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# ACTIONS OF SEROTONIN ANTAGONISTS ON DOG CORONARY ARTERY

ROBERT M. BRAZENOR and JAMES A. ANGUS *

*Baker Medical Research Institute, Melbourne, Victoria, Australia*

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Serotonin released from platelets may initiate coronary vasopasm in patients with variant angina. If this hypothesis is correct, serotonin antagonists without constrictor activity may be useful in this form of angina. We have investigated drugs classified as serotonin antagonists on dog circumflex coronary artery ring segments in vitro. Ergotamine, dihydroergotamine, bromocriptine, lisuride, ergometrine, ketanserin, trazodone, cyproheptadine and pizotifen caused non-competitive antagonism of serotonin concentration-response curves. In addition, ketanserin, trazodone, bromocriptine and pizotifen inhibited noradrenaline responses in concentrations similar to those required for serotonin antagonism. All drugs with the exception of ketanserin, cyproheptadine and pizotifen showed some degree of intrinsic constrictor activity. Methysergide antagonized responses to serotonin competitively but also constricted the coronary artery. The lack of a silent competitive serotonin antagonist precludes a definite characterization of coronary serotonin receptors at this time. However, the profile of activity observed for the antagonist drugs in the coronary artery differs from that seen in other vascular tissues. Of the drugs tested, ketanserin may be the most useful in variant angina since it is a potent 5HT antagonist, lacks agonist activity and has α-adrenoceptor blocking activity.

α-Adrenoceptors  Coronary artery  Ergot alkaloids  Serotonin antagonists  Serotonin receptors  Variant angina

## 1. Introduction

Ergometrine is used diagnostically to provoke episodes of spasm in large coronary arteries in patients with variant angina (Heupler et al., 1978). Recent studies suggest that ergometrine is a serotonin receptor agonist in canine coronary arteries in vitro (Brazenor and Angus, 1981; Müller-Schweinitzer, 1980; Sakenashi and Yonemura, 1980). This finding raises the possibility that serotonin itself, perhaps released from platelets at the site of a small fixed stenosis in the lumen of a coronary artery, could be one of the substances responsible for spasm in variant angina (Angus et al., 1982). If this hypothesis is correct, then specific and silent serotonin receptor antagonists may be useful in the treatment of vasospastic angina.

Using canine large coronary artery ring segments in vitro we have examined the activity of a wide range of drugs reported to be serotonin antagonists in other vascular tissues in an attempt to give a rational basis for the selection of a therapeutic agent for variant angina.

## 2. Materials and methods

### 2.1. General

Greyhound dogs of either sex weighing 20–30 kg were anaesthetized with sodium pentobarbitone (40 mg/kg i.v.). The hearts were removed and ring segments of circumflex coronary artery approximately 3 mm long were suspended in organ baths containing Krebs solution at 37°C. A detailed description of the method has been published previously (Brazenor and Angus, 1981). Six prepara-

* To whom all correspondence should be addressed.

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tions were run concurrently. After 1 h equilibration, potassium chloride (25 mM) was added to each bath to test the viability of each tissue. Force generated was measured by isometric strain gauge transducers (Grass FT03C). Following repeated washing, the tissues were equilibrated with or without a concentration of antagonist for a further one hour. A cumulative concentration (0.5 log unit increments) response curve to an agonist drug was then constructed for each tissue (Van Rossum, 1963).

### 2.2. *Analysis of results*

The magnitude of tissue responses expressed as development of g force were averaged for each concentration of agonist. The rings had some active force at rest as judged by the relaxation to glyceryltrinitrate, prostacyclin and slow $Ca^{2+}$ channel blockers. The $EC_{50}$ value of each agonist concentration-response curve was estimated from a linear regression of concentration (log units) against response levels between 20% and 80% of the maximum. $IC_{50}$ values were estimated as a measure of antagonist potency. This estimation involved measuring the difference between the maximum response to the agonist in untreated and antagonist-treated tissues which was expressed as a fraction of the difference between the control maximum and the response to the antagonist. Least squares linear regression of these ratios of each antagonist concentration level was then used to calculate the concentration causing a 50% depression of the agonist maximum ($IC_{50}$). Apart from the consideration required by the antagonist-induced changes in baseline the estimation of $IC_{50}$ is thus similar to the $pD_2'$ estimation for non-competitive antagonists described by Ariëns (1964).

