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The classification of β-adrenoceptors in isolated ring preparations of canine coronary arteries

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1 Relaxant responses to the β-adrenoceptor agonists isoprenaline, fenoterol, noradrenaline or procaterol were obtained on isolated ring preparations of canine coronary arteries contracted with KCl (20 mM) or 5-hydroxytryptamine (3 μM).

2 On left circumflex arterial preparations, Schild plots for the selective antagonists atenolol (β_1-selective) or ICI 118,551 (β_2-selective), when using noradrenaline or fenoterol as agonist, were superimposed. This suggested that only one subtype of β-adrenoceptor was involved in the responses.

3 The pA_2 values on left circumflex artery preparations were: atenolol, noradrenaline as agonist 6.98, fenoterol as agonist 6.71; ICI 118, 551, noradrenaline as agonist 6.66, fenoterol as agonist 7.04. These data indicated that the β-adrenoceptor subtype was β_1.

4 The relative potencies of isoprenaline: noradrenaline: fenoterol were left circumflex 100: 10.0: 2.3, left ventricular branch 100: 9.7: 2.0, septal branch 100: 10.9: 2.5. These data confirmed that β_1-adrenoceptors were involved in the responses of all three arterial preparations.

5 On preparations of left circumflex artery, left ventricular branch and septal branch, responses were obtained to high concentrations (1 to 100 μM) but not to low concentrations (0.001 to 0.1 μM) of procaterol. This observation confirmed the absence of β_2-adrenoceptors in these arteries.

6 Responses of left circumflex artery to isoprenaline were potentiated by the extraneuronal uptake inhibitor drugs, corticosterone and metanephrine.

7 It is concluded (a) that responses of canine left circumflex artery, left ventricular branch and septal branch are mediated by a homogeneous population of β_1-adrenoceptors, and (b) that modulation of responses to isoprenaline by extraneuronal uptake is not confined to responses mediated by β_2-adrenoceptors.

Introduction

The classification of the β-adrenoceptor subtype in coronary arteries as β_1 or β_2 has been controversial (Bevan *et al.*, 1980), and is still not resolved, despite a wealth of experimental data on the subject (Fiegl, 1983). In the present study, isolated ring preparations of three of the larger coronary arteries from dog, i.e. left circumflex artery, one of its ventricular branches and the septal branch of the left coronary artery, have been examined to see whether relaxant responses might be mediated by both β_1- and β_2-adrenoceptors, as has been described for other blood vessels (Cohen & Wiley, 1978; O'Donnell & Wanstall, 1981a; 1984). The method used has been described by O'Donnell & Wanstall (1981b). It involves determining whether Schild plots for a selective β-adrenoceptor antagonist, obtained using a β_1-selective agonist and a β_2-selective agonist, are superimposed (homogeneous β-adrenoceptor population) or separated (heterogeneous β-adrenoceptor population). The Schild plot data led us to conclude that relaxant responses of the coronary artery preparations examined were mediated only by β_1-adrenoceptors. This conclusion was confirmed by investigating responses to the highly β_2-selective agonist, procaterol (Minneman *et al.*, 1979).

Due to the lack of involvement of β_2-adrenoceptors, experiments have also been carried out to determine whether, for the left circumflex artery, responses to isoprenaline could be potentiated by extraneuronal uptake inhibitors (corticosterone and metanephrine). This was of interest since it has been postulated that β-adrenoceptor-mediated

responses to isoprenaline might be influenced by extraneuronal uptake only if the responses involve adrenoceptors of the β_2-subtype (Bryan *et al.*, 1981; O'Donnell & Wanstall, 1983; Osswald & Guimaraes, 1983). A preliminary report of some of these data was communicated to the 38th meeting of the Australian Physiological and Pharmacological Society, Melbourne, August, 1982 (Wanstall & O'Donnell, 1982).

Methods

Canine coronary arterial preparations

Greyhounds of either sex, weighing 25 to 35 kg, were killed with pentobarbitone i.v. The hearts were immediately removed and the coronary arteries dissected free from the myocardium. Three different vessels were used, the left circumflex artery, a small branch of the left circumflex artery (left ventricular branch) and the septal branch of the left coronary artery. The approximate external diameters of these 3 vessels were 2–3 mm, 1 mm and 1–1.5 mm respectively. The vessels were cleared and cut into rings 3–5 mm in length. Up to 12 preparations were obtained from each dog. Rings were either used immediately or were stored in Krebs solution at 4°C for 24 or 48 h. Retrospective analysis of the experimental data indicated that preparations used on three consecutive days did not differ from one another with respect to the magnitude of the induced contraction (to KCl or 5-hydroxytryptamine (5-HT) *vide infra*), the maximum relaxation to isoprenaline or the sensitivity to any of the β-adrenoceptor agonist or antagonist drugs used. Thus data from all preparations were pooled for analyses.

