THE EFFECT OF CAFFEINE ON EXCITATION-CONTRACTION COUPLING IN SKELETAL AND SMOOTH MUSCLE

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SUMMARY

1. For cockroach skeletal muscle, 2 mM caffeine considerably lowered the mechanical threshold without affecting the membrane potential. Contractions were induced by 8–10 mM caffeine.

2. In rat ileal smooth muscle, 1–10 mM caffeine inhibited spontaneous contractile behaviour, abolished spike activity and reduced KCl-induced contracture tension.

3. Enhanced spike activity associated with the KCl-induced phasic contraction was abolished by caffeine, the degree of caffeine-induced relaxation being proportional to the concentration employed. These relaxations were not accompanied by membrane hyperpolarization.

4. The present results accord with previous work which has shown that caffeine increases myoplasmic free calcium in the skeletal muscle and lowers it in the smooth muscle. It is suggested that caffeine releases bound calcium in the former muscle and promotes binding in the latter.

5. It is further suggested that in the smooth muscle caffeine may reduce the membrane permeability to calcium.

INTRODUCTION

At low concentrations (1–4 mM), caffeine enhances twitch tension in vertebrate skeletal muscle, while at higher concentrations (5–10 mM) caffeine brings about a contracture (Sandow, 1965), the contracture not being accompanied by a membrane depolarization (Axelsson & Thesleff, 1958). In mammalian muscle there is evidence that caffeine modifies tension by releasing calcium from the sarcoplasmic reticulum and by inhibiting reticular calcium uptake from the sarcoplasm (Herz & Weber, 1965; Weber, Herz & Reiss, 1966; Carvalho, 1968). A convincing correlation between caffeine action on reticulum and contracture-induction has now been established for mammalian muscle (Weber & Herz, 1968).

Far less is known about drug action on invertebrate skeletal muscle. A few reports have covered gross caffeine action on arthropod muscle (Huddart, 1969; Huddart & Abram, 1969; Bittar et al. 1974), but subcellular and gross fibre responses to caffeine

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have not been correlated. Even less is known about caffeine action on smooth muscle. Sakai & Iizuka (1972) have shown that caffeine abolishes spontaneous electrical and mechanical activity in toad bladder, and McFarland & Pfaffman (1972) have shown the same for guinea-pig taenia coli. However, with guinea pig taenia coli, Ito & Kuriyama (1971) found that caffeine evoked a small tension increase with a latency of 20–30 s, followed by a phasic contracture and then by a relaxation accompanied by a depolarization and block of spike generation. Both guinea-pig and rabbit taenia coli possess a significant amount of sarcoplasmic reticulum (Devine, Somlyo & Somlyo, 1972), suggesting that caffeine action on these muscles may resemble its action on vertebrate skeletal muscle.

In this present study we have compared caffeine action on an invertebrate skeletal muscle having an extensive sarcoplasmic reticulum with that on a smooth muscle devoid of sarcoplasmic reticulum. In a previous paper (Huddart & Syson, 1975), the subcellular actions of caffeine on these two muscles were described. Here we attempt to correlate the findings in order to unravel some of the confusion surrounding calcium mobilization in excitation-contraction coupling in these muscles.

MATERIALS AND METHODS

The skeletal muscle used throughout this study was the metathoracic extensor tibialis of the cockroach, *Periplaneta americana*. The smooth muscle was the longitudinal ileal muscle of the rat. Details of the cockroach preparation, its perfusion and methods of tension recording have already been published (Huddart, 1971), as have details of the ileum preparation and salines (Syson & Huddart, 1973; Huddart & Syson, 1975). Isolated preparations were allowed to equilibrate for 20 min in aerated Krebs' solution before recordings were made.

For tension recordings from the smooth muscle, the preparations were mounted vertically in a 50 ml organ bath, the contents of which could be rapidly exchanged (15 s) with fresh saline. The muscle strips were connected by cotton thread to the plate of a conventional isometric strain gauge, initial tension being adjusted to about 1 g. The gauge output was fed via an amplifier into a conventional ink-writing oscillograph.

For the simultaneous recording of membrane potential and tension, pieces of ileal muscle were mounted over short lengths of glass rod of diameter sufficient to prevent excessive movement of the preparation without overstretching. The mounted preparations were placed horizontally in a 15 ml bath, the contents of which could be quickly changed without disturbing the preparation. One end of the preparation was anchored to the glass rod while the other was attached to the strain gauge plate. All membrane potentials were recorded with glass micropipette electrodes filled with 3 M KCl. For the smooth muscle preparations, the microelectrodes were flexibly mounted on thin silver wire using the Woodbury & Brady (1956) technique and positioned over the preparation with the aid of a micromanipulator, penetration being observed through a microscope. The signal was fed via a high impedance probe into a Tektronix 502A oscilloscope. Permanent records were made with a Bell & Howell ultraviolet oscillograph in parallel with the oscilloscope.

