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Vasodilator actions of TRK-100, a new prostaglandin I_2 analogue

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1 TRK-100, a stable analogue of prostaglandin I_2 (PGI_2), relaxed isolated arteries of the dog precontracted with $PGF_{2\alpha}$ or K^+; the relaxation was in the order of mesenteric and renal > coronary and femoral > basilar and middle cerebral arteries. The relaxation by TRK-100 was not affected by treatment with atropine, propranolol, cimetidine, aminophylline, and indomethacin, but was suppressed by diphloretin phosphate, a prostaglandin antagonist.

2 Treatment with TRK-100 attenuated the contraction induced by $PGF_{2\alpha}$ and Ca^{2+} in mesenteric and basilar arteries previously exposed to Ca^{2+}-free medium, but did not significantly alter the contractile response to Ca^{2+} in the arteries exposed to Ca^{2+}-free medium and depolarized by excess K^+.

3 TRK-100 and nitroglycerin relaxed isolated mesenteric arteries to a similar extent; however, when continuously infused into mesenteric arteries in anaesthetized dogs, TRK-100 produced greater vasodilatation than nitroglycerin.

4 It is concluded that TRK-100 relaxes dog mesenteric and renal arteries more than cerebral arteries; the relaxation appears to derive from interference with the release of Ca^{2+} from intracellular stores and with the transmembrane Ca^{2+} influx through a receptor-operated channel. TRK-100 may vasodilate large and small mesenteric arteries and resistance vessels to a similar extent, whereas nitroglycerin preferentially dilates the large artery.

Introduction

Prostaglandin I_2 (PGI_2) synthesized from arachidonic acid by cyclo-oxygenase is a potent relaxant of vascular smooth muscle and a potent platelet antiaggregatory agent. However, PGI_2 is degraded rapidly in aqueous solutions *in vitro* and *in vivo*; therefore, its clinical use is limited. Although many PGI_2 analogues have been synthesized, including carboprostacyclin (Whittle *et al.*, 1980), ONO 41483 (Adaikan *et al.*, 1982; Yui *et al.*, 1985), HOE 892 (Schölkens *et al.*, 1983) and ciloprost (Skuballa & Vorbruggen, 1983), their oral usefulness has yet to be established. Recently, a stable analogue of PGI_2, TRK-100, was synthesized by replacing the exoenolether structure with a cyclopenta (b) benzofuran structure (Figure 1).

This analogue has a long biological half-life and is orally effective in inhibiting the aggregation of blood platelets (Sim *et al.*, 1985; Umetsu *et al.*, 1985) and lowering systemic blood pressure (Umetsu *et al.*, unpublished data). Further information on the cardiovascular actions of this drug has not been obtained. Therefore, the present study was undertaken to determine the characteristic features and the mechanism of action of TRK-100 in isolated arteries of the dog and in mesenteric vasculature of anaesthetized dogs.

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Methods

In vitro *experiments*

Mongrel dogs of either sex, weighing 6–15 kg, were anaesthetized with intravenous injections of pentobarbitone (30 mg kg^{-1}) and killed by bleeding from the

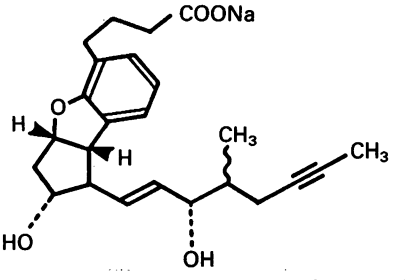

Figure 1 Chemical structure of TRK-100.

common carotid arteries. Middle cerebral, basilar, coronary, mesenteric, renal and femoral arteries (0.6–0.9 mm outside diameter) were isolated. The arteries were helically cut into strips 20–25 mm long. Each strip was vertically fixed between hooks in a muscle bath containing the nutrient solution, which was gassed with a mixture of 95% O_2 and 5% CO_2, and maintained at 37 ± 0.3°C. The hook fixing the upper end of the strips was connected to the lever of a force-displacement transducer (Nihon-Koden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g which was optimal for inducing the maximum contraction (Toda *et al.*, 1978). The composition of the bathing solution was (mM): NaCl 120, KCl 5.4, $NaHCO_3$ 25.0, $CaCl_2$ 2.2, $MgCl_2$ 1.0 and dextrose 5.6. The pH of the solution was 7.3–7.4. Before the start of experiments, preparations were allowed to equilibrate for 60–90 min in the control solution, during which time the medium was replaced every 10–15 min.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Nihon-Koden Kogyo Co.). The contractile response to 30 mM K^+ was first obtained; then, preparations were washed three times with fresh medium and equilibrated for 30–40 min. Before the dose-response curves for the vasodilators were obtained, arterial strips were partially contracted with $PGF_{2\alpha}$ or K^+. The vasodilators were added directly to the bathing medium in cumulative concentrations. At the end of each series of experiments, papaverine (10^{-4} M) was added to the bathing medium to attain the maximum relaxation. Relaxant responses induced by the vasodilators relative to papaverine-induced relaxation are presented. The median effective concentration (EC_{50}) of TRK-100 was calculated by taking the maximum relaxation induced by TRK-100 as 100%.The dissociation constant (K_B) of diphloretin phosphate (DPP) was calculated from the equation, K_B = [B]/dose-ratio − 1, where [B] is the concentration of DPP. The dose-ratio is the ratio of the EC_{50} of TRK-100 in the presence and absence of DPP. Preparations were pretreated for 20 to 30 min with pharmacological antagonists before the addition of TRK-100. Mesenteric arterial strips denuded of endothelium, were obtained by rubbing their intimal surface with a cotton pellet, and the removal of endothelium was determined by a loss of the acetylcholine-induced relaxation response.

