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Lack of a causal relationship between the vasodilator effect of papaverine and cyclic AMP production in the dog basilar artery

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1 The effects of papaverine and isoprenaline on smooth muscle cells of the dog basilar artery were investigated using radioimmunoassay, electrophysiological and isometric tension recording methods. For comparative purposes, the actions of these drugs on the guinea-pig basilar artery were also examined.

2 Papaverine and isoprenaline (1 μM and 10 μM) increased the amount of cyclic AMP in both dog and guinea-pig basilar arteries.

3 Papaverine (up to 100 μM) and isoprenaline (up to 1 μM) had no effect on the membrane potential and membrane resistance measured by recording the amplitudes of the electrotonic potentials in smooth muscle cells of the dog and guinea-pig basilar arteries.

4 The action potential evoked by outward current pulses after pretreatment with tetraethylammonium chloride (5–10 mM) was inhibited by papaverine (>1 μM) but not by isoprenaline (up to 10 μM) in smooth muscle cells of the dog and guinea-pig basilar arteries.

5 In the dog basilar artery, papaverine (>1 μM) consistently inhibited the contractions evoked by excess concentrations of $[K]_o$ (>20.2 mM) or 5-hydroxytryptamine (10 nM–10 μM), dose-dependently. Isoprenaline (1 μM) had only slight effects on the contraction evoked by low concentrations of 5-hydroxytryptamine (10 nM).

6 In the Ca^{2+}-free solution containing EGTA (2 mM), the contraction evoked by 5-hydroxytryptamine (10 μM) or caffeine (10 mM) was dose-dependently inhibited by papaverine (>1 μM). However, isoprenaline (1 μM) had no effect on these contractions.

7 These results indicate that the vasodilator actions of papaverine on the dog basilar artery are mainly due to inhibition of the voltage-dependent influx of Ca^{2+} and also to inhibition of the receptor-activated release of Ca^{2+} stored in the cell. Since isoprenaline increased the cyclic AMP to the same extent as papaverine but had no effect on the electrical and mechanical responses, the inhibitory actions of papaverine on this tissue may not be causaly related to the increased levels of cyclic AMP induced by inhibition of phosphodiesterase.

Introduction

The isoprenaline-induced relaxation of vascular smooth muscle tissues is linked to the production of adenosine 3′:5′-cyclic monophosphate (cyclic AMP; Bar, 1974; Hardman, 1981), i.e. cyclic AMP with cyclic AMP-dependent protein kinase inhibit the action of calmodulin thus preventing phosphorylation of the extracted myosin light chain kinase (Conti & Adelstein, 1981). This action of cyclic AMP has been confirmed using chemically skinned muscles and measuring the amplitude of the Ca^{2+}-induced contraction (Itoh *et al.*, 1981b; 1982; 1983). Cyclic AMP also produces an increase in the amount of Ca^{2+} stored in the cell and partly accelerates the Ca^{2+}-pump at the sarcolemmal membrane (Thorens & Haeusler, 1978; Bhalla *et al.*, 1978; Adelstein & Eisenberg, 1980; Itoh *et al.*, 1982). However, negative results on the accumulation of Ca^{2+} with respect to cyclic AMP levels have been found (Sands & Mascali, 1978; Kreye & Schlicker, 1980).

Papaverine has a potent vasodilator action which may be related to an increase in the amount of cyclic AMP, due to inhibition of phosphodiesterase (Bar, 1974). Isoprenaline increases the content of cyclic AMP through activation of adenylate cyclase, while

papaverine increases it in a passive manner. On the other hand, visceral smooth muscle is relaxed to a greater extent by papaverine than by isoprenaline, e.g. in the guinea-pig taenia coli, Tashiro & Tomita (1970) observed that papaverine and isoprenaline slightly hyperpolarized the membrane and inhibited the spontaneous electrical activity with no change in the ionic conductance of the membrane. However, the contraction evoked by depolarizing currents and modified ionic environments is inhibited more potently by papaverine than by isoprenaline. Brading *et al.* (1983) concluded that the inhibitory action of papaverine in the guinea-pig ureter is due to a block of the slow Na^+/Ca^{2+} channels responsible for the generation of the plateau component of the action potential. In the rat ileal smooth muscles, papaverine prevents the Ca^{2+} influx (Huddart & Saad, 1980).

Papaverine is commonly used to relax vascular tissues after they have been contracted by excess concentrations of K^+ or agonists. However, details on the mechanism by which this drug induces relaxation in vascular smooth muscle have not been documented.

We attempted to clarify the mechanism of this vasodilator effect of papaverine and to compare its actions with those of isoprenaline on the dog basilar artery. The effects of these agents were studied by measuring changes in the electrical properties of the membrane, the mechanical responses of tissues and their cyclic AMP content, i.e. to see whether or not the actions of these agents are due solely to the production of cyclic AMP. For comparative purposes, the effects of these drugs on the guinea-pig basilar artery were also observed.

Methods

Mongrel dogs of either sex, weighing 10–15 kg (age; 1.5–2 years) were anaesthetized with sodium pentobarbitone, (30 mg ml^{-1} intravenously) and exsanguinated. Guinea-pigs of either sex, weighing 300–350 g were decapitated, the brain was removed and the basilar artery was carefully removed. The basilar artery excised from either species was placed in Krebs solution (25°C). The tissue (diameter 1–1.5 mm for the dog and 0.3–0.6 mm for the guinea-pig) was carefully dissected from connective tissues under a binocular microscope, then mounted in an organ bath with a capacity of about 2 ml and superfused with warmed Krebs solution (35°C) at a flow rate of about 3 ml min^{-1}.

The ionic composition of the Krebs solution was as follows (mM): Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5. Potassium concentration was modified by replacing NaCl with KCl, isotonically. In Ca^{2+}-free solution, $CaCl_2$ was removed and 2 mM EGTA was added. The solution was bubbled with 97% O_2 and 3% CO_2, and the pH was maintained at 7.3–7.4.

Electrical responses of the membrane were recorded with a glass capillary microelectrode (Higenberg Glass, Frankfurt) filled with 3 M KCl, the tip resistance of which ranged between 40–80 MΩ. The microelectrode was impaled from the outer surface of the vessel.

