Br. J. Pharmac. (1979), **67**, 553–561

EFFECTS OF UPTAKE INHIBITORS ON RESPONSES OF SHEEP CORONARY ARTERIES TO CATECHOLAMINES AND SYMPATHETIC NERVE STIMULATION

F. BRINE[1], E.J. CORNISH & R.C. MILLER

Department of Pharmacology, Victorian College of Pharmacy, Parkville, Victoria, 3052, Australia

1 Transmural stimulation of intrinsic sympathetic nerves and exogenous catecholamines produce β_1-adrenoceptor mediated relaxant responses in strips of contracted sheep coronary artery.

2 The neuronal uptake inhibitors, metaraminol, cocaine and desipramine and the extraneuronal uptake inhibitor, cortisol, failed to potentiate responses to noradrenaline or sympathetic stimulation; responses to isoprenaline were enhanced by cortisol.

3 Oxytetracycline, which inhibits binding to connective tissue fibres, did not affect responses to noradrenaline or nerve stimulation.

4 17β-Oestradiol, caffeine and U0521 proved to be unsuitable compounds for studying catecholamine inactivation since they non-selectively potentiated responses to noradrenaline and isoprenaline.

5 It is concluded that catecholamine inactivation processes do not modify transmitter function in sheep coronary arteries.

Introduction

Coronary arteries receive a rich supply of sympathetic nerves which terminate at the adventitial-medial border (De la Lande, Harvey & Holt, 1974; Denn & Stone, 1976). The nerve terminals are separated from vascular smooth muscle cells by a large synaptic cleft reported as being between 40 and 700 nm wide (Lever, Mumtazuddin & Irvine, 1965; Malor, Griffin & Taylor, 1973). In addition to the potential neuronal uptake sites for catecholamines, fluorescence histochemistry indicates the presence of extraneuronal uptake and connective tissue binding sites (Clarke, Jones & Linley, 1969; Gillespie & Muir, 1970; De la Lande *et al.*, 1974). Both neuronal and extra-neuronal uptake of catecholamines have been demonstrated in isolated kitten coronary arteries by the use of radioactively labelled noradrenaline and isoprenaline respectively. Binding of isoprenaline to connective tissue was also found at a high amine concentration (Cornish, Goldie & Miller, 1978). The importance of these inactivation processes in modifying catecholamine responses in coronary arteries has only been studied previously with exogenous amines. There is general agreement that the neuronal uptake inhibitor, cocaine, does not sensitize isolated coronary arteries to noradrenaline (Nishioka, 1971; Kalsner, 1974; De la Lande *et al.*, 1974; Cornish *et al.*, 1978). Results with extraneuronal uptake inhibitors are equivocal. Potentiation of amine responses have been observed in coronary arteries of cattle (Kalsner, 1974; Kalsner, Frew & Smith, 1975) but not in those from rabbits and cats (De la Lande *et al.*, 1974; Cornish *et al.*, 1978).

The aim of the present investigation was to examine whether proven inhibitors of catecholamine uptake in the kitten coronary artery altered responses of sheep coronary arteries to exogenous catecholamines and sympathetic nerve stimulation.

Methods

The anterior descending branch of the left coronary artery was dissected from hearts of freshly slaughtered sheep and opened longitudinally. Incomplete cuts were made across the arterial sheet from alternate sides, creating a zig-zag strip in which any circularly arranged muscle fibres would have become orientated in an approximately longitudinal direction. This method has previously been described for guinea-pig tracheal preparations (Mylecharane & Raper, 1973). The tissue was placed under a tension of 0.5 g and bathed in oxygenated McEwen solution (1956) at 32°C. Changes in tension were monitored with a Grass force-displacement transducer (FT03C) coupled to a Grass model 79 polygraph. In experiments where

[1] Present address: Pharmacology Department, Commonwealth Serum Laboratories, Parkville, Victoria 3052, Australia.