### 2.3. *Drugs*

Drugs used in this study were kindly provided by the following companies: bromocriptine mesilate (Sandoz), ergometrine maleate (David Bull Laboratories), ergotamine tartrate (Sandoz), dihydroergotamine mesylate (Sandoz), ketanserin tartrate (Janssen), lisuride hydrogen maleate (Schering) and trazodone hydrochloride (Roussel). Other drugs used and their sources were: noradrenaline bitartrate (Sigma), serotonin creatinine sulphate (Calbiochem), propranolol hydrochloride (ICI), cocaine hydrochloride (Sigma), and 17$\beta$-oestradiol (Calbiochem). Drugs were prepared freshly each day in distilled water except in the case of noradrenaline which was diluted in 100 $\mu$M ascorbic acid. Equimolar amounts of tartaric acid were added to solutions of bromocriptine. Concentration-response curves to noradrenaline were constructed in the presence of EDTA (40 $\mu$M) and ($\pm$)-propranolol (1 $\mu$M).

## 3. Results

### 3.1. *Agonists*

Serotonin (5HT) was a potent constrictor of the dog coronary artery preparation ($EC_{50} = 0.05$ $\mu$M) and elicited a maximal contraction of $6.06 \pm 0.53$ g. Noradrenaline was somewhat less potent ($EC_{50} = 0.32$ $\mu$M) and elicited a significantly smaller maximal response ($3.45 \pm 0.41$ g) (fig. 1).

### 3.2. *Antagonists*

#### 3.2.1. *Effects on resting force*

The 5HT receptor antagonists studied in this paper can be classified as (a) ergot peptide alkaloids, (b) lysergic acid derivatives and (c) non-ergot compounds (fig. 2). Both the ergot peptides and the lysergic acid alkaloids showed intrinsic constrictor activity on the coronary artery. Ergometrine and lisuride, derivatives of lysergic and isolysergic acid, were the most effective drugs in this respect. These drugs were equipotent and at a concentration of 1 $\mu$M elicited a contraction of approximately 40% of the maximum response to 5HT. It was reported in a previous study that methysergide, another lysergic acid amide, also constricted the dog coronary artery although it was both less potent and elicited a smaller maximal contraction than ergometrine or lisuride (Brazenor and Angus, 1981).

The ergot peptides, ergotamine and dihydroergotamine, also constricted the coronary artery although the maximal constriction produced was

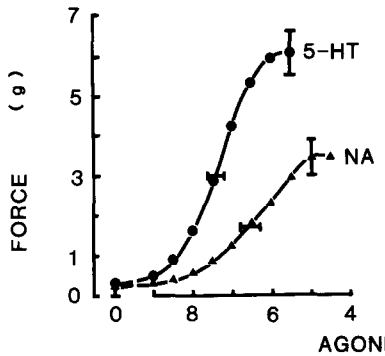


Fig. 1. Concentration-response curves to serotonin (5HT, n = 10) and noradrenaline (NA, n = 10) in dog circumflex coronary artery ring segments. Noradrenaline responses were obtained in the presence of propranolol (1 $\mu$M). All error bars represent $\pm$ 1 S.E.M. Horizontal bars are located at $EC_{50}$ values. *Ordinate*: change in isometric force (g); *abscissa*: agonist concentration (−log M). Only one agonist curve was obtained on each ring segment.

slightly smaller than that seen with the lysergic acid alkaloids. Both drugs elicited a constriction of approximately 35% of the maximal 5HT response although ergotamine was 10-fold more potent than dihydroergotamine. The third ergot peptide tested, bromocriptine, possessed only slight constrictor activity (less than 10% of the 5HT maximum) which was only apparent at the highest concentration (1 $\mu$M).