The ring preparations were mounted around two horizontal stainless steel pins (one fixed and one linked to a Statham Universal transducer, UC3 and UL5) in an organ bath containing Krebs solution at 37°C gassed with 95% O_2 and 5% CO_2. The resting tension of the preparations was adjusted to 2.5 to 3 g (left circumflex), 1.5 g (left ventricular branch) and 1 g (septal branch) and preparations were allowed to equilibrate for 2 to 3.5 h. At the completion of the equilibration period preparations were contracted with either KCl (0.1 ml of a 2 M solution of KCl added to 10 ml of Krebs solution in the organ bath) or 5-HT (3 μM). Cumulative concentration-response (relaxation) curves to β-adrenoceptor agonists (isoprenaline, fenoterol or noradrenaline) were obtained. At the end of each concentration-response curve the maximum relaxation to isoprenaline was established, and responses were expressed as a percentage of the isoprenaline maximum. Maximum responses to fenoterol or noradrenaline were shown to equal the maximum response to isoprenaline. The concentration of each agonist producing 50% of the maximum response (EC_{50}) was determined. Negative log EC_{50} values were used as an expression of the potency of agonists and were also used to calculate the relative potencies of the different agonists (see Results).

Schild plots for β-adrenoceptor antagonists

For these experiments, left circumflex arterial preparations contracted with KCl were used. During the equilibration period, these preparations were treated with phenoxybenzamine (50 μM) for 30 min followed by thorough washing with phenoxybenzamine-free Krebs solution, in order to block α-adrenoceptors, neuronal uptake and extraneuronal uptake. Cumulative concentration-response curves to fenoterol or noradrenaline were obtained in the absence (control) and presence of increasing concentrations of either atenolol or ICI 118,551. The contact time for the antagonists was 60 min. Values of concentration-ratio (CR) were calculated by dividing the agonist EC_{50} in the presence of antagonist by the agonist EC_{50} in the absence of antagonist. Preparations declined slightly in sensitivity to noradrenaline and fenoterol in successive concentration-response curves. Thus correction factors were obtained (on ring preparations from the same dogs as were used in the antagonist experiments), by carrying out experiments which were identical in design to the antagonist experiments, except that the antagonist was omitted. The correction factors were used to correct the experimentally determined CR values, as described by O'Donnell & Wanstall (1979). Plots of log (corrected $CR-1$) against log molar antagonist concentration (log [B]) were obtained. A linear least squares regression analysis (Colquhoun, 1971) was used to calculate the line of best fit through data points from a number of dogs and this line is referred to as a Schild plot. pA_2 values were obtained by extrapolation of the Schild plots to log $(CR-1)= 0$. 95% confidence limits of these values were calculated according to methods described by Colquhoun (1971).

Effects of procaterol

Concentrations of procaterol ranging from 0.001 μM to 100 μM were tested on preparations of left circumflex artery, left ventricular branch or septal branch contracted with either KCl (phenoxybenzamine-treated) or 5-HT (with phentolamine 10 μM, cocaine 10 μM and corticosterone 100 μM present for 30 min before and during the concentration-response curves). Responses were expressed as a percentage of the maximum response to isoprenaline.

Effects of extraneuronal uptake inhibitors on responses of left circumflex arterial preparations to isoprenaline

