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Responses of human, monkey and dog coronary arteries *in vitro* to carbocyclic thromboxane A_2 and vasodilators

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1 Carbocyclic thromboxane A_2 ($cTxA_2$), a stable analogue of TxA_2, and prostaglandin (PG) $F_{2\alpha}$ contracted helical strips of human, monkey and dog coronary arteries in a concentration-dependent manner. Apparent ED_{50} values for $cTxA_2$ were markedly less (1/58 in humans, 1/373 in monkeys and 1/397 in dogs) than those for $PGF_{2\alpha}$; maximum contractions induced by $cTxA_2$ and $PGF_{2\alpha}$ relative to those induced by 30 mM K^+ were approximately identical in the human and monkey arteries.

2 PGI_2 caused a concentration-related relaxation in human and dog coronary arteries maximally precontracted with $cTxA_2$ and in human, monkey and dog coronary arteries partially precontracted with $PGF_{2\alpha}$. The relaxation response was greatest in the dog arteries and least in the monkey arteries.

3 Contractions induced by $cTxA_2$ or $PGF_{2\alpha}$ and relaxations induced by PGI_2 were selectively antagonized by treatment with diphloretin phosphate.

4 Human coronary artery strips contracted with $cTxA_2$ responded to nitroglycerine with a rapid, transient relaxation and to verapamil with a slowly-developing, persistent relaxation, as did monkey and dog coronary artery strips.

5 Thromboxane (Tx) A_2 appears to be one of the most potent endogenous vasoconstrictors in human coronary arteries, if $cTxA_2$ acts on TxA_2 receptors. It is suggested that PGI_2, nitroglycerine and verapamil are effective vasodilators in human conduit coronary arteries maximally contracted with $cTxA_2$, although the extent and the duration of vasodilatation induced by these agents were quite different.

Introduction

Metabolites of arachidonic acid, formed by the catalyst cyclo-oxygenase, alter the blood supply to the heart, brain and other organs by contracting or relaxing vascular smooth muscle. Such an action on the vasculature, together with their effect on platelet adhesiveness and aggregation or blood coagulation, contributes to the physiological regulation of circulatory functions and can be related to pathogenesis of circulatory disturbances. Thromboxane (Tx) A_2 and prostaglandin (PG) $F_{2\alpha}$ are powerful vasoconstrictors that may be responsible for some vascular disorders, whereas PGI_2 (prostacyclin) counteracts the vasoconstriction and may regulate local circulation. Increased production of TxA_2 or an imbalance between TxA_2 and PGI_2 in the coronary circulation have been postulated to cause coronary vasospasm (Dusting *et al.*, 1979; Hirsh *et al.*, 1981; Tada *et al.*, 1981). Decreased production of PGI_2 by the atherosclerotic artery wall might be responsible for some of the clinical manifestations of ischaemic heart diseases (Dembinski *et al.*, 1977; D'Angelo *et al.*, 1978; Moncada & Vane, 1979). The coronary vasoconstrictor actions of the cyclo-oxygenase inhibitors, indomethacin and aspirin, have been demonstrated in patients with coronary artery diseases (Friedman *et al.*, 1981; Miwa *et al.*, 1981), suggesting a persistent liberation of vasodilator prostaglandins in the coronary circulation.

Despite such a possible involvement of the arachidonate metabolites in coronary vasospasm in patients with ischaemic heart diseases, actions and interactions of these compounds in primate coronary arteries have not been quantitatively analysed. Therefore, the present study was undertaken to compare the effects of carbocyclic TxA_2 ($cTxA_2$), a stable analogue of TxA_2 (Lefer *et al.*, 1980), and $PGF_{2\alpha}$ on human, monkey and dog isolated coronary arteries and those of PGI_2 on the arteries precontracted with

either $cTxA_2$ or $PGF_{2\alpha}$. Also the susceptibility of the arteries to the prostaglandin antagonist, diphloretin phosphate (DPP), or the coronary vasodilators, nitroglycerine and verapamil, was determined.

Methods

Ventral interventricular branches of the left epicardial coronary artery (1 to 1.5 mm outside diameter) were isolated from the human heart during autopsy, 4 to 8 h after death. The causes of death of the patients (aged 36, 37, 48, 54, 60, 63, 66, 66, 68, 69, 70, 77, 78, 80 and 80 years, males and 43, 44, 70 and 81 years, females) were cancers of the stomach, liver, kidney, pancreas and gall bladder, malignant lymphoma, pneumonia, broncho-obstructive lung disease, stroke and aortic rupture. Histological studies have demonstrated that endothelial cells are preserved in these human coronary arteries, and endothelial functions have been postulated to be retained (Toda, 1983). Japanese monkeys (*Macaca fuscata*) of either sex, weighing 6 to 11 kg, were anaesthetized with intramuscular injections of ketamine (25 to 40 mg kg^{-1}) and killed by bleeding from the carotid arteries. Mongrel dogs of either sex (8 to 15 kg body weight) were anaesthetized with intraperitoneal injections of sodium thiopentone (50 mg kg^{-1}) and also killed by bleeding from the carotid arteries.

