EFFECTS OF CAFFEINE ON THE MEMBRANE POTENTIALS AND CONTRACTILITY OF THE GUINEA PIG ATRIUM

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Summary 1. Effects of caffeine on the electrical and mechanical properties of the atrial muscle of guinea pig were investigated.

2. In normal Krebs solution, 3 mM caffeine caused a more than 5-fold potentiation of contraction. The potentiation, however, became smaller the higher the concentration, and 50 mM caffeine depressed the contraction somewhat in its later phase.

3. In 3 mM caffeine, the duration of action potential was prolonged up to 140% at a height of 80% of the spike, and in 50 mM caffeine, up to 230%, whereas the membrane slope resistance was decreased. The rate of rise of the action potential appeared enhanced at the later phase.

4. In sodium-free Tris-maleate Krebs solution, even 3 mM caffeine evoked a contracture, and the contracture tension tended to increase with concentration of caffeine.

5. By soaking the muscle in low calcium Tris-maleate Krebs solution the amplitude of caffeine contracture decreased, first rapidly and then slowly with time, suggesting the contributions of surface membrane and internal calcium store towards contractile activation.

6. These results are interpreted as indicating that:

a) Caffeine acts on both the surface membrane and the sarcoplasmic reticulum in the myocardium.

b) Enhancing the inward calcium movement, caffeine augments the contraction both directly and indirectly through the sarcoplasmic reticulum.

c) In normal Krebs solution, the amount of the accumulated calcium is small but is increased by sodium depletion.

Effects of caffeine on the mechanical properties of skeletal muscle were reviewed by SANDOW (1965). In low concentrations, up to about 2-4 mM, its sole effect is potentiation of twitch contraction. This effect has been extensively in-

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vestigated by various authors. Using different methods, they found that caffeine caused a prolongation of the active state (RITCHIE, 1954) and an increase in the rate of tension development (SANDOW *et al.*, 1965), but did not significantly alter the shape of the action potential (SANDOW *et al.*, 1964). In higher concentrations, above 5 mM, its predominant effect is to cause contracture.

Effects of caffeine on the mechanical and electrical properties of cardiac muscle have been investigated by several authors (SUZUKI, 1962; NAYLER, 1963; DE GUBAREFF and SLEATOR, 1965; CLARK and OLSON, 1968), and it is reported that caffeine altered the duration of action potential. However, little is analysed about the relationship between the alterations of action potential and contractility induced by caffeine, and the site of action of caffeine is not elucidated.

The present experiments were carried out to investigate the effects of caffeine on the electrical and mechanical properties of the auricular muscle of guinea pig. The main purpose was to find the mode and site of action of caffeine.

METHODS

Auricular muscles freshly isolated from the left atrium of guinea pig hearts were used. Each muscle strip of about 0.5 mm × 3 mm × 5 mm was immersed in 30 ml of Krebs solution. The chamber containing the solution was aerated with a mixture of 95% O₂ and 5% CO₂ and maintained at 37 ± 0.5°C. The composition of the solution was, in mm/liter: NaCl, 122.0; KCl, 4.75; NaHCO₃, 15.5; MgCl₂, 0.83; KH₂PO₄, 1.19; CaCl₂, 2.5; glucose, 11.5. The pH of the aerated solution was 7.4.

The strip was pinned at one end and attached at the other end to the sensitive arm of a resistance wire strain gauge. A pair of grid electrodes (10 × 20 mm surface, 10 mm apart) were used for field stimulation. The basal driving frequency was 1 shock/sec. The stimuli consisted of rectangular pulses, 5 msec in duration and less than twice the threshold voltage, which had been determined previously. The transmembrane potentials of single cells were recorded by inserting a flexibly mounted microelectrode into the muscle tissue. The tension of the strip was measured simultaneously with the recording of the action potentials.

Sodium-free solution was prepared by substituting sodium chloride and sodium bicarbonate in Tris-maleate.

