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Inhibitory effects of caffeine on contractions and calcium movement in vascular and intestinal smooth muscle

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1 The mechanism of the inhibitory effect of caffeine was investigated using vascular smooth muscle of rabbit aorta and intestinal smooth muscle of taenia isolated from guinea-pig caecum.

2 Caffeine, 0.5–10 mM, relaxed the sustained contraction induced by 65.4 mM KCl or 10^{-6} M noradrenaline in aorta, and by 45.4 mM KCl or 10^{-6} M carbachol in taenia. The inhibitory effect of caffeine on the high K^+-induced contraction was antagonized by external Ca^{2+} but not by the Ca^{2+} channel activators, Bay K 8644 (10^{-7} M) or CGP 28,392 (10^{-7} M). Forskolin (2×10^{-7} M) potentiated the inhibitory effect of caffeine on the noradrenaline-induced contraction but not on the high K^+- or carbachol-induced contraction. Caffeine induced a time- and concentration-dependent increase in the cyclic AMP content of aorta and forskolin caused a further augmentation.

3 $^{45}Ca^{2+}$ uptake was increased by high K^+ or noradrenaline in aorta and by high K^+ or carbachol in taenia. The increments were inhibited by caffeine at concentrations needed to inhibit muscle contractions.

4 $^{45}Ca^{2+}$ in the cellular releasable site in aorta was decreased either by noradrenaline or by caffeine. Simultaneous application of noradrenaline and caffeine did not induce an additive decrease.

5 In aorta treated with a Ca^{2+}-free solution, caffeine induced only a small contraction. Noradrenaline induced a greater contraction which was inhibited by caffeine. After washout of caffeine and noradrenaline, the second application of noradrenaline induced a transient contraction suggesting that caffeine does not deplete the noradrenaline-sensitive store. Caffeine did not inhibit Ca^{2+} accumulation by the noradrenaline-sensitive store.

6 It was concluded that caffeine has multiple sites of action in smooth muscle. Caffeine releases Ca^{2+} from a store which is apparently not sensitive to noradrenaline. Caffeine may inhibit noradrenaline-induced Ca^{2+} release. Caffeine itself induces only a small contraction possibly because it decreases the Ca^{2+} sensitivity of contractile filaments and/or increases Ca^{2+} extrusion. Further, caffeine seems to inhibit stimulated Ca^{2+} influx. Cyclic AMP may be only partly responsible for the inhibitory effect of caffeine.

Introduction

In skeletal (Endo, 1977) and cardiac muscles (Fabiato & Fabiato, 1977), caffeine increases the sensitivity to Ca^{2+} of the Ca^{2+}-induced Ca^{2+}-release mechanism and releases Ca^{2+} from sarcoplasmic reticulum, resulting in a muscle contraction. In smooth muscle, caffeine also releases cellular Ca^{2+} to induce a transient contraction (Leijten & van Breemen, 1984; Karaki *et al.*, 1987). Beside the contractile effect, caffeine has a potent inhibitory effect in various smooth muscles (Ito & Kuriyama, 1971; Sunano & Miyazaki, 1973; Nasu *et al.*, 1975; Poch & Umfahrer, 1976; Casteels *et al.*, 1977). Caffeine has been shown to inhibit the cyclic adenosine monophosphate (cyclic AMP) phosphodiesterase and increase cellular cyclic AMP concentration (Butcher & Sutherland, 1962). Since cyclic AMP inhibits smooth muscle contractions (Kroeger,

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1983), it may be reasonable to assume that the inhibitory effect of caffeine is mediated by cyclic AMP. However, it is not fully understood as yet if this is the sole mechanism of the caffeine-induced inhibition in smooth muscle. In the present study, we have examined the inhibitory effect of caffeine on the contractions in vascular smooth muscle of rabbit thoracic aorta and intestinal smooth muscle of guinea-pig taenia caeci.

Methods

Tissue preparations

Two muscle preparations were used. (1) Male New Zealand white rabbits (2.0–3.0 kg) were killed by rapid infusion of sodium pentobarbitone (50 mg kg^{-1}) and air into the ear vein. The thoracic aorta was rapidly removed and cut into spiral strips of 2–3 mm width. The adventitial layer was removed from the medial (smooth muscle) layer (Karaki & Urakawa, 1977) in order to avoid the possible involvement of endogenous catecholamines (Karaki *et al.*, 1984a), and muscle strips (10–15 mm long) were prepared. These aortic strips did not contain functionally intact endothelium. In a preliminary experiment, it was confirmed that the inhibitory effect of caffeine in rabbit aorta was not modified by vascular endothelium. (2) Albino male guinea-pigs, weighing 250–300 g, were killed by a blow on the neck and a section of taenia, 5–10 mm in length, was dissected from the caecum.

