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# Comparison of the effects of caffeine and procaine on noradrenergic transmission in the guinea-pig mesenteric artery

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**1** The effects of caffeine and procaine on noradrenergic transmission in the guinea-pig mesenteric artery were investigated by recording electrical responses of smooth muscle cells and by measuring the outflow of noradrenaline (NA) and 3,4-dihydroxyphenylglycol (DOPEG) induced by perivascular nerve stimulation.

**2** Caffeine possessed dual actions on the membrane, i.e., at low concentrations ($2.5 \times 10^{-4}$–$5 \times 10^{-4}$M), it hyperpolarized the membrane and decreased the membrane resistance and at high concentrations (over $2.5 \times 10^{-3}$M) it depolarized the membrane and increased the membrane resistance. Procaine (over $10^{-4}$M) consistently depolarized the membrane and increased the membrane resistance.

**3** The amplitude of the excitatory junction potential (e.j.p.) produced by perivascular nerve stimulation was increased by low concentrations of procaine ($2.5 \times 10^{-5}$–$10^{-4}$M) or high concentrations ($10^{-3}$–$5 \times 10^{-3}$M) of caffeine and was decreased by low concentrations of caffeine ($2.5 \times 10^{-5}$–$10^{-4}$M) or high concentrations of procaine ($5 \times 10^{-4}$–$10^{-3}$M). Higher concentrations of caffeine (over $5 \times 10^{-3}$M) induced a spike potential on the e.j.p., while higher concentrations of procaine (over $2.5 \times 10^{-3}$M) inhibited the generation of e.j.ps.

**4** Facilitation of e.j.ps produced by repetitive stimulation of perivascular nerves remained unchanged by caffeine, while it was enhanced by procaine at any given concentration (caffeine $2.5 \times 10^{-4}$–$10^{-3}$M; procaine $10^{-4}$–$10^{-3}$M).

**5** The membrane depolarization produced by exogenously applied NA ($10^{-5}$M) was not blocked by pretreatment with procaine.

**6** Conduction velocity of perivascular nerve excitation remained unchanged by application of caffeine (up to $5 \times 10^{-3}$M), and was reduced by application of procaine (over $2.5 \times 10^{-4}$M).

**7** Outflow of NA during perivascular nerve stimulation remained unchanged by caffeine ($10^{-4}$–$3 \times 10^{-3}$M), while it was enhanced by procaine (over $2.5 \times 10^{-4}$M). The outflow of DOPEG was slightly reduced by caffeine ($10^{-3}$–$5 \times 10^{-3}$M) and by lower concentrations of procaine ($10^{-4}$–$2.5 \times 10^{-4}$M) but was not altered by higher concentrations of procaine ($10^{-3}$–$5 \times 10^{-3}$M).

**8** It is concluded that in the guinea-pig mesenteric artery, high concentrations of caffeine (over $10^{-3}$M) increased the e.j.p. amplitude which might be due to an increase in membrane resistance of the smooth muscle cells. No marked effect of caffeine was observed on transmitter release from the nerve terminals. Procaine (over $2.5 \times 10^{-4}$M) increased transmitter release from perivascular nerves and blocked the re-uptake mechanism of released NA. The mechanisms underlying the decrease in e.j.p. amplitude by procaine remain to be determined.

## Introduction

Adrenergic transmission in vascular tissues can be estimated by recording the excitatory junction potentials (e.j.ps) induced by perivascular nerve stimulation from the smooth muscle cells (Kuriyama *et al.*, 1982). Release of transmitter requires Ca ions at the nerve terminals (Katz, 1969), and in vascular tissues the e.j.p. amplitude is dependent on the concentration of Ca ions in the superfusate (Kuriyama & Makita, 1983). Repetitive stimulation of perivascular nerves generates e.j.ps with a gradual increase in the

amplitude, i.e., facilitation of e.j.ps occurs (Kuriyama *et al.*, 1982). The facilitation of e.j.p. amplitude may be due to residual accumulation of Ca ions at the nerve terminal (Katz, 1969; McGraw *et al.*, 1982).

Caffeine and procaine have been used as tools to investigate excitation-contraction coupling mechanisms in striated (Endo, 1977) and smooth muscles (Itoh *et al.*, 1981), i.e., caffeine facilitates and procaine prevents release of Ca ions stored in the muscle cells. Caffeine also releases Ca ions stored in sympathetic nerve cells (Kuba, 1980).

We have examined the effects of caffeine and procaine on noradrenergic transmission in the guinea-pig mesenteric artery by recording e.j.ps from the smooth muscle cells and by measuring the outflow of noradrenaline (NA) and its metabolite, 3,4-dihydroxyphenylglycol (DOPEG), into the superfusate.

## Methods

Albino guinea-pigs of either sex, weighing about 200 g, were stunned and bled. Mesenteric vascular beds distributing to the ileal region were dissected in Krebs solution at room temperature.

Electrical responses of smooth muscle cells of the mesenteric artery were recorded by the methods described previously (Kuriyama & Suzuki, 1981); briefly, the mesenteric artery together with the vein or the lymphatic vessels, was mounted in a recording chamber and glass capillary microelectrodes filled with 3 M KCl (tip resistance, 40–80 MΩ) were used to impale the cells through the mesenteric membrane. The tissue was superfused with warmed (35°C) Krebs solution at a flow rate of about 3 ml $min^{-1}$. Electrotonic potentials were produced by the partition stimulating method (Abe & Tomita, 1968). Perivascular nerves were stimulated by drawing the proximal part of the artery into a suction electrode, and current pulses of 0.03–0.1 ms in duration and 30–100 V in intensity were supplied from an electric stimulator (Nihon Kohden SEN-3013).