Ventral interventricular and circumflex branches of the left coronary artery of medium size (0.6 to 0.8 mm outside diameter in monkeys and 0.7 to 0.8 mm in dogs) were rapidly removed from the monkey and dog heart. The arteries were cut into helical strips, approximately 20 mm long. The strip was fixed vertically between hooks in a muscle bath containing modified Ringer-Locke solution, which was aerated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37±0.3°C. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihon-kohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 2.0 g for human coronary artery strips, which was optimum for producing the maximum contraction. The mean value of the cross sectional area of the strips, calculated by the ratio of wet weight/length of the strips, was 0.707 ± 0.032 mm^2 ($n = 34$). Monkey and dog coronary artery strips were stretched and stabilized at optimum resting tensions of 1.0 and 1.5 g, respectively (Toda *et al.*, 1978; Toda, 1981). Constituents of the Ringer-Locke solution were as follows (mM): Na^+ 145, K^+ 5.4, Cl^- 132, Ca^{2+} 2.2, Mg^{2+} 1.0, HCO_3^- 25.0 and dextrose 5.6. The pH of the solution was 7.3 to 7.4. Before the start of experiments, all the strips were equilibrated in the bathing media for 60 to 90 min, during which time the Ringer-Locke solution was replaced every 10 to 15 min.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Nihon-kohden Kogyo Co.). The contractile response to 30 mM K^+ was obtained, then the preparations were

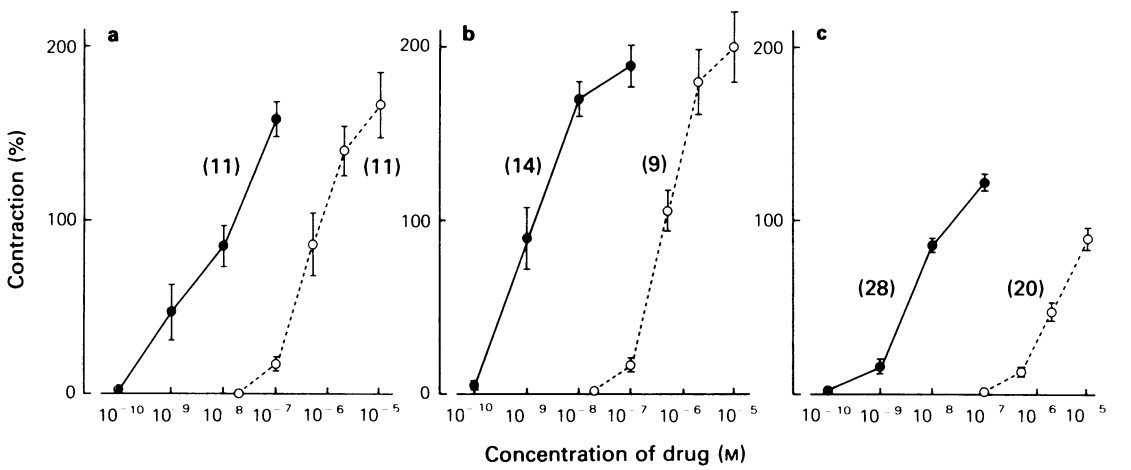

Figure 1 Concentration-response curves for carbocyclic thromboxane A_2 ($cTxA_2$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in (a) human, (b) monkey and (c) dog coronary arteries. Contractions induced by 30 mM K^+ were taken as 100%; mean absolute values in experiments with $cTxA_2$ and $PGF_{2\alpha}$ were 1392 ± 273 mg ($n = 11$) and 1223 ± 230 mg ($n = 11$), respectively, in the human arteries; 1203 ± 113 mg ($n = 14$) and 1288 ± 146 mg ($n = 9$), respectively, in the monkey arteries, and 1894 ± 162 mg ($n = 28$) and 1866 ± 160 mg ($n = 20$), respectively, in the dog arteries. Vertical lines represent s.e.mean. Numbers in parentheses indicate the number of preparations used. (●) Responses to $cTxA_2$; (○) responses to $PGF_{2\alpha}$.

repeatedly washed with fresh solution and equilibrated for 30 to 40 min. The K^+-induced contraction was obtained twice, and the second response was taken as a standard for contractions induced by test drugs. Concentration-response curves for $cTxA_2$, $PGF_{2\alpha}$, PGI_2, verapamil and nitroglycerine were obtained by adding the drugs directly to the bathing media in cumulative concentrations. Before the relaxant response to PGI_2, verapamil or nitroglycerine was obtained, the arterial strips had been precontracted with either $cTxA_2$, $PGF_{2\alpha}$ or K^+. Contractions induced by $PGF_{2\alpha}$ (0.2 to 0.5 μM) or K^+ (10 to 13 mM) were in a range between 30 and 45% of contractions induced by 30 mM K^+. At the end of each series of experiments, papaverine in a concentration of 100 μM was added to attain the maximum relaxation (Toda, 1974), which was taken as a standard for relaxation responses induced by the test drugs. To determine the effect of diphloretin phosphate (DPP), preparations were pretreated for 20 min with DPP before the response to agonists was obtained.

The results shown in the text, Figures and Tables are expressed as mean values ± s.e.mean. Statistical analyses were made using Student's paired and unpaired *t* test or Tukey's method for one-way analysis of variance (Wallenstein *et al.*, 1980).

