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Regional differences in the response to vasoconstrictor agents of dog and monkey isolated coronary arteries

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1 Contractile responses to vasoconstrictor agents were compared in helical strips of dog and monkey epicardial coronary arteries of different sizes. Contractions of large, medium and small arteries induced by KCl (30mM) were virtually identical.

2 Contractions induced by 5-hydroxytryptamine (5-HT) (10^{-9}–2×10^{-6} M) were in the order of large > medium > small arteries in dogs, and large = medium > small arteries in monkeys. Cinanserin suppressed these responses.

3 In contrast, contractions produced by angiotensin II (AII) (10^{-7} M) were in the order of small > medium > large arteries in dogs, and small > medium = large arteries in monkeys. Sar[1]-Ala[8]-angiotensin II markedly attenuated the peptide-induced contractions.

4 Contractions induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were significantly greater in large and medium sized arteries than in small arteries in dogs, while those of the arteries of different sizes isolated from monkeys did not differ. Contractions induced by carbocyclic thromboxane A_2 (c-TXA_2), in arteries of different sizes in dogs and monkeys, did not differ.

5 These results suggest that the sensitivity and/or the population of 5-HT receptors are greater in proximal coronary arteries than in distal arteries, while, in contrast, the sensitivity and/or population of AII receptors are greater in distal coronary arteries. Receptors for $PGF_{2\alpha}$ and c-TXA_2 appear to react to a similar degree in monkey arteries of different sizes, although receptors for $PGF_{2\alpha}$ appear to be fewer in distal coronary arteries in dogs.

Introduction

It is widely recognized (e.g. Oliva *et al.*, 1973; Oliva & Breckinridge, 1977; Yasue, 1980) that coronary spasm plays a key role in the patho-physiology of not only variant angina but also of other forms of ischaemic heart disease including some types of myocardial infarction. However, the precise mechanism by which coronary arterial spasm is triggered remains unknown, Vasoactive substances, such as catecholamines, are considered to contribute to cononary arterial spasm or vasoconstriction (Yasue *et al.*, 1976; 1979). Proximal and distal portions of coronary arteries respond differently to catecholamines, due to predominant α-adrenergic receptors in proximal arteries and predominant β-adrenergic receptors in distal arteries (Zuberbuhler & Bohr, 1965; Toda, 1981). Haddy *et al.* (1957) have shown that 5-hydroxytryptamine (5-HT) does not significantly change total vascular resistance, but increases the resistance of the large arteries in dog foreleg. It is suggested that angiotensin II (AII) acts mainly upon small blood vessels in the systemic vascular bed (Haddy *et al.*, 1962). However, responsiveness of proximal and distal portions of coronary arteries to angiotensin has not been fully clarified. The present study was thus undertaken to determine the contractile responses, to vasoconstrictor agents, such as KCl, 5-HT, AII, prostaglandin (PG) $F_{2\alpha}$ and carbocyclic thromboxane A_2 (c-TXA_2) (Lefer *et al.*, 1980), of helical strips of epicardial coronary arteries of different sizes, isolated from dogs and monkeys.

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Methods

Mongrel dogs of either sex, weighing 6 to 21 kg, were anaesthetized with intraperitoneal injections of thiopental (50mg kg^{-1}) and killed by bleeding from the common carotid arteries. Japanese monkeys of either sex, weighing 5 to 10 kg, were anaesthetized with intramuscular injections of ketamine