Two basilar or three mesenteric arterial strips were obtained from the same dogs. The contractile response to 30 mM K^+ was first obtained, and preparations were washed three times with fresh medium. After the equilibration period of 30–40 min, contractile responses to $PGF_{2\alpha}$ (10^{-5} M) or K^+ (30 mM) were obtained twice. The tonic contraction of the second responses was taken as a control. Arterial strips were then exposed to Ca^{2+}-free medium containing 0.1 mM EGTA for 60 min, during which time the medium was replaced twice every 20 min. After 40 min of exposure, one of the pair of strips was treated with TRK-100, and 20 min later (after 60 min exposure to Ca^{2+}-free medium), $PGF_{2\alpha}$ or K^+ was added; the other strip was left untreated before the addition of $PFG_{2\alpha}$ or K^+. When the response had stabilized, Ca^{2+} (2.2 mM for basilar arteries and $PGF_{2\alpha}$-stimulated mesenteric arteries, and 0.5 to 2.2 mM for K^+-depolarized mesenteric arteries) was added.

In vivo *experiments*

Mongrel dogs were anaesthetized with intravenous injections of pentobarbitone (30 mg kg^{-1}), and were ventilated with a respirator (Igarashi Ika-Kogyo Co., Tokyo; model B2). The blood flow probe (Nihon-Koden Kogyo Co.; inner diameter of 2.5–3.0 mm) was placed around the proximal, superior mesenteric artery, and blood flow was measured with an electromagnetic flowmeter (Nihon-Koden Kogyo Co.; MFV-2100). Into the site just distal to that of flow probe hooking, a needle (23 guage) was inserted, through which saline was infused continuously at a rate of 0.03 ml min^{-1}. Systemic arterial blood pressure was measured with a pressure transducer (Nihon-Koden Kogyo Co.; TP-200T) via a catheter inserted into the right brachial artery. The mesenteric blood flow and the systemic blood pressure were displayed on an ink-writing oscillograph (Sanei-Sokki Co., Tokyo). Local vascular resistance was calculated from an equation, systemic blood pressure/local blood flow. Drugs were injected in a volume of 50 µl through the needle inserted into the mesenteric artery, or continuously infused at a rate of 0.03 ml min^{-1} by a constant infusion pump (Harvard Apparatus, model 1975E; Dover, MA). The volume of the infusion media was 0.02–0.06% of local blood flow. The local blood concentration of drugs during continous infusion was obtained from the drug concentration in the infusion medium and the local blood flow rate, which stabilized during the infusion.

Data analysis and drugs used

Data are expressed as mean values ± s.e.mean. Comparisons were made by use of Student's paired and unpaired *t* test. Drugs used were TRK-100 (sodium (±)-4-[(1R, 2R, 3aS, 8bS)- 1, 2, 3a, 8b- tetrahydro-2-hydroxy-1-[(3S, 4RS)-3-hydroxy-4-methyl-oct-6-yne-(E)-1-enyl]-5-cyclopenta [b] benzofuranyl] butyrate; Toray-Kaken Pharmaceutical Co., Tokyo), nitroglycerin (Nihon-Kayaku, Tokyo), acetylcholine chloride (Daiichi Pharmaceutical Co., Tokyo) and $PGF_{2\alpha}$ (Toray-

Kaken Pharmaceutical Co., Tokyo), diphloretin phosphate (DPP; AB Leo, Helsingborg, Sweden), atropine sulphate (E. Merk, Darmstadt, FRG), (±)-propranolol HCl (Sumitomo Pharmaceutical. Co., Osaka, Japan), cimetidine (Fujisawa Pharmaceutical Co., Osaka), aminophylline (Nakarai Chemicals, Kyoto), indomethacin (Sigma, St Louis, USA) and EGTA [ethylene glycol-bis-(β-aminoethylether)-N, N'-tetra-acetic acid; Wakoh Pure Chemical Ind, Osaka]. TRK-100 and nitroglycerin were dissolved in saline, and $PGF_{2\alpha}$ in 0.1 mM phosphate buffer (pH 9.2).

Results

Relaxant responses induced by TRK-100 in isolated arteries

The addition of TRK-100 in concentrations ranging from 10^{-9} to 10^{-6} M produced a concentration-dependent relaxation in dog arterial strips partially contracted with $PGF_{2\alpha}$ (Figure 2a). The maximum relaxation was attained at 10^{-6} M TRK-100; further increase in the concentration to 3×10^{-6} M did not produce an additional relaxation. Mean values of the maximum relaxation and the median effective concentration (EC_{50}) with TRK-100 in different arteries are summarized in Table 1. On the basis of these values, the relaxant response was in the order of mesenteric and renal > coronary and femoral > basilar and middle cerebral arteries. Tachyphylaxis did not develop to repeated applications of TRK-100. Relaxation of mesenteric arteries induced by TRK-100 was not affected by treatment with 10^{-7} M atropine ($n = 3$), 10^{-6} M propranolol ($n = 3$), 10^{-5} M cimetidine ($n = 3$), 2×10^{-5} M aminophylline ($n = 3$) and 10^{-6} M indomethacin ($n = 3$).