Drugs used were carbocyclic thromboxane A_2 ($cTxA_2$), prostaglandins $F_{2\alpha}$ and I_2 ($PGF_{2\alpha}$ and PGI_2), diphloretin phosphate (DPP, Ono Pharmaceutical Co., Osaka, Japan), (±)-verapamil hydrochloride (Eisai Co., Ltd., Tokyo), nitroglycerine (Nippon Kayaku Co., Ltd, Tokyo) and histamine dihydrochloride (Katayama Chemical Co., Osaka).

Results

Contractile responses of human, monkey and dog coronary arteries to $cTxA_2$ and $PGF_{2\alpha}$

The addition of $cTxA_2$ in concentrations ranging from 0.1 to 100 nM produced a concentration-dependent, persistent contraction in human, monkey and dog coronary artery strips (Figure 1). Further increases in the concentration of $cTxA_2$ to 200 nM did not elicit an additional contraction. In 3 out of 11 human artery strips, spontaneous activities were provoked by 100 nM $cTxA_2$. Mean values of the maximum contraction induced by 100 nM $cTxA_2$ relative to that induced by 30 mM K^+ in the human, monkey and dog arteries were 158.2 ± 9.8% ($n = 11$), 187.5 ± 10.3% ($n = 14$) and 122.4 ± 4.8% (n =28, significantly different from humans and monkeys, $P < 0.001$), respectively. Contractions induced by 100 nM $cTxA_2$ were not reversed even after repeated washing for 2 to 4 h with fresh bathing solutions. However, when the arteries were contracted with $cTxA_2$ in concentrations lower than 10 nM, the resting level of tension was restored by repeated washing at intervals of 3 to 5 min for 1 to 2 h. The addition of $PGF_{2\alpha}$ (0.01 to 10 μM) also produced a concentration-related contraction; increasing the concentration to 30 μM produced no, or only a slight, additional contraction. The maximum contractions induced by 10 μM $PGF_{2\alpha}$ relative to those induced by 30 mM K^+ in the human, monkey and dog arteries were 166.3 ± 19.4% ($n = 7$), 208.0 ± 24.1% ($n = 9$) and 90.5 ± 6.8% ($n = 20$, significantly different from humans and monkeys, $P < 0.001$), respectively.

Table 1 Apparent ED_{50} values for carbocyclic thromboxane A_2 ($cTxA_2$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in human, monkey and dog coronary arterial strips

Artery	*$cTxA_2$* (nM)	*ED_{50}s $PGF_{2\alpha}$* (× 0.1 μM)	*$PGF_{2\alpha}/cTxA_2$*
Human	9.71 ± 2.29 (11)	5.64 ± 0.90 (11)	58.1
Monkey	1.46 ± 0.37 (14)	5.44 ± 0.76 (9)	373
Dog	5.04 ± 0.61 (28)	20.0 ± 1.71 (20)	397

Figures in parentheses indicate the number of preparations used. *F* ratios obtained from the analysis of variance (11.87 for $cTxA_2$ and 26.03 for $PGF_{2\alpha}$) are greater than the $P = 0.01$ critical values. The following were significantly different ($P < 0.01$) by Tukey's method: human vs. monkey and human vs. dog for $cTxA_2$, and human vs. dog and monkey vs. dog for $PGF_{2\alpha}$.

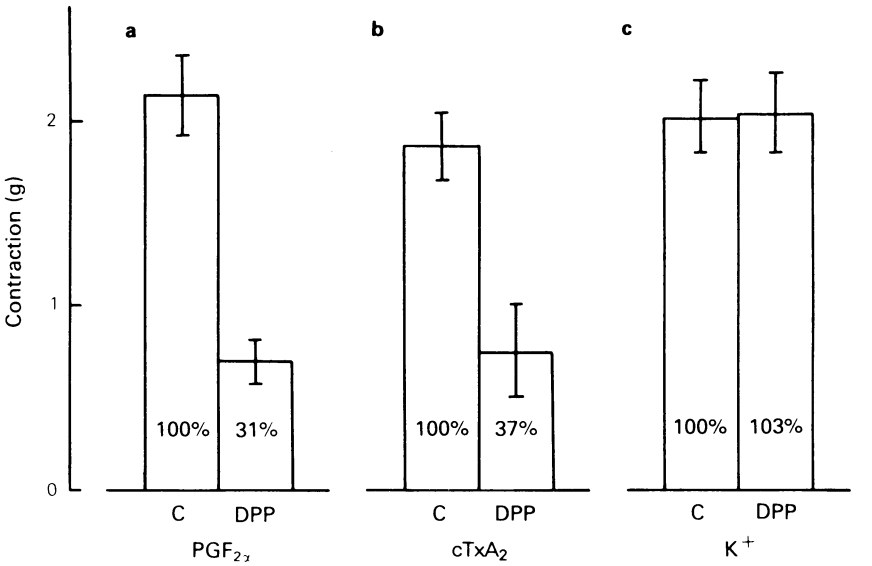

Figure 2 Contractile responses of dog coronary arteries to carbocyclic thromboxane A_2 ($cTxA_2$; 10 nM), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 2 μM) and K^+ (25 mM) before and after treatment with 10 μM diphloretin phosphate (DPP). Numbers of the preparations used were (a) 10, (b) 5 and (c) 6 for experiments with $PGF_{2\alpha}$ $cTxA_2$, and K^+, respectively. Numbers in the columns indicate % controls. Each column represents the mean contraction and vertical lines show s.e.mean.