Outflow of noradrenaline (NA) and its metabolite, 3,4-dihydroxyphenylglycol (DOPEG), into the superfusate were measured by the alumina adsorption method (Oishi *et al.*, 1983; Mishima *et al.*, 1984);

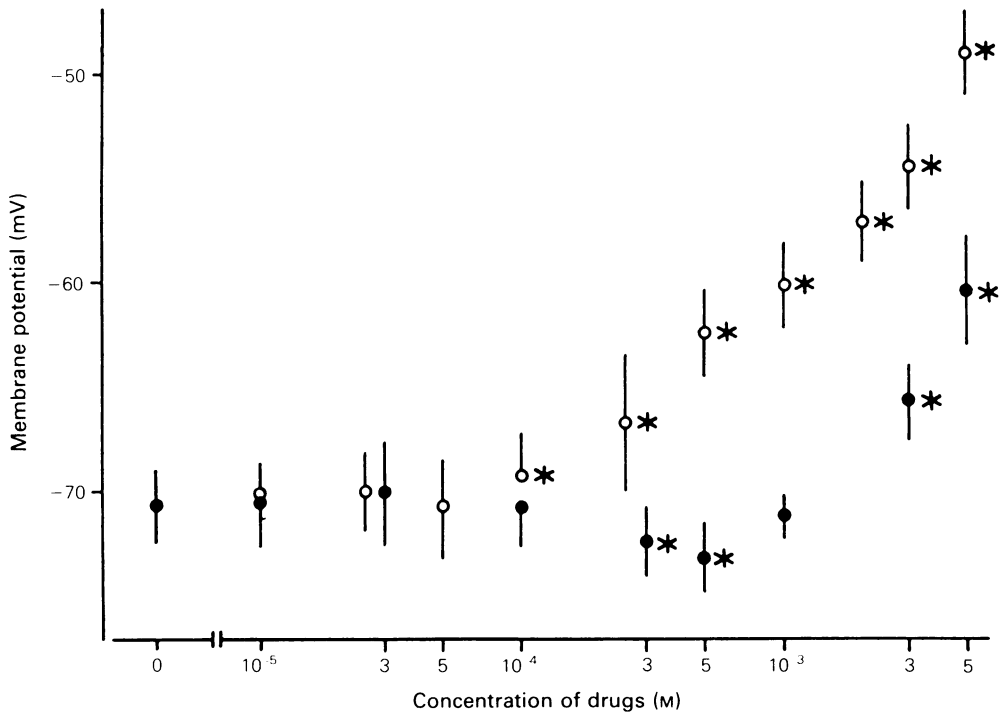


**Figure 1** Effects of caffeine (●) and procaine (○) on the membrane potentials of smooth muscle cells of the guinea-pig mesenteric artery. The membrane potentials were measured by impalements with electrodes of different cells, while caffeine ($10^{-5}$–$5 \times 10^{-3}$M) or procaine ($10^{-5}$–$5 \times 10^{-3}$M) was added for 20–30 min to the superfusate. Each point shows mean ($n = 7$–27); vertical lines show s.d. * Statistically significant from the control ($P < 0.05$).

briefly, a segment of artery (2.0–2.5 cm long) was mounted between a pair of Ag–AgCl wires (diameter, 0.5 mm, 3 cm long) fixed vertically at a distance of 1.5–2 mm, and the Krebs solution (35°C) was dripped onto the top of the tissue at a flow rate of 1 ml $min^{-1}$, using a perfusion pump (Tokyo Rikakikai, PO-1). The solution was collected into a conical test tube at the bottom of the tissue. Perivascular nerves were stimulated transmurally by current pulses of 0.2 ms in duration and 50 V in intensity.

To the collected solution was added 0.05 ml perchloric acid (60%). NA or DOPEG was measured by adsorption onto alumina and extracted with 0.1 N HCl (Oishi *et al.*, 1983). The extracted sample was examined by high-performance liquid chromatography. After the experiments, the tissue was blotted and weighed. The contents of NA and DOPEG in the samples were expressed as ng $g^{-1}$ wet weight of tissue.

Drugs used were caffeine (Wako), procaine HCl, guanethidine sulphate (Tokyo Kasei) and tetrodotoxin (Sankyo). All the drugs were freshly prepared for every experiment.

Experimental values were expressed as mean ± s.d., and statistical significance was tested using Student's $t$ test ($P < 0.05$).

## Results

### *Effects of caffeine and procaine on smooth muscle membrane*

The membrane potentials of smooth muscle cells of the guinea-pig mesenteric artery were measured by successive impalements of different cells with microelectrodes during application of caffeine or procaine (3–20 or 30 min). Figure 1 shows the concentration-response relationship of the effects of caffeine and procaine on the membrane potentials. Tetrodotoxin ($3 \times 10^{-7}$M) and guanethidine ($3 \times 10^{-6}$M) were added to the superfusate throughout the experiment, to prevent the involvement of transmitter released from perivascular adrenergic nerves in the membrane potential change. Caffeine hyper-