Solutions

The normal physiological salt solution (PSS) contained (mM): NaCl 136.9, KCl 5.4, glucose 5.5, $NaHCO_3$ 23.8, $CaCl_2$ 1.5, $MgCl_2$ 1.0 and ethylenediamine tetraacetic acid (EDTA) 0.01. The concentration of $CaCl_2$ was changed to 0.03 mM, 0.3 mM or 7.5 mM and $MgCl_2$ was omitted in some experiments. Isosmotic 65.4 mM K^+ PSS was made by substituting 60 mM NaCl in the normal PSS with equimolar KCl. Hyperosmotic 45.4 mM K^+ PSS was made by increasing the concentration of KCl to 45.4 mM. Ca^{2+}-free PSS was made by omitting $CaCl_2$ and adding 1 mM ethyleneglycol *bis* (β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) to the above solutions. These solutions were aerated with a mixture of 95% O_2 and 5% CO_2 at 37°C (pH 7.4).

Contractile tension

Muscle strips were attached to holders and suspended in baths containing 10 ml PSS. Muscle tension was recorded isometrically with a force-displacement transducer connected to a polygraph (Nihon Kohden). Passive tension of 1 g for aorta and 0.2 g for taenia was initially applied and tissues were allowed to equilibrate for 60 min before beginning the experimental period. Caffeine was cumulatively applied when the contractile tension induced by stimulants reached a steady level. The concentration of caffeine required to induce a 50% inhibition (IC_{50}) was calculated from the cumulative concentration-inhibition curves.

Noradrenaline-induced transient contraction in aorta was obtained as described by Karaki *et al.* (1979). After exposure of the muscle strips to Ca^{2+}-free PSS for 15 min, 10^{-6} M noradrenaline was added for 5 min to induce a transient contraction. Following a 10 min wash with Ca^{2+}-free PSS, tissues were again incubated with noradrenaline for 5 min. After washing the muscle strips for another 10 min with Ca^{2+}-free PSS, 1.5 mM Ca^{2+} was added for 15 min to load the Ca^{2+} store in the muscle. Muscle strips were then rinsed with Ca^{2+}-free PSS for 15 min followed by the application of 10^{-6} M noradrenaline. This procedure was repeated until steady transient contractions were obtained. Caffeine was added either 5 min before the first application of noradrenaline or 5 min before the addition of 1.5 mM Ca^{2+}.

Ca^{2+} influx and Ca^{2+} release

Ca^{2+} influx and release was measured as described by Karaki & Weiss (1979). To measure Ca^{2+} influx, muscle strips were allowed to equilibrate in normal PSS for 2 h and then incubated with $^{45}Ca^{2+}$ (1 μCi ml^{-1}) for 5 min. Caffeine was added 10 min before the $^{45}Ca^{2+}$ exposure. Agonists were added simultaneously with $^{45}Ca^{2+}$.

To measure Ca^{2+} release from a storage site, muscle strips were incubated in a Mg^{2+}-free, 0.03 mM Ca^{2+} PSS for 60 min followed by incubation in an identical solution containing $^{45}CaCl_2$ (0.25 μCi ml^{-1}) for 60 min. A cellular releasable site is relatively selectively loaded with $^{45}Ca^{2+}$ using this procedure (Karaki & Weiss, 1979; 1980; Karaki *et al.*, 1983). Noradrenaline (10^{-6} M) or caffeine (10 mM) was added for the last 10 min of the 60 min $^{45}Ca^{2+}$ loading period.

In both experiments, the strips were then washed for 30 min in an ice-cold lanthanum-substituted PSS containing $LaCl_3$ 73.8 mM, glucose 5.5 mM and tris (hydroxymethyl) aminomethane (Tris) 24.0 mM. This solution was adjusted to pH 6.8–6.9 at 0.5°C with 1N maleic acid. After the La^{3+}-wash period, muscle strips were removed from the holders, blotted, placed in scintillation vials and $^{45}Ca^{2+}$ was extracted overnight with 1 ml of 20 mM EGTA solution. Scintillation mixture (ACS II, Amersham, 1 ml) was added to each vial and radioactivity was counted with a liquid scintillation spectrometer (Beckman).

Cyclic AMP contents

After equilibration, muscle strips were treated with test solutions, removed from the bath, rapidly frozen in liquid N_2 and homogenized for 30 s in 6% trichloroacetic acid. Cyclic AMP in the extract was measured by radioimmunoassay.