TRK-100 (10^{-9} to 10^{-6} M) relaxed arterial strips contracted with K^+ (10 to 15mM) to a lesser extent than those contracted with $PGF_{2\alpha}$ (Figure 2b). The potency of the agent was the highest in mesenteric arteries and was the lowest in the basilar and middle cerebral arteries. EC_{50} values and maximum relaxant responses in the arteries are shown in Table 1. After responding to 10^{-6} M TRK-100, all the femoral arterial strips contracted from the relaxed state. In K^+-contracted mesenteric arteries, the concentration-response curve for TRK-100 was shifted to the right by treatment with 10^{-5} M diphloretin phosphate (DPP), a prostaglandin antagonist (Figure 3). The K_B value of DPP was $7.7 \pm 2.9 \times 10^{-7}$ M ($n = 7$). The inhibition was partially reversed by repeated washing.

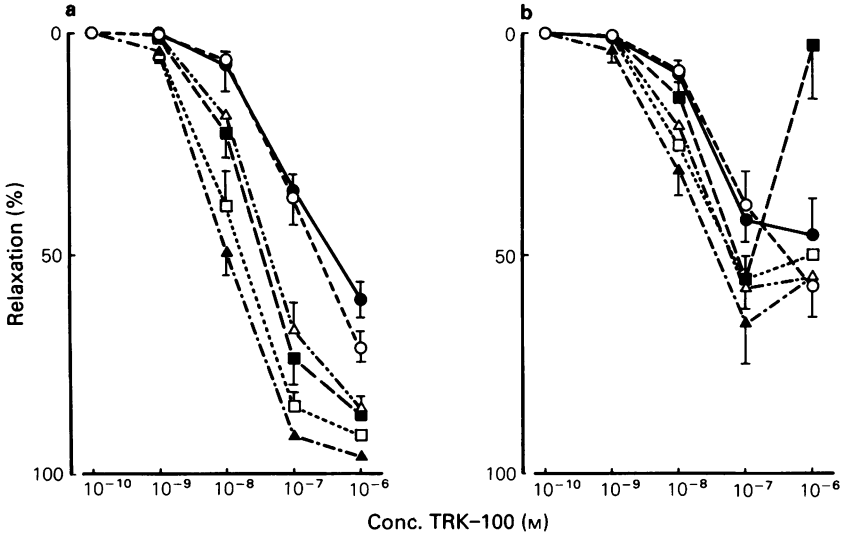

Figure 2 Concentration-response curves for TRK-100 in middle cerebral (○), basilar (●), coronary (△), mesenteric (▲), renal (□) and femoral (■) arteries contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$,a) or K^+ (b). Relaxation induced by papaverine (10^{-4} M) was taken as 100%. Mean absolute values in these arteries contracted with $PGF_{2\alpha}$ were 429 ± 116 mg ($n = 7$), 439 ± 53 mg ($n = 7$), 655 ± 139 mg ($n = 8$), 525 ± 54 mg ($n = 10$), 564 ± 61 mg ($n = 7$) and 676 ± 68 mg ($n = 9$), respectively, and the values in the K^+-contracted arteries were 546 ± 158 mg ($n = 7$), 558 ± 158 mg ($n = 8$), 503 ± 29 mg ($n = 7$), 349 ± 35 mg ($n = 10$), 423 ± 65 mg ($n = 8$) and 444 ± 72 mg ($n = 10$), respectively.

Table 1 The maximum relaxation and corresponding EC_{50} induced by TRK-100 on different arterial strips contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or K^+.

	EC_{50} ($\times 10^{-8}$ M)		*Maximum relaxation* (%)[a]	
Artery	*$PGF_{2\alpha}$-contracted*	*K^+-contracted*	*$PGF_{2\alpha}$-contracted*	*K^+-contracted*
Middle cerebral	13.7 ± 4.3 (7)	11.9 ± 5.4 (7)	70.8 ± 3.5 (7)	57.4 ± 8.0 (7)
Basilar	9.1 ± 1.3 (7)	4.7 ± 1.0 (8)	60.3 ± 4.3 (7)	45.4 ± 8.2 (8)
Coronary	5.5 ± 1.1 (8)	3.2 ± 0.8 (7)	84.9 ± 2.7 (8)	57.6 ± 7.3 (7)[d]
Mesenteric	1.5 ± 0.4 (10)[c]	2.0 ± 0.6 (10)	96.3 ± 1.0 (10)	65.6 ± 4.2 (10)[bd]
Renal	2.5 ± 0.8 (7)[c]	2.9 ± 0.5 (8)	91.3 ± 3.2 (7)	65.6 ± 4.8 (8)[cd]
Femoral	4.7 ± 1.0 (9)	4.0 ± 0.5 (10)	86.6 ± 3.3 (9)	55.7 ± 5.4 (10)[d]

Numbers in parentheses indicate the number of preparations used. [a] Relaxation relative to that induced by papaverine (10^{-4} M). Significantly different from values with middle cerebral arteries: [b] $P < 0.01$, [c] $P < 0.05$. Significantly different from values with basilar arteries: [d] $P < 0.01$.

