EFFECTS OF 5-HYDROXYTRYPTAMINE ON CANINE ISOLATED CORONARY ARTERIES

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- 1 The effects of 5-hydroxytryptamine (5-HT) were studied *in vitro* on proximal and distal portions of canine interventricular and circumflex coronary arterial strips. 5-HT produced concentration-related contractions in the proximal portion whether contracted previously with KCl or not. These responses were still present after either chemical sympathetic denervation or release of noradrenaline induced by K⁺-free salt solution. In contrast, the distal portions of coronary arteries did not respond to 5-HT.
- 2 Concentration-response curves to 5-HT exhibited a classical hyperbolic shape with a calculated Hill-coefficient of approximately 1.
- 3 Methysergide and phentolamine but not morphine shifted to the right and depressed the maximum of the dose-response curves to 5-HT.
- 4 It is concluded that the contractions produced by 5-HT in the proximal portion of the interventricular and circumflex coronary arteries are not due to the release of endogenous noradrenaline. The vessels appear to possess separate receptors for 5-HT and noradrenaline and the 5-HT responses belong to neither the M nor the D type.

Introduction

Coronary artery spasm is considered to be the result of a local and transient constriction of epicardial coronary arteries. Recently, attention has been focused on chemical induction of coronary artery spasm. Substances produced endogenously by either the vascular smooth muscle or the platelets may be important in influencing coronary vascular tone. Ergometrine, a substance used for eliciting controlled coronary arterial spasm in patients suspected of having Prinzmetal's variant form of angina, acts via the stimulation of 5-hydroxytryptamine (5-HT) receptors, thereby reinforcing sympathetic vascular tone (Müller-Schweinitzer, 1980). But it is generally accepted that 5-HT is a potent vasoconstrictor in several vascular beds except in the skeletal and coronary ones. Mena & Vidrio (1976) showed that 5-HT produces coronary dilatation in anaesthetized open chest mongrel dogs by directly stimulating specific 5-HT receptors.

A first aim of the present work was to characterize, *in vitro*, the receptors present in the dog coronary artery strips that might be stimulated by 5-HT. Zuberbühler & Bohr (1965) distinguished the small from the large coronary artery vessels in the dog and reported that whereas β_2 -adrenoceptors are predominant in small vessels, the α -adrenoceptors predominate on the large trunks. Secondly, we determined whether the circumflex and the interventricu-

lar artery exhibited a difference in their population of 5-HT receptors.

Methods

The circumflex and interventricular arteries were isolated from the heart of anaesthetized mongrel dogs (sodium pentobarbitone, 30 mg/kg i.v.). Strips were cut spirally from the proximal (diameter > 2 mm) and distal (diameter < 0.5 mm) portion of the two arteries, suspended in a 20 ml chamber containing Tyrode-bicarbonate solution maintained at 37°C and aerated with a mixture of 95% O₂ and 5% CO₂.

The strips were connected to an isotonic myograph transducer and subjected to a tension of 1.5 g for the proximal portion and 0.2 g for the distal portion and allowed to equilibrate for 60 min. Contractions were recorded on a polygraph (Narco Physiograph).

To verify the hypothesis of Mena & Vidrio (1976) (under *in vitro* conditions) the coronary relaxant effects of 5-HT were studied in the strips contracted by a high-potassium Tyrode solution, the composition of which was as follows (in mmol/l): NaCl 105, KCl 35, MgCl₂·6H₂O 0.49, NaHPO₄·12H₂O 0.14, glucose 5.55, NaHCO₃ 11.9, CaCl₂·2H₂O 0.92.

In one series of experiments, the release of en-

ogenous noradrenaline from adrenergic neurones was induced with K^+ -free physiological saline solution (PSS) (Bonaccorsi, Hermesmeyer, Smith & Bohr, 1977). In another series of preparations, sympathetic denervation was produced with 6-hydroxydopamine (6-OHDA) as described by Aprigliano & Hermesmeyer (1976).

Cumulative concentration-response curves to noradrenaline 5-HT and potassium chloride (KCl) were constructed as described by Van Rossum (1963). To study noradrenaline-induced contrac-

tions, the β_2 -adrenoceptors were blocked by addition of propranolol ($10 \mu M$) to the Tyrode solution. All responses to 5-HT or noradrenaline were expressed as fractions of the variation in length produced by a maximal dose and mean log concentration-response curves were calculated according to Ariens & Simonis (1964). The applicability of receptor occupancy theory to the response produced with 5-HT was examined using the procedure outlined by Goldstein, Aronow & Kalman, (1974).