To record mechanical responses circularly cut strips (1.0–1.5 mm outer diameter, 0.5 mm width for the dog) were prepared. Two needles were inserted into the internal lumen, one needle was fixed to the bottom of the bath and the other needle was connected to a mechanotransducer (Nihon Kohden; TB 612T) and recticorder (Nihon Kohden Recticorder; RJG (4024) Tokyo).

Assay of cyclic AMP

Dog basilar arteries, wet weight 9–12 mg, were excised and cut into three pieces. One was used for the control and the other two for testing the drugs. As the guinea-pig basilar artery is extremely small (below 1 mg wet weight), 21 guinea-pigs were killed and the pooled tissues were used to measure the cyclic AMP content (7 pieces for the control). Smooth muscle tissues excised from basilar arteries were prepared in cold Krebs solution, under a binocular microscope. The dissected tissues were then incubated in an organ bath (without any load); control tissues were kept at 37°C for 60 min in the bubbled Krebs solution. To observe the effects of isoprenaline or papaverine on their cyclic AMP content, different concentrations of these agents were applied for the final 3 min of the 60 min incubation period.

After 60 min incubation, the tissues were frozen in liquid nitrogen and homogenized in 6% trichloroacetic acid. The cyclic AMP contents of the extracts were measured using a radioimmunoassay kit (Yamasa Shoyu: Honma *et al.*, 1977).

Drugs

The following drugs were used; papaverine hydrochloride and 5-hydroxytryptamine hydrochloride (Sigma), isoprenaline hydrochloride, guanethidine sulphate and tetraethylammonium chloride (TEA; Tokyo-Kasei), caffeine (Wako), EGTA (ethyleneglycol-bos-β-aminoethylether) tetra N-tetraacetic acid (Dozin), and propranolol chloride (Sumitomo Kagaku).

Statistics

The results were expressed as the mean value ± standard deviation (s.d.; number of observations),

and statistical significance was assessed using Student's t test.

Results

Effects of isoprenaline or papaverine on cyclic AMP production

Figure 1 shows the effects of 1 μM and 10 μM papaverine or isoprenaline on the production of cyclic AMP in the dog basilar artery, and 10 μM papaverine or isoprenaline on that in the guinea-pig basilar artery. Papaverine (10 μM) or isoprenaline (10 μM) increased the cellular cyclic AMP content ($P < 0.05$) in both basilar arteries. In the dog basilar artery, 1 μM papaverine and isoprenaline caused the production of almost the same amount of cyclic AMP ($P < 0.05$). Whereas in the guinea-pig basilar artery, 10 μM papaverine increased the amount of cyclic AMP to a greater extent than 10 μM isoprenaline (control cyclic AMP content; 8.01 pmol mg^{-1} protein; plus papaverine or isoprenaline, 17.49 and 14.02 pmol mg^{-1} protein, respectively).

Effects of papaverine or isoprenaline on the membrane properties of the cerebral arteries

The resting membrane potentials of smooth muscle cells of the dog and guinea-pig basilar arteries were -49.0 ± 1.9 mV ($n = 91$) and -49.4 ± 2.4 mV ($n = 40$), respectively. These values were much the same as those previously observed in these tissues (Fujiwara *et al.*, 1982; Fujiwara & Kuriyama, 1983).

These membrane potentials remained unchanged on addition of papaverine (0.1–100 μM) or isoprenaline (0.01–1 μM) to the bathing medium, in both species (Table 1). On addition of 100 μM papaverine or 1 μM isoprenaline the membrane resistance, calculated by measuring the amplitude of the electronic potential evoked by the constant intensity of inward and outward current pulses (1.5 s pulse duration) or by various intensities of inward and outward current pulses (current-voltage relationship), was not affected.

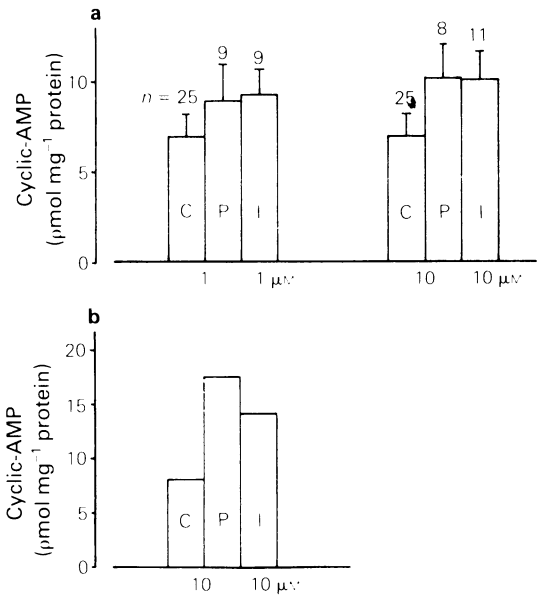

Figure 1 Effects of papaverine (1 and 10 μM) and isoprenaline (1 and 10 μM) on the production of cyclic AMP in (a) the dog and (b) guinea-pig basilar arteries. In (b) data of single experiment using 21 guinea-pig basilar arteries are shown. Vertical line indicate s.d. C: control, P: papaverine and I: isoprenaline.

Outward current pulses with different intensities produced rectification of the membrane. In some cells strong outward current pulses produced a small graded response, but a spike potential was never produced. However, these cells produced a spike potential in response to an outward current pulse when pretreated with 10 mM tetraethylammonium (TEA). TEA inhibited the ionic conductance of the membrane at 0.2 mm from the stimulating electrode, measured by applying the same intensity of inward current pulses (1.5 s duration) before and during the application of TEA (1.86 times the control) and depolarized the membrane (5.2 ± 2.3 mV, $n = 5$). Then outward current pulses produced a spike potential.

Table 1 Effects of papaverine and isoprenaline on the membrane potential in dog and guinea-pig basilar arteries

	Membrane potential (mV)	
	Dog basilar artery	*Guinea-pig basilar artery*
Control	-49.0 ± 1.9 ($n = 91$)	-49.4 ± 2.4 ($n = 40$)
Papaverine 100 nM	-48.7 ± 1.1 ($n = 10$)	-49.8 ± 4.0 ($n = 8$)
Papaverine 100 μM	-49.8 ± 1.8 ($n = 10$)	-48.6 ± 2.1 ($n = 14$)
Isoprenaline 1 μM	-48.8 ± 1.5 ($n = 9$)	-50.6 ± 1.8 ($n = 19$)

The results shown are the mean ± s.d. of n number of observations.