In mesenteric arterial strips, from which the endothelium was mechanically removed, relaxant responses induced by acetylcholine (10^{-8} to 10^{-5} M; the average maximal relaxation being 69.8 ± 11.6%, $n = 5$) were abolished or reversed to a contraction, whereas relaxant responses to TRK-100 were unaffected (Figure 4a). Concentration-response curves for TRK-100 in proximal and distal mesenteric arteries (mean cross sectional areas of 0.53 ± 0.03 and 0.22 ± 0.03 mm^2, respectively, $n = 11$) partially contracted with $PGF_{2\alpha}$ did not significantly differ (Figure 4b).

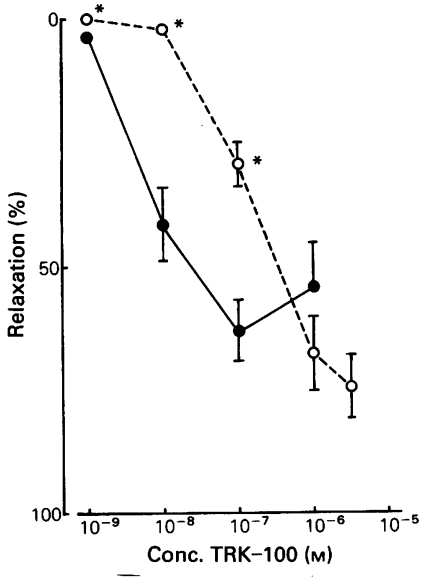

Figure 3 Modification by diphloretin phosphate (DPP) of the relaxation induced by TRK-100 in dog mesenteric arterial strips contracted with K^+ (10 to 15 mM). Relaxation induced by papaverine (10^{-4} M) was taken as 100%. Mean absolute values in control (●) and DPP (10^{-5} M)-treated arteries (○) were 347 ± 70 mg ($n = 7$) and 328 ± 61 mg ($n = 7$), respectively. Significantly different from controls, $^*P < 0.01$.

Modification by TRK-100 of contractions induced by $PGF_{2\alpha}$ and Ca^{2+} in mesenteric and basilar arteries exposed to Ca^{2+}-free media

The addition of 10^{-5} M $PGF_{2\alpha}$ elicited a rapid, phasic contraction followed by a tonic contraction in mesenteric arteries. The tonic contractions in the control medium averaged 1709 ± 177 mg ($n = 13$), which was 87.2 ± 7.7% of contractions induced by 30 mM K^+. These contractions were suppressed by 79.2 ± 2.7% following exposure of the arteries for 60 min to Ca^{2+}-free medium containing 0.1 mM EGTA. The addition of 2.2 mM Ca^{2+} produced triphasic responses, phasic contraction, relaxation and tonic contraction. Typical recordings of the response are shown in Figure 5. The contractions induced by $PGF_{2\alpha}$ and Ca^{2+} in the arteries previously exposed to Ca^{2+}-free medium were not affected by 10^{-8} M TRK-100 but moderately reduced by 10^{-7} M (Figures 5d and 6a). Exposure of mesenteric arteries to Ca^{2+}-free, EGTA-containing medium abolished the tonic contraction induced by 30 mM K^+. The addition of Ca^{2+} (0.5 to 2.2 mM) produced tonic, dose-dependent contractions. Treatment with TRK-100 10^{-7} and 10^{-6} M did not significantly reduce the contractions induced by Ca^{2+} (Figure 6b).

Contractions induced by 10^{-5} M $PGF_{2\alpha}$ in basilar arteries exposed to control medium averaged 992 ± 139 mg ($n = 9$), which was 85.2 ± 12.4% of contractions induced by 30 mM K^+. In the basilar arteries exposed for 60 min to Ca^{2+}-free medium containing 0.1 mM EGTA, the $PGF_{2\alpha}$-induced con-