0007-1188/79/120553-09 $01.00

responses to exogenous catecholamines were monitored, the tissue was set up in an organ bath and where responses to transmural stimulation were assessed, the preparation was suspended between platinum wire electrodes and superfused with the physiological salt solution at a rate of 3 ml/min.

In both types of preparation acetylcholine (5.5 μM) was used as a spasmogen and the relaxant effects of catecholamines or sympathetic nerve stimulation were monitored in the absence and presence of compounds known to influence catecholamine inactivation processes. A 30 min equilibration period to such compounds was allowed before re-establishment of adrenergic responses. Comparisons were made of the concentrations of catecholamines or frequencies of stimulation required to produce 20, 50 or 80% maximal relaxation (E_{max}) before and after a test compound.

The effects of noradrenaline and nerve stimulation were monitored in the presence of both neuronal and extraneuronal inhibitors. With the latter inhibitors, responses to isoprenaline were also assessed since unlike noradrenaline, isoprenaline is only a transportable substrate for the extraneuronal uptake process (Callingham & Burgen, 1966).

Drugs

The drugs used were acetylcholine chloride, (−)-adrenaline bitartrate, adenosine, caffeine, (±)-isoprenaline hydrochloride, (−)-noradrenaline bitartrate, 17β-oestradiol, oxytetracycline hydrochloride (Sigma Chemical Co.); cortisol sodium succinate (Glaxo), cocaine hydrochloride (May & Baker); desipramine hydrochloride, guanethidine sulphate, phentolamine mesylate (Ciba); dipyridamole, hexobendine dihydrochloride (Boehringer Ingelheim); metaraminol bitartrate (MSD); 3-methoxyisoprenaline (synthesized by Victorian College of Pharmacy); phenoxybenzamine hydrochloride (SKF); practolol hydrochloride, propranolol hydrochloride (ICI); tetrodotoxin (Calbiochem) and U0521 (3,4-dihydroxy-2-methylpropiophenone, Upjohn).

Concentrations of salts are expressed in terms of the base.

Results

Responses to catecholamines

Noradrenaline, adrenaline and isoprenaline, 0.1 to 1 μM, did not alter the basal tone of arterial strips but relaxed preparations previously contracted with acetylcholine. No significant differences were found between the maximal relaxant effects or the slopes of the concentration-response curves to the three amines. Preparations from different sheep differed considerably in their sensitivity to the catecholamines but the relative potencies of the three amines were much less variable. Molar potencies relative to (−)-noradrenaline (= 1) based on EC_{50} values were, for (−)-adrenaline 0.48 ± 0.13 and for (±)-isoprenaline 20.0 ± 4.3 (n = 6).

Relaxations were inhibited by propranolol (3 μM). Neural stimulation did not produce any contractile responses in the presence of the β-adrenoceptor antagonists, although such responses were demonstrated with high concentrations of exogenous noradrenaline (>2 μM). These were presumably due to stimulation of α-adrenoceptors since they were inhibited by phentolamine (3 μM) (Figure 1). The K_B for practolol (1.88 μM) using noradrenaline as the agonist was 0.13 ± 0.04 μM (n = 5).

Responses to transmural stimulation

Transmural stimulation at 40 V with 1 min trains of pulses of 0.5 to 1 ms duration did not affect the basal tone of the preparation. However, frequency-dependent relaxations were obtained in arteries contracted with acetylcholine (Figure 2). Maximal relaxations were obtained with frequencies between 10 and 20 Hz. Peak relaxations occurred 30 to 60 s after starting transmural stimulation and tone was rapidly regained when stimulation ceased. The relaxations were abolished following the addition of tetrodotoxin (2.9 μM), propranolol (3 μM) or guanethidine (20 μM) to the bathing fluid and were absent in tissues which had been stored at 6°C for 24 h. The relaxations were unaffected by dipyridamole (1 μM) or hexobendine (1 μM) although both of these drugs potentiated the relaxant effects of adenosine (0.5 μM) on the preparation. The responses to transmural stimulation were therefore presumed to be due to excitation of intrinsic sympathetic nerves.