Statistics

Results of the experiments are expressed as mean ± s.e.mean. Values were considered to be significantly different when P value was less than 0.05 by use of Student's t test.

Drugs and chemicals

The following drugs and chemicals were used: (−)-noradrenaline bitartrate (Wako), Bay K 8644 (4-[2-(trifluoromethyl) phenyl]-1, 4-dihydro-2, 6-dimethyl-3-nitropyridine-5-carboxylic acid methylester, donated by Bayer), CGP 28,392 (4-[2-(difluormethoxy) phenyl]-1, 4, 5, 7-tetrahydro-2-methyl-5-oxofuro [3,4-b] pyridine-3-carboxylic acid ethylester donated by Ciba-Geigy), carbamyl choline chloride (carbachol, Sigma), forskolin (Calbiochem), EDTA (Sigma), EGTA (Sigma), Tris (Sigma), cyclic AMP radioimmunoassay kit (Yamasa Shoyu) and $^{45}CaCl_2$ (New England Nuclear). Caffeine (Wako), up to 10 mM, was dissolved in PSS without changing pH.

Results

Resting tone

In aorta, 10 mM caffeine showed little effect on the resting tone. In taenia, 3 mM caffeine transiently increased the frequency of spontaneous rhythmic contractions and 10 mM caffeine induced a transient contraction which gradually returned to resting level (Figure 1).

Sustained contractions

When added during the high K^+-induced sustained contractions in aorta and taenia, caffeine inhibited the muscle contraction. In aorta, 1–10 mM caffeine sometimes induced a small, transient increase in muscle tension followed by an inhibition (Figure 1). Concentration-inhibition curves for caffeine on high K^+-induced contractions in aorta and taenia are shown in Figure 2. IC_{50} values for caffeine were 2.3 ± 0.2 mM ($n = 4$) for aorta and 1.9 ± 0.3 mM ($n = 4$) for taenia. When the concentration of external Ca^{2+} was decreased to 0.3 mM or increased to 7.5 mM from the control level of 1.5 mM, the concentration-inhibition curves for caffeine shifted to the left or to the right, respectively (Figure 2). Pretreatment of the muscle with 10^{-7} M Bay K 8644 did not modify the relaxant effects of caffeine on 65.4 mM KCl-induced contraction in aorta (Figure 3). Similar results were obtained with 10^{-6} M CGP 28,392 (data not shown).

Caffeine also inhibited the 10^{-6} M carbachol-induced contraction in taenia (Figure 1) with an IC_{50} of 3.7 ± 0.8 mM ($n = 7$). A decrease in the Ca^{2+} concentration to 0.3 mM augmented, whereas an increase to 7.5 mM did not change the inhibitory effect of caffeine (Figure 4). During these experiments, it was noted that although cumulative addition of 0.1–1.0 mM caffeine induced concentration-dependent inhibition, addition of 3 mM caffeine sometimes failed to decrease further the carbachol-induced contraction (Figure 1c); this

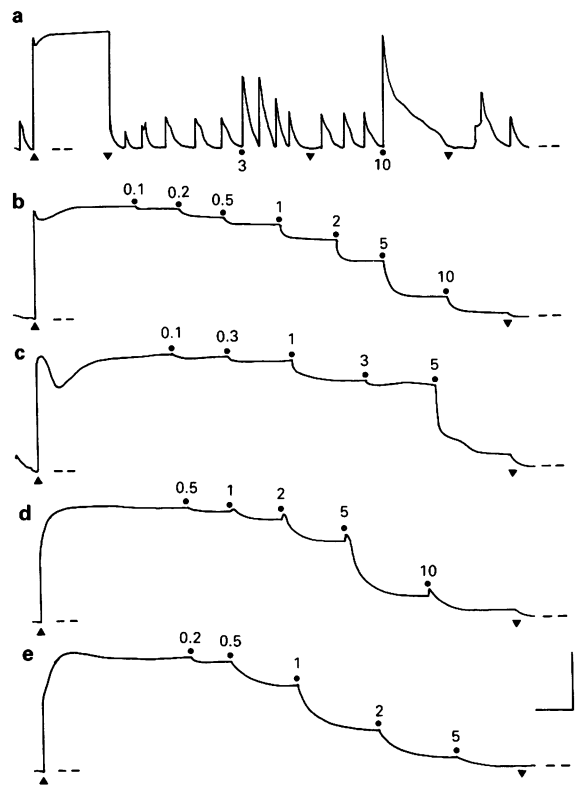

Figure 1 Effect of caffeine on the contractions in guinea-pig taenia caeci and rabbit aorta. (a) Effect of high K^+ and 3 and 10 mM caffeine on resting tone of taenia. (b and c) Inhibitory effect of caffeine on the high K^+- and 10^{-6} M carbachol-induced contractions in taenia, respectively. (d and e) Inhibitory effect of caffeine on the high K^+- and 10^{-6} M noradrenaline-induced contractions in aorta, respectively. Stimulant was added at ▲ and washout indicated by ▼. Vertical bar represents contractile force of 5 g for taenia and 0.5 g for aorta, and horizontal bar represents 10 min. Dashed line indicates the resting tension level.

may be due to a contractile effect of caffeine (Figure 1a).