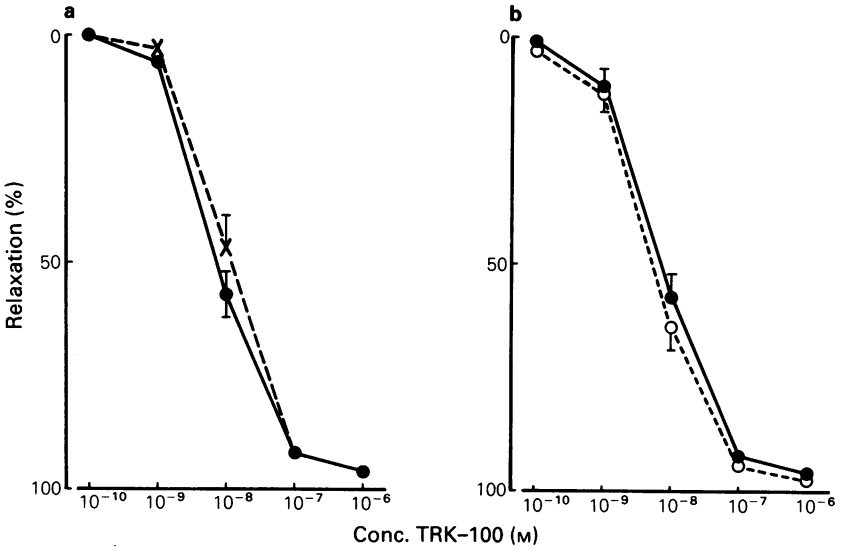

Figure 4 Concentration-response curves for TRK-100 in dog mesenteric arteries with or without endothelium (a) and in arteries of different sizes (b). Preparations were partially contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Relaxation induced by papaverine (10^{-4} M) was taken as 100%. Mean absolute values (a) in the arteries with (●) and without endothelium (X) were 464 ± 61 mg (n = 5) and 474 ± 40 mg (n = 5), respectively, and (b) in the proximal (●) and distal arteries (○) 580 ± 75 mg (n = 11) and 397 ± 67 mg (n = 11), respectively.

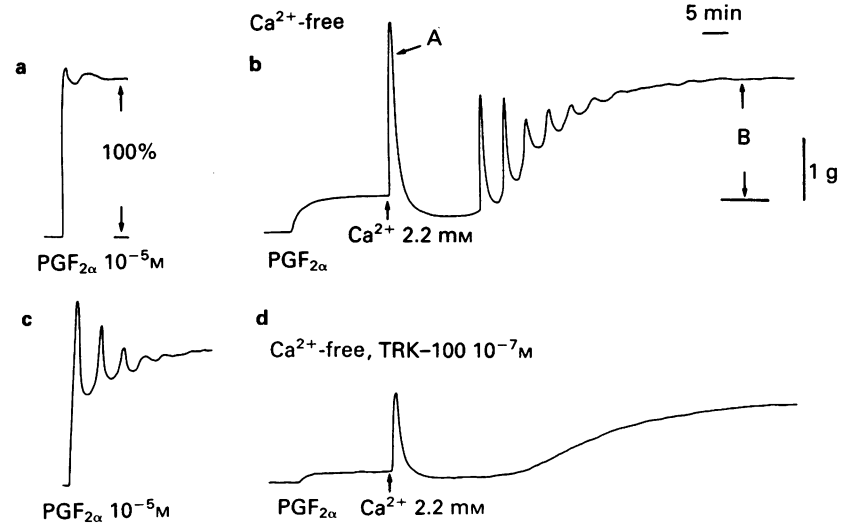

Figure 5 Responses of mesenteric arterial strips in Ca^{2+}-free, EGTA (0.1 mM)-containing medium to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and Ca^{2+} in the absence (b) and presence (d) of TRK-100. Two strips were obtained from the same dog. Contractile responses to 10^{-5} M $PGF_{2\alpha}$ were first obtained in control medium (a and c). Magnitudes of the $PGF_{2\alpha}$ (10^{-5} M)-induced contraction and of transient and persistent contractions induced by 2.2 mM Ca^{2+} (A and B, respectively, in (b)) were measured.