(40 mg kg^{-1}) and killed by bleeding from the common carotid arteries. The heart was rapidly removed. Ventral interventricular and circumflex branches (epicardial portion) of the left coronary artery of different sizes were isolated from the heart. The large coronary artery segment (dog: i.d. over 1.5 mm, monkey: i.d. over 1.0 mm) was dissected from proximal portions of the coronary artery. The small artery (both: i.d. below 0.3 mm) was dissected from the distal extramural portions of the coronary artery. The medium-sized artery (dog: i.d. 0.5–1.0 mm, monkey: i.d. 0.35–0.7 mm) was dissected from the portions between the large and small arteries. The arteries of different sizes were obtained from the same dogs or monkeys, and paired comparisons were made in the arteries of different sizes. The arterial segments were cleaned of extraneous tissue and cut into helical strips, approximately 20 mm long. Endothelial cells were expected to be intact, since acetylcholine produced a dose-dependent relaxation which was abolished by rubbing the surface of the endothalial cells (Furchgott & Zawadzki, 1980). Each helical strip was vertically fixed between hooks in a tissue bath containing modified Ringer-Locke solution, which was aerated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37 ± 0.5 °C. The composition of the nutrient solution was (mM): Na^+ 144.7; K^+ 5.4, Cl^- 131.5, Ca^{2+} 2.2, Mg^{2+} 1.0, HCO_3^- 25.0, dextrose 5.6. The pH of the solution was 7.3–7.4. Cross-sectional area was estimated by dividing the post-experimental wet weight of the strip by its length, a calculation that assumes a tissue density of 1. Mean values of the cross-sectional area of large, medium and small coronary arteries isolated from dogs were 1.17 ± 0.10 mm^2 (n = 31), 0.65 = 0.05 mm^2 (n = 31) and 0.22 ± 0.02 (n = 31), respectively (n = the number of donor animals). The resting tension of 1.5 g for medium sized arteries is optimal for inducing the maximum contraction (Toda *et al.*, 1978). Therefore, the resting tension was adjusted to 3.0 g for large arteries and 0.6 g for small arteries to match the tension per cross sectional area in arteries of different sizes. Mean values of the cross-sectional areas of large, medium and small coronary arteries isolated from monkeys were 0.49 ± 0.09 mm^2 (n = 13), 0.33 ± 0.04 mm^2 (n = 13) and 0.15 ± 0.02 mm^2 (n = 13), respectively. In monkey arterial strips of large, medium and small sizes, the resting tensions were thus adjusted to 1.5 g, 1.0 g and 0.5 g, respectively (Toda, 1981). Before starting the experiments, all preparations were allowed to equilibrate for 60 to 90 min in the bathing media, during which time the bathing solution was replaced every 15 min. At the end of the equilibration period, the resting tension was readjusted exactly. Isometric contractions were recorded on an ink-writing oscillograph (Sanei Sokki Co, Tokyo, Japan).

Test agents were added directly to the bathing media in a single concentration (KCl and AII) or in cumulative concentrations (5-HT, $PGF_{2\alpha}$ and c-TXA_2). The contractile response to KCl (30 mM) was obtained twice. The second response was taken as a standard for comparison on the contractile responses to the other agents. The developed tension/cross sectional area (g mm^{-2}) was presented throughout. Isolated dog arteries of medium-size were treated for 60 min with diphloretin phosphate or 20 min with the other blocking agents, before the response to vasoconstrictor agents was obtained.

Results shown in the text and figures represent the mean values ± s.e. One-way analysis of variance with repeated measures (Winer, 1971) and the Neuman-

Table 1 Maximum contractions (g mm^{-2}) induced by vasoconstrictors in coronary arteries of dogs and monkeys

	Dog					*Monkey*				
Artery size	*KCl*	*5-HT*	*AII*	*$PGF_{2\alpha}$*	*c-TXA_2*	*KCl*	*5-HT*	*AII*	*$PGF_{2\alpha}$*	*c-TXA_2*
Large	2.70 ±0.25	1.67 ±0.21	0.90 ±0.12	3.02 ±0.28	3.00 ±0.41	1.72 ±0.28	3.06 ±0.40	0.38 ±0.08	7.26 ±0.71	7.89 ±0.78
Medium	2.93 ±0.31	0.74[a] ±0.12	1.43[b] ±0.17	2.70 ±0.30	3.81 ±0.60	1.75 ±0.28	2.79 ±0.52	0.49 ±0.07	8.05 ±0.69	7.29 ±1.08
Small	2.46 ±0.38	0.12[ac] ±0.03	3.04[ac] ±0.52	1.23[ac] ±0.30	2.48 ±0.53	1.65 ±0.50	1.04[ad] ±0.28	1.20[ac] ±0.17	8.18 ±1.41	9.40 ±3.44
n	31	23	23	26	17	13	10	10	13	7

Concentrations of drugs used: KCl 30 mM, 5-hydroxytryptamine (5-HT) 5 × 10^{-7} or 2 × 10^{-6} M, angiotensin II (AII) 10^{-7} M, prostaglandin (PG) $F_{2\alpha}$ 10^{-5} M and carbocyclic thromboxane A_2 (c-TXA_2) 10^{-7} M.
[a]$P < 0.001$, [b]$P < 0.02$, significantly different from contraction in large artery.
[c]$P < 0.01$, [d]$P < 0.02$, significantly different from contraction in medium sized artery.