To make solutions in which the concentrations of free ionized calcium are stabilized at low levels, 2 mM of EGTA (Ethylene glycol bis (β-amino-ethyl ether)-N, N'-tetraacetic acid) was used as a chelating agent and pH of the solution was adjusted by Tris-maleate buffer (pH = 7.1). The concentration of the free ionized calcium [Ca⁺⁺] was calculated from the following equation presented by IMAI and TAKEDA (1967):

$$P_{Ca} = 2pH - 7.28 + \log \left[\frac{(\text{EGTA}) \text{ added}}{(\text{CaCl}_2) \text{ added}} - 1 \right].$$

RESULTS

Effects of caffeine on contractility

As shown in Fig. 1 Aa, b, and c, caffeine at a concentration of 3 mM in normal Krebs solution augmented the maximum twitch tension by an average of 5-fold (± 1 S.D. of 6 determinations). 10 mM caffeine produced a similar effect (Fig. 1 Ba, b, and c), but the degree of potentiation of twitch contraction was slightly less than in the case of 3 mM caffeine. When the concentration of caffeine was raised to 50 mM, a potentiation occurred, but much less prominently than in the cases of 3 mM and 10 mM caffeine (Fig. 1 Ca, b, and c). Moreover, the potentiation was followed by a depressed state and a slight contracture was often produced. Generally, the time course of the potentiation of twitch contraction under the influence of caffeine was very slow, in contrast with the skeletal muscle (LÜTTGAU and OETLIKER, 1968).

Fig. 1. Effects of 3 mM (A), 10 mM (B), and 50 mM (C) caffeine on the isometric contractile tension of the guinea pig atrium. Rate of stimulation: 1 shock/sec. a: Control records of isometric contractions. b: Records at 10–12 min (A), 16–17 min (B), and 4–5 min (C) after the application of caffeine. c: Records at 15 min (A), 23 min (B), and 20 min (C) after the application of caffeine. d: The control records of single isometric contraction. e: Records under the influence of caffeine. f: Superimposed tension tracings under the influence of 3 mM (1), 10 mM (2) and 50 mM (3) caffeine.

In calcium-free solution, the twitch contraction was markedly reduced and the potentiation of contraction induced by caffeine was very small, though the figures are not shown.

Figure 1, d and e, shows the time courses of twitch contraction before and during the influence of 3 mM (A), 10 mM (B), and 50 mM (C) caffeine. As can be seen, 3 and 10 mM caffeine produced a prominent increase in rate of rise and duration of contraction. When the concentration of caffeine was raised to 50 mM, a further increase in the duration of contraction was produced. However, the augmentation of peak tension as well as the rate of rise appeared rather depressed.

Figure 1 f indicates the superimposed tracings of the twitch tension in the presence of 3 mM (1), 10 mM (2) and 50 mM (3) caffeine.

Effects of caffeine on the electrical properties of the auricular cell

Resting potential. Resting potential was not significantly altered under the influence of 3 mM caffeine, but it decreased in the case of 50 mM (Table 1). The degree of decrease was not significantly different between the cases of presence or absence of contracture.

Fig. 2. Effects of caffeine on the transmembrane potentials and isometric contractile tensions of the guinea pig atrium. (A) The upper figure: control. The lower one: 10 min after the application of 3 mM caffeine. (B) The upper figure: control. The lower one: 5 min after the application of 50 mM caffeine. In each record, the upper tracing is the electrical time derivative of depolarization, middle tracing the action potential, and lower tracing the contractile tension. Note a marked prolongation of the APD and an increase in height of the plateau under the influence of caffeine.

Action potentials. Figure 2 shows simultaneous recordings of the action potential and isometric contractile tension. Under the influence of 3 mM and 50 mM caffeine, height and duration of the plateau increased. This increase during the influence of caffeine was slow, and the alterations of the action potential duration (APD) and height of the plateau were qualitatively parallel.

In Table 1 it was shown that 3 mM caffeine prolonged the duration of the

action potential up to a maximum of 140% when measured at a height of 80% from the resting level, whereas when measured at heights of 20% and 10% it was prolonged up to a maximum of 130%. On the other hand, 50 mM caffeine prolonged the duration of action potential measured at heights of 80, 20, and 10% of the spike much more than 3 mM caffeine, with values of a maximum of 230, 200, and 200%, respectively, when the drug potentiated the twitch. At the later stage, when 50 mM caffeine depressed the amplitude of twitch tension and induced a slight contracture, the action potential durations at heights of 80 and 20% of the spike were reduced to 160 and 140%, respectively.

