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Inhibitory effects of procaine on contraction and calcium movement in vascular and intestinal smooth muscles

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1 The effects of procaine on muscle tension and $^{45}Ca^{2+}$ movements were investigated in vascular smooth muscle of the rabbit aorta and intestinal smooth muscle of the taenia isolated from guinea-pig caecum.

2 Procaine (10 mM) induced a contraction in the taenia but had little effect on the resting tension in the aorta.

3 Procaine, 0.5–10 mM, relaxed the sustained contractions induced by 65.4 mM KCl and 10^{-6} M noradrenaline in the aorta, and by 45.4 mM KCl, 10^{-6} M carbachol and 10^{-6} M histamine in the taenia. The inhibitory effect of procaine on the high K^+-induced contractions was antagonized by external Ca^{2+} but not by the Ca^{2+} channel activators, Bay K 8644 and CGP 28,392.

4 $^{45}Ca^{2+}$ uptake was increased by high K^+ or noradrenaline in the aorta and by high K^+ or carbachol in the taenia. The increments were inhibited by procaine at the concentrations needed to inhibit the muscle contractions.

5 In a Ca^{2+}-free solution, noradrenaline and caffeine induced a transient contraction in the aorta, whereas a second application of each stimulant was almost ineffective. Addition of 1–10 mM procaine shortly before the first application of the stimulant inhibited the contraction. After washing the muscle with a Ca^{2+}-free solution without procaine, the second application of the stimulant induced a greater contraction than that in control muscle without procaine pretreatment.

6 Noradrenaline and caffeine released $^{45}Ca^{2+}$ from a cellular site in the aorta. Procaine inhibited the effects of these stimulants.

7 It was concluded that procaine may inhibit both the opening of Ca^{2+} channels and the release of Ca^{2+} from cellular stores and the former but not the latter effect may be attributable to a local anaesthetic action.

Introduction

Procaine inhibits smooth muscle contractions induced by noradrenaline, histamine, acetylcholine, high concentrations of K^+ and caffeine (Astrom, 1964; Somlyo *et al.*, 1969; Jacobs & Keatinge, 1974; Ito *et al.*, 1977; Weinstock & Weiss, 1979; Imai *et al.*, 1984; Karaki *et al.*, 1987). The inhibitory effect of procaine on high K^+-induced contraction is competitively antagonized by external Ca^{2+} (Feinstein, 1966; Somlyo & Somlyo, 1970; Kurihara & Sakai, 1976; Ishii & Shimo, 1984; Spedding & Berg, 1985). Since a larger portion of the smooth muscle contraction is due to Ca^{2+} influx (Karaki & Weiss, 1984, 1988), the inhibitory effect of procaine may be attributable to the inhibition of Ca^{2+} channels. However, Imai *et al.* (1984) showed in dog coronary artery that procaine did not inhibit the K^+-stimulated $^{45}Ca^{2+}$ influx at the concentration needed to inhibit the K^+-induced contraction.

Procaine also inhibits the transient contractions induced in a Ca^{2+}-free solution in various smooth muscles (Karaki *et al.*, 1979, 1987; Itoh *et al.*, 1981; Imai *et al.*, 1984). The transient contractions induced in a Ca^{2+}-free solution are attributable to release of

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Ca^{2+} from cellular stores and the effect of procaine on these contractions may be due to inhibition of Ca^{2+} release. In skeletal muscle, release of the Ca^{2+} store is triggered by a small amount of Ca^{2+} influx and procaine selectively inhibits this Ca^{2+}-induced Ca^{2+} release mechanism (Endo *et al.*, 1970; Ford & Podolsky, 1970; Endo, 1977). A similar mechanism has been demonstrated in smooth muscle (Saida, 1982; Itoh *et al.*, 1981; Imai *et al.*, 1984), although the physiological significance of this mechanism is not fully understood as yet (Karaki & Weiss, 1988).

In the present experiments, we examined the effects of procaine on smooth muscle contraction, transmembrane Ca^{2+} influx and release of Ca^{2+} in order to delineate the site of action of procaine. The results indicate that procaine inhibits both Ca^{2+} influx and Ca^{2+} release in smooth muscle.

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) surface in order to avoid the possible involvement of endogenous catecholamines, 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 procaine in the 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.