Keuls test for multiple comparisons (Zar, 1974) were used for statistical evaluation of the changes in the dose-response relationships induced by the drugs. Student's *t* test was used to evaluate the differences in the maximum contractions obtained to each drug in the different sized arteries. Drugs used were KCl, 5-hydroxytryptamine creatinine sulphate, angiotensin II (Protein Research Foundation, Osaka, Japan), prostaglandin $F_{2\alpha}$ (Ono Pharmaceutical Co, Osaka), cinanserin hydrochloride (Squibb and Sons, Princeton, USA), DPP (diphloretin phosphate, Ono Pharmaceutical Co), Sar^1-Ala^8-angiotensin II (Protein Research Foundation), carbocyclic thromboxane A_2 (Ono Pharmaceutical Co) and phentolamine mesylate.

Results

Contractile responses of the strips of large, medium and small coronary arteries, isolated from dogs and monkeys, to the vasoconstrictor agents are summarized in Table 1. Contractions induced by KCl (30 mM) when corrected for cross sectional areas were virtually identical in these coronary arteries.

Responses of dog coronary arteries of different sizes to 5-hydroxytryptamine

The addition of 5-HT in concentrations ranging from 10^{-9} to 2×10^{-6} M contracted coronary artery strips in a dose-dependent manner. However, a further increase in the concentration to 10^{-5} M did not enhance the response. The contractile responses to 5-HT per cross sectional area were in the order of large > medium > small arteries (Figure 1). Maximum contractions induced by 5-HT in these arteries

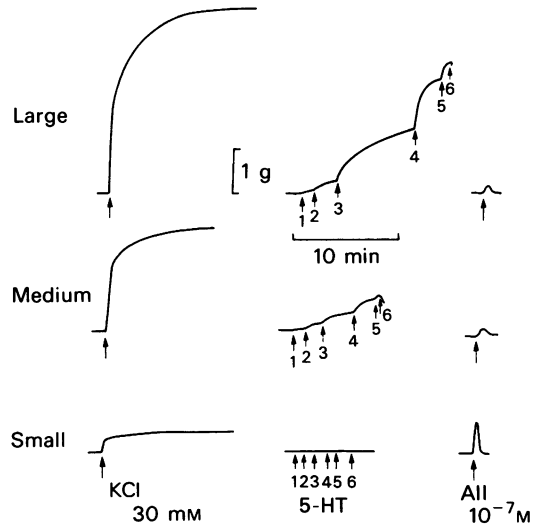

Figure 2 Typical recordings of the responses of dog coronary arteries of different sizes to KCl, 5-hydroxytryptamine (5-HT) and angiotensin II (AII). The strips were obtained from the same dog. The contractions to the vasoconstrictors were obtained from the same preparation. The following concentrations of 5-HT (represented in the figure by the number in parentheses) were used: 10^{-9} M (1), 5×10^{-9} M (2), 2×10^{-8} M (3), 10^{-7} M (4), 5×10^{-7} M (5), 2×10^{-6} M (6).