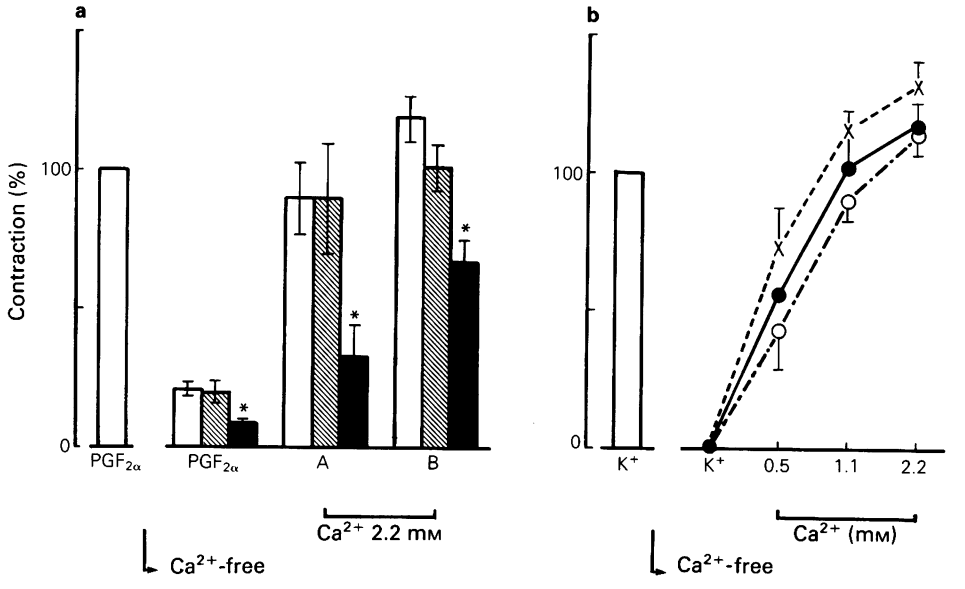

Figure 6 Modification by TRK-100 of contractile responses of mesenteric arterial strips in Ca^{2+}-free, EGTA (0.1 mM)-containing medium to (a) 10^{-5} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and (b) 30 mM K^+ and Ca^{2+}. A and B on the abscissa scale in (a) represent contractions induced by $PGF_{2\alpha}$ plus A and B, respectively, (see Figure 5 for explanation of A and B). In (a) open columns represent control ($n = 13$), hatched columns, TRK-100 10^{-8} M ($n = 12$) and solid columns, TRK-100 10^{-7} M ($n = 12$)-treated arteries. (b) Control (●, $n = 8$) and TRK-100 10^{-7} M (○, $n = 8$) and 10^{-6} M (X, $n = 6$)-treated arteries. Significantly different from controls, $*P < 0.01$. Contractions induced by 10^{-5} M $PGF_{2\alpha}$ (a) or 30 mM K^+ (b) in control medium were taken as 100%; mean absolute values were 1709 ± 177 mg ($n = 13$) and 1968 ± 306 mg ($n = 8$), respectively.

traction was attenuated by 87.4 ± 1.7% ($n = 9$). After the $PGF_{2\alpha}$-induced contraction was stabilized, Ca^{2+} (2.2 mM) elicited a rapid, transient contraction, relaxation and persistent contraction, as seen in the mesenteric arteries. Treatment for 20 min with 10^{-6} M TRK-100 significantly attenuated the contraction induced by $PGF_{2\alpha}$ in the Ca^{2+}-free medium and also the phasic and tonic contractions induced by Ca^{2+} (Figure 7a). This reduction was approximately identical to that seen in mesenteric arteries treated with 10^{-7} M TRK-100. Tonic contractions of basilar arteries induced by 30 mM K^+ were completely abolished by exposure for 60 min to Ca^{2+}-free, EGTA-containing medium. The addition of 2.2 mM Ca^{2+} to the K^+-depolarized arteries caused triphasic responses, phasic contraction, relaxation and tonic contraction. Treatment with 10^{-6} M TRK-100 did not affect these responses (Figure 7b).

Modification by TRK-100 and nitroglycerin of mesenteric vascular resistance in anaesthetized dogs

Intra-arterial bolus injections of TRK-100 (10 to 3000 ng kg^{-1}, 50 µl) caused a dose-related decrease in mesenteric vascular resistance (Figure 8a) without a significant alteration in the systemic blood pressure. A further increase in the dose lowered the blood pressure and did not produce an additional decrease in the mesenteric vascular resistance. Intra-arterial injections of nitroglycerin at doses higher than 300 ng kg^{-1} reduced the vascular resistance dose-dependently; the potency was 1/30 to 1/100 that of TRK-100. Mesenteric vascular resistance was also reduced by intra-arterial continuous infusions of TRK-100 and nitroglycerin (Figure 8b); the vasodilator effect of TRK-100 was appreciably greater than that of nitroglycerin.

Concentration-response curves for TRK-100 and nitroglycerin in isolated mesenteric arteries were identical (Figure 9a). The curves for TRK-100 *in vivo* (the concentrations estimated from the concentration and the rate of infusion of the drug and from blood flow, which stabilized during the drug infusion) and *in vitro* were also similar (cf. solid lines in Figure 9a and b). On the other hand, the reduction of vascular resistance induced by nitroglycerin was evidently less than that induced by TRK-100 (Figure 9a). Vasodilator actions of nitroglycerin were markedly greater in isolated