In acetylcholine-contracted preparations, stimulation with wider pulse widths (2 or 4 ms) and high frequencies (20 and 40 Hz) produced relaxations which could not be completely inhibited or were unaffected by tetrodotoxin, adrenergic antagonists or cold storage. These relaxations, therefore appear to involve a non-neural mechanism.

In experiments described below, pulse widths of 0.5 or 1 ms and frequencies up to 20 Hz were used in order to produce only sympathetically mediated responses.

Effects of uptake$_1$ inhibitors

Neuronal uptake inhibitors either did not alter, or inhibited rather than potentiated, dilator responses to noradrenaline and nerve stimulation. Metaraminol (1 μM) and cocaine (3 or 30 μM) produced non-significant

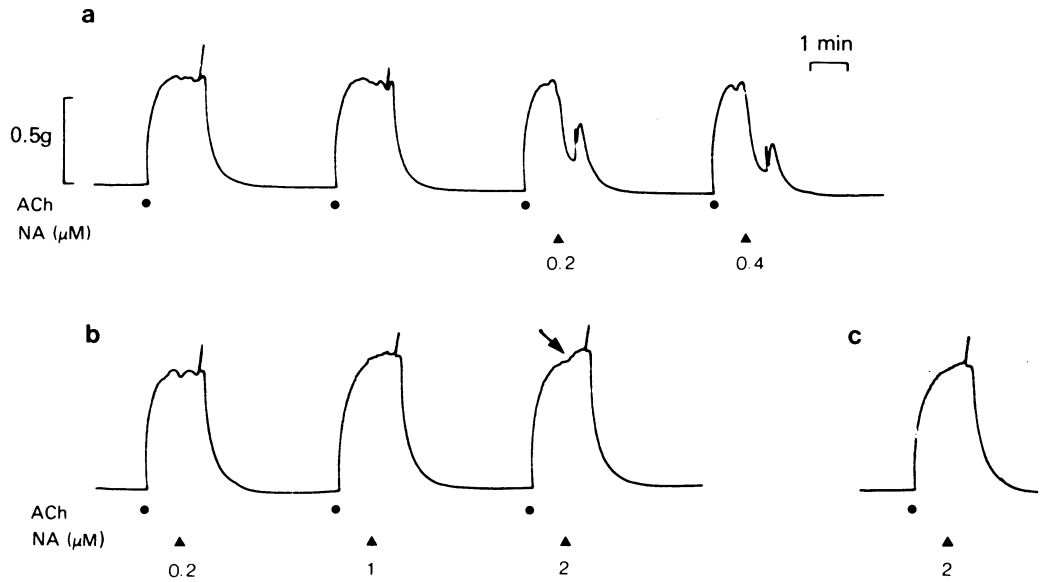

Figure 1 (a) Contractions of sheep coronary artery produced by acetylcholine, 5.5 μM (ACh) and superimposed relaxations produced by noradrenaline (NA). (b) Inhibition of relaxant responses to noradrenaline by propranolol, 3 μM, revealing a small contractile response to noradrenaline, 2 μM (at arrow). (c) Inhibition of this contractile response by phentolamine, 3 μM.