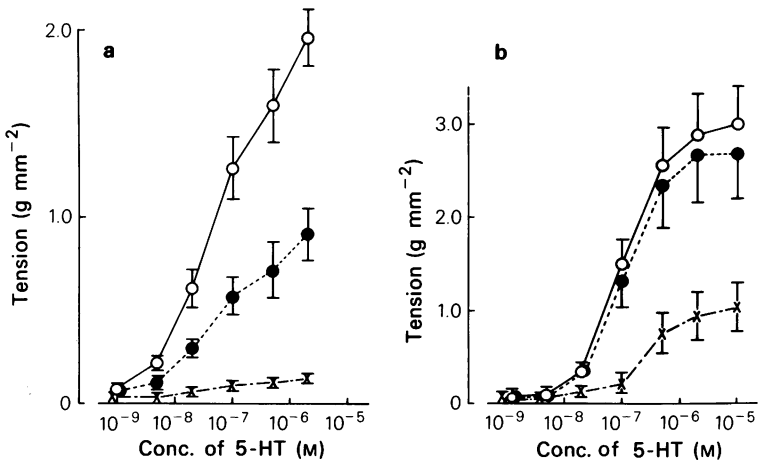

Figure 1 Dose-response curves to 5-hydroxytryptamine (5-HT) in strips of dog (a) and monkey (b) coronary arteries of different sizes (large (○), medium (●) and small (×)). In dogs, contractile responses induced by 5-HT in large arteries were significantly ($P < 0.001$) greater and those in small arteries were significantly ($P < 0.001$) smaller, than those in medium sized arteries. In monkeys, contractile responses in large and medium sized arteries were significantly ($P < 0.01$) greater than those in small arteries. Data are mean of 23 (dog) or 10 (monkey) preparations; vertical lines show s.e.mean.

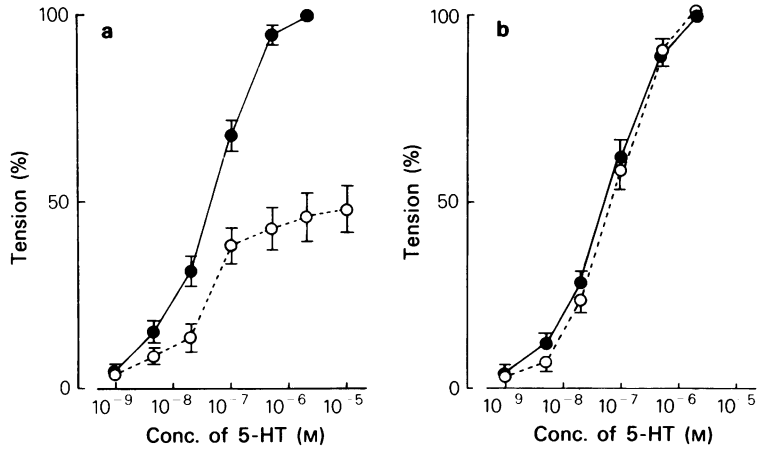

Figure 3 Modification by cinanserin 10^{-7} M (a) and phentolamine 10^{-7} M (b) of the responses of dog medium-sized coronary arteries to 5-hydroxytryptamine (5-HT). Contractile responses to 2×10^{-6} M 5-HT in control media (●) were taken as 100%; the mean absolute value was 1.29 ± 0.31 g mm^{-2} ($n = 7$). Contractile responses to 5-HT were significantly ($P < 0.001$) attenuated by treatment with cinanserin 10^{-7} M (○) but not by treatment with phentolamine 10^{-7} M (○). Data are mean of 7 (a) or 5 (b) preparations; vertical lines show s.e.mean.

are shown in Table 1. Apparent ED_{50} values for 5-HT in the large, medium and small arteries were 4.1 ± 0.7, 3.6 ± 0.8 and 3.4 ± 0.7 ($\times 10^{-8}$ M), respectively. Typical recordings in dog coronary arteries of different sizes are shown in Figure 2. Contractile responses to 5-HT were markedly reduced by treatment with cinanserin 10^{-7} M, a 5-HT antagonist, but were not significantly affected by treatment with phentolamine 10^{-7} M (Figure 3).

Responses of dog coronary arteries of different sizes to angiotensin II

AII (10^{-7} M) produced a rapidly-developing contraction, which persisted for several min. This concentration of AII was sufficient to produce a maximum contraction (Toda & Miyazaki, 1978). Tachyphylaxis developed when AII was repeatedly applied. Therefore, the responses to the first application of AII were