Caffeine inhibited the 10^{-6} M noradrenaline-induced contraction in aorta with an IC_{50} of

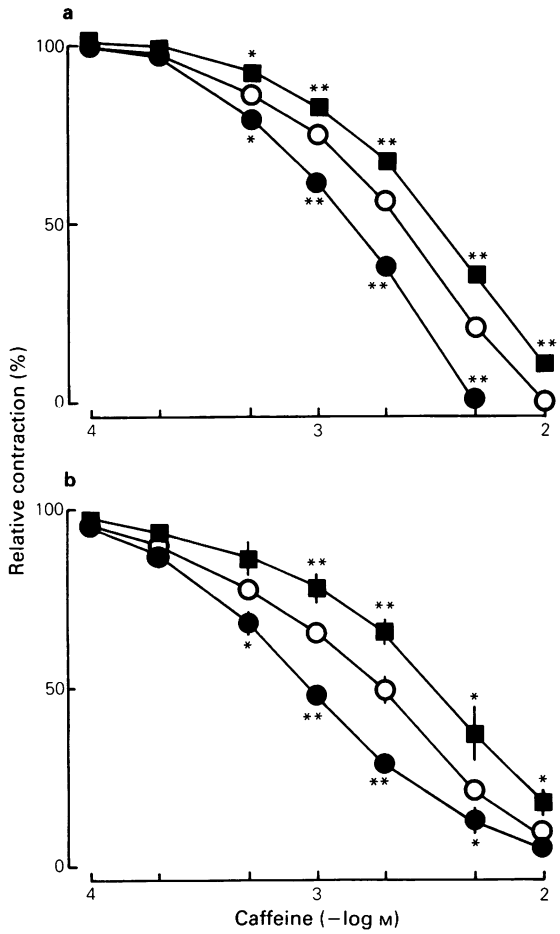

Figure 2 Inhibitory effect of caffeine on high K^+-induced contraction in rabbit aorta (a) and guinea-pig taenia (b) in the PSS with different Ca^{2+} concentrations; (■) 7.5 mM, (○) 1.5 mM and (●) 0.3 mM Ca^{2+}. One hundred % represents the tension level before the cumulative addition of caffeine. In aorta, actual contractile tension induced by high K^+ in a 1.5 mM Ca^{2+} PSS was 1.5 ± 0.2 g ($n = 8$) which increased to $106.4 \pm 2.1\%$ ($n = 4$, $P < 0.01$) in 7.5 mM PSS and decreased to $82.5 \pm 3.5\%$ ($n = 4$, $P < 0.01$) in 0.3 mM Ca^{2+} PSS. In taenia, actual contractile tension induced by high K^+ in a 1.5 mM Ca^{2+} PSS was 8.5 ± 0.9 g ($n = 8$) which increased to $113.8 \pm 5.2\%$ ($n = 4$, $P < 0.01$) in 7.5 mM PSS and decreased to $57.5 \pm 4.0\%$ ($n = 4$, $P < 0.01$) in 0.3 mM Ca^{2+} PSS. Means of 4 to 8 experiments are shown; vertical lines indicate s.e.mean. * and ** indicate values significantly different from the value in 1.5 mM Ca^{2+} PSS with $P < 0.05$ and $P < 0.01$, respectively.

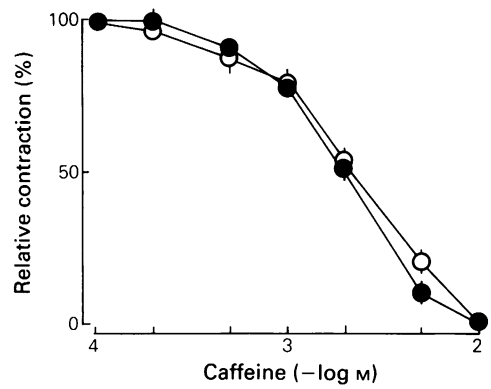

Figure 3 Effect of Bay K 8644 (●) on the caffeine-induced inhibition of noradrenaline-induced contraction in rabbit aorta. (○) Control responses. Bay K 8644 (10^{-7} M) was added 10 min before the cumulative addition of caffeine. One hundred % represents the tension level before the addition of caffeine. Actual contractile tension induced by high K^+ was 1.4 ± 0.3 g ($n = 4$) which increased to $110.2 \pm 1.5\%$ ($n = 4$, $P < 0.01$) in the presence of 10^{-7} M Bay K 8644. Means of 4 experiments are shown; vertical lines indicate s.e.mean.