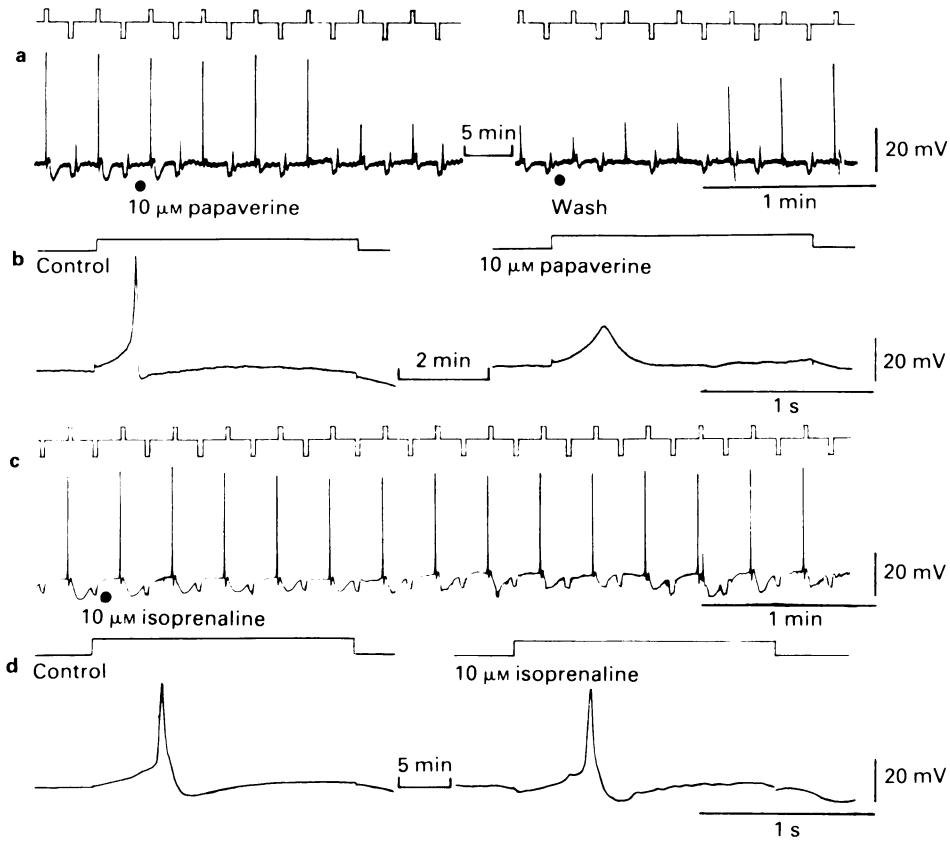

Figure 2 Effects of papaverine (10 μM) or isoprenaline (10 μM) on spike potentials in the guinea-pig basilar artery evoked by outward current pulses (1.5 s duration) after pretreatment with tetraethylammonium (5 mM). (a and b) Effect of papaverine. (c and d) Effect of isoprenaline.

Figure 2 shows the effects of papaverine and isoprenaline on the smooth muscle cell membrane of the guinea-pig basilar artery pretreated with 5 mM TEA. Outward current pulses (1.5 s pulse duration) produced a spike and, during the cessation of inward current pulses, an abortive spike appeared as an anodal break excitation (Figure 2a). When 10 μM papaverine was added, the amplitude of spike potential was markedly reduced. This inhibitory effect of papaverine was reversible. On the other hand, when 10 μM isoprenaline was added the spike amplitude was not inhibited (Figure 2C). In (Figure 2b and d) a comparison is made of the shape of the action potential evoked by an intensity just above the threshold intensity. Before and after the addition of isoprenaline, the shape and amplitude of the action potential were not modified (d), but after papaverine, the action potential ceased (b).

Figure 3 shows the effects of papaverine (100 μM) or isoprenaline 1 μM on the spike potentials evoked by outward current pulses in the dog basilar artery treated with 10 mM TEA. Papaverine but not isoprenaline inhibited the spike generation, as was also noted in the guinea-pig basilar artery.

Effects of papaverine or isoprenaline on the membrane potential and contraction evoked by excess concentrations of $[K]_o$ or 5-hydroxytryptamine (5-HT)

Figure 4 shows the effects of papaverine or isoprenaline on the K^+-induced depolarization and contraction in the dog basilar artery. Increased concentrations of $[K]_o$ depolarized the membrane, dose-dependently (control membrane potential; -49.0 ± 1.9 mV, $n = 91$; in 39.2 mM K^+, -29.4 ± 2.1 mV, $n = 13$; in 118 mM K^+, -6.1 ± 2.4 mV, $n = 10$). The minimum concentration of $[K]_o$ required to produce a contraction was 17.7 mM, and with 118 mM K^+, the contraction