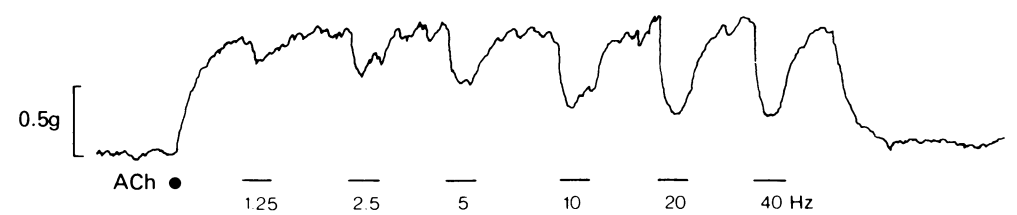

Figure 2 Relaxations of sheep coronary artery produced by different frequencies of transmural stimulation superimposed upon a contractile response to acetylcholine 5.5 μM (ACh). Horizontal bars indicate 1 min trains of pulses of 1 ms duration.

parallel shifts to the right of noradrenaline concentration-response curves (Table 1). Desipramine (0.1 μM) altered the gradient of the concentration-response line ($0.01 > P > 0.005$, paired t test) such that a shift to the right was produced at low noradrenaline concentrations. At a higher concentration, desipramine (1 μM) did not significantly affect noradrenaline responses. Neither desipramine (0.1 or 1 μM) nor cocaine (3 or 30 μM) significantly affected responses to sympathetic nerve stimulation (Table 1).

Effects of uptake$_2$ inhibitors

Extraneuronal uptake inhibitors produced a variety of changes in the sensitivity of arterial strips to catecholamines and nerve stimulation. Cortisol (40 μM) did not significantly alter the relaxations produced by noradrenaline or nerve stimulation but increased sensitivity to isoprenaline (Figure 3). The dose-ratio for isoprenaline based on EC_{50} values was 4.2 ± 0.9 ($n = 4$) (Table 1). No selective potentiation was found with 17β-oestradiol (18 or 37 μM). It increased sensitivity to noradrenaline 3.5 to 4 fold and to nerve stimulation about 2.5 fold (Figure 4, Table 1). In addition there was a significant increase in the slope of the concentration- and frequency-response curves. There was little difference between the effects produced by the two concentrations of 17β-oestradiol so the effects of isoprenaline were only tested with the lower concentration of the steroid. This concentration

increased sensitivity to isoprenaline (dose-ratio = 4.4 ± 0.7, $n = 8$) without changing the gradient of the concentration-response line (Figure 4).

3-Methoxyisoprenaline (1.34 μM) significantly inhibited responses to noradrenaline and was therefore unsuitable for analysis of uptake mechanisms. At the concentration used the depressant action of the compound is probably due to its weak β-adrenoceptor blocking activity (Bassett, 1971).

Caffeine (80 μM) did not alter relaxations to either noradrenaline or isoprenaline. However, at a very high concentration (500 μM) it produced parallel shifts to the left in the concentration-response lines for both catecholamines without significantly altering responses to nerve stimulation (Figure 5, Table 1). The dose-ratio for noradrenaline was 4.7 ± 1.1 ($n = 7$) and for isoprenaline was 3.7 ± 0.5 ($n = 6$).

Phenoxybenzamine (0.1 μM) did not affect responses to noradrenaline. Unlike the other compounds tested, higher concentrations of phenoxybenzamine inhibited the spasmogenic activity of aetylcholine. The effects of the drug on superimposed relaxations to catecholamines could therefore not be adequately assessed.

Effect of the catechol-O-methyltransferase (COMT) inhibitor, U0521

U0521 (60 or 120 μM) increased sensitivity to noradrenaline and also increased the slopes of the concentration-response curves ($P < 0.02$, paired t test) (Figure 6). The dose-ratios based on EC_{50} values were 2.1 ± 0.5, $n = 12$ and 3.4 ± 0.3, $n = 9$ respectively; U0521 (60 μM) also increased sensitivity to isoprenaline and caused an increase in the slope of the concentration-response line which was of borderline statistical significance ($0.1 > P > 0.05$, paired t test). The dose-ratio for EC_{50} values was 2.7 ± 0.6 ($n = 7$). In contrast, U0521 (120 μM) did not significantly change the frequency of stimulation required to produce a relaxation of 50% E_{max} although it increased the slope of the frequency-response line ($0.05 > P > 0.02$, paired t test, $n = 5$).