These experiments were carried out on preparations contracted with 5-HT. α-Adrenoceptors were blocked with phentolamine (10 μM), and neuronal uptake was blocked with cocaine (10 μM). Concentration-response curves to isoprenaline were obtained in the absence and presence of the extraneuronal uptake inhibitors (corticosterone (50 or 100 μM) or metanephrine (50 μM)). Potentiation was expressed in log units as the difference between the isoprenaline negative log EC_{50} values in the presence and absence of inhibitors. Repeated isoprenaline concentration-response curves in the absence of inhibitors were reproducible. In the experiments with corticosterone, the control isoprenaline concentration-response curves were obtained in the presence of absolute ethanol in a concentration corresponding to that which was present when the corticosterone was added. In the experiments with metanephrine, both isoprenaline and fenoterol (which is not potentiated by extraneuronal uptake inhibitors, O'Donnell & Wanstall, 1976) were examined on the same preparations. The negative log EC_{50} value for fenoterol was decreased by metanephrine, because of the weak β-adrenoceptor antagonist activity of metanephrine (Kenakin, 1980). This change in negative log EC_{50} value (in log units) was added to the potentiation value for isoprenaline, in order to correct it for the β-adrenoceptor antagonism by metanephrine (O'Donnell & Wanstall, 1983).

Drugs and solutions

Drugs used were: atenolol (I.C.I.); cocaine hydrochloride (Drug Houses of Australia); corticosterone (Sigma); fenoterol hydrobromide (Boehringer-Ingelheim); 5-hydroxytryptamine creatinine sulphate (Sigma); ICI 118,551 (erythro-DL-1 (7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol, I.C.I.); (±)-isoprenaline sulphate (Sigma); (±)-metanephrine hydrochloride (Calbiochem); (−)-noradrenaline acid tartrate (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French); phentolamine methanesulphonate (Regitine ampoules, Ciba); procaterol (Warner-Lambert).

Stock solutions (10 or 100 mM) of atenolol, fenoterol, ICI 118,551, isoprenaline, metanephrine and noradrenaline were made up in 0.01 M HCl and of cocaine and 5-hydroxytryptamine in deionized water. Procaterol (10 mM) was made up in Krebs solution and used immediately. Phenoxybenzamine was dissolved in absolute ethanol containing 0.01 M HCl to prepare a 100 mM stock solution. Corticosterone was dissolved in absolute ethanol to prepare a 50 mM stock solution. All dilutions of stock solutions were made in Krebs solution and kept on ice during the course of the experiment.

The composition of the Krebs solution was (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7, ascorbic acid 1.1.

Statistical Analyses

Mean values are quoted together with their standard errors (s.e.). Comparisons have been made using Student's *t* test or paired *t* test.

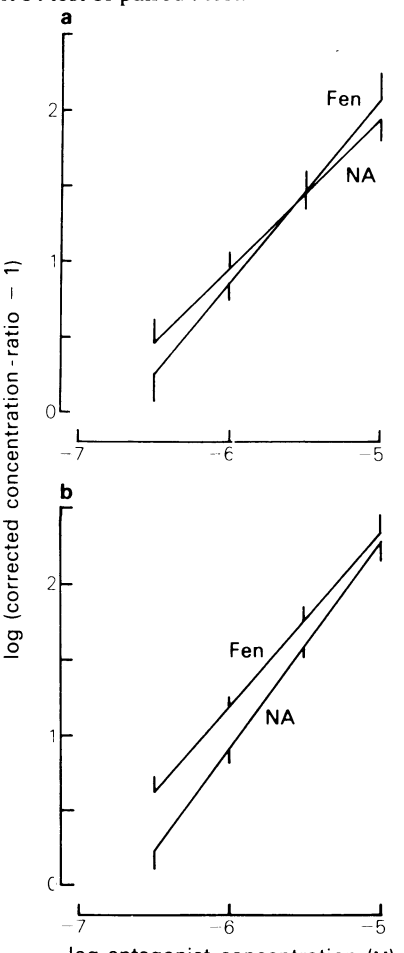

Figure 1 Schild plots for (a) atenolol, and (b) ICI 118, 551 on canine left circumflex artery preparations using noradrenaline (NA) or fenoterol (Fen) as agonist. The plots are calculated regression lines of best fit through data points from a number of animals. The vertical bars represent the s.e. of estimated values of log (concentration-ratio − 1) at points corresponding to the antagonist concentrations used.