1.3 ± 0.07 mM ($n = 4$). Changes in the external Ca^{2+} concentration to 0.3 mM or to 7.5 mM did not modify the effect of caffeine (Figure 4).

Pretreatment of the aorta with 2×10^{-7} M forskolin inhibited the 10^{-6} M noradrenaline-induced contraction to $79.0 \pm 3.5\%$ ($n = 8$). The inhibitory effect of caffeine on the noradrenaline-induced contraction was potentiated in the presence of forskolin, as shown in Figure 5. In contrast to this, 5×10^{-6} M forskolin showed little effect on the high K^+-induced contraction in aorta or the high K^+- or carbachol-induced contraction in taenia. Further, the inhibitory effects of caffeine on these contractions were not modified by forskolin (data not shown).

Transient contractions

In a Ca^{2+}-free PSS, 10^{-6} M noradrenaline induced a transient contraction in aorta (Figure 6). A second application of noradrenaline induced only a small contraction. Addition of 10 mM caffeine 5 min before noradrenaline either did not induce a contraction or induced only a small transient contraction. The noradrenaline-induced transient contraction was strongly inhibited by pretreatment with caffeine. Washing the muscle with caffeine- and Ca^{2+}-free PSS restored the transient contraction to a subsequent addition of noradrenaline (Figure 6). The magnitude of the transient contraction induced by the first application of noradrenaline in the presence of caf-

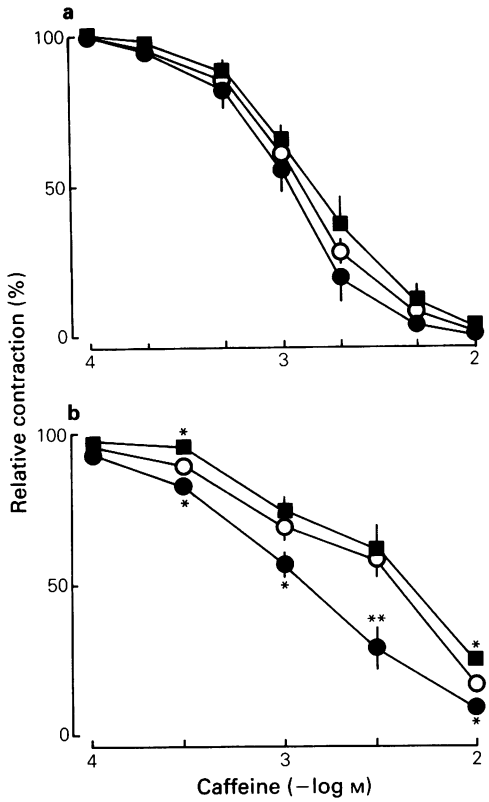

Figure 4 Inhibitory effect of caffeine on the 10^{-6} M noradrenaline-induced contraction in rabbit aorta (a) and carbachol-induced contraction in guinea-pig taenia (b) in the PSS with 0.3 mM (●), 1.5 mM (○) or 7.5 mM (■) Ca^{2+}. One hundred % represents the tension level before the cumulative addition of caffeine. In aorta, actual contractile tension induced by noradrenaline in a 1.5 mM Ca^{2+} PSS was 1.8 ± 0.2 g ($n = 8$) which did not change in 7.5 mM PSS but decreased to 89.0 ± 3.9% ($n = 4$) in 0.3 mM Ca^{2+} PSS. In taenia, actual contractile tension induced by carbachol in a 1.5 mM Ca^{2+} PSS was 9.3 ± 0.9 g ($n = 8$) which did not change in 7.5 mM PSS but decreased to 65.5 ± 4.5% ($n = 4$) in 0.3 mM Ca^{2+} PSS. Means of 4 to 8 experiments are shown; vertical lines indicate s.e.mean. * and ** indicate values significantly different from the value in 1.5 mM Ca^{2+} PSS with $P<0.05$ and $P<0.01$, respectively.

feine and that induced by the second application following washout of caffeine are shown in Figure 6. Caffeine induced a concentration-dependent inhibition of the first contraction. The second contraction, induced after removing caffeine, was slightly but significantly greater than the control which had not been pretreated with caffeine. When 5 mM caffeine was added only during the Ca^{2+} loading period, the transient contraction induced by 10^{-6} M noradrenaline was not affected, as has been found previously (Karaki *et al.*, 1979).