The non-ergot compounds ketanserin, cyproheptadine and pizotifen were devoid of constrictor activity over the concentration range tested (1–1000 nM). The triazolopyridine, trazodone, possessed slight constrictor activity with the highest concentration (10 $\mu$M) producing a maximal response approximately 18% of the 5HT maximum. Agonist activities of the various drugs are compared in table 1.

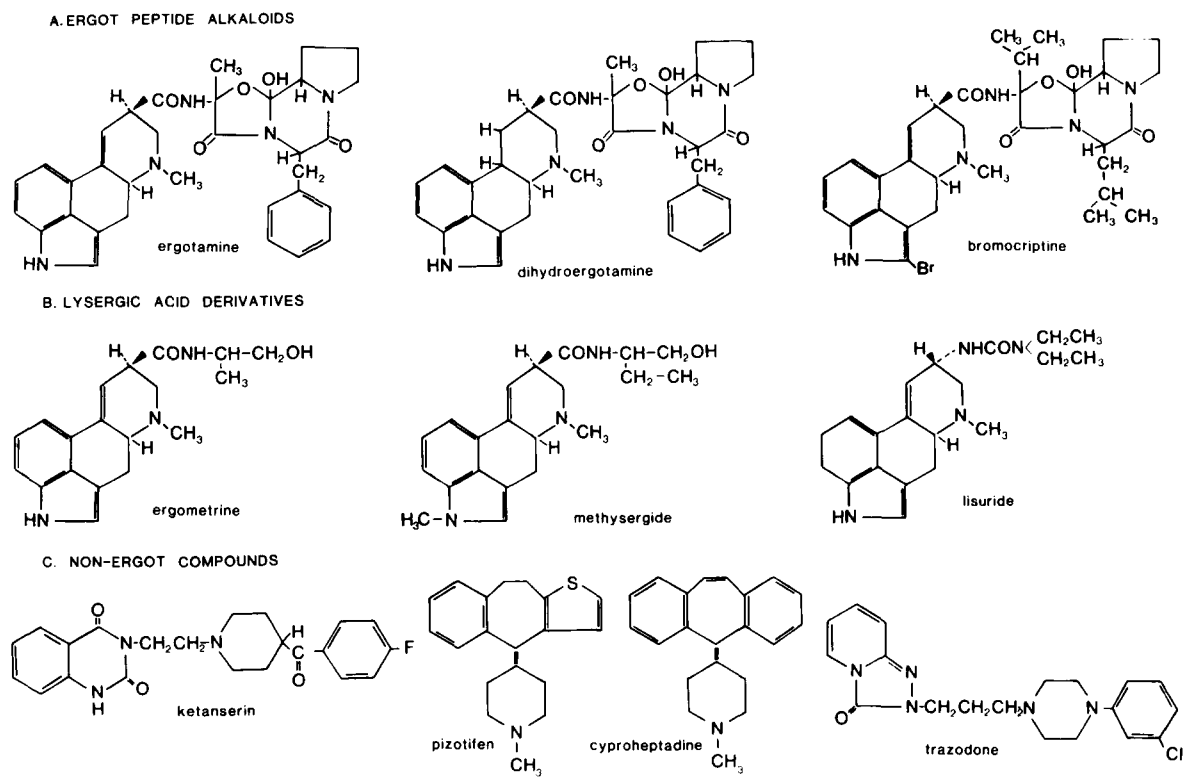


Fig. 2. Chemical structures of serotonin antagonists.

*3.2.2. 5HT antagonist activity*

The peptide alkaloids ergotamine (0.1–1.0 nM, n = 6) and dihydroergotamine (1–100 nM, n = 6) were the most potent inhibitors of 5HT found in this study. In both cases the antagonism was of a non-competitive nature indicated by a concentration-dependent depression of the maximum 5HT response without shift to the right of the $EC_{50}$. $IC_{50}$ values for these drugs are listed in table 1. Bromocriptine (0.01–1 μM, n = 6) depressed the 5HT curves but also displaced the curves to the right at least at higher concentrations. Bromocriptine had approximately one-hundredth the potency of the other peptide alkaloids in depressing the responses to 5HT.