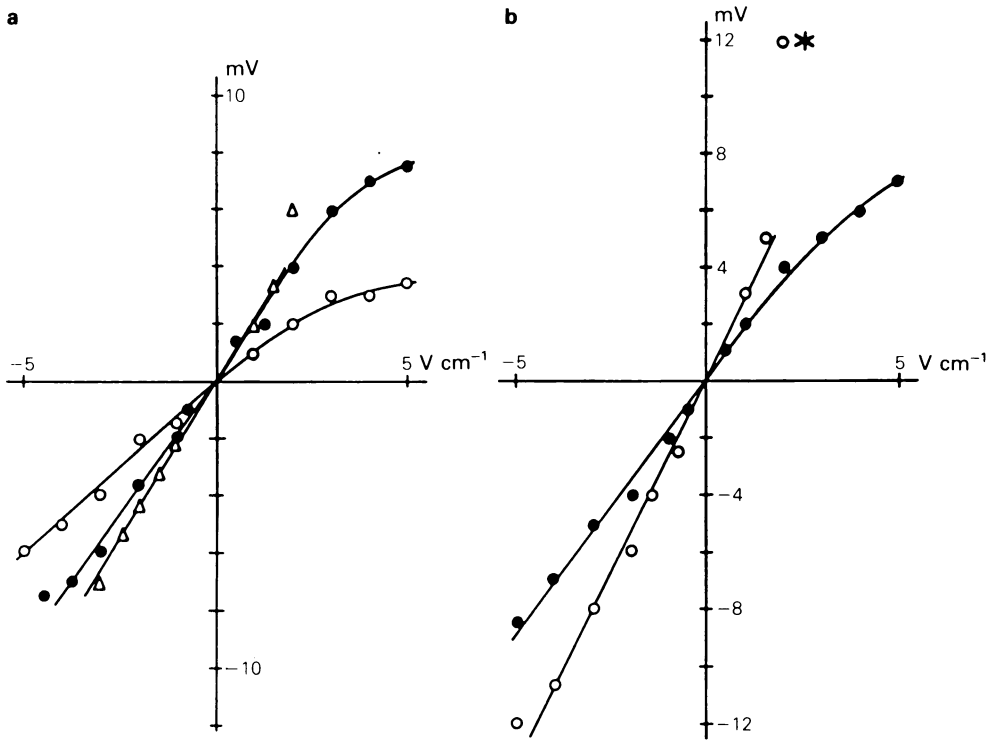


**Figure 2** Current-voltage relationships obtained during application of caffeine (a) or procaine (b). (a) Control (●); caffeine $5 \times 10^{-4}$M (○); caffeine $2 \times 10^{-3}$M (△); (b) Control (●); procaine $5 \times 10^{-4}$M (○). The relationships were obtained after the membrane potentials were shifted to the control level by current injection. The amplitudes of electrotonic potentials produced by inward (negative value) or outward (positive value) current pulses (1.5 s duration) were plotted. Depolarization or hyperpolarization is shown by a positive or negative value, respectively. * Spike potential was generated.

polarized the membrane at concentrations of $3 \times 10^{-4}$–$5 \times 10^{-4}$M and produced depolarization over $3 \times 10^{-3}$M. Procaine (over $10^{-4}$M) concentration-dependently depolarized the membrane. These effects of caffeine or procaine on the membrane potentials were reversed by washing for up to 20 min.

The amplitude of electrotonic potentials produced by application of inward and outward current pulses (1 s in duration) of constant intensity was decreased during hyperpolarization produced by $5 \times 10^{-4}$M caffeine and was increased during the depolarization produced by caffeine (over $2 \times 10^{-3}$M) or procaine (over $5 \times 10^{-4}$M).

Current-voltage relationships were observed during the hyperpolarization or depolarization induced by caffeine or procaine, by applying various intensities of current pulses (1.5 s in duration). Figure 2 shows the relationships recorded before and during the application of caffeine ($5 \times 10^{-4}$M, $2 \times 10^{-3}$M) or procaine ($5 \times 10^{-4}$M). As these concentrations of caffeine or procaine changed the membrane potential (Figure 1), the relationships were observed after the membrane potentials were shifted to the resting level by applying constant current. The figure shows that the relationship is steeper during application of $2 \times 10^{-3}$M caffeine or $5 \times 10^{-4}$M procaine, in comparison with the control, indicating that these concentrations of caffeine or procaine increase the membrane resistance. The lower concentration ($5 \times 10^{-4}$M) of caffeine decreased the slope of the current-voltage relationship, indicating a decrease in membrane resistance. During application of $2 \times 10^{-3}$M caffeine or $5 \times 10^{-4}$M procaine, strong outward current pulses (over $2\,V\,cm^{-1}$) generated spike potentials, therefore the current-voltage relationship was not observed with these intensities of outward current pulse.

*Effects of caffeine and procaine on excitatory junction potential*

Stimulation of perivascular nerves generated an excitatory junction potential (e.j.p.) in the smooth muscle cells of guinea-pig mesenteric artery (Kuriyama & Suzuki, 1981). The amplitude of the e.j.p. was nearly constant when the nerves were stimulated at low frequencies (below 0.1 Hz).

Figure 3 shows the concentration-response relationship of the effects of caffeine and procaine on

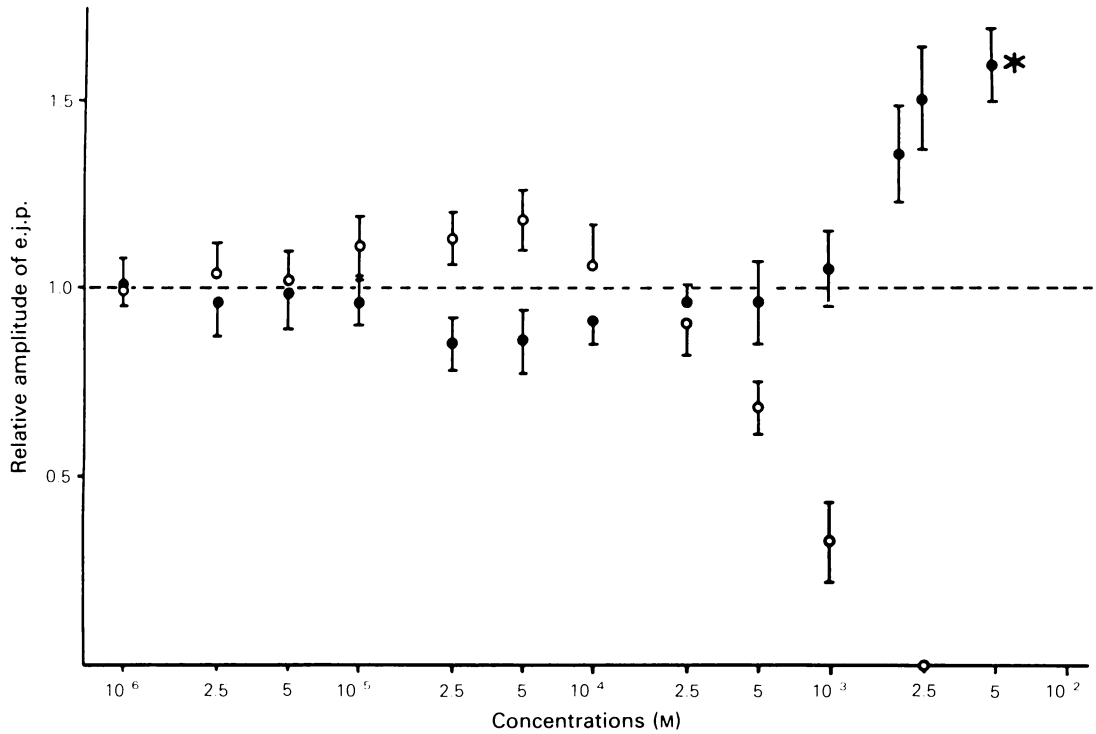


**Figure 3** Concentration-response relationship of the effects of caffeine (●) and procaine (○) on the e.j.p. amplitude. Perivascular nerves were stimulated at 0.05 Hz frequency while caffeine or procaine was applied for 5–10 min. The e.j.p. amplitude is expressed relative to the control (= 1.0), and each point is the mean ($n$ = 10–21); vertical lines show s.d. * Spike potential was generated on the e.j.p.