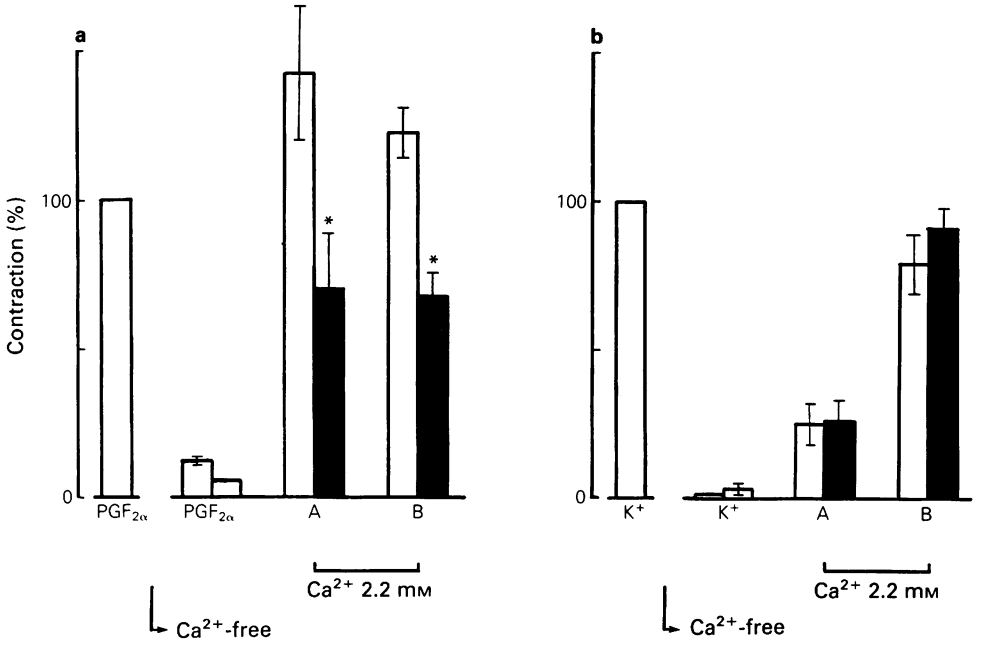

Figure 7 Modification by TRK-100 of contractile responses of basilar arterial strips in Ca^{2+}-free, EGTA (0.1 mM)-containing medium to (a) 10^{-5} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and (b) 30 mM K^+ and Ca^{2+}. A and B on the abscissa scale in (a) and (b) represent contractions induced by $PGF_{2\alpha}$ and K^+, respectively plus A and B (for explanation of A and B, see Figure 5). In (a) and (b) open columns represent control ($n = 9$) and solid columns represent TRK-100 10^{-6} M, ($n = 9$)-treated arteries. Significantly different from controls, $^*P < 0.01$. Contractions induced by 10^{-5} M $PGF_{2\alpha}$ and 30 mM K^+ in control medium were taken as 100%; mean absolute values were 992 ± 139 mg ($n = 9$) and 1551 ± 190 mg ($n = 9$), respectively.

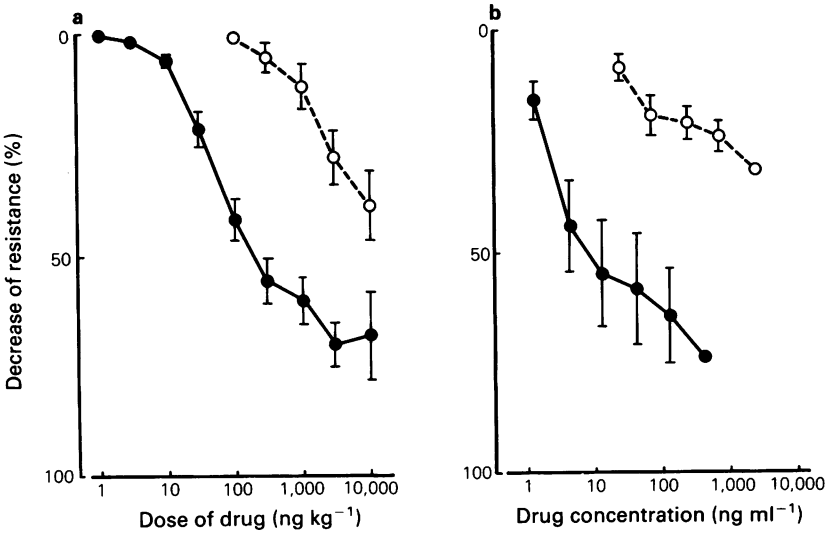

Figure 8 The effect of intra-arterial bolus injections (a) and continuous infusions (b) of TRK-100 (●) and nitroglycerin (○) on mesenteric vascular resistance in anaesthetized dogs. Vascular resistance before the drug injections was taken as 100%. Mean mesenteric vascular resistance before TRK-100 and nitroglycerin in (a) was 1.26 ± 0.17 ($n = 9$) and 1.27 ± 0.26 mmHg ml^{-1} min^{-1} ($n = 9$), respectively, and in (b) 1.29 ± 0.18 ($n = 7$) and 1.14 ± 0.33 mmHg ml^{-1} min^{-1} ($n = 5$), respectively.

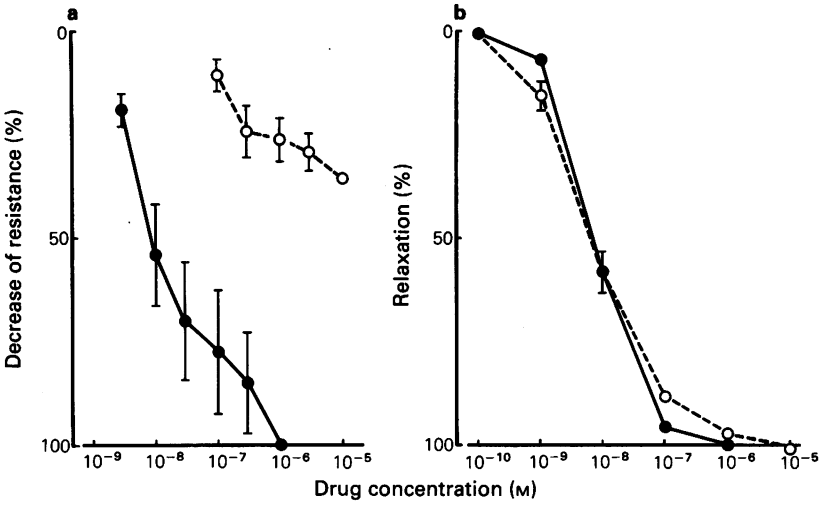

Figure 9 (a) The effect of continuous infusions of TRK-100 (●, $n = 7$) and nitroglycerin (○, $n = 5$) on mesenteric vascular resistance in anaesthetized dogs. (b) The effect of these drugs (●, TRK-100, $n = 11$ and ○, nitroglycerin, $n = 7$) on the tone of isolated mesenteric arteries precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Maximum responses to 10^{-6} M TRK-100 in these preparations were taken as 100%. In (a) the mean absolute value was 1.29 ± 0.18 mmHg ml^{-1} min^{-1} ($n = 7$) and in (b) was 580 ± 75 mg ($n = 11$).

arteries than in mesentery *in vivo* (cf. broken lines in Figure 9a and b).