Contractile tension

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

Noradrenaline- or caffeine-induced transient contraction was obtained by the method described by Karaki *et al.* (1979) and Karaki (1987) (see Figure 4). This experiment was done at 23°C because caffeine induces greater contraction at low temperature, possibly because Ca^{2+} leakage from storage sites is less (Karaki *et al.*, 1987). After exposure of the muscle strips to Ca^{2+}-free physiological salt solution (PSS) for 15 min, 10^{-6} M noradrenaline or 10 mM caffeine was added for 5 min to induce the first transient contraction. Following a 10 min wash with Ca^{2+}-free PSS, the stimulant was applied for 5 min a second time. 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 addition of the agonist. This procedure was repeated until the transient contraction induced by the first application of the stimulant became constant. Procaine was added either 5 min before the first application of the stimulant or 5 min before the addition of 1.5 mM Ca^{2+}.

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

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

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

In both of the experiments, muscle strips were then washed to remove extracellular $^{45}Ca^{2+}$ 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 1 N 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).

In a preliminary experiment, we tried to determine the effects of procaine on cytosolic free Ca^{2+} concentrations using a fluorescent dye, fura 2, by a method

described by Ozaki *et al.* (1987). However, since fura 2-Ca^{2+} fluorescence was strongly inhibited by procaine, we were not able to use this technique in the present experiments.

Statistics

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

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. $MgCl_2$ 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*(beta-aminoethyl-ether)-N,N,N',N'-tetraacetic acid (EGTA) to the isosmotic 65.4 mM K^+ PSS. These solutions were aerated with 95% O_2 and 5% CO_2 mixture at 37°C (pH 7.4).

Drugs and chemicals

The following drugs and chemicals were used: procaine hydrochloride (Sigma), caffeine (Wako), (−)-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-difluoromethoxy) phenyl]-1,4,5,7-tetrahydro-2-methyl-5-oxofuro-[3, 4b]-pyridine-3-carboxylic acid ethylester, donated by Ciba-Geigy), carbamyl choline chloride (carbachol, Sigma), histamine dihydrochloride (Sigma), EDTA (Sigma), EGTA (Sigma), tris (Sigma) and $^{45}CaCl_2$ (New England Nuclear).

Results

Resting tone

In the rabbit aorta, addition of 1–10 mM procaine did not change the resting tone of the muscle. In the guinea-pig taenia, 1 mM procaine did not change or slightly augmented the spontaneous rhythmic contractions. However, 10 mM procaine induced a contraction followed by an inhibition of all the spontaneous activities. Washing the muscle with normal PSS without procaine induced a large after-contraction in the taenia, as shown in Figure 1.

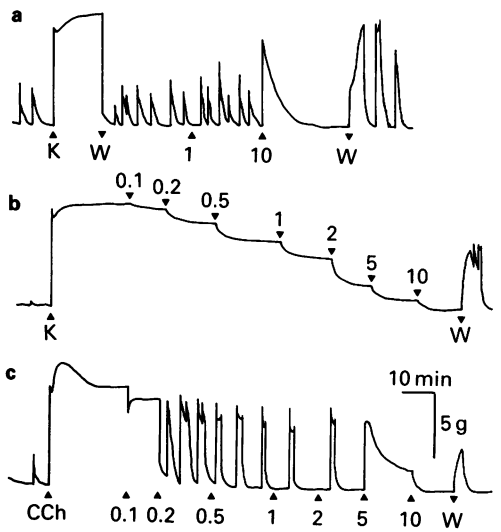

Figure 1 Effects of procaine on the muscle tone in guinea-pig taenia. (a) High K^+ (K, 45.4 mM) induced a sustained contraction. Although 1 mM procaine showed little effect, 10 mM procaine induced a contraction followed by an inhibition of all the spontaneous contractions. After removing procaine, a large after contraction was induced. (b) Effect of cumulative addition of procaine during the contraction induced by 45.4 mM K^+. (c) Effect of cumulative addition of procaine during the sustained contraction induced by 10^{-6} M carbachol (CCh). Numbers indicate mM concentration of procaine. W: washout.

Sustained contractions

Cumulative application of procaine during the sustained contractions induced by high K^+ decreased the muscle tension (Figure 1b). Concentration-inhibition curves for procaine on high K^+-induced contractions in the aorta and the taenia are shown in Figure 2. IC_{50} values for procaine were 5.4 ± 0.2 mM (n = 4) for the aorta and 0.9 ± 0.2 mM (n = 4) for the 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 procaine shifted to the left and to the right, respectively, in both of the preparations.

Pretreatment of the muscle with 10^{-7} M Bay K 8644 or 10^{-6} M CGP 28,392 did not modify the relaxing effects of procaine on the 65.4 mM K^+-induced contraction in the aorta (data not shown).