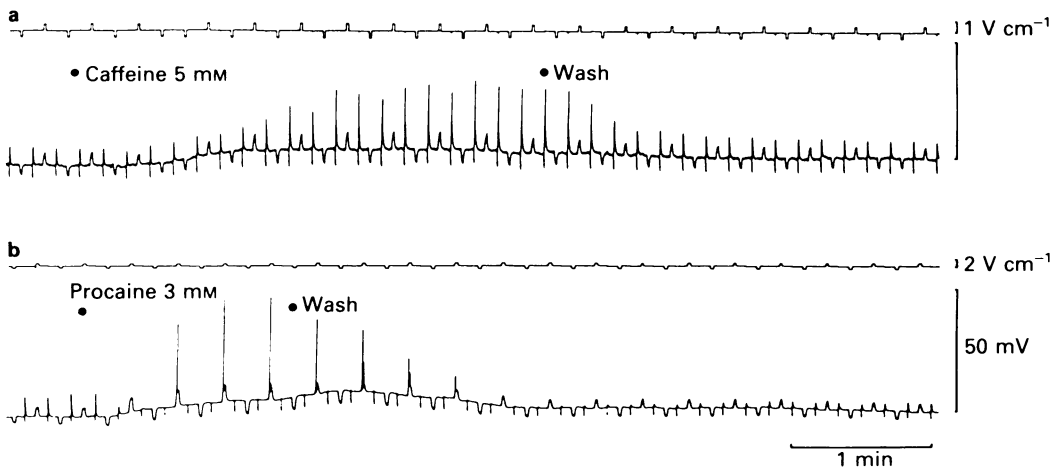


**Figure 4** Effects of caffeine (5 mM) or procaine (3 mM) on electrotonic potentials and e.j.ps. Constant intensities of inward and outward current pulses (1 s in duration; (a) 1 V cm$^{-1}$; (b) 0.5 V cm$^{-1}$) and brief current pulses (0.5 ms in duration; 50 V intensity) were alternately applied at 0.1 Hz frequency. In each record, upper and lower traces indicate current monitor and membrane potential, respectively.

amplitudes of the e.j.ps generated by 0.05 Hz frequency stimulation. Application of caffeine or procaine showed dual effects on the amplitude of e.j.ps, i.e., $2.5 \times 10^{-5}$–$10^{-4}$M caffeine decreased and over $2.5 \times 10^{-3}$M caffeine enhanced the e.j.p. amplitude, or $10^{-5}$–$10^{-4}$M procaine enhanced and $2.5 \times 10^{-4}$–$10^{-3}$M procaine decreased the e.j.p. amplitude. Application of over $5 \times 10^{-3}$M caffeine generated a spike potential on the e.j.p., while that of over $2.5 \times 10^{-3}$M procaine ceased the generation of e.j.ps. These effects of caffeine and procaine on the e.j.p. amplitude were reversed by washing for up to 20 min.

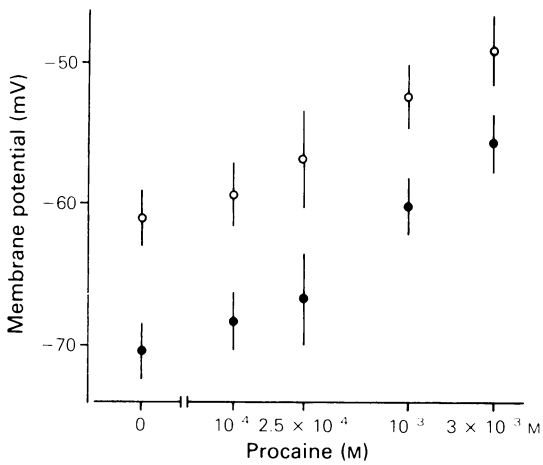


**Figure 5** Effects of procaine on membrane depolarizations produced by noradrenaline $10^{-5}$M. Tissue was equilibrated with procaine for 10–20 min, then noradrenaline $10^{-5}$M was added for 10–15 min. Mean of 6–21 observations is shown; vertical lines show s.d. Procaine alone (●); procaine plus noradrenaline (○).

Experiments were carried out to record electrotonic potentials and e.j.ps from the same cell during application of caffeine or procaine, to investigate whether or not the changes in e.j.p. amplitudes are related to the changes in the membrane resistance of the smooth muscle cells of the guinea-pig mesenteric artery. Constant intensities (0.5–1.0 V cm$^{-1}$) of inward and outward current pulses (1 s in duration) and of brief current pulses (0.05 ms in duration, 50 V intensity) were applied alternately at 0.1 Hz frequency, while caffeine ($5 \times 10^{-3}$M) or procaine ($3 \times 10^{-3}$M) was applied (Figure 4). These concentrations of caffeine or procaine depolarized the smooth muscle membrane (Figure 1), and the amplitude of the electrotonic potentials was increased. Spike potentials were generated by outward current pulse during application of $3 \times 10^{-3}$M procaine. The amplitude of e.j.p. was increased and generated a spike potential on the e.j.p. during application of $5 \times 10^{-3}$M caffeine, while the generation of e.j.p. was abolished by the application of $3 \times 10^{-3}$M procaine. Thus, while the application of $5 \times 10^{-3}$M caffeine or $3 \times 10^{-3}$M procaine increased membrane resistance, the former enhanced and the latter blocked the e.j.p. in the guinea-pig mesenteric artery.