Discussion

TRK-100, a stable analogue of PGI_2, relaxed a variety of dog arteries contracted with $PGF_{2\alpha}$ or K^+; the relaxation potency was in the order of mesenteric and renal > coronary and femoral > basilar and middle cerebral arteries. Similar results were also obtained with PGI_2 (Toda, 1980). PGI_2 has been demonstrated to increase blood flow in mesentery to a greater extent than that in the brain (Schölkens *et al.*, 1982) possibly indicating a regional difference in the response of resistance vessels to PGI_2. Relaxations induced by TRK-100 were not reduced by effective concentrations of atropine, propranolol, cimetidine, aminophylline and indomethacin, suggesting that muscarinic receptors, β-adrenoceptors, histamine H_1-receptors, histamine H_2-receptors, P_1-purinoceptors and prostaglandin related mechanisms are not involved. However, the relaxant response was attenuated by treatment with DPP, a prostaglandin antagonist (Eakins *et al.*, 1973), in a concentration (10^{-5} M) sufficiently to inhibit significantly relaxations induced by PGI_2 but not those by adenosine (Toda 1984). These findings indicate that PGI_2 and TRK-100 share the same site for vasodilator actions. Vasodilatation induced by substances, such as acetycholine, bradykinin, substance P, histamine and angiotensin II, is mediated by vasodilator substances released from the endothelium, including endothelium-derived relaxing factor (EDRF, Furchgott, 1983) and PGI_2 (Toda, 1984). In the present study, acetylcholine-induced relaxant responses were abolished by removal of endothelium. However, relaxant responses induced by TRK-100 were not dependent on endothelium, as was the response to PGI_2 (Toda, 1984).

TRK-100 relaxed dog arterial strips precontracted with $PGF_{2\alpha}$ to a greater extent than those precontracted with K^+. Treatment with TRK-100 attenuated the contraction induced by Ca^{2+} in mesenteric and basilar arteries previously exposed to Ca^{2+}-free medium and stimulated by $PGF_{2\alpha}$ but did not significantly alter the contraction in the arteries stimulated by excess K^+. Contractile responses to K^+ are induced by an increase in the transmembrane Ca^{2+} influx associated with depolarization of the cell membrane, whereas the increased Ca^{2+} influx due to $PGF_{2\alpha}$ is possibly mediated by a receptor-operated process (Toda, 1982). TRK-100 appears to interfere more predominantly with the Ca^{2+}-influx due to the receptor-operated process than with the voltage-dependent Ca^{2+} influx. $PGF_{2\alpha}$-induced contractions are also associated with the release of Ca^{2+} from intracellular stores via activation of the receptors; the contraction induced by $PGF_{2\alpha}$ in the arteries exposed to Ca^{2+}-free medium is

probably as a result of this mechanism. Such a contraction was suppressed by TRK-100, suggesting that this compound interferes with the release of Ca^{2+} from its storage sites.

Relaxation induced by TRK-100 was less in cerebral arteries than in mesenteric arteries. Also the attenuation of the contraction induced by Ca^{2+} in Ca^{2+}-free medium evoked by 10^{-7} M TRK-100 in the mesenteric artery was approximately identical to that induced by 10^{-6} M TRK-100 in the basilar artery. The mechanism underlying the greater inhibition by TRK-100 of the release and influx of Ca^{2+} through the receptor-operated process in mesenteric arteries cannot be determined from the present study.

TRK-100 and nitroglycerin relaxed isolated mesenteric arteries to a similar extent. However, when continuously infused into mesenteric arteries in anaesthetized dogs, the PGI_2 analogue produced greater vasodilatation than nitroglycerin. TRK-100 elicited similar vasodilator responses in strips of proximal and distal mesenteric arteries and in the mesentery *in vivo*. These findings indicate that TRK-100 relaxes smooth muscle of large and small arteries and arterioles similarly, whereas nitoglycerin relaxes the arteries more than the arterioles. On the other hand, hydralazine up to 10^{-4} M does not relax dog isolated mesenteric arteries, but markedly dilates resistance vessels (Toda *et al.*, 1985). These data indicate that different vasodilators can alter the tone of smooth muscle in arteries, from the proximal to the distal portion, and of resistance vessels to a different extent. According to Levenson *et al.* (1985), vasodilator actions on large arteries are postulated to be important in the treatment of hypertension, because of increased arterial compliance.

In summary, TRK-100, a PGI_2 analogue, relaxes dog mesenteric and renal arteries more than cerebral arteries, the relaxant response may be identical in the large and small arteries and resistance vessels. The vasodilatation appears to derive from interference with the release of Ca^{2+} from intracellular stores and its transmembrane influx via the receptor-operated process.

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