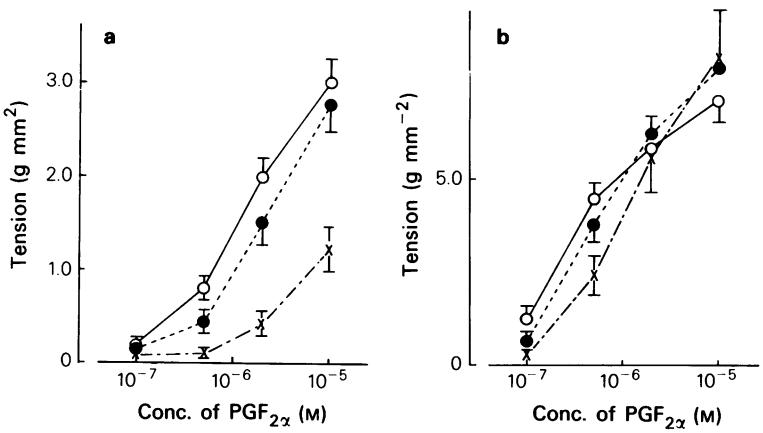

Figure 4 Dose-response curves to prostaglandin (PG)$F_{2\alpha}$ in strips of dog (a) and monkey (b) coronary arteries of different sizes (large (○), medium (●) and small (×)). Contractile responses induced by $PGF_{2\alpha}$ in large and medium sized arteries were significantly ($P < 0.01$) greater than in small arteries in dogs, but no regional differences were observed in monkey arteries. Data are mean of 26 (a) or 13 (b) preparations; vertical lines show s.e.mean.

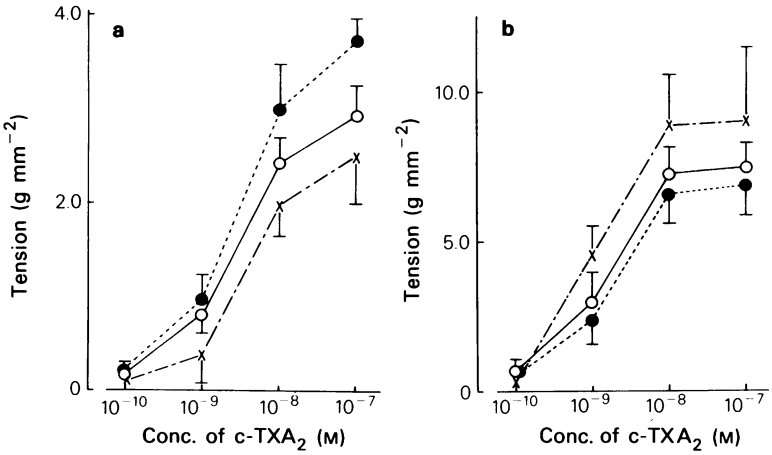

Figure 5 Dose response curves to carbocyclic thromboxane A_2 (c-TXA_2) in strips of dog (a) and monkey (b) coronary arteries of different sizes (large (○), medium (●) and small (×)). There was no difference in the contractile responses to c-TXA_2 in the arteries of different sizes. Data are mean of 17 (a) or 7 (b) preparations; vertical lines show s.e.mean.

compared. The contractile responses to AII (10^{-7} M) were in the order of small > medium > large arteries in dogs (Table 1). Contractions of dog coronary arteries induced by AII were depressed from 1.52 ± 0.41 to 0.35 ± 0.09 g mm^{-2} (77.2 ± 8.0 % inhibition, $n = 6$) by treatment with Sar^1-Ala^8-AII 5×10^{-9} M, an AII antagonist, but were not affected by treatment with phentolamine 10^{-7} M ($n = 5$).

Responses of dog coronary arteries of different sizes to prostaglandin $F_{2\alpha}$ and carbocyclic thromboxane A_2

$PGF_{2\alpha}$ (10^{-7}–10^{-5} M) induced dose-dependent contractions of the coronary arteries. The contractile responses of large and medium-size arteries from dogs were significantly greater than those of small arteries (Figure 4). The maximum contractions are shown in Table 1. Apparent ED_{50} values for $PGF_{2\alpha}$ in large, medium and small arteries were 1.1 ± 0.5, 1.5 ± 0.5 and 3.2 ± 0.2 ($\times 10^{-6}$ M), respectively. Contractile responses to $PGF_{2\alpha}$ were attenuated by treatment with DPP 10^{-5} M, a prostaglandin antagonist. Dose-response curves to c-TXA_2 (10^{-10}–10^{-7} M) in large, medium and small arteries did not differ (Figure 5). Contractile responses to c-TXA_2 were attenuated by treatment with DPP 10^{-5} M.