Table 1. Changes in the membrane potential produced by caffeine. The basal driving frequency was 1/sec. Mean values (from 2-7 series of experiments) are given with the standard deviation.

Concentration of caffeine (mm/liter)	RP† (mV)	AP†† (mV)	APD (msec)			Maximum rate of rise (V/sec)	Peak tension (%)
			80% level	20% level	10% level		
Control	86±5	108±18	23±5	57±2	75±3	79±3	100
3	85±9	109±18	33±3	73±5	96±8	91±12	548±97
Control	80±6	97±20	19±2	61±5	77±5	171±29	100
50*	70±7	100±9	44±7	98±32	122±40	110±26	224±40
50**	67±9	92±8	30±8	83±26	130±45	46±21	48±8

* contracture absent, ** contracture present,

† resting potential, †† action potential.

Membrane resistance. The membrane resistance changes before and during the influence of 3 mM caffeine were measured by the partition chamber method (KAMIYAMA and MATSUDA, 1966) with two pulse generators, one for ordinary stimulation and the other for repetitive small and short square pulses of anodal current. The results obtained are illustrated in Fig. 3. In the experiment illustrated by Fig. 3B, a few action potentials were superimposed by synchronizing the time base with the upstroke of the spike. The upper edge of the single (Fig. 3A) or the superimposed traces (Fig. 3B) corresponds to an action potential in the absence of applied current, while the lower edge represents action potential displaced by anodal current. The amplitude of the voltage changes is proportional to the square root of membrane resistance. Three mM caffeine decreased the membrane slope resistance measured at 10 and 20 msec after the upstroke of the spike up to 72 and 84%, respectively, when the corresponding normal values are expressed as 100%. Similarly, 50 mM caffeine decreased the membrane slope resistance measured at 3, 10, and 20 msec after the upstroke of the spike, with values of 80, 82, and 69% respectively.

Rising phase of the action potential. Figure 4 a and b, shows the records of the rising phase of the atrial action potential of the guinea pig before and during the influence of 3 mM and 50 mM caffeine. In this case, the potentiation of twitch

Fig. 3. Changes in membrane resistance during action potential in auricular fiber. (A) a: control. b: 15 min after addition of 3 mM caffeine. (B) a: control. b: 20 min after addition of 50 mM caffeine. Records of several sweeps are superimposed. Anodal pulses of 10 msec duration at 30 cps (A) and 20 cps (B) were applied. In each record, the upper tracing is the monitor of current, middle tracing the action potential, and lower tracing the contractile tension.

contraction was four- and two-fold at 3 mM and 50 mM caffeine, respectively. As is illustrated in the figure, 3 mM caffeine accelerated the fast upstroke, but 50 mM caffeine decreased it (*cf.* Table 1). On the other hand, the rate of slower rise of the action potential was increased by both 3 mM and 50 mM caffeine. In Fig. 4 c, in which the rate of depolarization, dV/dt , has been plotted against the time which elapsed after the point of the fastest upstroke, it is clear that throughout this later phase the potential rises faster in the presence of caffeine. Taking the ratio of the ordinates, in 3 mM or 50 mM caffeine and in the control, the value was 1.4 in the former and 1.5 in the latter for 1 and 0.5 msec, respectively. In the same figure, the rate of repolarization was plotted as the negative value of dV/dt from the records of whole action potential, which correspond to the records of rising phase of the action potential, although the figures are not shown. The figure indicates that the rate of repolarization at 10 or 20 msec from the upstroke of the spike was depressed markedly in the presence of caffeine, especially in the case of 50 mM caffeine.

Fig. 4. Effects of 3 mM (A) and 50 mM (B) caffeine on the rising phase of the action potential. (a) The control records. (b) Those under the influence of caffeine. (c) The values (dV/dt) of the control (filled circles) and in the presence of caffeine (open circles) plotted against the time after the point of $(dV/dt)_{max}$. In each record, the upper tracing is the electrical time derivative of depolarization, middle tracing the action potential, and lower tracing the tension.