After establishing concentration-response curves

Figure 1 Effects of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) on isolated proximal and distal portions from interventricular coronary artery perfused with Tyrode high-potassium solution (a) or with Tyrode bicarbonate solution (b, c).

to agonists, a predetermined concentration of antagonist was added to the strips. Thirty minutes later the concentration-response curves were re-established in the presence of antagonists. The nature and degree of antagonism were quantified as described by Van Rossum (1963). The results were expressed as the mean $\pm$ s.e. mean. Differences between means were compared by Student's *t* test or Dunnett's test for unpaired series; *P* values of 0.05 or less were considered to be significant.

Drugs

Drugs used in this study were: noradrenaline hydrochloride (Sigma Chemical Co.), 5-hydroxytryptamine creatinine sulphate (5-HT) (Koch-Light), methysergide bimalate (Sandoz), phentolamine hydrochloride (Ciba Geigy), morphine hydrochloride, propranolol hydrochloride (I.C.I. Pharma). Noradrenaline was dissolved in distilled water containing ascorbic acid (0.2 mg/ml). Methysergide and 5-HT was dissolved in distilled water and phentolamine in 0.9% w/v NaCl solution (saline).

Results

Effects of 5-hydroxytryptamine on isolated coronary arteries: comparison of K⁺-contracted and control strips

In the proximal portions of the K⁺-contracted strips, 5-HT did not relax the tonic contraction but elicited concentration-dependent contractions. In the distal portions, 5-HT was inactive over a concentration range of 1.0 nM to 3.16 μ M (Figure 1a). In control strips, 5-HT elicited concentration-dependent contractions in the proximal portion over the concentration range of 1 nM to 1 μ M (Figure 1b), without modifying the basal length of the distal portion over a concentration range of 1 nM to 0.1 mM (Figure 1b). In contrast, noradrenaline caused contractions in both portions of the control strips (1 nM to 1 mM) (Table 1) (Figure 1c).

Effect of 6-hydroxydopamine and K⁺ deprivation

The 6-hydroxydopamine (6-OHDA) treatment reduced, but did not eliminate contractions induced by 5-HT (Figure 2a). The EC₅₀s after sympathetic denervation were 0.3 μ M for the interventricular artery and 0.08 μ M for the circumflex artery. Similar results were obtained when the artery strips were exposed to K⁺-free PSS (Figure 2b). The EC₅₀s for response to 5-HT were 0.026 μ M for the interventricular artery and 0.016 μ M for the circumflex artery. The values were not significantly different from those obtained in Tyrode bicarbonate solution. After 6-OHDA treatment and K⁺ deprivation the tissues were unresponsive to tyramine.

Concentration-response curves to 5-hydroxytryptamine in the proximal portion of the interventricular artery

The concentration-response curves to 5-HT, and noradrenaline exhibited a classical hyperbolic shape as opposed to the sigmoid curve obtained with potassium (Figure 3a). The experimental concentration-response curve for 5-HT was compared with the theoretical curve calculated by replacing the KA with the experimental EC₅₀ in the equation of Clark (1937) as rearranged by Ariens & Simonis (1964) and was found to be superimposed (Figure 3b). The ratio of concentrations producing 16% and 84% of maximum contractions was near the theoretical values 1:27, proposed by Clark (1937) for a bimolecular interaction. The classical Hill plot, log R/(R_m - R) (R: response in mm of shortening; R_m is the maximal response) versus log (5-HT) gave a straight line with a slope near 1.0. These results indicate that interaction of 5-HT with its receptor on the proximal portion of the interventricular artery strips could be analysed by applying the mass-action principle. The parameter of 'affinity', i.e. the ability to combine with its receptor, and intrinsic activity as elaborated by Ariens & Simonis (1964) could therefore be used. The plot of fractional response against the logarithm of the 5-HT and noradrenaline concentrations in the

Table 1 Effects of 5-hydroxytryptamine (5-HT) and noradrenaline on proximal portion of canine coronary arteries

Compound	Maximal shortening (mm)		EC ₅₀ (μ M)		pD ₂ values	
	Interventricular artery	Circumflex artery	Interventricular artery	Circumflex artery	Interventricular artery	circumflex artery
5-HT	0.95 $\pm$ 0.14 (n = 31)	0.43 $\pm$ 0.09 (n = 9)	0.053 $\pm$ 0.005	0.068 $\pm$ 0.02	7.27 $\pm$ 0.07 (n = 31)	7.17 $\pm$ 0.13 (n = 9)
Noradrenaline	0.51 $\pm$ 0.06 (n = 16)	0.53 $\pm$ 0.06 (n = 5)	15 $\pm$ 6	6.1 $\pm$ 2	5.09 $\pm$ 0.13 (n = 16)	5.21 $\pm$ 0.12 (n = 5)

**P* < 0.05, Dunnett's test.

Figure 2 Effects of 5-hydroxytryptamine (5-HT) and tyramine on proximal portion from interventricular coronary artery: (a) before and after 6-hydroxydopamine (6-OHDA) treatment; (b) before and after K⁺-free PSS treatment.

proximal portion of the interventricular artery strips are shown in Figure 3d. The calculated pD₂ values indicate that 5-HT and noradrenaline did not have the same 'affinity' for the receptor.