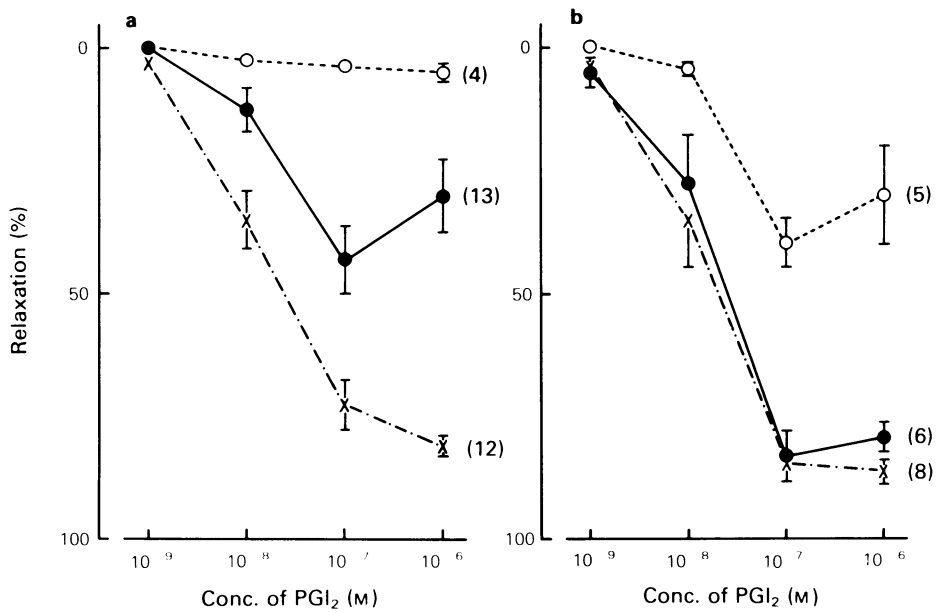

Figure 3 Concentration-response curves for prostaglandin I_2 (PGI_2) in human (●), monkey (○) and dog (×) coronary arteries precontracted with 0.1 μM carbocyclic thromboxane A_2 ($cTxA_2$) (a) or 0.2 to 0.5 μM $PGF_{2\alpha}$ (b). Relaxation responses induced by 100 μM papaverine were taken as 100%; mean absolute values for the human, monkey and dog arteries precontracted with $cTxA_2$ were 1603 ± 225 mg ($n = 13$), 1913 ± 593 mg ($n = 4$) and 2132 ± 291 mg ($n = 12$)*, respectively, and those for the arteries precontracted with $PGF_{2\alpha}$ were 545 ± 84 mg ($n = 6$), 853 ± 107 mg ($n = 5$) and 671 ± 82 mg ($n = 8$), respectively. * Data obtained from the previous study (Toda, 1982b). Numbers in parentheses indicate the number of preparations used. Vertical lines represent s.e.mean.

Contractions induced by 10 μM $PGF_{2\alpha}$ were completely reversed by repeated washing of preparations for 30 to 40 min. Mean values of the apparent median effective concentration (ED_{50}) for $cTxA_2$ and $PGF_{2\alpha}$ are summarized in Table 1. The ED_{50} values for $cTxA_2$ were in the order of monkey < dog < human arteries, whereas the values for $PGF_{2\alpha}$ were in the order of human = monkey < dog arteries. The ratio of ED_{50} values ($PGF_{2\alpha}/cTxA_2$) was appreciably less in human coronary arteries.

The contractile response to 10 nM $cTxA_2$ and 2 μM $PGF_{2\alpha}$ was inhibited by 63.4 ± 12.5% ($P < 0.01$) and 68.8 ± 4.2% ($P < 0.001$), respectively, following treatment with DPP in a concentration of 10 nM in dog coronary arteries. On the other hand, contractions induced by 25 mM K^+ were not influenced by 10 μM (Figure 2) or 30 μM DPP ($n = 3$). Human coronary artery strips were more resistant to DPP than dog arteries, and a concentration of 30 μM was required to attenuate significantly the response to $cTxA_2$ and $PGF_{2\alpha}$. Inhibitions of the response to 10 nM $cTxA_2$ and 0.5 and 2 μM $PGF_{2\alpha}$ averaged 78.3 ± 3.8% ($n = 4$), 75.8 ± 8.1% ($n = 5$) and 43.4 ± 6.5% ($n = 5$), respectively. Contractions induced by histamine (0.5 to 10 μM) were not attenuated by 30 μM DPP ($n = 2$).