Relationship between electrical and mechanical properties in the presence of caffeine

In order to see the relationship between the electrical and mechanical changes, the per cent increase in duration of the action potential (APD) measured at a height of 80% of the spike and the per cent augmentation of the peak tension induced by 3 mM (A) and 50 mM (B) caffeine are plotted in Fig. 5, where the normal values are expressed as 100%. As seen in the figure, in the presence of 3 mM caffeine, the peak tension increased markedly from 100% to a maximum of 760%, while APD was prolonged less markedly, to a maximum of 140%. With 50 mM caffeine, the peak tension reached a maximum of 300% and APD a maximum of 190%. However, during the depressed state of twitch contraction under the influence of 50 mM caffeine, the contraction was decreased even when the action potential was prolonged (Fig. 5C).

Figure 6 shows the relationship between the time to peak tension and APD. The time to peak tension was plotted as a measure of the duration of contraction since the changes of the time to peak tension and the duration of contraction induced by caffeine were almost parallel. As can be seen, plots of the time to peak

tension against action potential duration appeared linear with 3 mM, 10 mM, and 50 mM caffeine, and indicate that the APD and time to peak tension increase together as the concentration of caffeine is raised.

Fig. 5. Relationship between contraction amplitude and action potential duration at a height of 80% of the spike in the presence of 3 mM (A) and 50 mM caffeine (B: the potentiating stage, and C: depressive stage). Continuous lines indicate the increase in equal percentages of the contraction and action potential duration. Plots in A were measured starting immediately after the application of caffeine until several minutes after the maximum potentiation of twitch contraction, plots in B starting about 5 min after the application of caffeine until the maximum potentiation, and plots in C for several minutes after the maximum potentiation.

Characteristics of caffeine contracture in sodium-free solution

General behavior of caffeine contracture. It was reported that in the frog ventricle caffeine contracture was easily induced in sucrose-Ringer solution lacking

Fig. 6. Relationship between the time to peak tension (TPT) and action potential duration (APD) at a height of 80% of the spike in the presence of 3 mM (filled circles), 10 mM (filled triangles), and 50 mM caffeine (open circles).

Fig. 7. Time course of the caffeine-induced contracture of guinea pig atrial fibers after soaking the muscle for 20–30 min in Tris-maleate Krebs solution. (a) 3 mM caffeine. (b) 50 mM caffeine. The bathing solution was exchanged three times with caffeine-containing Tris-maleate Krebs solution, in order to expect the complete replacement. Mechanical artifacts were marked in such a procedure.

sodium ions (SUZUKI, 1962). Further examination was carried out in sodium-free Tris-maleate Krebs solution in order to investigate the characteristics of the caffeine-induced contracture of the guinea pig atrium.

Figure 7 shows the recordings of tension elicited in auricular muscle fibers bathed in sodium-free (Tris) Krebs solution to which 3 mM (a) or 50 mM (b) caffeine had been added. Caffeine was applied 20–30 min after the substitution of sodium chloride and sodium bicarbonate to Tris-maleate, since transient contracture occurred in the sodium-free solution. As seen in the figure, even 3 mM caffeine, as well as 50 mM caffeine, evoked a distinct contracture in sodium-free solution. The rate of rise and the peak contracture tension, however, increased markedly as the caffeine concentration was increased.

Role of external calcium. In order to find the source of calcium which contributes to the caffeine contracture in sodium-free solution, the behavior of caf-

feine contracture was investigated in sodium-free and calcium-depleted solution. Figure 8 shows an example of the effects of the depletion of calcium on caffeine contracture. The solution in which the muscle was soaked contained 6.1×10^{-7} M free ionized calcium and was adjusted to the ratio of calcium and EGTA added to Tris-maleate Krebs solution. In this experiment, each application of 50 mM caffeine lasted about 5 min, with 5 min (from A to B), 10 min (from B to C), and 15 min (from C to D) intervals between applications. Contracture tension induced by the second application (B) of caffeine was reduced markedly. In the right hand figure, the peak tension evoked by 50 mM caffeine was plotted against the soaking time. As can be seen, caffeine contractures declined rapidly during the first 5 min, then slowly. After 5 min of soaking in 6.1×10^{-7} M Ca^{++} , the contractile response to 50 mM caffeine decreased to about 30% of the initial value.