Effect of oxytetracycline

Oxytetracycline (100 μM), which was reported by Powis (1973) to inhibit binding of catecholamines to

Table 1 Effects of uptake inhibitors on dilator responses to noradrenaline (NA), isoprenaline (Iso) or nerve stimulation in sheep coronary arteries expressed in terms of the ratio of values obtained in the absence and presence of inhibitors at 50% E_{max}

Inhibitor	*Conc.* (μM)	*Ratios at* 50% E_{max} *NA*	*Iso*	*Hz*
Metaraminol	1	0.7 ± 0.1† (18)		
Cocaine	3	0.6 ± 0.3† (9)		1.4 ± 0.2 (5)
	30	0.2 ± 0.05† (4)		0.90 ± 0.2 (4)
Desipramine	0.1	0.7 ± 0.1* (7)		1.1 ± 0.2 (5)
	1	1.1 ± 0.3 (7)		1.1 ± 0.2 (4)
Cortisol	40	1.8 ± 0.5 (7)	4.2 ± 0.9† (4)	1.2 ± 0.5 (6)
17β-Oestradiol	18	3.6 ± 0.8*† (12)	4.4 ± 0.7† (8)	2.5 ± 0.2*† (6)
	37	3.9 ± 1.2*† (7)		2.4 ± 0.3*† (6)
3-Methoxy-isoprenaline	1.3	0.3 ± 0.05† (4)		
Caffeine	80	1.6 ± 0.4 (3)	1.5 ± 0.6 (4)	
	500	4.7 ± 1.1† (7)	3.7 ± 0.5† (6)	1.1 ± 0.1 (5)
Phenoxybenzamine	0.1	1.6 ± 0.2 (4)		

Values are shown together with s.e. mean. Numbers in parentheses indicate number of experiments.
* Indicates a significant change in the gradient of the concentration-response line and or frequency-response line;
† Significant increases or decreases in sensitivity ($P < 0.05$, paired t test).