Effects of procaine pretreatment on membrane depolarization produced by exogenously applied noradrenaline (NA) were observed, to determine whether blockade of e.j.p. by procaine was due to decreased sensitivity of α-adrenoceptors in the smooth muscle membrane. In the absence of procaine, NA ($10^{-5}$M) depolarized the membrane by about 9 mV,

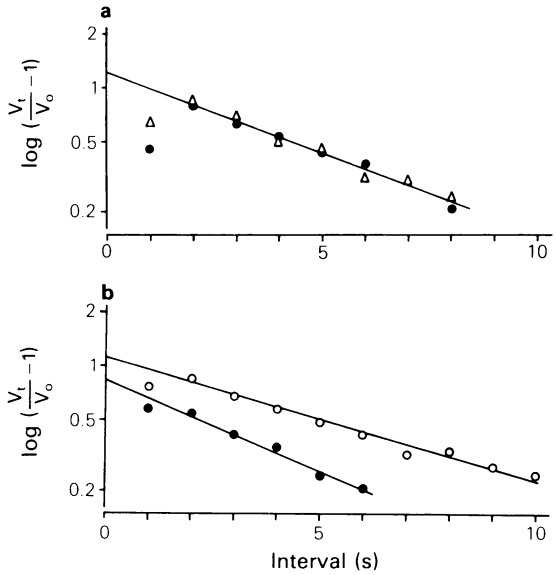


**Figure 6** Effects of caffeine (a) or procaine (b) on the e.j.p. amplitude. Perivascular nerves were stimulated twice at various intervals, and the e.j.p. amplitude produced by the second stimulus (Vt) was expressed relative to the first (Vo), as a function of (Vt/Vo − 1) on a logarithmic scale. (a) Control (●); caffeine 0.25 mM (△). The regression line is given by y = exp (− 0.21t); (b) control (●); procaine 0.1 mM (○). The regression line for control, y = exp (− 0.24t); procaine 0.1 mM, y = exp (− 0.16t); where y is the value determined by log (Vt/Vo − 1), and t is time.

**Table 1** Effects of caffeine and procaine on (a), time constant of the decay in e.j.p. amplitude and (b), conduction velocity of perivascular nerves in the guinea-pig mesenteric artery

| | *(a) Time constant of e.j.p. facilitation* (s) | *(b) Conduction velocity* ($cm\ s^{-1}$) |
|---|---|---|
| Control | 4.8 ± 0.9 (16) | 25.1 ± 3.1 (27) |
| Caffeine $2.5 \times 10^{-4}$ M | 4.7 ± 0.4 ( 3) | 25.7 ± 1.7 ( 3) |
| $10^{-3}$ M | 4.8 ± 1.5 ( 5) | 26.4 ± 3.7 ( 5) |
| Procaine $10^{-4}$ M | 6.3 ± 0.9 ( 4)* | 24.5 ± 3.2 ( 6) |
| $2.5 \times 10^{-4}$ M | 6.2 ± 0.5 ( 4)* | 21.9 ± 2.2 ( 5)* |
| $10^{-3}$ M | | 12.3 ± 1.4 ( 8)* |

The time constant of the decay in e.j.p. amplitude was calculated from the relationship shown in Figure 6. The conduction velocity of perivascular nerve excitation was calculated from relationship between the latency of e.j.ps and the distance between the recording and stimulating electrodes.
* Statistically significant from the control value ($P < 0.05$).
Mean ± s.d. is shown (number of observations in the parenthesis).

and the amplitude remained unchanged during procaine-induced depolarization (Figure 5). This NA-induced depolarization could be blocked by application of prazosin ($10^{-6}$M) (Kuriyama & Makita, 1983). Thus, the sensitivity of α-adrenoceptors in the smooth muscle membrane was not changed by procaine.

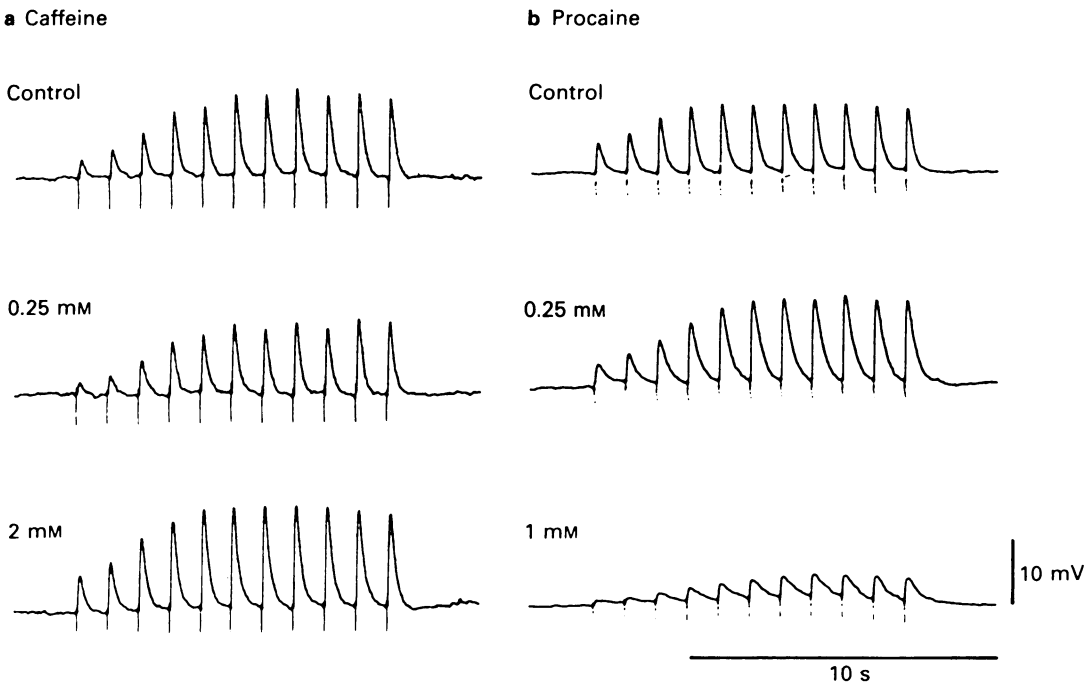


**Figure 7** Effects of caffeine (0.25 and 2 mM) or procaine (0.25 and 1 mM) on the e.j.ps generated by perivascular nerve stimulation at 1 Hz frequency. Each response was recorded from a different cell.