Relaxant responses to PGI_2, nitroglycerine and verapamil of coronary arteries precontracted with $cTxA_2$ or $PGF_{2\alpha}$

The addition of PGI_2 (1 nM to 1 μM) relaxed human and dog coronary arteries precontracted with 100 nM $cTxA_2$ in a concentration-dependent manner (Figure 3a). The relaxation response developed rapidly, and after the maximum relaxation was attained, the tension tended to return slowly to the level present before the addition of PGI_2 (Figure 4). Relaxation responses of dog arteries were appreciably greater than those of human arteries. Apparent ED_{50} values for PGI_2 in these arteries did not differ significantly (Table 2). On the other hand, monkey coronary arteries precontracted with $cTxA_2$ did not significantly respond to PGI_2 with a relaxation. In the arteries partially precontracted with $PGF_{2\alpha}$ (0.2 to

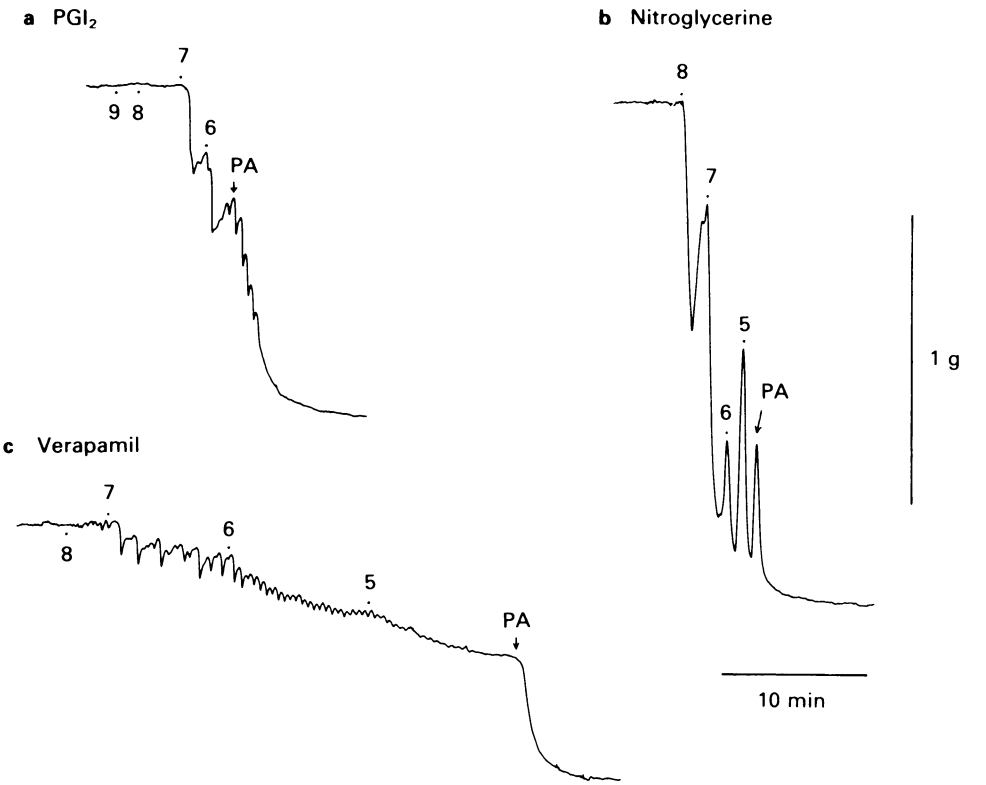

Figure 4 Relaxant responses to (a) prostaglandin I_2 (PGI_2), (b) nitroglycerine and (c) verapamil of human coronary artery strips precontracted with 0.1 μM carbocyclic thromboxane A_2 ($cTxA_2$). The preparations were obtained from the same branch of the artery. Concentrations: 0.001 to 1 μM PGI_2; 0.01 to 10 μM nitroglycerine; 0.01 to 10 μM verapamil. PA = 100 μM papaverine.

Table 2 Apparent ED_{50} values for prostaglandin I_2 (PGI_2), verapamil and nitroglycerine in human, monkey and dog coronary artery strips contracted with carbocyclic thromboxane A_2 ($cTxA_2$) or $PGF_{2\alpha}$

	ED_{50}s			
Artery	*PGI_2 (× 10 nM) $cTxA_2$-contracted*	*PGI_2 (× 10 nM) $PGF_{2\alpha}$-contracted*	*Verapamil (× 100 nM) $cTxA_2$-contracted*	*Nitroglycerine (× 10 nM) $cTxA_2$-contracted*
Human	3.08 ± 0.64 (13)	2.06 ± 0.57 (6)	3.90 ± 0.82 (9)	6.17 ± 2.37 (10)
Monkey	x	2.70 ± 0.08 (5)	1.38 ± 0.46 (8)	
Dog	1.91 ± 0.43* (12)	1.85 ± 0.44 (8)	1.80 ± 0.24* (14)	2.06 ± 0.48 (10)

Figures in parentheses indicate the number of preparations used. x, ED_{50} value could not be calculated. * Data obtained from the previous study (Toda, 1982b). The F ratio obtained from the analysis of variance (6.592 for verapamil) is greater than the $P = 0.01$ critical value. The following were significantly different ($P < 0.01$) by Tukey's method: human vs. monkey, and human vs. dog. The values for PGI_2 and nitroglycerine are not statistically significant ($P > 0.05$) between humans, monkeys and dogs.