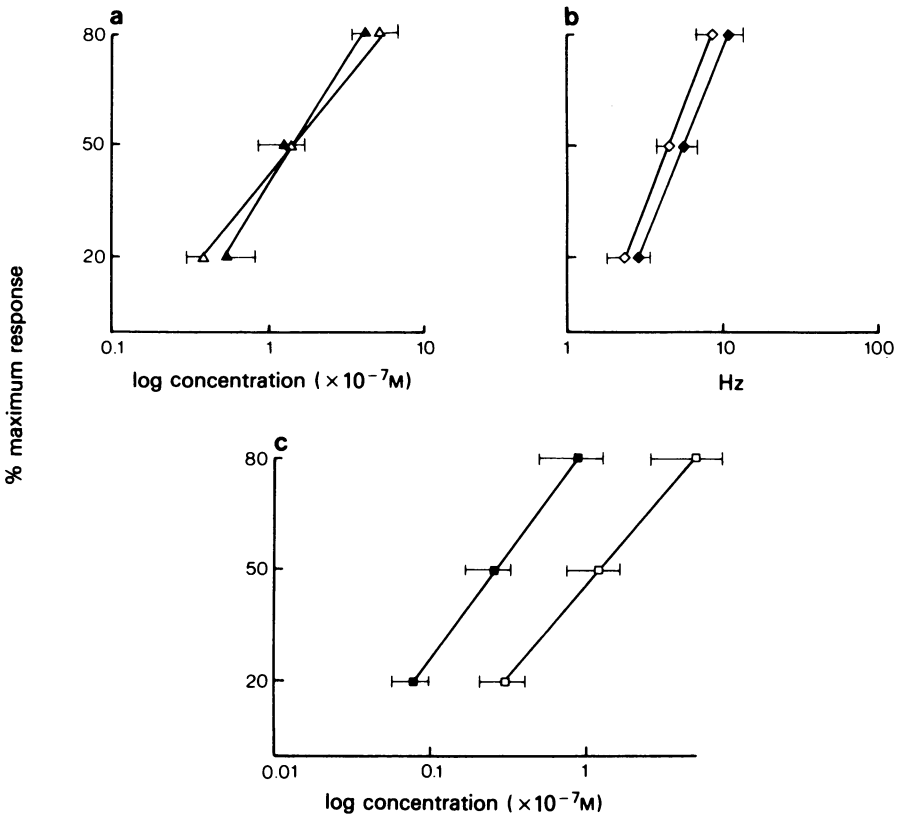

Figure 3 Concentration-response lines (a, c) and frequency-response lines (b) for dilator responses to noradrenaline (a), isoprenaline (c) and nerve stimulation (b) in untreated sheep coronary arteries (open symbols) and in arteries exposed to cortisol 40 μM (closed symbols). Horizontal lines represent s.e. mean.

connective tissues, had no significant effect on noradrenaline-induced relaxations of sheep coronary arteries ($n = 5$) nor did it affect responses to nerve stimulation ($n = 5$).

Discussion

The sheep coronary artery cut into a zig-zag strip and constricted with acetylcholine proved to be a very simple preparation for studying responses to transmural stimulation of the sympathetic nerves. Previous attempts to stimulate such nerves in isolated coronary arteries have generally been unsuccessful (Nishioka, 1971; Toda, 1975); however, more recently it has been reported that adrenergic responses can be elicited in the isolated left descending coronary artery of the dog when stimulating electrodes are placed close to the aortic root (Borda, Schuchleib & Henry, 1977).

Two types of experiment with catecholamines indicated that there is a predominance of β_1-adrenoceptors in the isolated coronary artery of the sheep. Firstly, comparison of agonist potency ratios gave a rank order for coronary relaxant activity of isoprenaline > noradrenaline > adrenaline which is consistent with the presence of β_1-type receptors (Furchgott, 1972). Secondly the K_B value (0.13 μM) found for practolol (1.88 μM) using noradrenaline as agonist is of the same order as that previously obtained for inotropic responses to isoprenaline in kitten atria (0.28 μM) (Cornish & Miller, 1974). Atria are generally assumed to contain mainly β_1-type receptors. In isolated coronary arteries from other species β_1-adrenoceptors also appear to predominate (Baron, Speden & Bohr, 1972; Drew & Levy, 1972; Cornish & Miller, 1974). In the sheep coronary artery the presence of α-adrenoceptors could only be demonstrated with high concentrations of noradrenaline. The neuronal