A pair of stimuli at various intervals was applied, to observe the effects of caffeine and procaine on facilitation of the e.j.ps. The relative amplitudes of e.j.p. produced by the second stimuli (Vt − Vo/Vo) were plotted on a logarithmic scale against the period of intervals (t), where Vo and Vt were the amplitudes of the first and the second e.j.ps, respectively (Mallart & Martin, 1967). As shown in Figure 6, the relationship was linear, and caffeine ($2.5 \times 10^{-4}$M) did not alter while procaine ($10^{-4}$M) decreased the slope of the relationship. The experiments were repeated in different tissues by applying various concentrations of caffeine or procaine, and the time constant of the decay of e.j.p. amplitude observed during applications of these drugs is summarized in Table 1. Caffeine ($2.5 \times 10^{-4}$ or $10^{-3}$M) did not alter the time constant of the decay of the e.j.p. amplitude, while procaine ($10^{-4}$ or $2.5 \times 10^{-4}$M) increased it.

Effects of caffeine and procaine on the e.j.ps generated by a train of stimulation to perivascular nerves were observed. Application of repetitive stimulation of nerves at 0.1–1 Hz frequency generated e.j.ps with successively increasing amplitude and plateaued at 5–7 s (Kuriyama & Suzuki, 1981). Figure 7 shows effects of caffeine or procaine on the amplitude of e.j.ps generated by a train of nerve

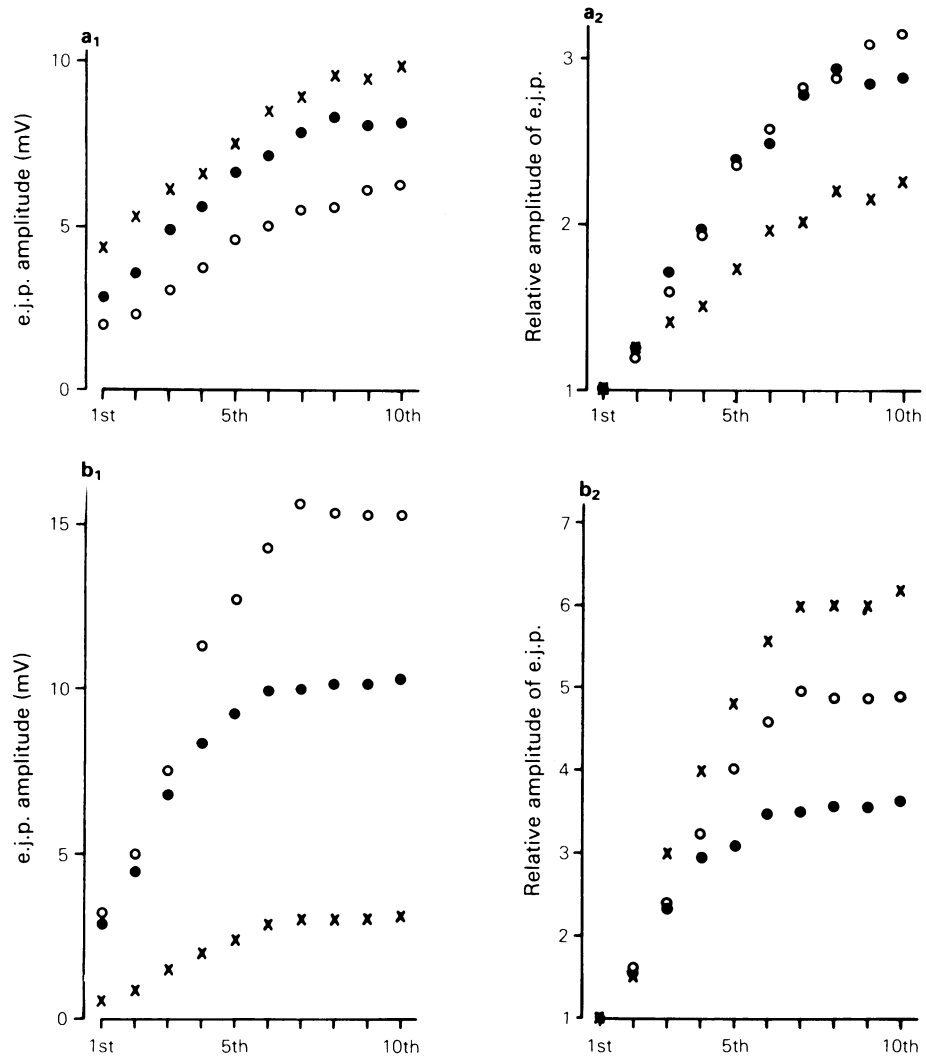


**Figure 8** Effects of (a) caffeine (0.1 and 2 mM) or (b) procaine (0.05 and 1 mM) on the e.j.p. amplitudes produced by perivascular nerve stimulation for 10 times at 1 Hz frequency. The e.j.p. amplitudes ($a_1$ or $b_1$) were plotted as relative value to the first of the train, in each condition ($a_2$ or $b_2$). (a) Control (●), caffeine 0.1 mM (○), caffeine 2 mM (x); (b) control (●), procaine 0.05 mM (○), procaine 1 mM (x). Each point is a mean value ($n = 5-7$). The s.d. was less than 10% of each value.