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

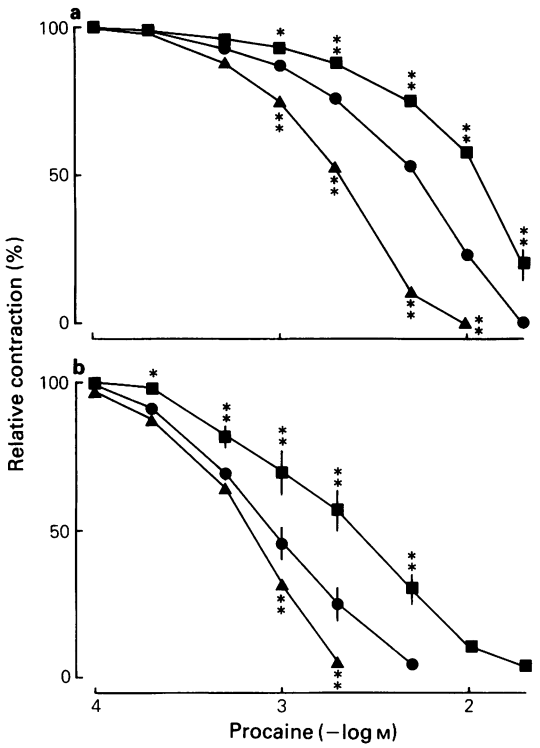

Figure 2 Concentration-inhibition curves for procaine on high K^+-induced contraction in rabbit aorta (a) and guinea-pig taenia (b) at different Ca^{2+} concentrations: (■) 7.5, (●) 1.5 and (▲) 0.3 mM Ca^{2+}. Experiments were done as shown in Figure 1b. Each point represents the mean of 4–6 experiments and s.e.mean is shown by a vertical line when it is greater than the symbol. * and **, significantly different from the value in the presence of 1.5 mM Ca^{2+} with $P < 0.05$ and 0.01, respectively.

2.1 ± 0.1 mM ($n = 8$). Changes in the concentration of external Ca^{2+} to 0.3 mM or to 7.5 mM did not modify the concentration-inhibition curves for procaine (Figure 3).

In the taenia, procaine inhibited the 10^{-6} M carbachol-induced contraction at concentrations higher than 0.1 mM and the maximum inhibition was obtained at 10 mM (Figure 1c). Similar results were obtained with the 10^{-6} M histamine-induced contraction. Since these contractions became oscillatory on the addition of procaine (Figure 1c), concentration-inhibition curves were not constructed.

Transient contractions

In a Ca^{2+}-free PSS at 23°C, addition of 10^{-6} M noradrenaline or 10 mM caffeine induced a transient contraction in the aorta. The second application of

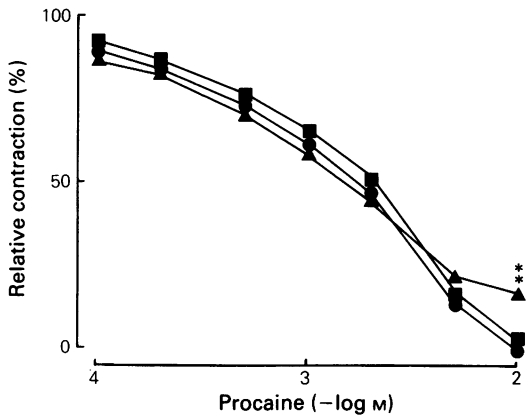

Figure 3 Concentration-inhibition curves for procaine on 10^{-6} M noradrenaline-induced contraction in rabbit aorta at different Ca^{2+} concentrations: (■) 7.5, (●) 1.5 and (▲) 0.3 mM Ca^{2+}. Each point represents the mean of 4–8 experiments. The s.e.mean was smaller than each symbol. ** Significantly different from the value in the presence of 1.5 mM Ca^{2+} with $P < 0.01$.

noradrenaline induced a small contraction whereas the second application of caffeine did not induce a detectable contraction. Addition of 10 mM procaine 5 min before the first application of the stimulant strongly inhibited the transient contraction. After washing the muscle with Ca^{2+}-free PSS without procaine, the second application of the stimulant induced a transient contraction larger than that in the control (Figure 4). The magnitude of the transient contraction induced by the first application of the stimulant in the presence of procaine and that induced by the second application in the absence of procaine are shown in Figure 5. Procaine, 1–10 mM, induced concentration-dependent inhibition of the first contraction. However, the second contraction, induced after removing procaine, was greater when the concentration of procaine was higher. It was also noted that recovery of the second caffeine-induced contraction was not as great as the second noradrenaline-induced contraction (Figure 5).