Table 1 pA_2 values and slopes of Schild plots for atenolol and ICI 118,551 on canine left circumflex artery using fenoterol or noradrenaline as agonists

	Agonist	*Slope of Schild plot ± s.e.*	*pA_2 value (95% confidence limits)*
Atenolol	Noradrenaline	0.99 ± 0.15 (4, 12)[a]	6.98 (6.63, 7.63)
	Fenoterol	1.22 ± 0.18 (4, 10)	6.71 (6.40, 7.29)
ICI 118,551	Noradrenaline	1.36 ± 0.14* (5, 10)	6.66 (6.45, 6.99)
	Fenoterol	1.13 ± 0.14 (4, 9)	7.04 (6.77, 7.51)

[a]Number of animals, number of data points.
* Slope significantly greater than 1.0 ($0.05 > P > 0.01$) but not significantly different from that of the corresponding plot using fenoterol as agonist.
Preparations were treated with phenoxybenzamine (50 μM for 30 min followed by wash out) and contracted with KCl (20 mM).

Results

Schild plots for atenolol and ICI 118,551 on preparations of left circumflex coronary artery

For both atenolol and ICI 118,551, the Schild plot obtained when using noradrenaline as agonist was superimposed on that obtained when using fenoterol as agonist (Figure 1). The pA_2 values obtained from these Schild plots are shown in Table 1 together with 95% confidence limits. For each of the antagonists, the pA_2 value obtained using noradrenaline as agonist, was not significantly different from that obtained using fenoterol as agonist ($P > 0.05$).

Potencies and relative potencies of isoprenaline, noradrenaline and fenoterol on preparations of left circumflex artery, left ventricular branch and septal branch

The mean negative log EC_{50} values for isoprenaline, noradrenaline and fenoterol are summarized in Table 2. These have been used to calculate the relative potencies of the agonists (isoprenaline = 100). The negative log EC_{50} values and relative potency values were the same on each of the vessels examined (Table 2). Noradrenaline was 4 to 5 times more potent than fenoterol. The mean maximum relaxations to isoprenaline (expressed as a % of the KCl-

Table 2 Potencies (mean negative log EC_{50} values) and relative potencies of isoprenaline (Iso) noradrenaline (NA) and fenoterol (Fen) on canine left circumflex artery, left ventricular branch and septal branch

	Mean negative log EC_{50} ± s.e.			*Relative potency*		
	Iso	*NA*	*Fen*	*Iso*	*NA[a]*	*Fen*
Left circumflex	8.20 ± 0.13 (6)	7.50 ± 0.11 (8)	6.57 ± 0.11 (8)	100	10.0	2.3
Left ventricular branch	8.01 ± 0.13 (5)	7.30 ± 0.13 (4)	6.31 ± 0.09 (4)	100	9.7	2.0
Septal branch	7.80 ± 0.13 (6)	7.14 ± 0.19 (4)	6.20 ± 0.11 (4)	100	10.9	2.5

[a]Half the experimental value to allow for the fact that (−)-noradrenaline was used, in contrast to (±)-isoprenaline.
The numbers in parentheses represent the number of animals.
Preparations were treated with phenoxybenzamine (50 μM for 30 min followed by wash out) and contracted with KCl (20 mM).