0.5 μM), the addition of PGI_2 caused a concentration-related relaxation, the magnitude being approximately the same in human and dog arteries but significantly less in monkey arteries (Figure 3b). Apparent ED_{50} values for PGI_2 in the arteries from different species were quite similar (Table 2). Greater relaxation responses were induced by PGI_2 in human coronary arteries precontracted with $PGF_{2\alpha}$ than in the $cTxA_2$-contracted arteries; mean values of the maximum relaxation induced by 0.1 μM PGI_2

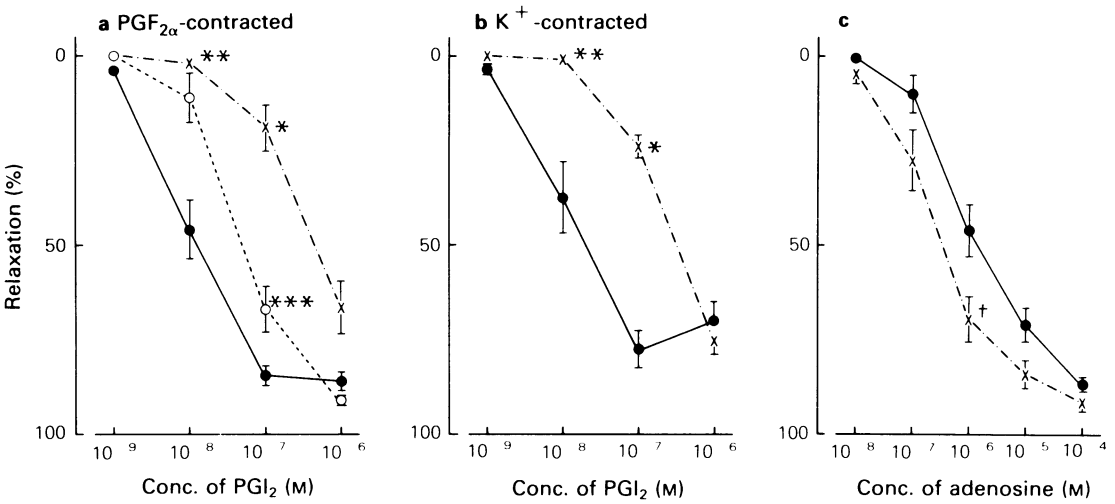

Figure 5 Modification by diphloretin phosphate (DPP) of the relaxation induced by prostaglandin I_2 (PGI_2) (a and b) or adenosine (c) in dog coronary artery strips precontracted with either $PGF_{2\alpha}$ (0.2 to 0.5 μM) (a and c) or K^+ (10 to 13 mM) (b). Relaxations induced by 100 μM papaverine were taken as 100%; mean absolute values in control and DPP (3 and 10 μM)-treated arteries precontracted with $PGF_{2\alpha}$ were 779 ± 104 mg ($n = 7$), 628 ± 59 mg ($n = 4$) and 637 ± 36 mg ($n = 7$), respectively, those in control and DPP (10 μM)-treated arteries precontracted with K^+ were 603 ± 85 mg ($n = 7$) and 671 ± 129 mg ($n = 7$), respectively, and those in control and DPP (10 μM)-treated arteries in response to adenosine were 552 ± 98 mg ($n = 6$) and 422 ± 77 mg ($n = 6$), respectively. The strips treated with DPP were contracted with higher concentrations (0.7 to 2 μM) of $PGF_{2\alpha}$. Significantly different from controls, $^*P < 0.001$; $^{**}P < 0.01$; $^{***}P < 0.02$; $^\dagger P < 0.05$. (a) Control responses to PGI_2 (●); plus DPP 3 μM (○), 10 μM (×); $n = 7, 4$ and 7, respectively. (b) Control responses to PGI_2 (●); plus DPP 10 μM (×); $n = 7$ for both. (c) Control responses to adenosine (●); plus DPP 10 μM (×); $n = 6$ for both (n = number of preparations used). Vertical lines represent s.e.mean.

in the arteries contracted with $PGF_{2\alpha}$ and $cTxA_2$ were 84.5 ± 5.0% (n = 6) and 42.5 ± 5.9% ($n = 13$) (significantly different, $P < 0.001$), respectively.

The relaxation induced by PGI_2 in dog coronary arteries partially precontracted with $PGF_{2\alpha}$ or K^+ was significantly attenuated by treatment with 3 or 10 μM DPP (Figure 5a, b). Mean ED_{50} values for PGI_2 in $PGF_{2\alpha}$- and K^+-contracted arteries were 11.6 ± 3.2 nM ($n = 7$) and 13.6 ± 3.5 nM ($n = 7$), respectively. DPP (10 μM) reduced the response to these concentrations of PGI_2 by 95.0 and 96.7%, respectively. Adenosine-ineduced relaxations tended to be potentiated by 10 μM (Figure 5c) and 30 μM DPP ($n = 3$). Relaxation responses of human coronary arteries contracted with $PGF_{2\alpha}$ induced by PGI_2 were not influenced by 10 μM DPP but significantly attenuated by 30 μM DPP. Relaxation responses induced by 10 nM PGI_2 relative to those induced by 100 μM papaverine before and after treatment with this concentration of DPP were 77.0 ± 7.1% and 44.0 ± 5.0% ($n = 4$), respectively (41.3 ± 10.1% inhibition, significantly different, $P < 0.05$).

Human and dog coronary arteries precontracted with $cTxA_2$ responded to nitroglycerine (1 nM to 10 μM) with a rapidly-developing relaxation (Figure 4). In 3 out of 10 human artery strips contracted with $cTxA_2$, spontaneous activity was induced when the arteries were moderately or markedly relaxed by nitroglycerine (Figure 4). Quantitative data on nitroglycerine-induced relaxation responses in the human and dog arteries are summarized in Figure 6a. The apparent ED_{50} value tended to be less in dog arteries than in human arteries, although the difference was not statistically significant.