When 5 mM procaine was added only during the Ca^{2+} loading period, the 10^{-6} M noradrenaline-induced transient contraction was inhibited by only $20.3 \pm 1.5\%$ ($n = 4$), as has been found by Karaki *et al.* (1979).

Ca^{2+} influx

The resting Ca^{2+} influx in the aorta was 99.8 ± 3.2 nmol g^{-1} wet weight ($n = 6$). High K^+ (65.4 mM) and 10^{-6} M noradrenaline increased the Ca^{2+} influx to 183.1 ± 21.5 nmol g^{-1} ($n = 6$) and to 140.7 ± 3.5 nmol g^{-1} ($n = 6$), respectively. Addition

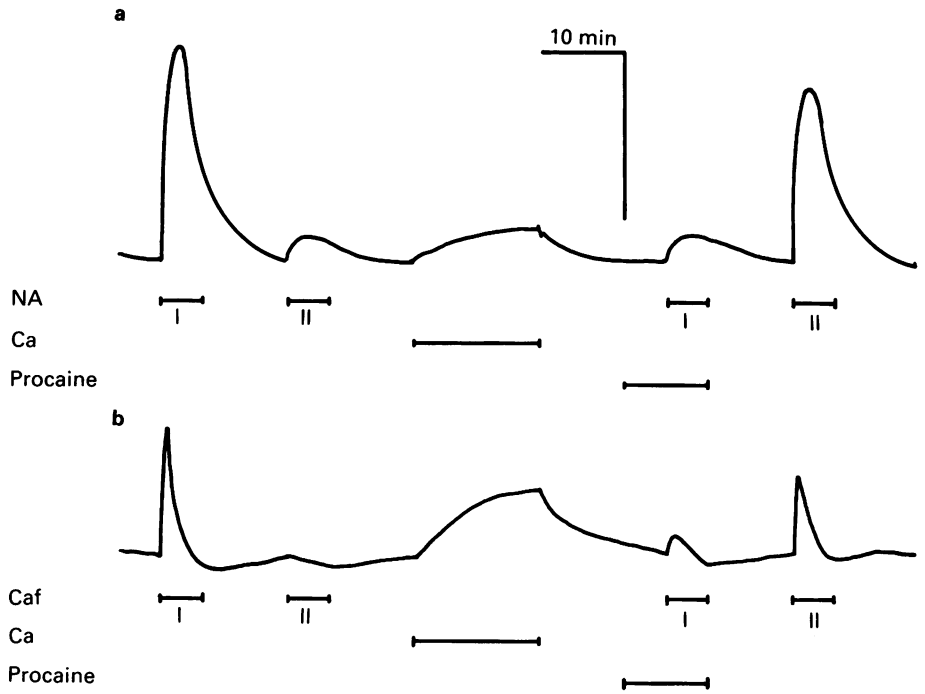

Figure 4 Inhibitory effect of procaine on the transient contraction induced by (a) 10^{-6} M noradrenaline (NA) and (b) 10 mM caffeine (Caf) (I) in rabbit aorta in Ca^{2+}-free PSS. Procaine (10 mM), added 5 min before the addition of 10^{-6} M noradrenaline or 10 mM caffeine, inhibited the contraction. After washing out procaine, the second application of noradrenaline or caffeine (II) induced a greater contraction than the second contraction without procaine-pretreatment. Vertical scale indicates 1 g for (a) and 0.5 g for (b).

of 5 mM procaine did not change the resting Ca^{2+} influx. However, 5 mM procaine significantly decreased the Ca^{2+} influx activated by 65.4 mM KCl or 10^{-6} M noradrenaline (Figure 6).

In the taenia, the resting Ca^{2+} influx was 111.8 ± 6.2 nmol g^{-1} ($n = 6$). Procaine (10 mM) increased the resting Ca^{2+} influx to 139.4 ± 4.5 nmol g^{-1} ($n = 6$). High K^+ (45.4 mM) and 10^{-6} M carbachol increased Ca^{2+} influx to 185.2 ± 6.7 nmol g^{-1} ($n = 6$) and to 176.9 ± 6.5 nmol g^{-1} ($n = 6$), respectively. The increase in Ca^{2+} influx due to 45.4 mM K^+ or 10^{-6} M carbachol was inhibited by 10 mM procaine (Figure 6).