Effects of caffeine on Ca^{2+} influx

The resting Ca^{2+} influx in aorta was 85.4 nmol g^{-1} wet weight $5\ min^{-1}$; 65.4 mM K^+ and 10^{-6} M noradrenaline increased the Ca^{2+} influx to 146.5 nmol g^{-1} and to 128.4 nmol g^{-1}, respectively. Addition of 5 mM caffeine did not change the resting Ca^{2+} influx. However, 5 mM caffeine significantly decreased the Ca^{2+} influx activated by 65.4 mM KCl or 10^{-6} M noradrenaline (Figure 7).

In taenia, the resting Ca^{2+} influx was 116.8 nmol g^{-1} $5\ min^{-1}$; 45.4 mM K^+ and 10^{-6} M carbachol increased it to 180.2 nmol g^{-1} and to 179.5 nmol g^{-1}, respectively. The increments were inhibited by 10 mM caffeine (Figure 7).

Ca^{2+} release

Rabbit aorta accumulated 68.3 ± 2.7 nmol Ca^{2+} g^{-1} wet weight ($n = 6$) under a condition favourable for loading a releasable site with $^{45}Ca^{2+}$. Addition of 10^{-6} M noradrenaline or 10 mM caffeine decreased the Ca^{2+} content to 52.9 ± 1.5 nmol g^{-1} ($n = 6$) or to 47.8 ± 1.6 nmol g^{-1} ($n = 6$), respectively. Thus, caffeine released more Ca^{2+} (20.8 nmol g^{-1}) than

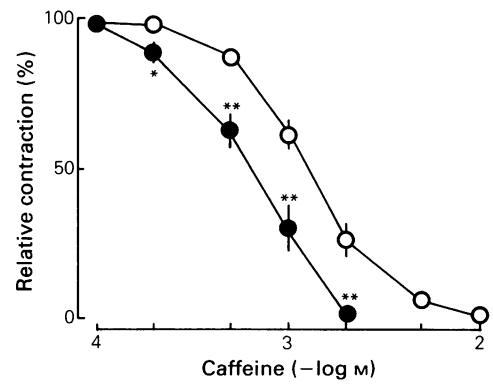

Figure 5 Effect of forskolin (●) on the caffeine-induced inhibition of noradrenaline-induced contraction in rabbit aorta. (○) Control responses. Forskolin (2×10^{-7} M) was added 30 min before the cumulative addition of caffeine. One hundred % represents the tension level before the addition of caffeine. Actual contractile tension induced by noradrenaline was 1.8 ± 0.3 g ($n = 4$) which decreased to 80.1 ± 3.3% ($n = 4$, $P<0.01$) in the presence of 2×10^{-7} M forskolin. Means of 4 experiments are shown; vertical lines indicate s.e.mean. * and ** indicate values significantly different from the value in the absence of forskolin with $P<0.05$ and $P<0.01$, respectively.

noradrenaline did (15.4 nmol g^{-1}, $P<0.05$). Simultaneous addition of 10^{-6} M noradrenaline and 10 mM caffeine did not show an additive effect (47.6 ± 0.9 nmol g^{-1}, $n=6$, or 20.7 nmol g^{-1} was released).

Cyclic AMP contents

Effects of caffeine on the cyclic AMP content of aorta are shown in Table 1. Caffeine caused a time- and concentration-dependent increase in the cyclic AMP contents of the aorta. Addition of forskolin in combination with 10 mM caffeine further increased the cyclic AMP content. Caffeine also increased the cyclic AMP content in the presence of high K^+ or noradrenaline.