The lysergic acid alkaloids ergometrine (0.01–0.1 μM, n = 5) and lisuride (1–100 nM, n = 8) also caused marked depression of the 5HT concentration-response curve. Lisuride and ergometrine were equipotent with respect to this 5HT antagonist activity (table 1). Some rightward displacement of the 5HT curves was also observed. These findings are in contrast to results obtained in a previous study using the lysergic acid amide, methysergide. Methysergide (10 nM–1 μM) was a partial agonist in the coronary artery and caused a parallel shift to the right of the 5HT concentration-response curves without affecting the maximum response indicating a competitive interaction (Brazenor and Angus, 1981). The effects of the ergot peptide alkaloids ergotamine and bromocriptine and the lysergic acid alkaloid, lisuride, on the 5HT concentration-response curve are shown in fig. 3.

Of the non-ergot compounds ketanserin (1–100 nM, n = 8) acted as a silent non-competitive antagonist of 5HT with a potency approximately equal to that of ergometrine and lisuride (table 1). This pattern of non-competitive 5HT antagonism without concomitant constrictor activity was observed for cyproheptadine and the structurally related pizotifen in a previous study (Brazenor and Angus, 1981). The effect of cyproheptadine (1 μM) on the constrictor responses elicited by the ergot alkaloids was examined in the present investigation. It was found that the constriction elicited by ergometrine (n = 3) dihydroergotamine (n = 3) and ergometrine (n = 6) could be blocked by cyproheptadine which suggests that the agonist activity

TABLE 1

Summary of some actions of serotonin antagonists in dog coronary artery. Constrictor activity: calculated as % of maximum response to 5HT; 0 = no activity; * = 1–20%; ** = 20–40%; *** = 40–60%. $IC_{50}$ values: Concentration (nM) of antagonist that reduces maximum constrictor response to 5HT by 50% (non-competitive antagonism). [a] Apparent $K_B$ for competitive antagonism (Brazenor and Angus, 1981). α-Adrenoceptor antagonism: 0 = no activity; S = surmountable; NS = non-surmountable inhibition.

| Antagonist | Constrictor activity | 5HT antagonism ($IC_{50}$, nM) | α-Adrenoceptor antagonism |
|---|---|---|---|
| *(A) Ergot* | | | |
| *(i) Peptide alkaloids* | | | |
| Ergotamine | ** | 0.5 | 0 |
| Dihydroergotamine | ** | 0.8 | 0 |
| Bromocriptine | * | 100 | NS |
| *(ii) Lysergic acid alkaloids* | | | |
| Ergometrine | *** | 3.2 | 0 |
| Lisuride | *** | 4.2 | 0 |
| Methysergide | ** | 10.5 [a] | 0 |
| *B. Non-ergot* | | | |
| Ketanserin | 0 | 13.3 | NS |
| Trazodone | * | 562 | NS |
| Cyproheptadine | 0 | 2.2 | 0 |
| Pizotifen | 0 | 1.6 | S |

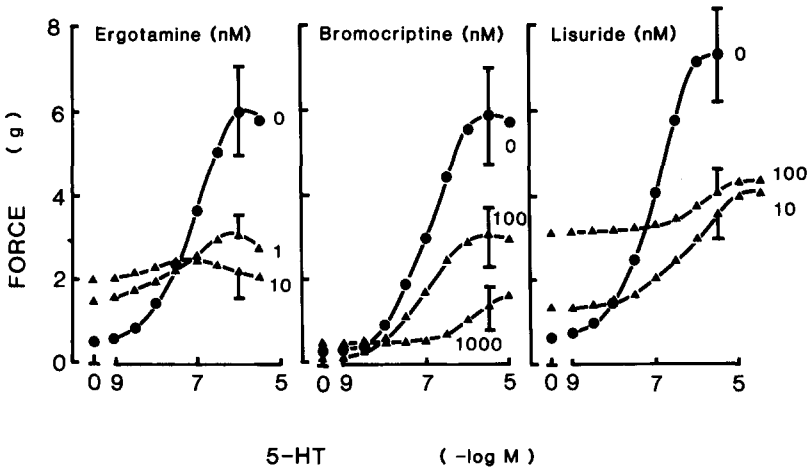


Fig. 3. Effects of ergotamine (n=5, left) bromocriptine (n=6, center) and lisuride (n=8, right) on concentration-response curves to 5HT in dog coronary artery. Points at left of each curve represent the initial constrictor response to the antagonist prior to the addition of 5HT. Only one 5HT curve was obtained, with or without antagonist present, on each ring segment. *Ordinate*: change in isometric force (g); *abscissa*: 5HT concentration (−log M). Error bars are ±1 S.E.M.

of both the ergot peptides and the lysergic acid derivatives is due to stimulation of 5HT receptors.