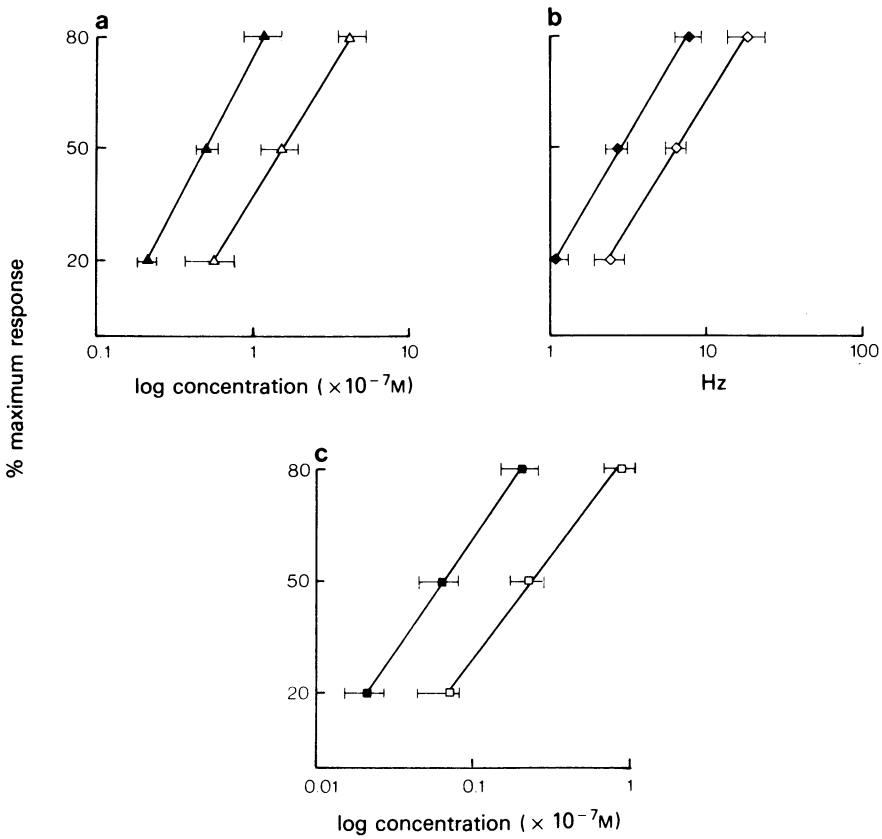

Figure 4 Concentration-response lines (a, c) and frequency-response lines (b) for dilator responses to noradrenaline (a), isoprenaline (c) and nerve stimulation (b) in untreated sheep coronary arteries (open symbols) and in arteries exposed to 17β-oestradiol 18 μM (closed symbols). Horizontal lines represent s.e. mean.

uptake inhibitors metaraminol, cocaine and desipramine all failed to potentiate responses to noradrenaline and nerve stimulation. Phenoxybenzamine could not be tested at an effective concentration for inhibition of neuronal (or extraneuronal) uptake since it markedly reduced responses to the spasmogen, acetylcholine. The present results support observations on cattle, rabbit and pig coronary arteries that cocaine does not alter responses to noradrenaline (Nishioka, 1971; Kalsner, 1974; De La Lande *et al.*, 1974).

Sensitization of tissues to noradrenaline by neuronal uptake inhibitors seems to be inversely related to the size of the neuroeffector junction (Verity, 1971). In coronary arteries large synaptic clefts, often >100 nm have been reported (Lever *et al.*, 1965; Malor *et al.*, 1973). Thus the results in coronary arteries with neuronal uptake inhibitors are, perhaps, not surprising.

In a variety of species extraneuronal uptake sites have been observed in coronary arteries by fluorescence histochemistry (Clarke *et al.*, 1969; Gillespie & Muir, 1970; De la Lande *et al.*, 1974). In the sheep coronary artery the extraneuronal uptake inhibitor, cortisol, selectively potentiated responses to isoprenaline. This steroid has previously been found to inhibit isoprenaline, but not noradrenaline accumulation in kitten coronary arteries and atria and to potentiate atrial responses to isoprenaline but not to noradrenaline or adrenaline (Kaumann, 1972; Cornish *et al.*, 1978). The results thus indicate that extraneuronal uptake sites are present in the sheep coronary artery but that these do not significantly influence responses to neurally released or exogenous noradrenaline.

17β-Oestradiol is known to be a weak neuronal as well as an extraneuronal uptake inhibitor (Iversen & Salt, 1970; Cornish *et al.*, 1978). In view of the