The various caffeine salines were prepared from 50 mM caffeine stock solution made
Fig. 1. The relationship between contracture tension and external potassium in cockroach extensor tibialis muscle treated with normal salines (filled circles) and salines containing 2 mM caffeine (open circles). Note the considerable lowering of the mechanical threshold in the responses to caffeine-containing salines. n = 5.

up in the appropriate saline. The KCl contracture salines were derived by isotonic substitution of Na by K.

RESULTS

Potassium-induced contracture tension

Drug-induced modifications to the relationship between depolarization and tension in muscle can be most conveniently studied by examining contracture tension developed at different KCl levels. Fig. 1 summarizes the results of five experiments on cockroach skeletal muscle in which contracture tension was examined over a wide KCl range. In this muscle a sharp mechanical threshold was seen at about $-15$ mV, with an external potassium concentration of about 60 mM.

Potassium-induced contractures of rat ileal smooth muscle, and simultaneous records of membrane potential, are shown in Fig. 2. Potassium concentrations below 50 mM induced contractions which were accompanied by an increase in spike fre-
Fig. 2. Potassium-induced contractures in rat ileal smooth muscle. Membrane potential (upper traces) and tension (lower traces), at (a) 15 mM-KCl, (b) 50 mM-KCl and (c) 100 mM-KCl. The horizontal calibration bar indicates zero potential. Calibrations (a) and (b) 20 mV, 0.5 g and 10 s; (c) 20 mV, 0.5 g and 5 s.

frequency and decrease in spike amplitude as the membrane depolarized. At potassium concentrations around 50 mM the membrane depolarized sufficiently to block spike conduction, with a sustained depolarization of about 20 mV. Above 50 mM, potassium-induced contractions typically consisted of a phasic and a tonic response, as previously reported in guinea-pig taenia coli (Shimo & Holland, 1966) and in frog sartorius (Costantin, 1971). However, the delayed and protracted tonic response is only revealed when the response is recorded on a slow time course (Syson & Huddart,
Termination of spike activity was associated with the transition from phasic to tonic contraction. A sustained depolarization of about 30 mV was achieved. Maximal contracture activation was achieved with considerably less depolarization in the smooth muscle (about 20–25 mV) than in the skeletal muscle (Fig. 1).

**The effect of caffeine on rhythmicity and KCl-induced contracture tension**

With the skeletal muscle, 2 mM caffeine was without effect on resting or action potentials, but caused a considerable enhancement of KCl-induced contracture tension, representing a significant lowering of the mechanical threshold (Fig. 1). At


<table>
<thead>
<tr>
<th>Caffeine concentration (mM)</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in tension (g ± S.E.) (resting tension = 1 g)</td>
<td>-0.5 ± 0.02</td>
<td>-0.6 ± 0.04</td>
<td>-1.0 ± 0.1</td>
<td>-1.1 ± 0.1</td>
</tr>
<tr>
<td>Spontaneous tension generation (% control ± S.E.)</td>
<td>52.7 ± 15.2</td>
<td>47.8 ± 18.1</td>
<td>8.4 ± 3.0</td>
<td>0</td>
</tr>
<tr>
<td>Abolition of spike activity (% control)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Abolition of slow wave activity (% control ± S.E.)</td>
<td>0</td>
<td>0</td>
<td>50 ± 22.2</td>
<td>90 ± 10.0</td>
</tr>
<tr>
<td>Depolarization (mV ± S.E.)</td>
<td>0</td>
<td>0</td>
<td>5 ± 3.1</td>
<td>9 ± 5.3</td>
</tr>
<tr>
<td>Tissue recovery (% control rhythmicity)</td>
<td>100</td>
<td>100</td>
<td>83.3 ± 16.6</td>
<td>70.0 ± 15.2</td>
</tr>
</tbody>
</table>

Fig. 5. The relationship between contracture tension and external potassium in rat ileal smooth muscle, treated with normal salines (filled circles) and with salines containing 2 mM caffeine (squares). Each point represents the mean ± S.E. (n = 9–12). Note the significant increase in mechanical threshold in the responses to caffeine-containing salines.
Caffeine action on skeletal and smooth muscle

Fig. 6. The effect of 100 mM-KCl upon membrane potential (upper trace) and tension (lower trace) in a rat ileal smooth muscle pretreated for 1 min with 2 mM caffeine. The horizontal calibration bar indicates zero potential. Calibrations, 20 mV, 0.5 g and 10 s.

Fig. 7. The relationship between relaxation of tonic contracture tension in rat ileal smooth muscle and caffeine concentration. Contractures were induced with 100 mM KCl. On the vertical scale, 100% represents relaxation back to resting tension. Each point represents the mean ± S.E. (n = 6).

Concentrations from 6–10 mM, caffeine induced strong contractures (Fig. 3), again with no significant effect on membrane potential.

Application of 1–10 mM caffeine to spontaneously active preparations of the smooth muscle resulted in an immediate reduction of tension and abolition of spike activity (Fig. 4). With concentrations above 5 mM, spontaneous rhythmic contractions were abolished, as were the associated slow waves of membrane depolarization. Return to fresh saline for 5–10 min restored the previous electrical and mechanical activity, although recovery was somewhat incomplete after exposure to caffeine concentrations above 5 mM. These results are summarized in Table 1.