Responses of monkey coronary arteries of different sizes to the various agents

5-HT (10^{-9}–10^{-5} M) also contracted monkey coronary arteries in a dose-dependent manner. The maximum contractions obtained in large, medium and small arteries are summarized in Table 1. The contractile responses of large and medium-size arteries to 5-HT were significantly greater than those of small arteries (Figure 1). Apparent ED_{50} values for 5-HT in large, medium and small arteries were 1.1 ± 0.2, 1.1 ± 0.2 and 1.8 ± 0.3 ($\times 10^{-7}$ M), respectively. Contractile responses to AII (10^{-7} M) were in the order of small > medium = large sized arteries in monkeys (Table 1). Contractile responses to $PGF_{2\alpha}$ of the different sizes of arteries did not differ (Figure 4); the maximum contractions are shown in Table 1. Apparent ED_{50} values for $PGF_{2\alpha}$ in large, medium and small arteries were 3.1 ± 0.6, 7.3 ± 1.3 and 9.5 ± 0.3 ($\times 10^{-7}$ M), respectively in monkeys. Dose-response curves to c-TXA_2 in large, medium and small arteries did not differ significantly (Figure 5).

Discussion

The present study revealed that contractile responses (g mm^{-2} cross sectional area) of dog and monkey arteries of different sizes to 5-HT and AII clearly differed, while the responses to KCl were virtually identical. Contractions induced by KCl are attributed mainly to the influx of Ca^{2+} across the muscle cell membrane, which is depolarized, but not to activation of specific receptors. The degree of contraction induced by KCl may thus be considered to reflect the amount of vascular smooth muscle in these coronary arteries.

Contractile responses to 5-HT, $PGF_{2\alpha}$ and AII were significantly attenuated by cinanserin, DPP and Sar^1-Ala^8-AII, respectively, suggesting that the responses are mediated by activation of their specific receptors. Contractile responses to 5-HT were found to be in the order of large > medium > small arteries in dogs, and large = medium > small arteries in

monkeys; in contrast, the responses to AII in small arteries were significantly greater than those in large arteries. It appears that the sensitivity and/or population of 5-HT receptors are inversely related to the distance of coronary arteries from the aortic orifice, whereas the sensitivity and/or population of AII receptors are directly related.

Porquet *et al.* (1982) found that 5-HT induced contractions in the proximal portions of canine coronary arteries, and that both methysergide and phentolamine attenuated these responses. However, the concentration of phentolamine used may have been high enough to cause a non-selective inhibition of drug-induced contractions. In our preparations, contractile responses to 5-HT were not affected by phentolamine at a concentration sufficient to attenuate markedly the contraction induced by noradrenaline in propranolol-treated dog coronary arteries (Hayashi & Toda, 1982), but were suppressed by cinanserin, a specific 5-HT antagonist.

5-HT preferentially contracted conduit coronary arteries; the contraction was selectively antagonized by cinanserin. Ergonovine also causes intense contractions of isolated coronary arteries which are mediated exclusively by 5-HT receptors (Müller-Schweinitzer, 1980; Brazenor & Angus, 1981). Ergonovine provokes coronary arterial spasm in patients with variant angina (e.g. Heupler *et al.*, 1978), and the ergonovine-induced spasm resembles spontaneously occurring spasm. These findings may indicate that 5-HT is one of the endogenous substances responsible for the generation of epicardial large coronary artery spasm.

AII mainly constricts small vessels rather than large vessels in the foreleg and mesenteric vascular beds (Haddy *et al.*, 1962). In our study, AII also produced a greater contraction in small coronary arteries than in large arteries. AII increases coronary vascular resistance, but does not appear to play a rôle in the pathogenesis of coronary vasospasm.

TXA_2 is a potent, endogenous vasoconstrictor. TXA_2 and c-TXA_2, a stable analogue of TXA_2, are considered to induce vasoconstriction by the same mechanism of action (Lefer *et al.*, 1980; Smith *et al.*, 1981). TXA_2 increases coronary vascular resistance and is postulated to be responsible for coronary vasospasm (Tada *et al.*, 1981). In our study, c-TXA_2, in low concentrations, produced a marked contraction of coronary arteries, but there was no significant regional difference in the contractions per cross sectional areas in large, medium and small arteries. TXA_2 may intensely constrict coronary arteries of localized areas wherever it is synthesized and released.

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References

BRAZENOR, R.N. & ANGUS, J.A. (1981). Ergometrine contracts isolated canine coronary arteries by a serotonergic mechanism; no role for alpha adrenoceptors. *J. Pharmac. exp. Ther.*, **218,** 530–536.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288,** 373–376.

HADDY, F.J., FLEISHMAN, M. & EMANUEL, D.A. (1957). Effect of epinephrine, norepinephrine and serotonin upon systemic small and large vessel resistance. *Circulation Res.*, **5,** 247–251.