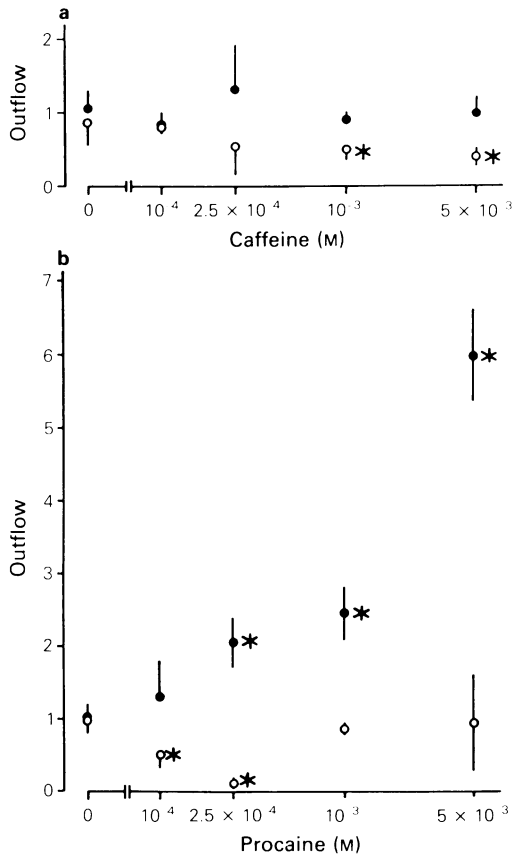


**Figure 9** Effects of caffeine (a) or procaine (b) on the outflows of noradrenaline (NA) (●) and DOPEG (○) induced by perivascular nerve stimulation. The nerve stimulation (2 Hz, 240 pulses) was applied twice, with a 30 min interval, while caffeine ($10^{-4}$–$5 \times 10^{-3}$M) or procaine ($10^{-4}$–$5 \times 10^{-3}$M) was applied 10 min before application of the second stimulation of the nerves. The amounts of NA or DOPEG outflow induced by the second nerve stimulation were expressed relative to the first. Each point shows mean ($n = 4$–16) and vertical line, s.d. * Statistically significant ($P < 0.05$) from the control value.

stimulation at 1 Hz frequency. Applications of $2.5 \times 10^{-4}$M caffeine or $10^{-3}$M procaine decreased while $2 \times 10^{-3}$M caffeine or $2.5 \times 10^{-4}$M procaine enhanced the e.j.p. amplitudes measured during full facilitation.

These effects of caffeine and procaine on the e.j.p. amplitudes were quantified by the plot of e.j.p. amplitude relative to the first of the train in each condition. Application of $10^{-4}$M caffeine decreased the e.j.p. amplitudes produced by a train of perivascular nerve stimulation at 1 Hz frequency while $2 \times 10^{-3}$M caffeine enhanced it (Figure 8 $a_1$). When the e.j.p. amplitudes were plotted relative to the first of the train, $2 \times 10^{-3}$M caffeine decreased the facilitation process of e.j.ps whereas $10^{-4}$M caffeine was ineffective (Figure 8 $a_2$). Procaine $5 \times 10^{-5}$M and $10^{-3}$M increased and decreased the train of e.j.p. amplitudes respectively (Figure 8 $b_1$). However, both of these concentrations of procaine enhanced the facilitation process of e.j.ps (Figure 8 $b_2$).

Conduction velocity of excitation of perivascular nerves was estimated from the relationship between the latency of e.j.p. and the distance between the recording and stimulating electrodes. The relationship was linear and the conduction velocity of the nerve excitation was calculated to be $25.1 \pm 3.1\,\mathrm{cm\,s^{-1}}$ ($n = 27$) in the guinea-pig mesenteric artery. Procaine ($2.5 \times 10^{-4}$ or $10^{-3}$M) reduced the conduction velocity while caffeine ($2.5 \times 10^{-4}$ or $10^{-3}$M) was ineffective (Table 1).

*Outflow of noradrenaline and DOPEG*

Effects of caffeine and procaine on the outflow of NA or DOPEG into the perfusate were observed in the guinea-pig mesenteric vascular bed. Perivascular nerves were stimulated (2 Hz frequency, 240 stimuli) twice at a 30 min interval, and perfusates were collected for 5 min periods. Caffeine or procaine was applied 10 min before the second nerve stimulation, and the amount of NA or DOPEG in the perfusate was expressed relative to that produced in the first.

In the absence of nerve stimulation, there was a spontaneous outflow of NA and DOPEG (NA, $2.6 \pm 1.3\,\mathrm{ng\,g^{-1}}$ wet weight of tissue, $n = 28$; DOPEG, $8.6 \pm 3.7\,\mathrm{ng\,g^{-1}}$, $n = 28$). Application of caffeine ($10^{-4}$–$5 \times 10^{-3}$M) did not change the level of the spontaneous outflow of NA and DOPEG. Upon nerve stimulation, the outflow of NA and DOPEG increased to $5.5 \pm 1.9\,\mathrm{ng\,g^{-1}}$ ($n = 28$) and $11.5 \pm 4.4\,\mathrm{ng\,g^{-1}}$ ($n = 28$), respectively. Application of caffeine ($10^{-4}$–$5 \times 10^{-3}$M) did not change the NA-outflow induced by the nerve stimulation. The outflow of DOPEG induced by the nerve stimulation was significantly reduced ($P < 0.05$) (Figure 9 a).

Procaine ($10^{-4}$–$5 \times 10^{-3}$M) increased the spontaneous outflow of NA by 2–4 times the control while that of DOPEG was decreased 0.5–0.9 times the control. Procaine ($2.5 \times 10^{-4}$–$5 \times 10^{-3}$M) increased the NA outflow induced by nerve stimulation, in a concentration-dependent manner (Figure 9 b). The DOPEG outflow induced by nerve stimulation was decreased with application of $10^{-4}$–$2.5 \times 10^{-4}$M procaine, and remained unchanged with application of $10^{-3}$–$5 \times 10^{-3}$M procaine.