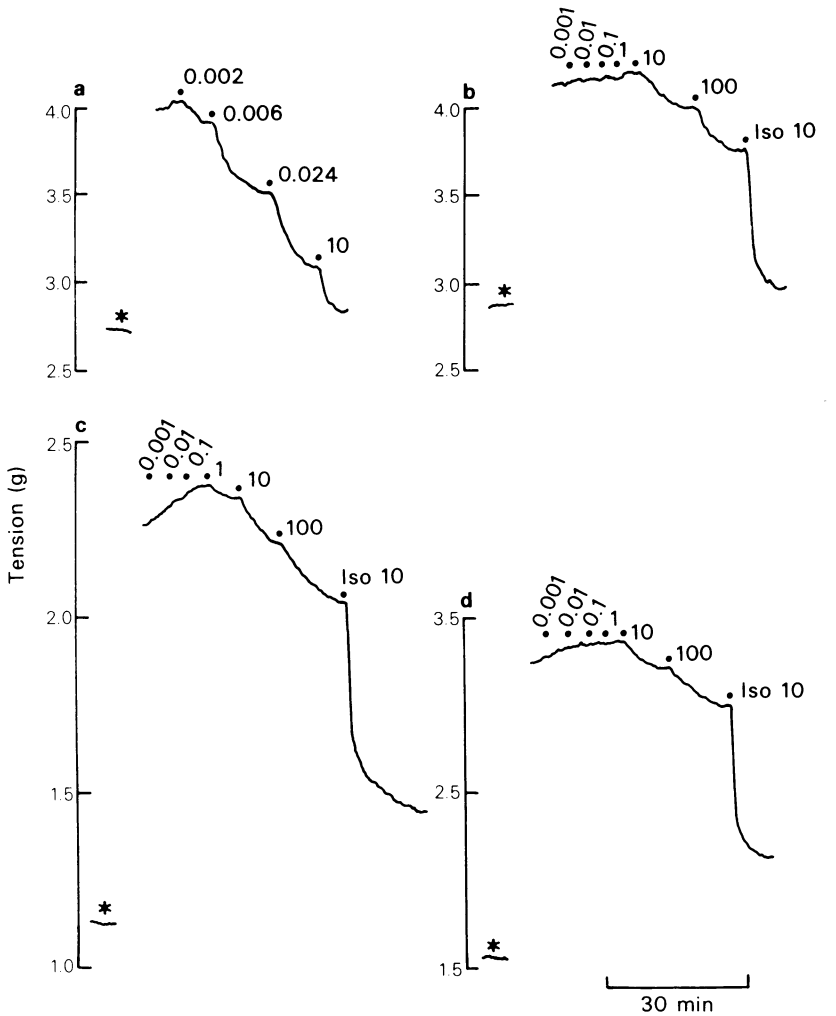

Figure 2 Responses to isoprenaline (a) or procaterol (b,c and d) on preparations of canine left circumflex artery (a,b), septal branch (c) and left ventricular branch (d) treated with phenoxybenzamine (50 μM) and contracted with KCl (20 mM). Concentrations are in μM. The resting tension before the addition of KCl is shown (*). At the end of each procaterol concentration-response curve a supramaximal concentration of isoprenaline (10 μM) was added.

induced contraction) were 78 ± 6.7% (12), 85 ± 4.4% (5) and 63 ± 11.4% (6) on left circumflex, left ventricular branch and septal branch preparations, respectively.

Effects of procaterol on preparations of left circumflex artery, left ventricular branch and septal branch

No responses to procaterol, in the concentration range 0.001 to 0.1 μM, were obtained on any of the three vessel types examined, whether the preparations were contracted with KCl or with 5-HT. Small relaxant responses were obtained to procaterol in concentrations of 1 to 100 μM (Figure 2). The response to 100 μM procaterol was less (30–64%) than the maximum response to isoprenaline, but it was not established whether this was the maximum response to procaterol, since concentrations higher than 100 μM were not tested.

Effects of corticosterone and metanephrine on responses of left circumflex artery to isoprenaline

On preparations contracted with 5-HT, responses to isoprenaline were significantly potentiated by both corticosterone and metanephrine ($P < 0.01$; paired t test). The mean potentiation (in log units) by corticosterone (50 μM) was 0.26 ± 0.04 (6) and by cor-

ticosterone (100 μM) 0.55 ± 0.08 (6). The mean potentiation by metanephrine 50 μM (when corrected for the weak β-adrenoceptor antagonist activity of metanephrine, see Methods) was 0.47 ± 0.07 (5). The mean maximum relaxation to isoprenaline on 5-HT-contracted preparations was 85 ± 4.3% (16) which was similar to that obtained on KCl-contracted preparations.

Discussion

There is already evidence in the literature for the involvement of each subtype of β-adrenoceptor (β_1 or β_2) in responses of coronary blood vessels to β-adrenoceptor agonists (see Fiegl, 1983). It has also been suggested that coronary vascular β-adrenoceptors might have properties intermediate between those of β_1- and β_2-adrenoceptors (Ross & Jorgenson, 1970; Cornish & Miller, 1975; Parratt, 1980; Fiegl, 1983).

Most of the evidence for the presence of β_1-adrenoceptors has been obtained from experiments on isolated coronary vessels, and was based largely (a) on the rank order of potency of isoprenaline, noradrenaline, adrenaline and salbutamol, and (b) on the potency of the β_1-selective antagonist, practolol, against the non-selective agonist isoprenaline (see Fiegl, 1983). These data on isolated vessels have been obtained on large, rather than resistance, coronary vessels. In contrast, most of the evidence for β_2-adrenoceptor-mediated responses has been obtained in studies in which total coronary blood flow has been measured, either *in vivo* or in perfused isolated hearts. Therefore, the data probably relate to responses of the smaller resistance vessels. The evidence for β_2-adrenoceptors in these experiments was based on the ability to obtain responses to β-adrenoceptor agonists after administration of an antagonist which blocked β_1-adrenoceptors, e.g. practolol.

