viscosity on the twitch contraction, it is concluded that the recruitment of a coupling process by caffeine in the hypertonic solution is much larger than in the normal medium. It is in agreement with the conclusion on the caffeine-induced contracture (Caputo, 1966).

It was observed that the twitch tension was decreased also in the hypotonic

solution. Although, in this case, the shape of the action potential is changed, the action potential has an overshoot potential and the duration at the half value of the spike potential was not appreciably different from that of the control (Table 2). From this result, it is hardly suggested that the action potential itself may bring about the reduction of twitch tension. Again, as previously pointed out in the result of tetanus tension elicited, it seems probable that the contractility of the muscle fibre is unchanged by this hypotonicity. If it is so, it will be expected that the hypotonicity impairs, in some way, a link of excitation with contraction, and results in diminution of the tension output.

It was observed that addition of caffeine to the hypotonic medium potentiated the twitch tension without any change of action potential just as observed in the hypertonic solution while the contractility remained. This may suggest that the potentiation by caffeine in the hypotonic solution is also due to the recruitment of an excitation-contraction link. The foregoing result shows that the potentiation under the hypotonic condition is less than that under the control condition.

In summary, a process of excitation-contraction coupling may be impaired by both hypertonicity and hypotonicity and be recruited by caffeine in larger amount under the former condition and in less under the latter.

In the frog skeletal muscle fibre, it has been discussed that the transverse tubular system (T-system) and the sarcoplasmic reticulum which composes triad with the T-system on the Z-line may play some roles as links of excitation with contraction (Sandow, 1965). The continuity of the surface membrane to the T-system in frog twitch fibre was illustrated in an electron micrograph using a new fixative (Birks, 1965). Furthermore, Huxley (1964), Birks (1965); and Endo (1964), using both fixed and living frog muscles, respectively, presented the results that the particles or a fluorescent dye applied to the bathing solution seemed to diffuse into or enter only the tubule of the T-system from the external fluid, and, thus, the T-system might continue from the external medium. It is, therefore, expected that caffeine, besides acting on the surface membrane, may enter the tubule and recruit a process of the excitation-contraction coupling like other potentiators such as NO_3^- and Zn^{++} (Hodgkin and Horowicz, 1960; Isaacson and Snadow, 1963). The delayed potentiation on application of caffeine and the recovery from potentiation (Fig. 1) may agree with the explanation.

An electron micrograph represents that the transverse tubule is swollen in a hypertonic solution (Freygang, *et al.*, 1964) whereas the muscle fibre is shrunk (Fig. 7). This makes possible that the potentiator such as caffeine in the external medium may diffuse into the tubule in a larger amount under the hypertonic condition. On the other hand, if the fibre is immersed in the hypotonic solution, it swells clearly as illustrated in Fig. 7. It is very probable that the swelling is induced by water absorption through the semipermeable sarcolemma, which may be considered to invaginate to form the T-system from the papers cited above (Birks, Huxley, Endo). Unfortunately, we have no information about the structure of the T-system of the fibre exposed in the hypotonic solution, but it may be probable that

the tubule will be shrunk by the swelling of the fibre and the potentiator will enter in less amount.

Although, it may not be neglected that caffeine acts on or through the surface membrane (Axelsson and Thesleff, 1958; Sandow, 1965), it is considerable that caffeine may enter the T-system and easily access to the internal structure such as the sarcoplasmic reticulum and enhance the internal free calcium which is thought to be available for contraction mechanism (Herz and Weber, 1965). The present result suggests that such entrance of caffeine to the T-system may be regulated by the volume change of the tubule caused by external tonicity and, as a result the effect of the drug on a process of the excitation-contraction coupling is changed. Besides the volume change of the T-system, it is conceivable that the coupling effect may be regulated by distance from surface to centre of the fibre which is varied by the tonicity.

The author suggested previously the possibility of the entrance of caffeine to the tubule from the result of the delayed contracture induced by caffeine in a single muscle fibre immersed in Ca-free K_2SO_4 Ringer's solution (Yamaguchi, 1965). The present result will also confirm this possibility.

Summary

1. Caffeine-induced twitch potentiation in a single fibre or two isolated from the frog skeletal muscle was investigated by means of an mechano-electronic transducer tube under the hypertonic ($2 \times$ Na) and under the hypotonic ($1/2 \times$ Na) conditions with consideration of the electrical characteristics of the membrane recorded by means of intracellular micro-electrodes.

2. Both under the hypertonic and the hypotonic conditions the twitch tensions were decreased and caffeine potentiated the tension developed in larger amount in the hypertonic solution and in less in the hypotonic than it developed in the normal one.

3. In the magnitude of the resting potential, there were no differences between the control value and those obtained in isosmotic, hypertonic and hypotonic solutions with or without caffeine. In the hypertonic solution, the duration of action potential elicited was reduced than that obtained in the normal solution, but its amplitude was not changed from the control value. On the other hand, it was observed that the action potential elicited in the hypotonic solution had particularly prolonged rising and falling phases. Caffeine did not cause any change on the shape of action potential elicited in both the hypertonic and in the hypotonic solutions whereas the drug had a tendency to reduce the duraton of action potential elicited in the normal medium.

4. It was presented by the measurement of the diameter that the volume of the fibre was increased by 20 percent in the hypotonic solution and decreased by about 25 percent in the hypertonic solution.

5. From these results, a possible explanation of the varied action of

caffeine on a link of excitation with contraction under the osmotically anomalous conditions, was given.

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