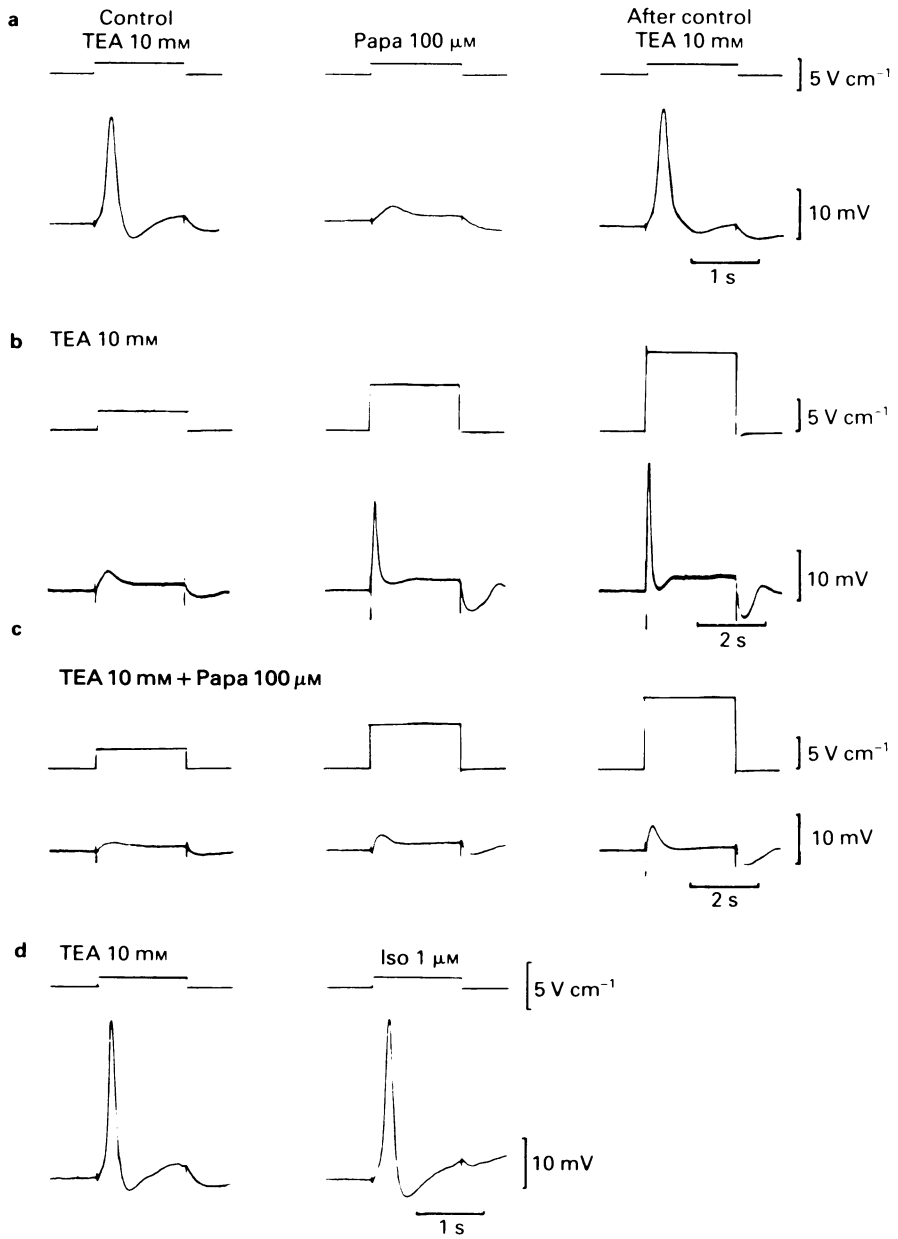

Figure 3 Effects of papaverine (Papa; 100 μM) or isoprenaline (iso; 1 μM) on the spike potential in the dog basilar artery evoked by outward current pulses (1.5 s pulse duration in (a) and (d), and 3 s pulse duration in (b) and (c) after pretreatment with 10 mM tetraethylammonium (TEA).

reached a maximum. In Figure 4b the amplitude of contraction evoked by 118 mM K^+ was defined as a relative tension of 1.0. Application of 10 μM papaverine or 1 μM isoprenaline did not modify the membrane potential, in the presence of any given concentration of $[K]_o$. On the other hand, in the presence of papaverine in concentrations above 1 μM, the contraction evoked by any given concentration of K^+ (above 20.2 mM) was inhibited, dose-dependently and 100 μM papaverine inhibited markedly the contraction evoked by 118 mM K^+. However, isoprenaline 1 μM did not affect the con-

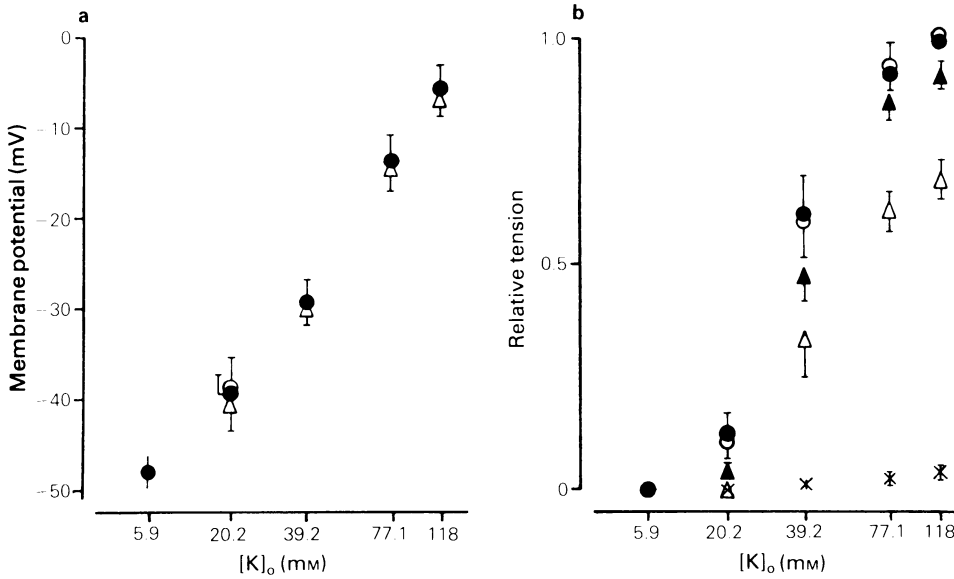

Figure 4 Effects of papaverine or isoprenaline on the K^+-induced depolarization and contraction in the dog basilar artery. The amplitude of contraction evoked by 118 mM K^+ was taken as a relative tension of 1.0. Vertical lines indicate 2 × s.d. or s.d.; $n = 5-8$. During applications of excess concentrations of $[K]_o$, the tissues were pretreated with guanethidine (1 μM). (a) Membrane potentials induced by $[K]_o$, (●) control, in the presence of 10 μM papaverine (△) or 1 μM isoprenaline (○). (b) Relative tensions, control (●), in the presence of papaverine 1 μM (▲), 10 μM (△), 100 μM (×) or isoprenaline 1 μM (○).