The other non-ergot derivative used in the present study, trazodone (0.1–10 μM, n = 6) was also a weak non-competitive antagonist of 5HT. The effects of the non-ergot compounds ketanserin and trazodone on 5HT concentration-response curves are shown in fig. 4.

*3.2.3. α-Adrenoceptor antagonist activity*

A feature of many 5HT antagonists is their ability to antagonize 5HT and α-adrenoceptor mediated responses in a similar concentration range. For this reason the effect of the highest concentration of each drug used in the 5HT antagonist studies on the concentration-response curve to noradrenaline was investigated in the presence of β-adrenoceptor blockade. Of the peptide alkaloids, neither ergotamine nor dihydroergotamine (0.1 μM, n = 9) affected the $EC_{50}$ or the maximum of the noradrenaline concentration-response curves. Because the marked constrictor effects of these ergot compounds could obscure antagonism of noradrenaline responses, the inter-

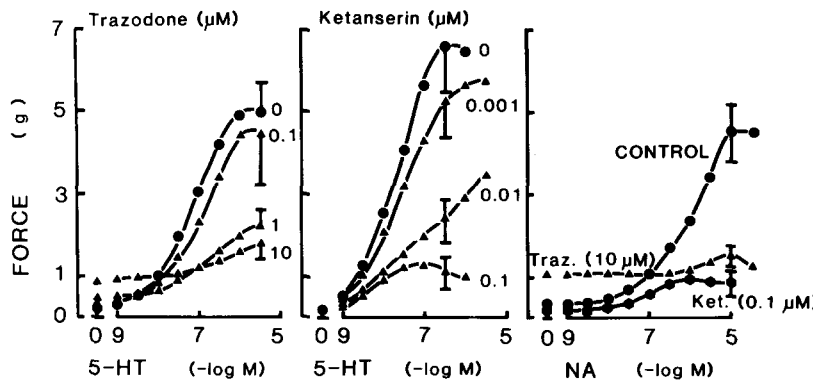


Fig. 4. Effect of trazodone (left, n=6) and ketanserin (center, n=8) on concentration-response curves to 5HT. *Right panel*: effect of trazodone (Traz. 10 μM, n=16) and curves to (−)-noradrenaline (NA). *Ordinate*: change in isometric force (g); *abscissa*: agonist concentration (−log M). Error bars are ±1 S.E.M. Values at left of each graph are initial constrictor response to the antagonist prior to the addition of agonist. Only one agonist curve was obtained on each ring segment.