Ca^{2+} release

Rabbit aorta accumulated 68.3 ± 2.7 nmol Ca^{2+} g^{-1} tissue ($n = 6$) under conditions designed to load the cellular releasable site with $^{45}Ca^{2+}$. Noradrenaline (10^{-6} M) and caffeine (10 mM) decreased the amount of this Ca^{2+} store to 52.9 ± 1.5 nmol g^{-1} ($n = 6$) and 47.8 ± 1.6 nmol g^{-1} ($n = 6$), respectively. In the presence of 10 mM procaine, the aorta accumulated 52.4 ± 1.8 nmol Ca^{2+} g^{-1} tissue ($n = 6$). Neither noradrenaline nor caffeine decreased the amount of Ca^{2+} in the presence of procaine (53.1 ± 2.1 nmol g^{-1}, $n = 6$ and 48.6 ± 1.9 nmol g^{-1}, $n = 6$, respectively).

Discussion

In the rabbit aorta, procaine did not change the resting tone. In the guinea-pig taenia, however, 10 mM procaine induced a contraction followed by an increase in Ca^{2+} influx. Procaine has been shown to depolarize the smooth muscle membrane (Jacobs & Keatinge, 1974; Ito *et al.*, 1977) by inhibiting potassium permeability (Casteels *et al.*, 1977; Imaizumi & Watanabe, 1982) and this may be the reason for the contractile effect of procaine (see also Kuriyama, 1981). Several minutes after the addition of procaine, however, muscle contraction was inhibited. Washing the muscle with normal PSS without procaine induced an after-contraction. These results may indicate that the site of the inhibitory effect of procaine is different from the site of the contractile effect.

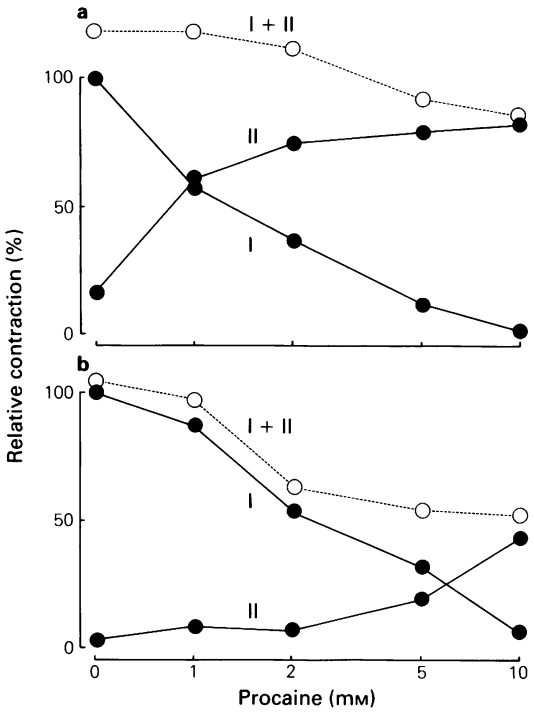

Figure 5 Concentration-inhibition curves for procaine on the transient contraction induced by 10^{-6} M noradrenaline (a) and 10 mM caffeine (b) in rabbit aorta. Experiments were done as shown in Figure 4. I: magnitude of the first contraction induced in the presence of procaine. II: magnitude of the second contraction induced after removing procaine. I + II: total of I and II; 100% represents the magnitude of the first transient contraction in the absence of procaine.