HADDY, F.J., MOLNER, J.I., BORDEN, C.W. & TEXTER, E.C. Jr. (1962). Comparison of direct effects of angiotensin and other vosoactive agents on small and large blood vessels in several vascular beds. *Circulation*, **25,** 239–246.

HAYASHI, S. & TODA, N. (1982). Response of isolated dog coronary arteries to tyramine. *Jap. J. Pharmac.*, **32,** 47–54.

HEUPLER, F.A. Jr. PROUDFIT, W.L., RAZAVI, M., SHIREY, E.K., GREENSTREET, R. & SHELDEN, W.C. (1978). Ergonovine maleate provocative test for coronary arterial spasm. *Am. J. Cardiol.*, **41,** 631–641.

LEFER, A.M., SMITH, E.F.III, ARAKI, J., SMITH, J.B., AHARONY, D., CLAREMON, D.A., MAGOLDA, R.L. & NICOLAOU, K.C. (1980). Dissociation of vasoconstrictor and platelet aggregatory activities of thromboxane by carbocyclic thromboxane A_2, a stable analog of thromboxane A_2. *Proc. natn. Acad. Sci. U.S.A.*, **77,** 1706–1710.

MÜLLER-SCHWEINITZER, E. (1980). The mechanism of ergometrine-induced coronary arterial spasm: *in vitro* studies on canine arteries. *J. cardiovasc. Pharmac.*, **2,** 645–655.

OLIVA, P.B. & BRECKINRIDGE, F.C. (1977). Arteriographic evidence of coronary arterial spasm in acute myocardial infarction. *Circulation*, **56,** 366–374.

OLIVA, P.B., POTTS, D.E. & PLUSS, R.G. (1973). Coronary arterial spasm in Prinzmetal's angina. Documentation by coronary arteriography. *N. Engl. J. Med.*, **288,** 745–751.

PORQUET, M.F., POURRIAS, B., & SANTAMARIA, R. (1982). Effects on 5-hydroxytryptamine on canine isolated coronary arteries. *Br. J. Pharmac.*, **75,** 305–310.

SMITH, E.F.III, LEFER, A.M. & NICOLAOU, K.C. (1981). Mechanism of coronary vasoconstriction induced by carbocyclic thromboxane A_2. *Am. J. Physiol.*, **240,** H493–497.

TADA, M., KUZUYA, T., INOUE, M., KODAMA, K., MISHIMA, M., YAMADA, M., INUI, M. & ABE, H. (1981).

Elevation of thromboxane B_2 levels in patients with classical and variant angina pectoris. *Circulation*, **64,** 1107–1115.

TODA, N., HATANO, Y. & HAYASHI, S. (1978). Modification by stretches of the mechanical response of isolated cerebral and extracerebral arteries to vasoactive agents. *Pflügers Arch.*, **374,** 73–77.

TODA, N. & MIYAZAKI, M. (1978). Regional and specific difference in the response of isolated arteries to angiotensin II. *Jap. J. Pharmac.*, **28,** 495–497.

TODA, N. (1981). Response of isolated monkey coronary arteries to catecholamines and to transmural electrical stimulation. *Circulation Res.*, **49,** 1228–1236.

WINER, B.J. (1971). Statistical principles in experimental design. New York: McGraw-Hill.

YASUE, H. (1980). Pathophysiology and treatment of coronary arterial spasm. *Chest*, **78 (Suppl),** 216–223.

YASUE, H., OMOTE, S., TAKIZAWA, A., NAGAO, M., MIWA, K. & TANAKA, S. (1979). Exertional angina pectoris caused by coronary arterial spasm: Effects of various drugs. *Am. J. Cardiol.*, **43,** 647–652.

YASUE, H., TOUYAMA, M., KATO, H., TANAKA, S. & AKIYAMA, F. (1976). Prinzmetal's variant angina as a manifestation of alpha-adrenergic receptor-mediated coronary artery spasm: Documentation by coronary arteriography. *Am. Heart. J.*, **91,** 146–155.

ZAR, J.H. (1974). Biostatistical analysis. Englewood Cliffs NJ: Prentice-Hall.

ZUBERBUHLER, R.C. & BOHR, D.F. (1965). Responses of coronary smooth muscle to catecholamines. *Circulation Res.*, **16,** 431–440.

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