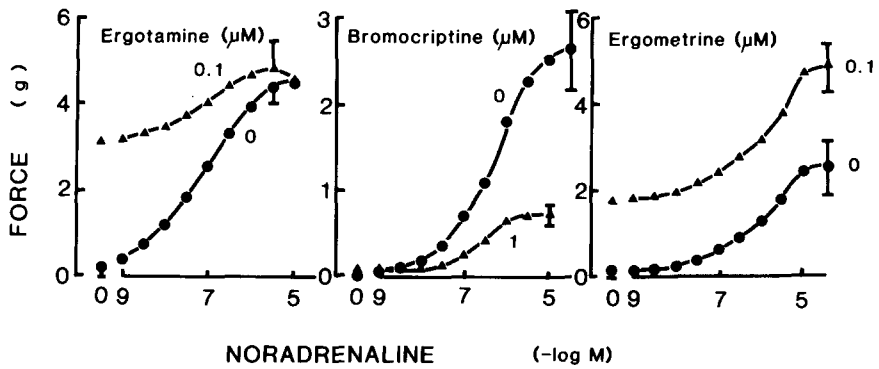


Fig. 5. Effect of ergotamine (0.1 μM, n=9) bromocriptine (1 μM, center, n=7) and ergometrine (0.1 μM, ring, n=8) on (−)-noradrenaline concentration-response curves. *Ordinate*: change in isometric force (g); *abscissa*: noradrenaline concentration (−log M). Values at left of each graph are the constrictor responses to the antagonist prior to the addition of noradrenaline. Error bars are ±1 S.E.M. Only one agonist curve was obtained, with or without antagonist, on each ring segment.

action between ergotamine and dihydroergotamine with noradrenaline was investigated in the presence of cyproheptadine (1 μM), a concentration which has been shown not to affect α-adrenoceptor mediated responses (Brazenor and Angus, 1981). Under these conditions, no α-adrenoceptor antagonist activity was observed for dihydroergotamine or ergotamine (both n = 3). The possibility that α-adrenoceptor antagonist activity of these alkaloids might be masked by possible uptake blocking properties was also considered. However pretreatment of tissues with cocaine (10 μM) or 17 β-oestradiol (5 μM) had no effect on either the maximum or $EC_{50}$ of the noradrenaline concentration-response curve which suggests that neither neuronal nor extraneuronal uptake significantly affected the responses to noradrenaline in this tissue.

In contrast with the other ergot peptide alkaloids, bromocriptine (1 μM, n = 7) depressed the maximal response to noradrenaline by approximately 75% without affecting the $EC_{50}$ which suggests a non-competitive α-adrenoceptor antagonist activity. Of the lysergic acid alkaloids neither lisuride (n = 16) nor ergometrine (n = 8) had any effect on the magnitude or $EC_{50}$ of the noradrenaline concentration-response curves. The interaction of the ergot compounds as exemplified by ergotamine, bromocriptine and ergometrine with noradrenaline are shown in fig. 5. Ketanserin (0.1 μM, n = 16) depressed the maximum response to noradrenaline by approximately 80% without affecting the $EC_{50}$ suggesting non-competitive α-adrenoceptor antagonism. Non-competitive antagonism was also observed for trazodone (10 μM, n = 16) which caused a 70% depression of the noradrenaline concentration-response curve. The effects of ketanserin and trazodone are shown in fig. 4.

## 4. Discussion

The most satisfactory method of receptor classification involves the estimation of the dissociation constant of a specific, silent, competitive antagonist (Waud, 1968). None of the 5HT-receptor antagonists examined in the present study showed competitive kinetics in the dog coronary preparation. All drugs showed non-competitive 5HT-receptor antagonist properties although some rightward displacement of 5HT curves did occur with some antagonists. The drugs tested showed considerable variation with respect to antagonist potency, intrinsic constrictor activity and specificity but no obvious correlation between structure and activity could be obtained. In general, the ergot peptide alkaloids and lysergic acid derivatives possessed greater constrictor activity and less α-adrenoceptor blocking activity than the other compounds tested. In many cases the profile of activity shown by the antagonist drugs in the

coronary artery differed in some respects from that reported in other tissues.

Of the peptide alkaloids, ergotamine and dihydroergotamine were extremely potent non-competitive 5HT antagonists. In addition both drugs possessed considerable intrinsic constrictor activity and can therefore be described as non-competitive dualists (Van Rossum, 1962; Ariëns, 1964). Similar activity has been reported for ergotamine in canine basilar arteries (Toda et al., 1976). Müller-Schweinitzer and Weidmann (1978) who also used canine basilar arteries reported $IC_{50}$ ($pD_2'$) values for ergotamine and dihydroergotamine similar to those found in the coronary artery in the present study. The other ergot peptide alkaloid bromocriptine, possessed very little constrictor activity on the coronary artery but behaved as a non-competitive 5HT antagonist with approximately one-hundredth the potency of the other ergot peptides. This is in accordance with a report by Flückiger (1976), that bromocriptine causes little constriction of canine basilar artery or femoral vein but produces a non-competitive 5HT antagonism which is less pronounced than that caused by dihydroergotamine.