## Discussion

The present experiments revealed that in the smooth muscle cells of the guinea-pig mesenteric artery, caffeine and procaine showed effects similar to those seen in the guinea-pig abdominal aorta (Kajiwara, 1982) or basilar artery (Fujiwara & Kuriyama, 1983), i.e., caffeine had dual actions on the membrane: low concentrations ($2.5 \times 10^{-4}$–$5 \times 10^{-4}$M) hyperpolarized the membrane and reduced the membrane resistance and high concentrations (over $3 \times 10^{-3}$M) depolarized the membrane and increased the membrane resistance; procaine (over $10^{-4}$M) depolarized the membrane and increased the membrane resistance. In skeletal muscles, caffeine releases Ca from the sarcoplasmic reticulum by enhancing the Ca-induced Ca release mechanism and this release is antagonized by procaine (Endo, 1977). Similar effects of caffeine and procaine can be seen in the guinea-pig mesenteric artery (Itoh *et al.*, 1981). In many excitable tissues, the K-conductance is controlled by Ca on the internal surface of the membrane (the 'Meech' effect; Meech, 1978). The hyperpolarization induced by low concentrations of caffeine may be due to an increase in K-conductance of the membrane as a result of the mobilization of Ca at the plasma membrane. Increased concentrations of caffeine further mobilize Ca stored in the cell and also facilitate extrusion of Ca from the cytoplasm due to activation of a Ca-dependent ATPase (Blayney *et al.*, 1978), thus reducing Ca at the myoplasmic membrane and consequently lowering the K-permeability of the membrane. Procaine, on the other hand, immobilizes Ca at the myoplasmic membrane and this would suppress the K-conductance (Fujiwara & Kuriyama, 1983).

However, these effects of caffeine on the smooth muscle cells do not apply to perivascular nerve endings, as caffeine did not enhance the facilitation process of e.j.ps and NA-outflow during perivascular nerve stimulation. Caffeine releases Ca from the intracellular stored sites in the sympathetic (Kuba, 1980) or the dorsal root ganglion cells (Neering & McBurney, 1984). Synaptosomes isolated from the rat brain accumulate Ca in the smooth endoplasmic reticulum which possesses properties similar to the sarcoplasmic reticulum in muscles (McGraw *et al.*, 1982). Therefore, caffeine may release Ca from the stored sites in nerve terminals, and this would enhance the release of transmitters (Katz, 1969). However, in the present experiments the effects of caffeine on the e.j.p. amplitude and on the nerve-stimulation induced NA-outflow were observed after the tissues had been treated for a long period (5–10 min) with caffeine. Muscle contractions produced by caffeine are transient, and continued application of caffeine relaxes the muscle tissues which had been previously contracted by high-potassium or sodium-free solutions (Itoh *et al.*, 1983). This suggests that caffeine releases Ca ions stored in the cells and keeps intracellular Ca concentrations at low levels. Neering & McBurney (1984) have shown that in the dorsal root ganglion cells, the caffeine-induced Ca release is transient, and intracellular Ca stores once depleted of Ca by caffeine cannot be re-filled until the next firing of nerves. Thus, caffeine reduces Ca concentrations in the nerve terminals and Ca ions required for transmitter release may be supplied by influx from the external media associated with the nerve action potential. Therefore, the enhancement of the e.j.p. amplitude by caffeine may be due to increase in the membrane resistance of smooth muscle cells.

In the guinea-pig mesenteric artery, procaine depressed the e.j.p. amplitude, yet increased the membrane resistance of the smooth muscle cells. Procaine reduced the conduction velocity of perivascular nerve excitations, presumably due to its anaesthetic action, but the amount of NA outflow induced by nerve stimulation was increased. These effects of procaine on adrenergic transmission were similar to those seen with cocaine (Kuriyama & Suyama, 1983). Depression of the e.j.p. amplitude by cocaine or procaine may not be due to inhibition of α-adrenoceptors at the postjunctional membrane, since these drugs did not inhibit the depolarizations produced by exogenously applied NA. When the effects of procaine were compared to those of cocaine, the facilitation of the e.j.p. was enhanced by the former but not by the latter. Procaine ($2.5 \times 10^{-4}$–$10^{-3}$M) reduced the e.j.p. amplitude produced by single stimuli, but repetitive stimulation of nerves enhanced the facilitation of e.j.ps and, at $2.5 \times 10^{-4}$M, finally produced e.j.ps larger than those seen in the absence of procaine. These concentrations of procaine depolarized the smooth muscle membrane, and this would reduce the e.j.p. amplitude. However, the depolarization may not be the main reason for the inhibition of e.j.p. amplitude, because high concentrations of caffeine depolarized the membrane and increased the e.j.p. amplitude. Alternatively, procaine may selectively inhibit NA receptors located at the junctional region (Hirst & Neild, 1981), as has been postulated for cocaine (Kuriyama & Suyama, 1983). It is unlikely that the e.j.p. amplitude was decreased due to activation of the α-autoinhibition mechanism operated at the prejunctional membrane (Langer, 1977; Starke, 1977), because activation of the α-autoinhibition mechanism by NA decreases the e.j.p. amplitude and depresses the facilitation processes (Kuriyama & Makita, 1983).

Procaine increased the NA outflow and decreased the DOPEG outflow in the guinea-pig mesenteric artery, the effect being similar to that of cocaine (Kuriyama & Suyama, 1983). It is accepted that NA released from adrenergic nerve terminals is, in part, taken up into the nerves, and this re-uptake is inhibited

by cocaine. These effects of cocaine can be estimated by measurement of NA and DOPEG outflows into the perfusate (Graefe & Henseling, 1983), i.e., blockade by cocaine of the re-uptake of released NA leads to a decrease in DOPEG outflow. Thus, the enhancement of NA outflow by procaine may be due, in part, to inhibition of the re-uptake mechanism of the released NA.

In conclusion, in the guinea-pig mesenteric artery, caffeine, at low concentrations, decreased and, at high concentrations, enhanced the e.j.p. amplitude, due in part to changes in membrane resistance of the smooth muscle cells. Procaine facilitated the adrenergic transmission and increased the NA-outflow, presumably due to inhibition of re-uptake of released NA. However, the mechanisms or the sites of action of procaine in depressing the e.j.p. amplitude remain to be determined.

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