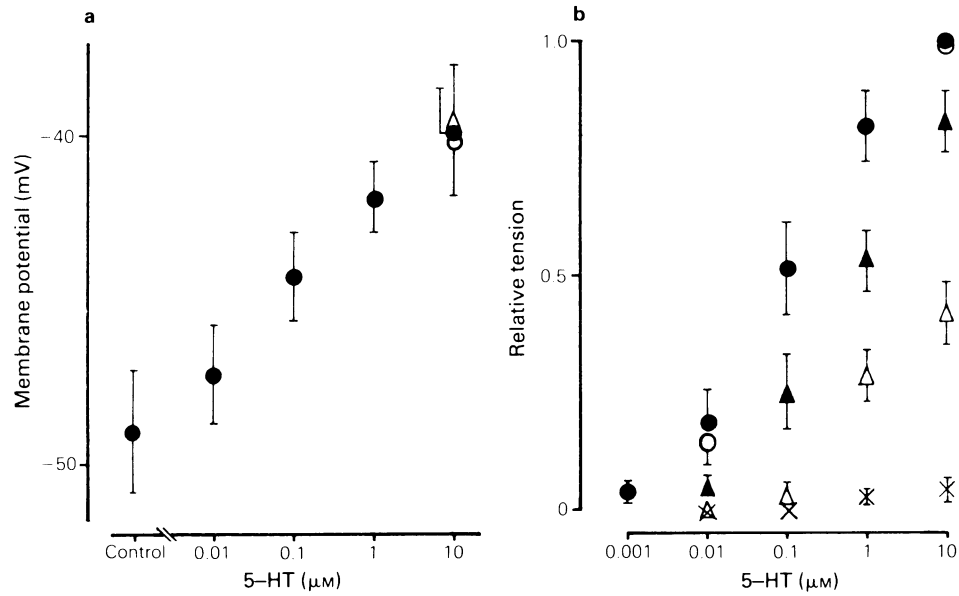

Figure 5 Effects of papaverine or isoprenaline on the 5-hydroxytryptamine (5-HT)-induced depolarization and contraction in the dog basilar artery. The contraction evoked by 10 μM 5-HT was taken as a relative tension of 1.0. Vertical lines indicate 2 × s.d.; $n = 5-7$. (a) Membrane potentials induced by 5-HT, control (●), in the presence of 10 μM papaverine (△) or 1 μM isoprenaline (○). (b) Relative tensions, control (●) in the presence of papaverine 1 μM (▲), 10 μM (△), 100 μM (×) or isoprenaline 1 μM (○).

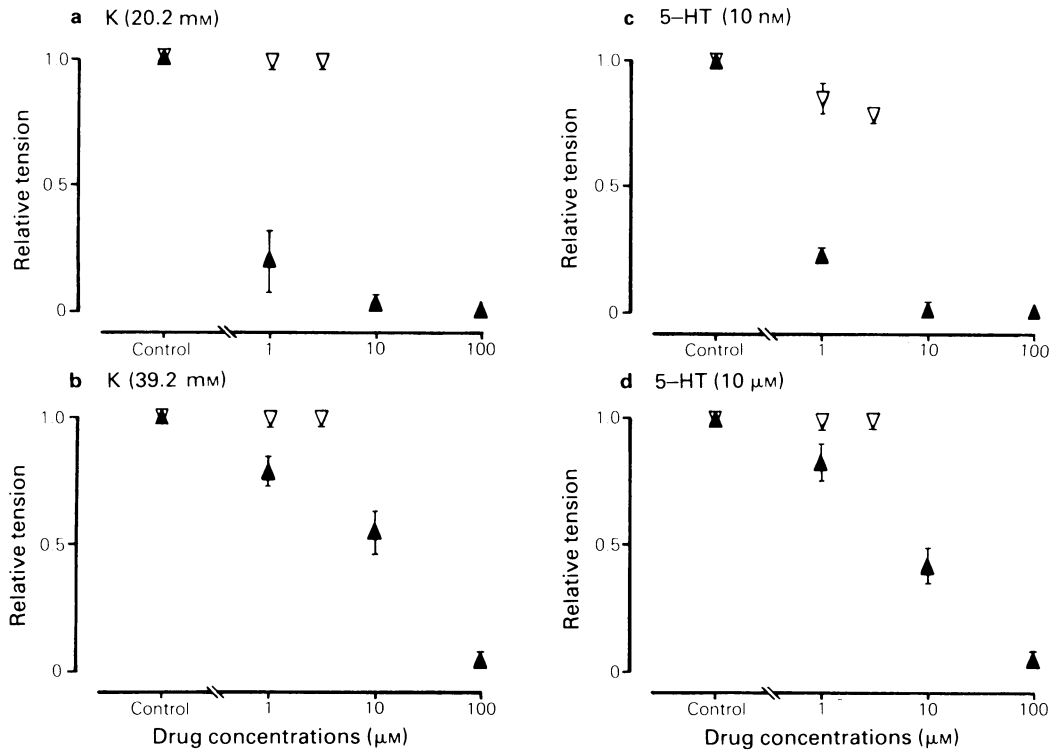

Figure 6 Effects of papaverine or isoprenaline on the K- and 5-hydroxytryptamine (5-HT)-induced contraction in the dog basilar artery. Contractions produced by (a) 20.2 mM K^+, (b) 39.2 mM K^+, (c) 10 nM 5-HT and (d) 10 μM 5-HT. The amplitudes of contraction evoked by K^+ or 5-HT in the absence of papaverine or isoprenaline were taken as a relative tension of 1.0, in (a and b) and (c and d), respectively. Vertical lines indicate 2 × s.d.; $n = 5-8$. In the case of excess concentrations of $[K]_o$, guanethidine was added. In (a–d) contractions in presence of papaverine (▲) or isoprenaline (▽).