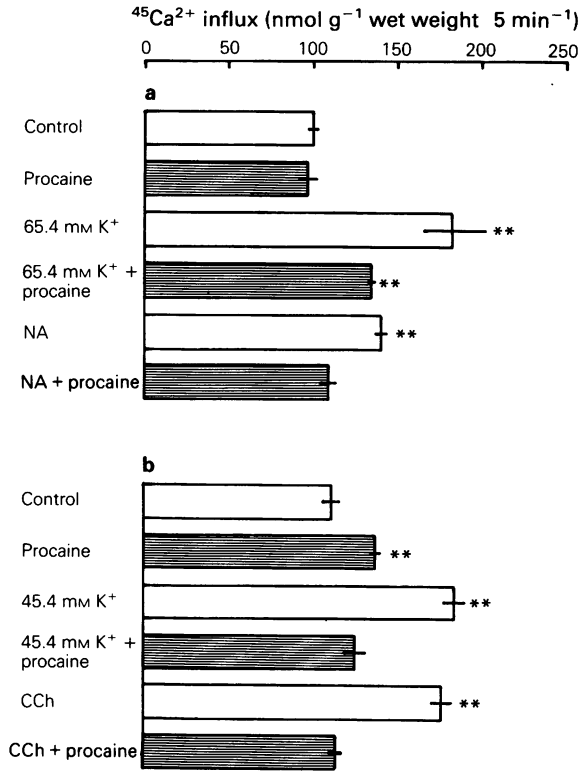

Figure 6 Effects of procaine (hatched columns) on $^{45}Ca^{2+}$ influx in (a) rabbit aorta and (b) guinea-pig taenia. Concentration of procaine was 5 mM for aorta and 10 mM for taenia. Each column represents the mean of 6 experiments; vertical lines indicate s.e.mean. ** Significantly different from control with $P < 0.01$. NA, 10^{-6} M noradrenaline and CCh, 10^{-6} M carbachol.

When added during the sustained contraction induced by high K^+ in the aorta and taenia, procaine showed an inhibitory effect. The procaine-induced inhibition was antagonized by increasing the Ca^{2+} concentration in the medium, as has been found previously (see Introduction). Further, procaine inhibited the high K^+-stimulated Ca^{2+} influx at the concentration needed to inhibit the contraction. These results suggest that procaine may directly or indirectly inhibit the voltage-dependent Ca^{2+} channels in these smooth muscles. In dog coronary artery, however, Imai *et al.* (1984) showed that procaine inhibited the high K^+-induced contraction but not the high K^+-stimulated Ca^{2+} influx. We do not have available data to explain the differences but point out the differences in the preparations and experimental procedures used; in the present study, procaine was added 10 min before the 5 min incubation with high K^+ and $^{45}Ca^{2+}$, whereas, in the experiments by Imai *et al.* (1984), procaine was added simultaneously with high K^+ and $^{45}Ca^{2+}$ and incubated for 30 min.

The inhibitory effect of procaine was not reversed by Bay K 8644 or CGP 28,392, dihydropyridines which stimulate Ca^{2+} entry through Ca^{2+} channels (Schramm *et al.*, 1983; Truog *et al.*, 1984). Spedding & Berg (1984) found that Bay K 8644 antagonized the effects of verapamil, diltiazem and dihydropyridine Ca^{2+} channel blockers but not the diphenylalkylamine Ca^{2+} channel blockers. Therefore, the mode of action of procaine on the voltage-dependent Ca^{2+} channel may be different from that of verapamil, diltiazem and dihydropyridine Ca^{2+} channel blockers. Ahn *et al.* (1988) also found that the inhibitory effects of caffeine on high K^+-induced contractions in rabbit aorta and guinea-pig taenia were antagonized by external Ca^{2+} but not by Bay K 8644 or CGP 28,392.

Ca^{2+} channel blockers inhibit not only high K^+-induced contractions but also the carbachol-induced contraction in the taenia (Karaki & Weiss, 1984). Procaine also inhibited the carbachol-induced contraction and $^{45}Ca^{2+}$ influx in the taenia. In contrast to these contractions, noradrenaline-induced contraction in rabbit aorta is relatively insensitive to Ca^{2+} channel blockers (Karaki & Weiss, 1984). However, procaine inhibited this contraction and the inhibitory effect was not antagonized by external Ca^{2+}. Further, procaine inhibited the $^{45}Ca^{2+}$ influx stimulated by noradrenaline. These results suggest that procaine may inhibit the receptor-linked Ca^{2+} channel in smooth muscles. The non-selective inhibitory effect of procaine on Ca^{2+} channels may result from disarrangement of phospholipids and alteration in membrane properties induced by its local anaesthetic action, as has been suggested by Spedding & Berg (1985).

In a Ca^{2+}-free solution, noradrenaline induced a transient contraction and the second application of noradrenaline was almost ineffective, possibly because the cellular Ca^{2+} store was depleted by the first noradrenaline application (Karaki *et al.*, 1979). Procaine inhibited the first transient contraction induced by noradrenaline. The second contraction induced after removing procaine was greater than that of control muscle. These results are consistent with the previous suggestion that procaine inhibits the noradrenaline-induced release of cellular Ca^{2+} and thus preserves the Ca^{2+} store (Karaki *et al.*, 1979). Similar results were obtained with 10 mM caffeine although the recovery of the caffeine-induced contraction after removing procaine was not so great as the noradrenaline-induced contraction. The $^{45}Ca^{2+}$ release experiments confirmed that both noradrenaline and caffeine mobilize Ca^{2+} from cellular stores and procaine inhibits the Ca^{2+} release. Although procaine itself decreased the amount of Ca^{2+} accumulated by the aorta, the amount of Ca^{2+} in the noradrenaline-releasable store does not seem to be decreased by procaine, because the procaine-pretreatment completely preserved the Ca^{2+} store which was released by the second application of noradrenaline. However, procaine may release a portion of the caffeine-releasable Ca^{2+} store because the pretreatment with procaine did not completely preserve the contraction due to the second application of caffeine.