Antagonists

In the proximal portions for the coronary artery strips, methysergide (10 nM, 100 nM) and phentolamine (1 μM, 10 μM) shifted the curve for 5-HT to the right and caused concentration-dependent reduction in the maximal response. The pD₂' values calculated according to Van Rossum were 5.47 ± 0.14 (*n* = 5) for methysergide and 4.64 ± 0.15 for phentolamine. Morphine (10 μM) did not antagonize the effects of 5-HT. Methysergide (10 nM) did not modify significantly the concentration-response curve to noradrenaline whereas phentolamine (10 nM) shifted it to the right and reduced the maximum of these curves.

Discussion

Under *in vitro* conditions, 5-HT was found to produce contractile responses only in the proximal and not the distal segment of coronary arteries with or without exposure to high potassium concentration. These results confirm that within the same vessel the reactivity to spasmogens may change with their vascular diameter (Morishita, 1977). The contraction produced by 5-HT was not due to endogenous noradrenaline, since it persisted unchanged after either sympathetic denervation with 6-OHDA or depletion of neuronal noradrenaline stores by exposing the vessels to K⁺-free salt solution.

Noradrenaline contracted both portions of the interventricular coronary artery. Whereas the pD₂ values were not statistically different, the max-

imum shortening was greater in the proximal than in the distal portion. These results are in agreement with Morishita's suggestion that the distal portion of the coronary artery is thinly populated with α-adrenoceptors.

The effects of 5-HT were further characterized by measuring concentration-responses curves and by calculating the relation between stimulus and effect, according to Van Rossum (1968). The results of these experiments indicated that the responses elicited by 5-HT are mechanistically consistent with the receptor occupancy theory.

One difficulty in any pharmacological experiment aimed at the characterization of 5-HT responses in vascular smooth muscles is the lack of suitable 5-HT receptors antagonists. Methysergide had been shown to be a specific 5-HT receptor antagonist on smooth muscle. The contractile effect of 5-HT on coronary arteries was inhibited by methysergide but not in a competitive manner. Although it has been assumed that part of the contractile effect of 5-HT might involve stimulation of α-adrenoceptors, a moderate concentration of the α-antagonist, phentolamine, producing a clear shift of the concentration-response curve to noradrenaline, did not affect 5-HT-induced constriction. Similar observations have been made for isolated intracranial vessels from cats and man (Edvinsson, Hardebo & Owman, 1978). The contractile effect of 5-HT on proximal portion of coronary arteries appears to be mediated by 5-HT receptors but these receptors are pharmacologically different from the classical D receptors since they are not competitively blocked by methysergide.

The aetiology of coronary vasospasm is still uncertain but it is likely that liberation of 5-HT and/or other vasoactive substances from platelets aggregating at small endothelial lesions may contribute to it (Schwartz & Stone, 1977). Recently, Van Nueten & Van Houtte (1980) showed that anoxia caused

Figure 3 Analysis of the concentration-response relation. (a) Concentration-response curves to 5-hydroxytryptamine (5-HT), noradrenaline (NA) and potassium for proximal portion of coronary artery; abscissa scale: % of maximal concentration; ordinate scale: % of maximal effect. (b) Concentration-response curve to 5-HT for proximal portion of coronary artery: experimental (solid line); and theoretical (dotted line) curves. (c) Hill plot for concentration-response curve. (d) Replicate concentration-response curve for the action of 5-HT and noradrenaline (NA) on proximal portion of coronary artery; abscissa scale: concentration of 5-HT or noradrenaline; ordinate scale: % of maximal response. The values plotted are the means $\pm$ s.e. mean.

potentiation of constriction induced by 5-HT in isolated coronary arteries. The decrease of coronary resistance described by Mena & Vidrio (1976) in anaesthetized dogs was obtained in the intact coronary vascular bed perfused by normally oxygenated blood. The overall coronary resistance was then predominantly determined by resistance vessels (R_2). Epicardial coronary arteries (R_1) are conductance vessels and make only a small contribution to the overall coronary resistance. Since after atheroma formation, a decline in R_2 may maintain adequate

coronary blood flow, then R_1 resistance of epicardial vessels becomes a variable that can significantly affect total coronary vascular resistance. In this situation the 5-HT receptors localized on the proximal portion of large coronary vessels, may become very sensitive to 5-HT liberation by the platelets and thus strongly control blood flow to the atheromatous coronary region.

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