A feature common to methysergide and cyproheptadine is their ability to block 5HT receptor and α-adrenoceptor mediated responses (Görlitz and Frey, 1973). Peptide ergot alkaloids in particular usually show dual blockade of responses to 5HT and catecholamines (Müller-Schweinitzer and Weidmann, 1978). It was therefore surprising to find no evidence of blockade of noradrenaline-mediated constrictor response by ergotamine or dihydroergotamine in the coronary artery. Although bromocriptine antagonized the effect of noradrenaline, this blockade was of a non-competitive nature and is in contrast with the competitive α-adrenoceptor antagonist properties of this drug in femoral vein (Flückiger, 1976). At present, no clear explanation for these results can be offered although these results may indicate that the mechanisms mediating constrictor responses to α-adrenoceptor agonists differ in the coronary artery from other vascular tissues. This does not, however, imply that the α-adrenoceptor itself is different from that in other tissues. It has been shown that the α-adrenoceptor of coronary artery can be blocked irreversibly with benextramine or competitively with prazosin. An estimation of the antagonist dissociation constant for prazosin yields a value which is not different from that obtained in other vascular preparations (Brazenor and Angus, 1981).

The lysergic acid compounds, ergometrine and lisuride, showed no α-adrenoceptor blocking activity in the coronary artery. Both ergometrine and lisuride behaved as non-competitive dualists at 5HT receptors in this tissue and differed from the peptide alkaloids only in that they had slightly more powerful constrictor effects but were slightly less potent as antagonists of 5HT. The actions of ergometrine and lisuride contrast with those of the structurally related compound methysergide which has been shown to be a partial agonist in coronary artery (Brazenor and Angus, 1981). Although ergometrine also shows non-competitive dualist behaviour in canine basilar artery (Müller-Schweinitzer, 1980) the pharmacodynamic properties of the lysergic acid alkaloids in the coronary artery may differ from those in other tissues. For example, methysergide has been shown to be a non-competitive dualist in dog saphenous vein but a silent competitive antagonist in dog femoral artery (Apperley et al., 1980). Similarly, Podvalova and Dlabac (1972) describe lisuride as an antagonist of 5HT on the rat stomach strip but make no mention of any intrinsic agonist activity.

The non-ergot 5HT antagonists also show different properties in the dog coronary artery compared with other tissues. Ketanserin, a silent non-competitive antagonist of 5HT in the coronary artery is reported to be a competitive 5HT antagonist in rat caudal artery, dog gastrosplenic, internal carotid and basilar arteries (Van Nueten et al., 1981). Cyproheptadine and pizotifen are also silent non-competitive antagonists in coronary artery. However, these drugs are reported to be competitive 5HT antagonists in dog femoral artery and rabbit aorta (Apperley et al., 1976, 1980).

The differences in the nature of the interaction between 5HT and various antagonist drugs in different tissues has been explained by some authors by postulating the existence of 5HT receptor sub-types (Apperley et al., 1980). These tissue differences however need not necessarily reflect

differences in the nature of the receptor. If the cellular mechanisms mediating the events subsequent to receptor activation differ between tissues, it is possible that 5HT antagonists may act in certain tissues by depressing the translation of receptor stimulation into a biological response rather than by interaction with the 5HT receptor itself. Until specific, silent, competitive antagonists of 5HT in coronary artery can be identified, it remains uncertain whether the effects of the antagonists used in this study are indicative of 5HT receptors in the coronary artery different from those of other vascular tissues.

It is interesting to speculate on the possible usefulness of the antagonist drugs as therapeutic agents assuming that endogenous 5HT is involved in coronary vasospasm. The high agonist activity of the ergot alkaloids tends to suggest that these drugs would be least useful. Indeed, the agonist activity or ergometrine is exploited to evoke coronary vasospasm for diagnostic purposes. Of the remaining antagonists, ketanserin may be the logical choice in view of its high antagonist potency, lack of agonist activity and its $\alpha$-adrenoceptor blocking activity which may be of some importance if sympathetic neural mechanisms are involved in variant angina.

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