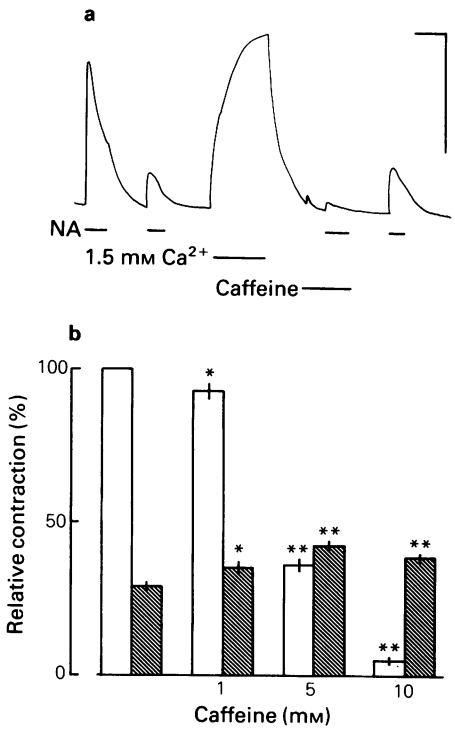

Figure 6 The effect of caffeine on the noradrenaline (NA)-induced transient contraction in rabbit aorta incubated in a Ca^{2+}-free, high K^+ solution. (a) Caffeine (10 mM) was added shortly before addition of noradrenaline after Ca^{2+} loading. The vertical bar represents 0.5 g contraction and the horizontal bar indicates 5 min. For further details see text. (b) The inhibitory effect of caffeine on a transient contraction in rabbit aorta. The experiment was performed as illustrated in (a). Open column represents the magnitude of the first transient contraction in the presence or absence of caffeine. The shaded column represents the magnitude of the second transient contraction obtained after wash out of caffeine. One hundred % represents the contractile tension induced by the first application of noradrenaline in the absence of caffeine. Means of 4 to 8 experiments are shown; vertical lines indicate s.e.mean. * and ** indicate values significantly different from the value in the absence of caffeine with $P<0.05$ and $P<0.01$, respectively.

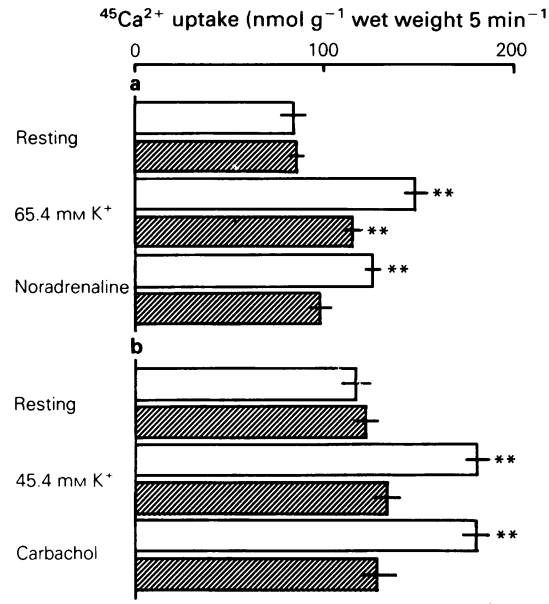

Figure 7 $^{45}Ca^{2+}$ uptake in rabbit aorta (a) and guinea-pig taenia (b). ** indicates values significantly different from resting control ($P<0.01$). Open column represents $^{45}Ca^{2+}$ uptake in the absence of caffeine and shaded column represents the value in the presence of 5 mM caffeine in aorta and 10 mM caffeine in taenia.

Discussion

Caffeine induced a transient contraction in resting taenia and high K^+-contracted aorta suggesting that caffeine has a dual effect on smooth muscle contraction and that there is a time lag between onset of contractile effect and onset of inhibitory effect. Caffeine inhibited the sustained contractions induced by high K^+ in both the aorta and taenia and this inhibitory effect was antagonized by external Ca^{2+}. Caffeine also inhibited the $^{45}Ca^{2+}$ influx stimulated by high K^+. These results are similar to those obtained with Ca^{2+} antagonists (Karaki, 1987a). The inhibitory effect of caffeine was not antagonized by Bay K 8644 or CGP 28,392, dihydropyridines which facilitate Ca^{2+} entry through the Ca^{2+} channel (Schramm *et al.*, 1983; Truog *et al.*, 1984; Karaki *et al.*, 1986). Spedding & Berg (1984) found that Ca^{2+} channel activators antagonized the effects of verapamil, diltiazem and dihydropyridine Ca^{2+} antagonists but not the diphen-

Table 1 Effect of caffeine on the cyclic AMP content of rabbit aorta

Caffeine		*Cyclic AMP content* (pmol g^{-1} wet weight)		
Concentration (mM)	*Incubation period* (min)	*Control*	*65.4 mM K^+*	*10^{-6} M Noradrenaline*
0	—	120.9 ± 9.6 (11)	134.2 ± 15.0 (6)	135.6 ± 6.1 (6)
1	20	167.6 ± 12.4 (6)**		
5	20	212.9 ± 23.4 (6)**		
10	1	222.7 ± 36.5 (6)**		
10	10	231.7 ± 10.9 (4)**		
10	20	261.3 ± 21.5 (10)**	239.9 ± 32.3 (6)**	225.2 ± 21.1 (5)**
10	20 (with forskolin)†	576.7 ± 54.3 (6)**		