With the smooth muscle, 2 mM caffeine reduced potassium contracture tension and shifted the depolarization/tension relationship to the right (Fig. 5), i.e. it increased the
Fig. 8. The effect of 10 mM caffeine on a preparation of rat ileal smooth muscle previously depolarized and contracted by 50 mM KCl. The caffeine can be seen to bring about a relaxation of tension (lower trace) without hyperpolarization of the membrane potential (upper trace). The horizontal calibration bar indicates zero potential. Calibrations, 20 mV, 0.5 g and 10 s.

mechanical threshold, in complete contrast to the situation in skeletal muscle (Fig. 1). A typical KCl contracture in the presence of 2 mM caffeine is shown in Fig. 6. Not only was contracture tension reduced (compare with Fig. 2), but there was no increased spike activity during the onset of depolarization.

Of great interest here is that the final level of membrane depolarization attained at each potassium level was unaffected by the presence of caffeine; it appears that caffeine uncoupled contractile activity from membrane potential depolarization.

Further study of caffeine-induced relaxation of KCl-induced contractures in smooth muscle

At concentrations from 0.5 to 10 mM, caffeine caused an immediate relaxation of smooth muscle preparations that had been induced into contracture with 100 mM KCl salines. The caffeine caused no significant hyperpolarization of the membrane. The degree of relaxation was proportional to the caffeine level (Fig. 7). A typical response to KCl and then caffeine is shown in Fig. 8. Control preparations, which showed rhythmic spontaneous contractions were also relaxed upon application of 0.5–10 mM caffeine, with no membrane hyperpolarization.

DISCUSSION

It is clear that caffeine action on the excitation-contraction coupling mechanism of the smooth muscle is very different from its action on the skeletal muscle. These experiments show that caffeine acts in an excitatory manner on the skeletal muscle, enhancing normal contractility and inducing contracture. In complete contrast to this, caffeine inhibits spontaneous rhythmic contraction of the smooth muscle and abolishes the membrane slow wave and spike activity. The intracellular recording techniques used here confirm the supposition of Somlyo & Somlyo (1968) that the action of caffeine is to inhibit the generation of spike action potentials in certain smooth muscles. These authors employed extracellular recording techniques and alternative explanations were possible for their results.

In smooth muscle, the production of the action potential that initiates contraction
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involves an inward movement of calcium from the extracellular space (Holman, 1958; Bulbring & Kuriyama, 1963; Bennett, 1967; Nonomura, Holta & Oshashi, 1966; Brading, Bulbring & Tomita, 1969), and this calcium may act as a trigger for the release of membrane-bound calcium, in a manner similar to that proposed for skeletal muscle (Bianchi & Bolton, 1967; Sandow, 1970). The observed caffeine-induced inhibition of spike electrogenesis and associated contractions may therefore result from a reduction in membrane calcium permeability. The observation that caffeine reduces the size of both phasic and tonic components of the KCl-induced contracture supports the hypothesis that caffeine reduces smooth muscle membrane calcium permeability. Reduction of the phasic contracture, which has been shown to be related to release of membrane-bound calcium (Shimo & Holland, 1966), is almost certainly caused by the caffeine-induced abolition of the spikes which normally accompany this contracture. As in the case of suppression of natural spontaneous rhythmic contractile activity, spike abolition and related calcium influx inhibition may prevent the release of subcellular bound calcium. The tonic contracture response on the other hand is believed to derive its activator calcium from extracellular sources (Shimo & Holland, 1966). Since caffeine reduces this contracture response without affecting membrane depolarization level, this strongly supports the hypothesis that caffeine has blocked the usual rise in membrane permeability to calcium during the tonic contracture. The observation that caffeine will relax a previously evoked tonic contracture without hyperpolarization is further evidence for this hypothesis.

The nature of 'membrane-bound calcium' is of crucial importance in interpreting the differing action of caffeine on the skeletal and the smooth muscle. Whereas in skeletal muscle caffeine induces a rise in myoplasmic free calcium by inhibition of sarcoplasmic reticular Ca\textsuperscript{2+} ATPase binding sites (Carvalho, 1968; Huddart & Williams, 1974), its action on smooth muscle suggests a fall in myoplasmic free calcium. In a previous study (Huddart & Syson, 1975) it was shown that the membrane vesicular fraction of ileal smooth muscle (there is no organized sarcoplasmic reticulum) acted to release and bind calcium during the contraction-relaxation cycle. Isolated vesicular fractions were found to respond to caffeine application by an increase in calcium binding, causing a fall in myoplasmic free calcium and a relaxation of the preparation. These findings, considered with those reported here, suggest that caffeine action on smooth muscle may be twofold: firstly, a decrease in membrane calcium permeability, resulting in reduced fibre calcium influx with related abolition of spikes and phasic contractures; and secondly, a stimulation of intracellular calcium binding promoting a reduction of the tonic contracture. As to how interrelated these two phenomena may be in vivo it is difficult to tell.

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REFERENCES


A. J. Syson and H. Huddart