As for the mechanism of Ca^{2+} release due to noradrenaline, it has recently been shown that activation of α-adrenoceptors may enhance phosphoinositide metabolism and the inositol-1,4,5-trisphosphate thus formed may release Ca^{2+} (Somlyo *et al.*, 1985; Hashimoto *et al.*, 1986). In contrast to this, caffeine releases Ca^{2+} by activating the Ca^{2+}-induced Ca^{2+}-release mechanism (Saida, 1982; Itoh *et al.*, 1981; Karaki *et al.*, 1987). Although procaine is an inhibitor of the latter mechanism (Endo, 1977), it is not known how procaine inhibits the noradrenaline-induced Ca^{2+} release. In the guinea-pig taenia, procaine induces a sustained increase in spike discharges although muscle contraction is inhibited (Ishii & Shimo, 1984). Such an 'uncoupling effect' of procaine may result from the inhibition of the Ca^{2+}-induced Ca^{2+} release mechanism (Ishii & Shimo, 1984; Karaki & Weiss, 1988). The inhibitory effect of procaine on Ca^{2+} release does not seem to be the result of a local anaesthetic action because lidocaine does not have such an effect at the concentration needed to inhibit high K^+- or noradrenaline-induced sustained contraction (Karaki *et al.*, 1987).

Smooth muscle contractions are attributable to Ca^{2+} influx through voltage-dependent and receptor-linked Ca^{2+} channels and also to release of Ca^{2+} stores (see Brading, 1981; Karaki & Weiss, 1984, 1988). The present results suggest that the non-selective inhibitory effect of procaine may be due to inhibition of Ca^{2+} influx through these Ca^{2+} channels and also inhibition of Ca^{2+} release. The former, but not the latter effect of procaine is probably due to its local anaesthetic action.

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References

AHN, H.Y., KARAKI, H. & URAKAWA, N. (1988). Inhibitory effects of caffeine on contractions and calcium movement in vascular and intestinal smooth muscle. *Br. J. Pharmacol.*, **93**, 267–274.

ASTROM, A. (1964). Influence of some local anaesthetics upon the adrenaline contraction of isolated strips of rabbit aorta. *Acta. Physiol. Scand.*, **60**, 30–38.

BRADING, A.F. (1981). How do drugs initiate contraction in smooth muscles? *Trends Pharmacol. Sci.*, **26**, 261–265.

CASTEELS, R., KITAMURA, K., KURAYAMA, H. & SUZUKI, H. (1977). The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.*, **271**, 41–61.

ENDO, M. (1977). Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.*, **57**, 71–108.

ENDO, M., TANAKA, M. & OGAWA, T. (1970). Calcium-induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibers. *Nature*, **228**, 34–36.

FEINSTEIN, M.B. (1966). Inhibition of contraction and calcium exchangeability in rat uterus by local anesthetics. *J. Pharmacol. Exp. Ther.*, **152**, 516–524.

FORD, L.E. & PODOLSKY, R.J. (1970). Regenerative calcium release within muscle cells. *Science*, **167**, 58–59.

HASHIMOTO, T., HIRATA, M., ITOH, Y., KANMURA, Y. & KURIYAMA, H. (1985). Inositol 1,4,5-trisphosphate activates pharmacomechanical coupling in smooth muscle of the rabbit mesenteric artery. *J. Physiol.*, **370**, 605–618.

IMAI, S., NAKAZAWA, M., IMAI, H. & NABATA, H. (1984). Effects of procaine on the isolated dog coronary artery. *Arch. Int. Pharmacodyn.*, **271**, 98–105.

IMAIZUMI, Y. & WATANABE, M. (1982). Effect of procaine on potassium permeability of canine tracheal smooth muscle. *Pflügers Arch. Eur. J. Physiol.*, **394**, 144–149.

ISHII, T. & SHIMO, Y. (1984). Inhibitory effects of procaine on the contractile responses of guinea-pig taenia caecum to acetylcholine, substance P and potassium chloride. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326**, 175–180.