traction evoked by any concentration of $[K]_o$ tested. Throughout these experiments, 1 μM guanethidine (prevents release of noradrenaline from nerve terminals) and 0.1 μM phentolamine (prevents activation of the α-adrenoceptor by 1 μM isoprenaline) were present in the bathing solutions. 5-Hydroxytryptamine (5-HT) depolarizes the smooth muscle membrane of the dog basilar artery and evokes a contractile response. Figure 5 shows the effects of papaverine and isoprenaline on the 5-HT-induced depolarization and peak amplitude of contraction (phasic contraction). The membrane was marginally depolarized by 10 nM 5-HT (control membrane potential, -49.0 ± 1.9 mV; plus 5-HT, -47.3 ± 1.5 mV, $n = 10$, $P < 0.05$). Increased concentrations of 5-HT depolarized the membrane, dose-dependently (plus 10 μM 5-HT, membrane potential $= -39.8 \pm 1.1$ mV, $n = 20$). However, a contraction could be evoked by as little as 10 nM 5-HT. As can be seen from Figure 5, 10 μM papaverine or 1 μM isoprenaline did not modify the membrane potential in the presence of any given concentration of 5-HT (membrane potential in 10 μM 5-HT; -39.8 ± 1.1 mV; plus 10 μM papaverine, -39.6 ± 1.9 mV, $n = 10$; plus 1 μM isoprenaline, -40.2 ± 1.6 mV, $n = 10$), but papaverine in concentrations above 1 μM consistently inhibited the contractions evoked by 5-HT (10 nM–10 μM). On the other hand, 1 μM isoprenaline did not affect the contractions to different concentrations of 5-HT, except those induced by 10 nM 5-HT. Figure 6 summarizes the effects of papaverine or isoprenaline on the contractions produced by K^+ (20.2 mM and 39.2 mM) or 5-HT (10 nM and 10 μM). Papaverine in concentrations above 1 μM consistently inhibited the contraction evoked by both stimulants and the inhibition was more marked in the case of the contraction evoked by a lower concentration of $[K]_o$ or 5-HT (a vs b and c vs d). Isoprenaline (up to 3 μM) did not modify the K^+-induced contraction but slightly inhibited the contraction evoked by 10 nM 5-HT.

The effects of papaverine or isoprenaline on the

tonic component of the K^+- or 5-HT-induced contraction were also observed. Papaverine in concentrations above 1 μM consistently and dose-dependently inhibited the tonic response, but such inhibition was not observed on addition of isoprenaline. Papaverine (100 μM) inhibited the tonic response evoked by 5-HT (10 μM) to a greater extent than that evoked by 39.2 mM $(K]_o$.

Figure 7 shows the effects of papaverine or isoprenaline on the twitch contraction evoked by direct muscle stimulation (0.5 s pulse duration; 40 V intensity, single pulse, every 2 min) in the dog basilar artery. TEA (10 mM) increased the resting tension and enlarged the twitch contraction (a). When 1 μM papaverine was added, the amplitude of contraction evoked by electrical stimulation was reduced (b) and 10 μM papaverine reduced both the resting tension and amplitudes of twitch contraction (c), while isoprenaline (10 μM) did not modify the contraction (d).

Effects of papaverine on the contraction evoked by 5-hydroxytryptamine (5-HT) in Ca-free solution

The K^+-induced contraction in the guinea-pig basilar artery seems to be mainly due to the voltage-dependent influx of Ca^{2+} whereas the 5-HT-induced contraction is largely caused by an influx of Ca^{2+} through activation of receptors (i.e. receptor activated Ca^{2+} influx) and in part the release of Ca^{2+} from the cell store (Fujiwara & Kuriyama, 1983). The effects of papaverine or isoprenaline on the 5-HT- or caffeine-induced contraction in Ca^{2+}-free EGTA (2 mM)-containing solution were observed in the dog basilar artery (Figure 8). The K^+-induced contraction ceased 3.5 min after superfusion with the Ca^{2+}-free solution, while the phasic response of the contraction to 5-HT was reduced in amplitude (0.68 times the control; $n = 5$) and the tonic response ceased (Figure 8a, b and d). The contraction induced

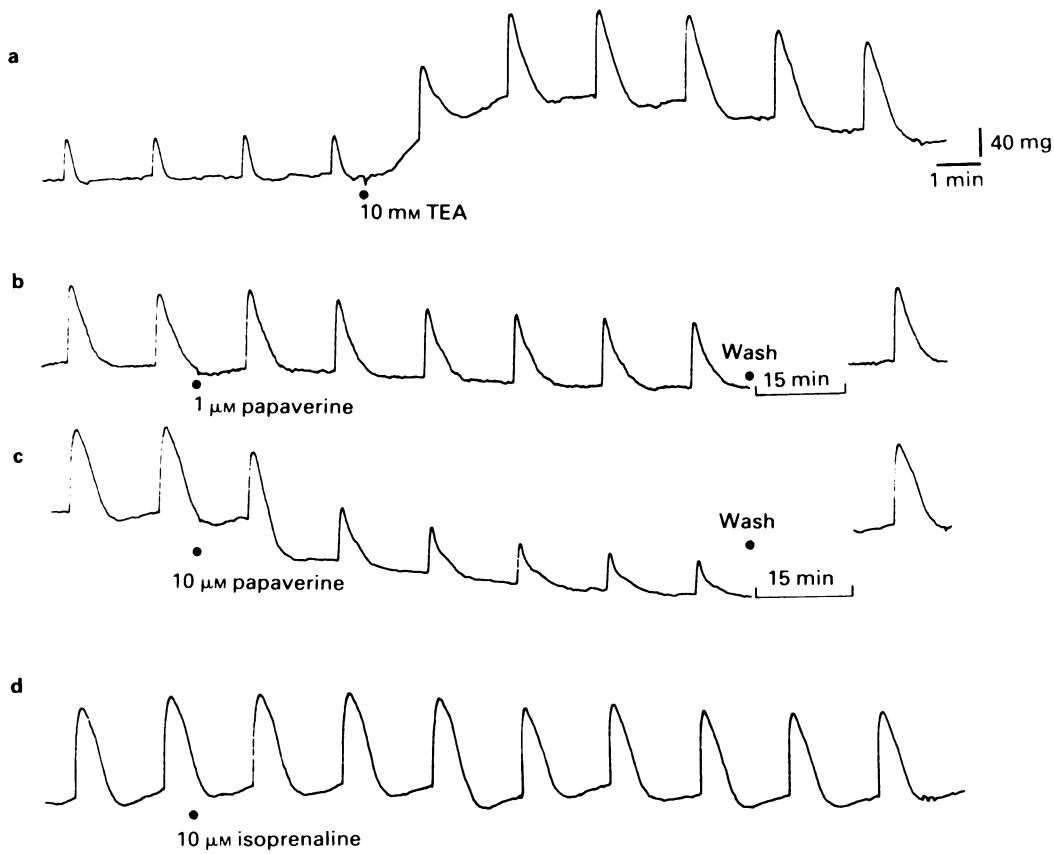

Figure 7 Effects of papaverine or isoprenaline on the twitch contraction evoked by direct muscle stimulation (40 V, 0.5 s, 1 pulse) of the dog basilar artery. (a) Control and after treatment with tetraethylammonium (TEA) 10 mM. (b) The effect of 1 μM papaverine, (c) the effect of 10 μM papaverine and (d) the effect of 10 μM isoprenaline. (b–d) Recorded in the presence of TEA.