Fig. 8. Effect of low (6.1×10^{-7} M) calcium solution on caffeine contracture. The muscle was equilibrated for about 30 min in a calcium-depleted Tris-maleate Krebs solution. The first addition of 50 mM caffeine (A) resulted in a large and slowly declining tension. Subsequent challenges with caffeine, after 5 min (B), 15 min (C), and 30 min (D) from (A), resulted in gradually smaller and briefer tensions. The right-hand figure shows a plot of the peak tensions evoked by 50 mM caffeine against the time after the muscle was soaked in low calcium Tris-maleate solution.

Minimum concentration of calcium ion required to induce the tension development by caffeine. Effects of caffeine (3 mM and 50 mM) on the mechanical activities at various concentrations of calcium ion were investigated in sodium-free Krebs solution. Caffeine was added 30 min after the immersion of the calcium-deficient solution.

Table 2 shows the presence or absence of caffeine contracture at various concentrations of calcium ion ($\geq 2.4 \times 10^{-6}$ — 6.4×10^{-9} M) in Tris-maleate Krebs solution. Three mM caffeine did not produce tension development when the concentration of calcium ion was 1.9×10^{-7} M, and the minimum concentration required to produce tension development by 3 mM caffeine was 2.4×10^{-6} M Ca^{++} . With 50 mM caffeine, the minimum concentration of calcium ion was 1.9×10^{-7} M.

Table 2. Dependence of the contracture on the external Ca^{++} concentration which was adjusted with calcium-EGTA buffers (pH about 7.1; temp. about 37°C).

Composition of the EGTA buffer		Total number of strips tested	Number of strips undergoing caffeine contracture	
[Ca] added [EGTA] added	Molar concentration of Ca^{++}		caffeine concentration 3 mM	50 mM
≥ 0.95	$\geq 2.4 \times 10^{-6}$	3	3	3
0.61	1.9×10^{-7}	1	0	1
0.34	6.2×10^{-8}	2	0	0
0.05	6.4×10^{-9}	1	0	0

DISCUSSION

In skeletal muscle, caffeine at a concentration of 1–4 mM produces a marked augmentation of twitch contraction without altering significantly the action potential (SANDOW *et al.*, 1964; SANDOW, 1965). Our results on the auricular muscle, however, indicated that caffeine at comparable concentrations increased the duration and height of action potential considerably and decreased the membrane slope resistance. These results are in agreement with those on the guinea pig atrium (DE GUBAREFF and SLEATOR, 1965) and on the kitten papillary muscle (CLARK and OLSON, 1968). In our results, these effects of caffeine on the action potential were found to be closely associated with the potentiation of the twitch contraction.

The increase in height and duration of the action potential, when considered with the decrease of membrane slope resistance, strongly suggests an enhancement of some inward movement of positive charges or outward movement of negative charges. Recent voltage clamp studies (ROUGIER *et al.*, 1968, 1969; MASCHER and PEPER, 1969) have revealed that a slow channel allowing the penetration of both sodium and calcium ions exists in cardiac cells and this current is related to the positive plateau level of the myocardial action potential. Thus, as the positive charges, sodium and/or calcium ions could be considered. As for the negative charges, on the other hand, chloride ions would be sole candidate. The plateau potential in our case, however, appeared much higher than chloride equilibrium potential (-111 mV in rabbit atrium, DE MELLO, 1961; -80 mV in sheep Purkinje fibers, DUDEL *et al.*, 1967), and since it was elevated due to caffeine, the possibility of increase of the chloride outward movement is hardly conceivable and could be neglected, although the exact chloride equilibrium potential in the guinea pig atrium is not known.

NAYLER (1963) found an increase of calcium influx when caffeine was applied in the cardiac muscle of toad, and recent voltage clamp experiments on dog ventricular muscle have shown that calcium current contributes to the activation

of twitch contraction (REUTER and BEELER, 1969; BEELER and REUTER, 1970). In the guinea pig ventricle also, the calcium current was found to be responsible for the rate of rise and amplitude of twitch contraction (OCHI and TRAUTWEIN, 1971). Thus, the prolongation and augmentation of action potential, as well as twitch contraction observed under the influence of caffeine, could be considered mainly due to an increase of the calcium inward current. Though the estimation should be proved directly, the finding that caffeine actually increased the rate of rise in the late rising phase of the action potential supports this view.