ITO, Y., SUZUKI, H. & KURIYAMA, H. (1977). Effects of caffeine and procaine on the membrane and mechanical properties of the smooth muscle cells of the rabbit main pulmonary artery. *Jpn. J. Physiol.*, **27**, 467–481.

ITOH, T., KURIYAMA, H. & SUZUKI, H. (1981). Excitation-contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. *J. Physiol.*, **321**, 513–535.

JACOBS, A. & KEATINGE, W.R. (1974). Effects of procaine and lignocaine on electrical and mechanical activity of smooth muscle of sheep carotid arteries. *Br. J. Pharmacol.*, **51**, 405–411.

KARAKI, H. (1987). Use of tension measurements to delineate the mode of action of vasodilators. *J. Pharmacol. Methods*, **18**, 1–21.

KARAKI, H., AHN, H.Y. & URAKAWA, N. (1987). Caffeine-induced contraction in vascular smooth muscle. *Arch. Int. Pharmacodyn.*, **285**, 60–71.

KARAKI, H., KUBOTA, H. & URAKAWA, N. (1979). Mobilization of stored calcium for phasic contraction induced by norepinephrine in rabbit aorta. *Eur. J. Pharmacol.*, **56**, 237–245.

KARAKI, H. & WEISS, G.B. (1979). Alterations in high and low affinity binding of ^{45}Ca in rabbit aortic smooth muscle by norepinephrine and potassium after exposure to lanthanum and low temperature. *J. Pharmacol. Exp. Ther.*, **211**, 86–92.

KARAKI, H. & WEISS, G.B. (1984). Calcium channels in smooth muscle. *Gastroenterology*, **87**, 960–970.

KARAKI, H. & WEISS, G.B. (1988). Calcium release in smooth muscle. *Life Sci.*, **42**, 111–122.

KURIHARA, S. & SAKAI, T. (1976). Inhibitory effects of procaine on the electrical and mechanical activities of the smooth muscle cells of the guinea pig urinary bladder. *Jpn. J. Physiol.*, **26**, 503–516.

KURIYAMA, H. (1981). Excitation-contraction coupling in various visceral smooth muscles. In *Smooth Muscle.* ed. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 171–197. London: Edward Arnold.

OZAKI, H., SATO, K., SATOH, T. & KARAKI, H. (1987). Simultaneous recordings of calcium signals and mechanical activity using fluorescent dye fura 2 in isolated strips of vascular smooth muscle. *Jpn. J. Pharmacol.*, **45**, 429–433.

SAIDA, K. (1982). Intracellular Ca release in skinned smooth muscle. *J. Gen. Physiol.*, **80**, 191–202.

SCHRAMM, M., TOWART, G.T.R. & FRANCHOWIAK, G. (1983). Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. *Nature*, **303**, 535–537.

SOMLYO, A.V., BOND, M., SOMLYO, A.P. & SCAPA, A. (1985). Inositol trisphosphate-induced calcium release and contraction in vascular smooth muscle. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 5231–5235.

SOMLYO, A.P. & SOMLYO, A.V. (1970). Vascular smooth muscle, pharmacology of normal and hypertensive vessels. *Pharmacol. Rev.*, **22**, 249–353.

SOMLYO, A.V., VINALL, P. & SOMLYO, A.P. (1969). Excitation-contraction coupling and electrical events in two types of vascular smooth muscle. *Microvasc. Res.*, **1**, 354–373.

SPEDDING, M. & BERG, C. (1984). Interactions between a 'calcium channel agonist', Bay K 8644, and calcium antagonists differentiate calcium antagonist subtype in K^+-depolarized smooth muscle. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 69–75.

SPEDDING, M. & BERG, C. (1985). Antagonism of Ca^{2+}-induced contractions of K^+-depolarized smooth muscle by local anaesthetics. *Eur. J. Pharmacol.*, **108**, 143–150.

TRUOG, A.G., BRUNNER, H., CRISCIONE, L., FALLERT, M., KUHINS, H., MEIER, M. & ROGG, H. (1984). CGP 28392, a dihydropyridine Ca^{2+} entry stimulator. In *Calcium in Biological Systems.* ed. Rubin, P.R., Weiss, G.B. & Putney, J.W. Jr. pp. 441–449. New York: Plenum.

WEINSTOCK, M. & WEISS, C. (1979). Effects of procaine and extracellular calcium concentration on response of rat stomach fundus muscle to acetylcholine and 5-hydroxytryptamine. *Br. J. Pharmacol.*, **65**, 593–599.

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