In $cTxA_2$-contracted human, monkey and dog coronary arteries, verapamil (10 nM to 10 μM) produced slowly-developing relaxation responses, which levelled off 10 to 30 min later (Figure 4). In the human arteries which generated spontaneous contractile responses following stimulation by low concentrations of $cTxA_2$ or $PGF_{2\alpha}$, the responses were abolished when the arteries were maximally contracted with 0.1 μM $cTxA_2$. In these preparations, verapamil in low concentrations (10 to 100 nM) sufficient to produce a slight relaxation regenerated the spontaneous contractile responses. Increasing the concentrations of verapamil to 1 to 10 μM abolished the spontaneous activity ($n = 3$). Concentration-relaxation response curves for verapamil in human, monkey and dog coronary arteries are shown in Figure 6b. Maximum relaxation responses were greatest in dog arteries and least in monkey arteries.

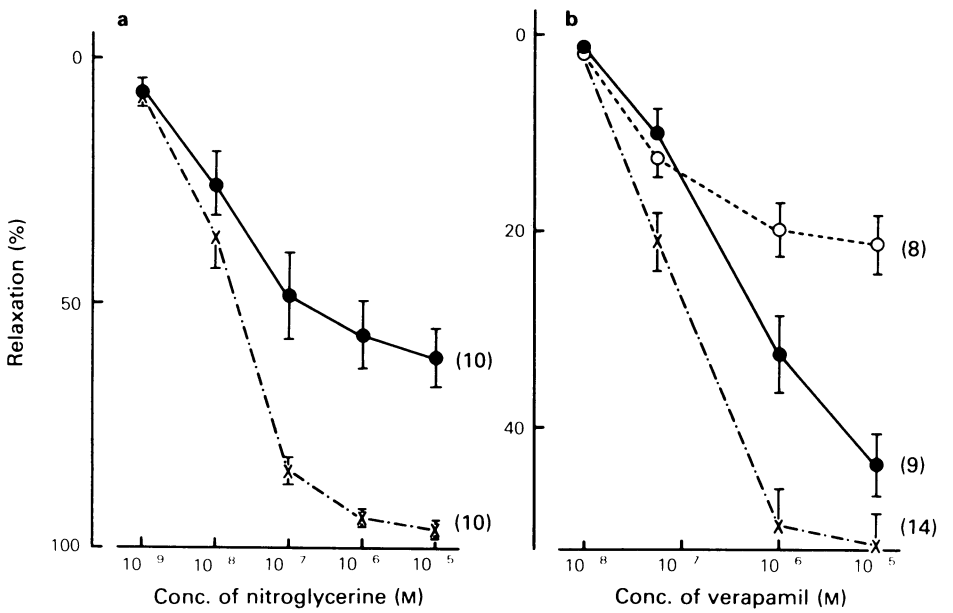

Figure 6 Concentration-response curves for nitroglycerine (a) and verapamil (b) in human (●), monkey (○) and dog (×) coronary arteries precontracted with 0.1 μM carbocyclic thromboxane A_2 ($cTxA_2$). Relaxation responses induced by 100 μM papaverine were taken as 100%; mean absolute values in the human and dog arteries in response to nitroglycerine were 1533 ± 281 mg ($n = 10$) and 2262 ± 363 mg (n = 10), respectively, and those in the human, monkey and dog arteries in response to verapamil were 1644 ± 388 mg ($n = 9$), 1582 ± 328 mg ($n = 8$) and 1955 ± 194 mg ($n = 14$)*, respectively. *Data obtained from the previous study (Toda, 1982b). Numbers in parentheses indicate the number of preparations used. Vertical lines represent s.e.mean.

The apparent ED_{50} value for verapamil was significantly less in monkey and dog arteries than in human arteries (Table 2).

Discussion

$cTxA_2$ and $PGF_{2\alpha}$ contracted human, monkey and dog coronary arteries in a concentration-dependent manner; the contractile responses were attenuated by DPP, a prostaglandin antagonist (Sanner, 1974). Contractile responses induced by K^+ in a submaximum concentration (25 mM) were not affected by DPP. Similar selective antagonism by DPP and polyphloretin phosphate against the contractile response of dog cerebral and mesenteric arteries to $cTxA_2$, $PGF_{2\alpha}$, PGE_2 and PGD_2 has been previously demonstrated (Toda & Miyazaki, 1978; Toda, 1982a, b). $cTxA_2$ and $PGF_{2\alpha}$ appear to share the same mechanism underlying the contraction of coronary artery smooth muscle. Maximum contractile responses induced by $cTxA_2$ and $PGF_{2\alpha}$ relative to those induced by 30 mM K^+ were in the order of human = monkey > dog arteries. The affinity of $cTxA_2$ for receptors, based on apparent ED_{50} values, was in the order of monkey > dog > human arteries, whereas the affinity of $PGF_{2\alpha}$ was in the order of monkey = human > dog arteries. Such a difference in the order of affinities of $cTxA_2$ and $PGF_{2\alpha}$ in coronary arteries of different species may indicate that the receptors for $cTxA_2$ and $PGF_{2\alpha}$ are not identical, or relative affinities of $cTxA_2$ to $PGF_{2\alpha}$ differ in these mammals.