The experiments on isolated vessels, in which β_1-adrenoceptors were detected, and those on whole hearts, in which β_2-adrenoceptors were detected, were not designed to reveal a minor population of the other subtype of β-adrenoceptor, if present. Consequently it has never been clearly established whether or not both β-adrenoceptor subtypes are involved in the response of any particular region of the coronary vascular bed. If both subtypes of β-adrenoceptor should co-exist in any of the vessels of the coronary vasculature, this could be one reason why there are differing opinions in the literature on the classification of coronary vascular β-adrenoceptors. The only data to date which have been interpreted as suggesting that both β_1- and β_2-adrenoceptors may be involved in responses of a coronary vessel are those of Vatner *et al.*, (1982) in conscious dogs.

In the present study evidence has been obtained that, in at least three of the larger coronary vessels of the dog, relaxation to β-adrenoceptor agonists is mediated only by β_1-adrenoceptors. The evidence for this in the left circumflex artery preparations is (1) the Schild plots for atenolol (β_1-selective) or for ICI 118,551 (β_2-selective), when using two different agonists, noradrenaline (β_1-selective) and fenoterol (β_2-selective), were superimposed, suggesting that responses were mediated by a homogeneous population of β-adrenoceptors; (2) the pA_2 values for both antagonists were close to values previously obtained on β_1-adrenoceptors (see O'Donnell & Wanstall, 1983); (3) noradrenaline was more potent than fenoterol and (4) the β_2-selective agonist, procaterol, produced responses only in concentrations higher than 1 μM. Procaterol has previously been shown to be able to detect a small minority of β_2-adrenoceptors in a tissue containing predominantly β_1-adrenoceptors, and the concentrations which activate β_2-adrenoceptors are in the range 0.001 to 0.1 μM (Hedberg & Mattson, 1981).

On the two other vessels studied (the left ventricular branch and septal branch) the relative potencies of isoprenaline, noradrenaline and fenoterol were the same as those obtained on the left circumflex artery, and responses to procaterol were obtained only at high (> 1 μM) concentrations. Thus the data indicated that these smaller branches had the same β-adrenoceptor population as the larger left circumflex artery (i.e. β_1 only), even though smaller branches have been shown to differ from the left circumflex artery in other respects, e.g. in smaller branches the adrenergic innervation is less dense than in the left circumflex artery (Denn & Stone, 1976) and α-adrenoceptors are sparse (Morishita, 1979) or absent (Cohen *et al.*, 1983).

Since β_1-adrenoceptors are more sensitive than β_2-adrenoceptors to the neurotransmitter, noradrenaline, and in canine coronary arteries, are activated by noradrenaline released from sympathetic nerves by electrical stimulation (Toda & Hayashi, 1982; Cohen *et al.*, 1983), then canine coronary vascular β_1-adrenoceptors can be regarded as functionally innervated receptors. Furthermore, responses of these receptors to neuronally released noradrenaline can be modulated by neuronal uptake (Toda & Hayashi, 1982) i.e. neuronal uptake can act as a dissipation mechanism for agonists acting on these β_1-adrenoceptors. We have investigated whether responses of the β_1-adrenoceptors in the left circumflex artery could be modulated by another dissipation mechanism, namely extraneuronal uptake, since extraneuronal uptake has been postulated to be more closely associated with β_2- than with β_1-adrenoceptors (Bryan *et al.*, 1981; Osswald & Guimaraes, 1983). In fact, potentiation, by ex-

traneuronal uptake inhibitors, of responses known to be mediated exclusively by β_1-adrenoceptors has not previously been described. Although extraneuronal uptake inhibitors potentiated responses of coronary arteries of sheep to isoprenaline (Brine *et al.*, 1979) and of ox to noradrenaline (Kalsner, 1974; Kalsner *et al.*, 1975), the absence of a minor population of β_2-adrenoceptors in the preparations from these two species has not yet been experimentally established. In the present study, responses of the canine left circumflex artery to isoprenaline (mediated exclusively by β_1-adrenoceptors, *vide supra*) were potentiated by corticosterone and by metanephrine. This suggests that modulation of β-adrenoceptor-mediated responses by extraneuronal uptake is not confined to responses mediated by β_2-adrenoceptors, as had previously been suggested (Bryan *et al.*, 1981; O'Donnell & Wanstall, 1983; Osswald & Guimaraes, 1983).