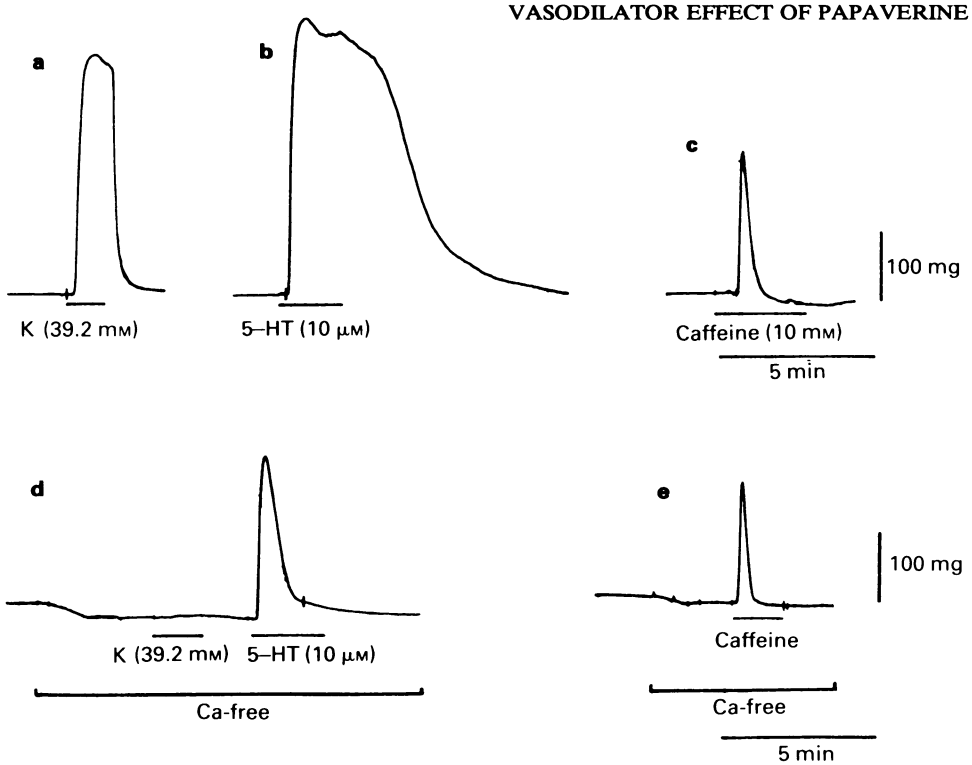

Figure 8 Contractile responses of the dog basilar artery to 39.2 mM K^+, 10 μM 5-hydroxytryptamine (5-HT) or 10 mM caffeine in normal Ca^{2+} (2.5 mM)-containing Krebs solution (a–c) and their effects in Ca^{2+}-free solution (containing 2 mM EGTA) (d and e). In Ca^{2+}-free solution (d) 39.2 mM K^+ did not evoke a contraction but 5-HT produced a transient phasic contraction which was reduced in amplitude. (e) Shows that removing Ca^{2+} from the solution had little effect on the response to caffeine.

by 10 mM caffeine was only slightly reduced in amplitude (Figure 8c and e).

Figure 9 shows the effects of papaverine (1–100 μM) or isoprenaline (1 μM) on the contraction evoked in the dog basilar artery by 10 mM caffeine or 10 μM 5-HT in Ca^{2+}-free EGTA-containing solution. In Ca^{2+}-free solution, the amplitude of the phasic contraction evoked by 10 μM 5-HT was reduced to 0.68 times the control value ($n = 5$) observed in normal (2.5 mM) Ca^{2+}-containing solution and the tonic contraction ceased; the amplitude of the phasic contraction evoked by 10 mM caffeine was reduced to 0.62 times the control ($n = 3$). Both these contractions were inhibited by addition of papaverine (more than 1 μM). However, isoprenaline (1 μM) had no effect on the 5-HT-induced contraction in Ca^{2+}-free solution (Figure 9c). Figure 9d shows the effects of papaverine on the contractions to 5-HT or caffeine in Ca^{2+}-free solution. There were no differences between the inhibitory effects of papaverine on the contractile responses to either of these agents.

Discussion

Various observations have been made on the inhibitory mechanism of action of papaverine on smooth muscle tissues; however, there is not yet a consensus of opinion. Although papaverine increases the amount of cyclic AMP by inhibiting phosphodiesterase (Kukovetz & Poch, 1970; Poch & Kukovetz, 1971; Bar, 1974; Uruno *et al.*, 1974; Takayanagi *et al.*, 1978), a number of inconsistencies in the relationship between inhibition of phosphodiesterase and the relaxant effect of papaverine have been found (Andersson *et al.*, 1977; Polson *et al.*, 1978).