$cTxA_2$ is the most potent vasoconstrictor among substances ever tested in isolated human coronary arteries, including noradrenaline, adrenaline, acetylcholine, histamine (Toda, 1983), 5-hydroxytryptamine and angiotensin II (unpublished data). $cTxA_2$ in low concentrations (1 to 100 nM) also contracts helical strips of dog and monkey cerebral, mesenteric, renal and femoral arteries (Toda, 1982b) and isolated perfused cat coronary arteries (Smith *et al.*, 1981). Coronary vasospastic actions of TxA_2 in the guinea-pig isolated perfused heart have been postulated (Terashita *et al.*, 1978). TxA_2, generated by adding PGH_2 (255 nM) to platelet particles, causes moderate contractions of bovine and porcine isolated coronary arteries (approximately 50 and 30%, respectively, of contractions induced by 40 mM K^+) (Ellis *et al.*, 1977). On the other hand, in human, monkey and dog coronary artery strips, $cTxA_2$ (0.1 μM) produced contractions 158.2, 187.5 and 122.4%, respectively, relative to those induced by 30 mM K^+. Whether the different efficacy of TxA_2 and $cTxA_2$ is due to the instability of TxA_2 (Hamberg *et al.*, 1975), to species difference or to different mechanisms of action remains to be ascertained.

PGI_2 relaxed human and dog coronary arteries maximally precontracted with $cTxA_2$ and also human, monkey and dog coronary arteries partially precontracted with $PGF_{2\alpha}$. The relaxation response did not persist for long, probably due to a rapid degradation of PGI_2 in the artificial bathing fluids (Moncada *et al.*, 1976). This does not exclude a possible role for PGI_2 endogenously released from the vascular wall, as an inhibitor of the vasoconstriction and platelet aggregation caused by substances such as TxA_2, 5-hydroxytryptamine and angiotensin II. The present study revealed that human coronary arteries even when intensely precontracted with high concentrations of $cTxA_2$ significantly relaxed in response to PGI_2. The PGI_2-induced relaxation was obtained in coronary arteries precontracted not only with $cTxA_2$ or $PGF_{2\alpha}$ but also with K^+, suggesting that the relaxation is not due to antagonistic actions of PGI_2 against $cTxA_2$ and $PGF_{2\alpha}$ but rather to a non-selective vasodilator action. The PGI_2-induced relaxation of dog and human arteries was significantly attenuated by DPP, whereas the relaxation induced by adenosine was potentiated. The actions of prostaglandins appear to be selectively antagonized by DPP.

Relaxation responses of coronary arteries precontracted with $cTxA_2$, induced by PGI_2 were in the order of dog > human > monkey whereas those induced by nitroglycerine were in the order of dog > human and those induced by verapamil, dog > human > monkey. Vasodilator responses may be greater in dog coronary arteries than in the primate arteries maximally precontracted. Such a lower susceptibility of human coronary arteries to vasodilators does not appear to derive from age-related and *post mortem* changes, since coronary arteries freshly excised from the heart of young adult monkeys (3 to 7 years old) responded to the agents with a lesser magnitude of relaxation as compared to that seen in human coronary arteries and with a similar relaxation to that obtained in the arteries from an adult monkey (older than 7 years) which had been dead for some time (unpublished data).

Relaxant responses to PGI_2 were appreciably less in monkey coronary arteries than in the dog and human arteries. PGI_2 increases cellular cyclic AMP, which would be expected to participate in vasodilatation (Dembinska-Kiec *et al.*, 1979; Miller *et al.*, 1979). However, other vasodilator agents, such as isoprenaline and papaverine, which also increase cellular cyclic AMP by activating adenylate cyclase or inhibiting cyclic AMP phosphodiesterase, relax monkey, dog and human coronary arteries to a similar extent (unpublished data). Therefore, the relative unresponsiveness of monkey coronary arteries to PGI_2 does not appear to be due to an inability of those arteries to produce cellular cyclic AMP but to a

decreased sensitivity of their PGI_2 receptors.

Nitroglycerine rapidly relaxed human and dog coronary arteries precontracted with $cTxA_2$. On the other hand, relaxation responses induced by verapamil, a Ca^{2+}-antagonist, in human, monkey and dog coronary arteries developed slowly. Similar relaxation responses were elicited by another Ca^{2+}-antagonist, nifedipine, in cat coronary arteries precontracted with 0.29 μM $cTxA_2$ (Toward & Perzborn, 1982). These characteristic features of the response appear to reflect the clinical usefulness of nitroglycerine in relieving acute, vasospastic anginal attacks and of Ca^{2+}-antagonists as prophylactic drugs. In association with the persistent relaxation responses induced by high concentrations of verapamil, spontaneous rhythmic activity induced by $cTxA_2$ was suppressed, as demonstrated by Weinheimer *et al.* (1983). However, such a suppression of the spontaneous responses was not always the case with the Ca^{2+}-antagonist, as the spontaneous activity could be provoked when the contracted level of tension was slightly reduced by low concentrations of verapamil (Figure 4). The spontaneous activity could not be induced when the arteries were contracted or relaxed to an approximately maximum extent, and the magnitude and the rate of this activity may be dependent upon the arterial tension maintained. Further studies are required to determine whether or not such spontaneous activity is induced *in situ* in human conduit coronary arteries and is related to coronary artery vasospasm.

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