In conclusion, the experiments described in this paper have confirmed the presence of β_1-adrenoceptors in three of the larger coronary arteries from dog and, in addition, have shown that, in these three arteries, there is no minor population of β_2-adrenoceptors. The evidence in the literature for β_2-adrenoceptors in the resistance vessels seems to be good (Fiegl, 1983). Thus it remains possible that vessels smaller than those examined in the present study could contain a heterogeneous β-adrenoceptor population, or even a homogeneous β_2-adrenoceptor population, depending on whether the transition from β_1-adrenoceptors in the larger vessels to β_2-adrenoceptors in the resistance vessels is gradual or abrupt. β_1-Adrenoceptors have now been demonstrated in canine left circumflex artery not only *in vitro* (Baron *et al.*, 1972; this study) but also *in vivo*. Vatner *et al.* (1982) recently showed that the calibre of large coronary arteries could be regulated by β_1-adrenoceptors in conscious dogs. Thus one could speculate that cardio-(β_1-)selective antagonists might attenuate coronary vasodilatation mediated by endogenous noradrenaline and/or adrenaline, at least in the larger coronary arteries. Admittedly, attenuation of vasodilatation of the large coronary vessels may be unimportant in normal hearts, since the arterioles, rather than the large vessels, determine coronary vascular resistance and hence blood flow to the myocardial tissue. However, in ischaemic hearts, the arterioles in ischaemic regions are probably maximally dilated, due to hypoxia, and would no longer be the only vessels controlling coronary vascular resistance (Winbury *et al.*, 1969). In this case, the calibre of the large vessels would become important in determining blood flow to ischaemic areas, and any attenuation of large vessel dilatation could then be regarded as disadvantageous.

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References

BARON, G.D., SPEDEN, R.N. & BOHR, D.F. (1972). Beta-adrenergic receptors in coronary and skeletal muscle arteries. *Am. J. Physiol.*, **223,** 878–881.

BEVAN, J.A., BEVAN, R.D. & DUCKLES, S.P. (1980). Adrenergic regulation of vascular smooth muscle. In *Handbook of Physiology Section 2 The Cardiovascular System Vol. II Vascular Smooth Muscle* ed. Bohr, D.F., Somlyo, A.P. & Sparks, H.V. pp. 515–566, Bethesda: American Physiological Society.

BRINE, F., CORNISH, E.J. & MILLER, R.C. (1979). Effects of uptake inhibitors on responses of sheep coronary arteries to catecholamines and sympathetic nerve stimulation. *Br. J. Pharmac.*, **67,** 553–561.

BRYAN, L.J., COLE, J.J., O'DONNELL, S.R. & WANSTALL, J.C. (1981). A study designed to explore the hypothesis that *beta*-1 adrenoceptors are 'innervated' receptors and *beta*-2 adrenoceptors are 'hormonal' receptors. *J. Pharmac. exp. Ther.*, **216,** 395–400.

COHEN, M.L. & WILEY, K.S. (1978). Beta$_1$ and beta$_2$ receptor mechanisms in rat jugular veins: differences between norepinephrine and isoproterenol-induced relaxation. *Life Sci.*, **23,** 1997–2006.

COHEN, R.A., SHEPHERD, J.T. & VANHOUTTE, P.M. (1983). Prejunctional and postjunctional actions of endogenous norepinephrine at the sympathetic neuroeffector junction in canine coronary arteries. *Circulation Res.*, **52,** 16–25.

COLQUHOUN, D. (1971). In *Lectures on Biostatistics*, Bath: Pitman Press.

CORNISH, E.J. & MILLER, R.C. (1975). Comparison of the β-adrenoceptors in the myocardium and coronary vasculature of the kitten heart. *J. Pharm. Pharmac.*, **27,** 23–30.

DENN, M.J. & STONE, H.L. (1976). Autonomic innervation of dog coronary arteries. *J. appl. Physiol.*, **41,** 30–35.

FIEGL, E.O. (1983). Coronary Physiology. *Physiol. Rev.*, **63,** 1–205.

HEDBERG, A. & MATTSON, H. (1981). Beta adrenoceptor interaction of full and partial agonists in the cat heart and soleus muscle. *J. Pharmac. exp. Ther.*, **219,** 798–808.