In the sarcoplasmic reticulum isolated from frog skeletal muscle, it is well known that release of calcium ions is larger the higher the concentration of caffeine (WEBER and HERZ, 1968). In the crayfish muscle fibers, it has been found that the rate of rise and peak tension increase markedly as the caffeine concentration is increased (CHIARANDINI *et al.*, 1970). They investigated the effect of caffeine in the range of concentrations between 1 and 80 mM. In the present experiments on the guinea pig atrium, however, the potentiation of twitch contraction was smaller the larger the concentration of caffeine over 3 mM. This could not be explained simply by the action of caffeine on the surface membrane.

WOOD and his co-workers (1969) proposed a hypothesis that the cardiac action potential has a dual effect: (1) to fill calcium stores; and (2) to release the calcium ions from the stores. The release was thought to contribute also to contraction and presumably to be larger when more calcium was accumulated. Thus, when this hypothesis is applied to our case, the stores can be sufficiently filled with more calcium ions which result from the increased calcium current by caffeine unless the calcium uptake of the stores is inhibited. On the isolated reticulum from the frog skeletal muscle, however, TANIGUCHI and NAGAI (1970) reported that more than 10 mM caffeine does inhibit the calcium uptake. If the mechanism in the guinea pig auricle is similar, 3 mM caffeine does not inhibit the filling of the stores, whereas 50 mM caffeine does. Hence, the present results, that the potentiation of twitch contraction was larger the smaller the concentration of caffeine, can be explained by the amount of stored calcium which may be affected by caffeine.

In the present experiments it was found that caffeine, 50 mM in concentration, evoked only a slight contracture in the auricular muscle, while it is known that 5–10 mM caffeine produces a contracture which reaches the height of maximum potassium contracture in skeletal muscle (LUDIN *et al.*, 1966). In sodium-free solution, however, a contracture of the auricular muscle was produced with 3 mM caffeine and contracture tension became larger as the concentration was increased. The latter results are compatible with those of the frog skeletal muscle (LÜTTGAU and OETLIKER, 1968) and of the crayfish muscle (CHIARANDINI *et al.*, 1970). Under sodium-free conditions, a large cellular uptake of calcium does occur in myocardium (REUTER and SEITZ, 1968; LANGER, 1968) and an increasing number of binding sites are occupied. Thus, it is presumed in the present case that the

amount of stored calcium is greater in the sodium-free solution and caffeine acts on the store in the atrium to produce contracture, as in the case of the skeletal muscle. This estimation may be supported by the facts that under normal conditions the calcium storage capacity is considerably less in myocardium than in skeletal muscle (FANBURG *et al.*, 1964; HASSELBACH, 1964).

The soaking experiment showed that after 5 min of soaking the contractile response to 50 mM caffeine decreased rapidly. During this time the calcium in the extracellular fluid is thought to have been largely removed by soaking in the low Ca^{++} solution (WINEGRAD and SHANES, 1962), since the half-time of the rapidly exchangeable component of the calcium of the guinea pig atrium is 4.5 ± 0.2 min. This may explain the rapid decrease of the contractile response to 50 mM caffeine in the present experiments, suggesting at least contribution of the surface membrane towards contractile activation. Under these conditions, however, calcium in the deep sites, probably in the sarcoplasmic reticulum, remains and is probably involved in the response in 50 mM caffeine, because the half-time of the slow component of the calcium washout curve is 86 (or 168) min in guinea pig atria (WINEGRAD and SHANES, 1962). Thus it is probable that caffeine acts on the sarcolemma as well as the sarcoplasmic reticulum in the myocardium.

In conclusion, from the effects of caffeine in the various ionic environment it is proposed that the effects on the cardiac muscle are fundamentally the same as those on the skeletal muscle. However, the modification of calcium current through the surface membrane affects the contractility both directly and indirectly via the sarcoplasmic reticulum, thus causing the responses different from those of the skeletal muscle.

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