In the dog basilar artery, the action of papaverine was found to be related to the inhibition of Ca^{2+} influx at the sarcolemmal membrane and the inhibition of Ca^{2+} release from its storage site. Papaverine inhibited the action potential and contraction evoked by excess concentrations of $[K]_o$ or 5-HT. The spike potential evoked by outward current pulses in the presence of TEA in the dog and guinea-pig basilar arteries has been found to be due to the Ca^{2+} spike

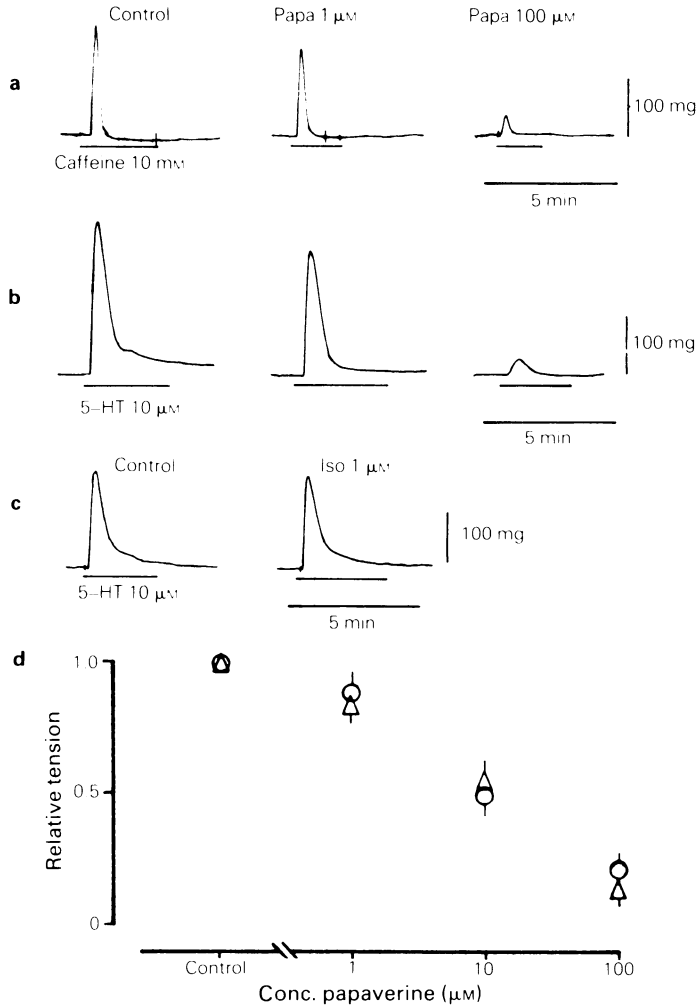

Figure 9 Effects of papaverine (Papa; a and b) or isoprenaline (Iso; c) on the caffeine (10 mM)- or 5-hydroxytryptamine (5-HT)-induced contraction of the dog basilar artery in Ca^{2+}-free EGTA-containing solution. Ca-free solution was applied 3.5 min, and isoprenaline or papaverine 3 min, before application of the stimulant. (a–c) Actual mechanical responses. (d) Effects of papaverine (1 μM–100 μM) on the contractions evoked by 10 mM caffeine (○) or 10 μM 5-HT (△). The contractions evoked in Ca^{2+}-free solution before addition of papaverine were defined as relative tensions of 1.0. Vertical bars indicate s.d.; $n = 3–5$.

(Fujiwara *et al.*, 1982; Fujiwara & Kuriyama, 1983), and the contraction evoked by excess concentrations of K^+ to influx of Ca^{2+} by activation of the voltage-dependent Ca^{2+} channel. Both phenomena ceased in Ca^{2+}-free EGTA-containing solution, or on treatment with papaverine. In the dog basilar artery, we used the caffeine- and 5-HT-induced contractions as indicators of intracellular Ca^{2+} mobilization. The contraction evoked by 5-HT was largely the result of influx of Ca^{2+} and in part to release of Ca^{2+} stored in the cell, since a small amplitude contraction could still be evoked in Ca^{2+}-free EGTA-containing solution. In visceral smooth muscles from various species, the receptor activated by agonists increases the free Ca^{2+} in the cell by the following mechanisms; (1) the receptor activated voltage-dependent Ca^{2+} influx; (2) voltage less dependent influx of Ca^{2+} and (3) release of Ca^{2+} stored in the cell (Bolton, 1979). In the guinea-pig basilar artery, 5-HT produced a contraction with depolarization of the membrane, and this depolarization was accompanied by an increase in the membrane resistance. The 5-HT-induced con-

traction was inhibited (almost totally) in Ca^{2+}-free solution. Therefore, the receptor-activated voltage less dependent influx of Ca^{2+} probably plays a major role in the generation of this contraction (Fujiwara & Kuriyama, 1982). In the dog basilar artery, 5-HT depolarized the membrane, and a small amplitude of contraction was maintained in Ca^{2+}-free solution, which must be due to the release of Ca^{2+} from its storage site in the cell. Hence, the contraction induced by 5-HT can be as a result of either of the above three mechanisms. Caffeine produces a contraction in Ca^{2+}-free solution by activation of a Ca^{2+} release mechanism in the storage site (Itoh *et al.*, 1981b; 1982). Papaverine reduced the amplitude of contraction evoked by 5-HT or caffeine in the presence or absence of Ca^{2+}. This means that papaverine inhibits not only the influx of Ca^{2+} but also the release of Ca^{2+} stored in the cell. The inhibition by papaverine of the caffeine- or 5-HT-induced contractions in Ca^{2+}-free solution may not be related to the production of cyclic AMP as isoprenaline increased cyclic AMP to much the same extent as papaverine, but isoprenaline did not inhibit the contractions to caffeine or 5-HT in the Ca^{2+}-free solution. In the pig coronary artery, papaverine had no effect on the action of calmodulin based on the Ca^{2+}-induced contraction in saponin-treated skinned muscle (Itoh *et al.*, 1981a). However, it is not certain, whether the inhibition of 5-HT- or caffeine-induced contractions by papaverine is due to a reduction in the Ca^{2+} stored in the cell or to an inhibition of the release of Ca^{2+}.

In the dog and guinea-pig basilar arteries, papaverine consistently inhibited the spike generation and contractions evoked by various procedures. This effect was mostly as a result of inhibition of the voltage-dependent influx of Ca^{2+}, but may also in part be due to inhibition of the receptor operated Ca^{2+} releasing mechanisms. Hence, it is concluded that in the dog, papaverine seems to inhibit the contraction of the basilar artery by a mechanism that is unrelated to its inhibitory actions on phosphodiesterase, and that this drug acts as a non-selective inhibitor of Ca^{2+} influx in this tissue.

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