KALSNER, S. (1974). Sensitization of noradrenaline responses by inhibitors of extraneuronal uptake in a coronary artery preparation. *Br. J. Pharmac.*, **51,** 453–455.

KALSNER, S., FREW, R.D. & SMITH, G.M. (1975). Mechanism of methylxanthine sensitisation of norepinephrine responses in a coronary artery. *Am. J. Physiol.*, **228,** 1702–1707.

KENAKIN, T.P. (1980). Errors in the measurement of agonist potency ratios produced by uptake processes: A general model applied to beta-adrenoceptor agonists. *Br. J. Pharmac.*, **71,** 407–417.

MINNEMAN, K.P., HEDBERG, A. & MOLINOFF, P.B. (1979). Comparison of beta adrenergic receptor subtypes in mammalian tissues. *J. Pharmac. exp. Ther.*, **211,** 502–508.

MORISHITA, H. (1979). Distribution and characterization of the adrenoceptors in dog coronary arteries. *Arch. int. Pharmacodyn.*, **239,** 195–207.

O'DONNELL, S.R. & WANSTALL, J.C. (1976). The contribution of extraneuronal uptake to the trachea-blood vessel selectivity of β-adrenoceptor stimulants *in vitro* in guinea-pigs. *Br. J. Pharmac.*, **57,** 369–373.

O'DONNELL, S.R. & WANSTALL, J.C. (1979). The importance of choice of agonist in studies designed to predict β_2:β_1 adrenoceptor selectivity of antagonists from pA_2 values on guinea-pig trachea and atria. *Naunyn-Schmiedebergs Arch. Pharmac.*, **308,** 183–190.

O'DONNELL, S.R. & WANSTALL, J.C. (1981a). Demonstration of both β_1- and β_2-adrenoceptors mediating relaxation of isolated ring preparations of rat pulmonary artery. *Br. J. Pharmac.*, **74,** 547–552.

O'DONNELL, S.R. & WANSTALL, J.C. (1981b). Pharmacological approaches to the characterization of β-adrenoceptor populations in tissues. *J. Auton. Pharmac.*, **1,** 305–312.

O'DONNELL, S.R. & WANSTALL, J.C. (1983). Relaxation of cat trachea by β-adrenoceptor agonists can be mediated by both β_1- and β_2-adrenoceptors and potentiated by inhibitors of extraneuronal uptake. *Br. J. Pharmac.*, **78,** 417–424.

O'DONNELL, S.R., & WANSTALL, J.C. (1984). *Beta*$^{-1}$- and *Beta*$^{-2}$-adrenoceptor-mediated responses in preparations of pulmonary artery and aorta from young and aged rats. *J. Pharmac. exp. Ther.*, (in press).

OSSWALD, W. & GUIMARAES, S. (1983). Adrenergic mechanisms in blood vessels: morphological and pharmacological aspects. *Rev. Physiol. Biochem. Pharmac.*, **96,** 53–122.

PARRATT, J.R. (1980). Effects of adrenergic activators and inhibitors on the coronary circulation. In *Handbook of Experimental Pharmacology*, Vol. LIV. ed. Szekeres, L. pp. 735–822, Berlin: Springer Verlag.

ROSS, G. & JORGENSEN, C.R. (1970). Effect of a cardioselective beta-adrenergic blocking agent on the heart and coronary circulation. *Cardiovasc. Res.*, **4,** 148–153.

TODA, N. & HAYASHI, S. (1982). Responses of canine coronary arteries to transmural electrical stimulation and nicotine. *Eur. J. Pharmac.*, **80,** 73–81.

VATNER, S.F., HINTZE, T.H. & MACHO, P. (1982). Regulation of large coronary arteries by β-adrenergic mechanisms in the conscious dog. *Circulation Res.*, **51,** 56–66.

WANSTALL, J.C. & O'DONNELL, S.R. (1982). A dog isolated blood vessel preparation with only β_1-adrenoceptors. *Proc. Aust. Physiol. Pharmac. Soc.*, **13,** 162P.

WINBURY, M.M., HOWE, B.B. & HEFNER, M.A. (1969). Effect of nitrates and other coronary dilators on large and small coronary vessels: an hypothesis for the mechanism of action of nitrates. *J. Pharmac. exp. Ther.*, **168,** 70–95.

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