†Forskolin (2×10^{-7} M) was added 30 min before the addition of caffeine. Forskolin alone increased the cyclic AMP content to 157.3 ± 11.1, $n = 6$ ($P < 0.05$). **Significantly greater than the value in the absence of caffeine.

ylalkylamine Ca^{2+} antagonists. Therefore, caffeine may directly inhibit the Ca^{2+} channel as diphenylalkylamine Ca^{2+} antagonists do, or may indirectly inhibit the Ca^{2+} channel by a mechanism yet to be determined.

Caffeine inhibited the carbachol-induced contraction in taenia as do Ca^{2+} antagonists (Karaki *et al.*, 1984b; Karaki & Weiss, 1984). Since the inhibitory effect of caffeine on carbachol-induced contraction in taenia was antagonized by external Ca^{2+}, and the carbachol-stimulated $^{45}Ca^{2+}$ influx was inhibited by caffeine, caffeine may inhibit the carbachol-induced contraction by a similar mechanism to that for the inhibition of high K^+-induced contraction.

Caffeine inhibited the noradrenaline-induced contraction in aorta and this effect was not antagonized by external Ca^{2+}. Noradrenaline-induced contraction in rabbit aorta is composed of an initial transient contraction, which is due to Ca^{2+} release, followed by a sustained contraction which is largey due to Ca^{2+} influx (Karaki & Weiss, 1984; Karaki, 1987a). Caffeine inhibited noradrenaline-stimulated Ca^{2+} influx. Since the noradrenaline-induced contraction and Ca^{2+} influx in rabbit aorta are resistant to Ca^{2+} antagonists (Karaki & Weiss, 1984; Karaki, 1987a), caffeine may inhibit the noradrenaline-stimulated Ca^{2+} influx by a mechanism which is different from that of Ca^{2+} antagonists.

Caffeine induced a time- and concentration-dependent increase in cyclic AMP content. Forskolin potentiated the effect of caffeine to increase the cyclic AMP content. However, forskolin did not modify the inhibitory effect of caffeine on the high K^+-induced contraction. Similarly, forskolin did not change the inhibitory effect of caffeine in the taenia. It has been found that forskolin and dibutyryl cyclic AMP inhibit the contraction induced by 10^{-6} M noradrenaline more strongly than that induced by 65.4 mM K^+ in rabbit aorta (Karaki, 1987a). These results suggest that the inhibitory effect of caffeine on the contractions sensitive to Ca^{2+} antagonists is not mediated by cyclic AMP. Since forskolin potentiated the inhibitory effect of caffeine on the noradrenaline-induced contraction, this effect may be mediated, at least partly, by cylic AMP.

Caffeine also inhibited the noradrenaline-induced transient contraction in the absence of external Ca^{2+}. After washout of caffeine, the second application of noradrenaline induced a significantly greater contraction than that in control muscle. Further, addition of caffeine during the Ca^{2+} loading period did not inhibit the following noradrenaline-induced contraction (present result and Karaki *et al.*, 1979). These results suggest that caffeine does not deplete noradrenaline-sensitive Ca^{2+} stores but inhibits noradrenaline-induced Ca^{2+} release. By measuring the amount of Ca^{2+} in the cellular releasable site, it was found that caffeine mobilized more Ca^{2+} than noradrenaline. However, the caffeine-induced contraction was much smaller than that induced by noradrenaline. Simultaneous addition of caffeine and noradrenaline induced a similar contraction and a similar Ca^{2+} mobilization to that induced by caffeine alone (present results and Karaki, 1987b). These results suggest that (1) caffeine releases cellular Ca^{2+} from a store that is not sensitive to noradrenaline, (2) caffeine inhibits noradrenaline-induced Ca^{2+} release, and (3) caffeine inhibits contractions due to Ca^{2+} release by decreasing the sensitivity to Ca^{2+} of contractile filaments and/or increasing Ca^{2+} extrusion.

It is concluded that caffeine has multiple sites of action in smooth muscle. It seems to release Ca^{2+} from a store which is different from that sensitive to noradrenaline and to inhibit noradrenaline-induced Ca^{2+} release. Caffeine itself induces only a small contraction possibly because it decreases the Ca^{2+}

sensitivity of contractile filaments and/or increases Ca^{2+} extrusion. Further, caffeine seems to inhibit stimulated Ca^{2+} influx. Cyclic AMP may be only partly responsible for the inhibitory effect of caffeine.

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