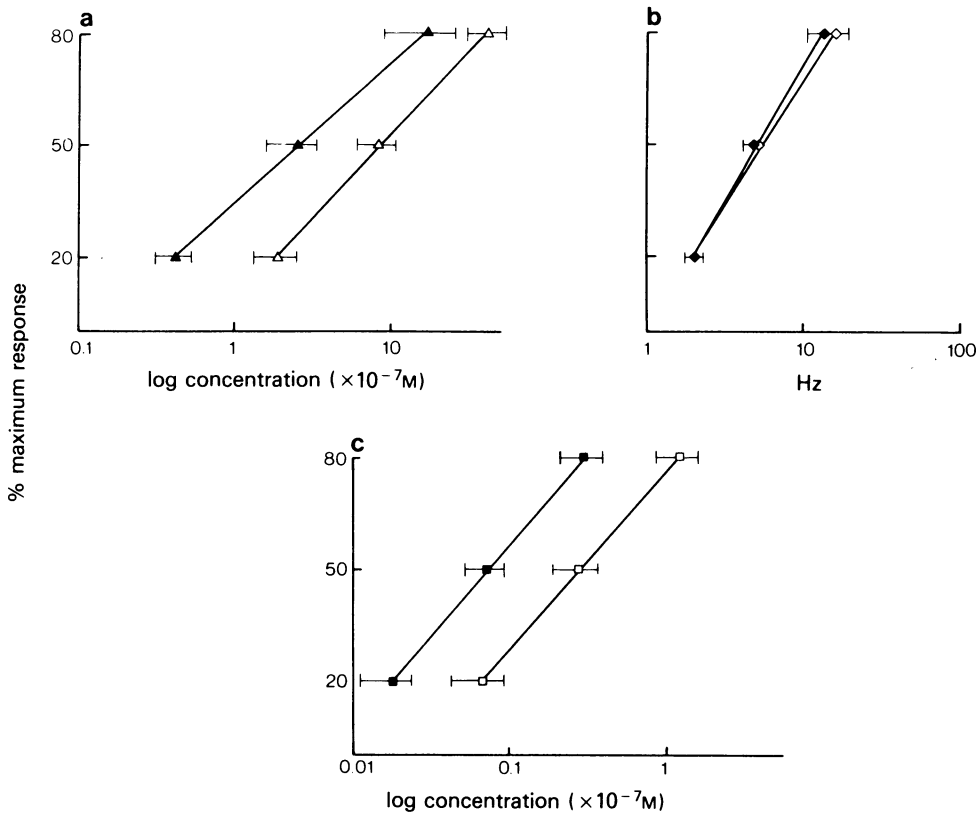

Figure 5 Concentration-response lines (a, c) and frequency-response lines (b) for dilator responses to noradrenaline (a), isoprenaline (c) and nerve stimulation (b) in untreated sheep coronary arteries (open symbols) and in arteries exposed to caffeine 500 μM (closed symbols). Horizontal lines represent s.e. mean.

results obtained with the more potent extraneuronal inhibitor cortisol and the lack of effect of other neuronal inhibitors it was expected that 17β-oestradiol would enhance responses to isoprenaline but not affect those to noradrenaline or nerve stimulation. The finding that the steroid produced an increase in sensitivity to noradrenaline, isoprenaline and nerve stimulation suggests that in addition to its effects on extraneuronal uptake 17β-oestradiol either sensitizes β-adrenoceptor mediated effects in the artery or non-selectively enhances relaxant responses. The effects found with 17β-oestradiol in the sheep coronary artery were not observed in perfused kitten coronary artery preparations (Cornish *et al.*, 1978) but if they do occur in other species the enhanced responses to noradrenaline found in cattle coronary arteries in the presence of 17β-oestradiol (Kalsner, 1974) may be unrelated to uptake inhibition.

Caffeine (80 μM) was an effective inhibitor of extraneuronal uptake in tracer experiments with the kitten coronary artery (Cornish *et al.*, 1978) but this concentration did not significantly alter responses of the sheep coronary artery to isoprenaline (or noradrenaline). This could be due to species differences in its potency as an inhibitor; however, in the presence of a higher concentration of caffeine (500 μM) responses to both noradrenaline and isoprenaline were enhanced while those to nerve stimulation were unaffected. Kalsner *et al.* (1975) reported that this concentration of caffeine increased responses of coronary arteries of cattle to noradrenaline, an effect they attributed to block of extraneuronal uptake. This is unlikely to be the sole explanation for the results obtained in the present experiments since caffeine's effects are clearly different from those of the selective extraneuronal uptake inhibitor cortisol which only in-

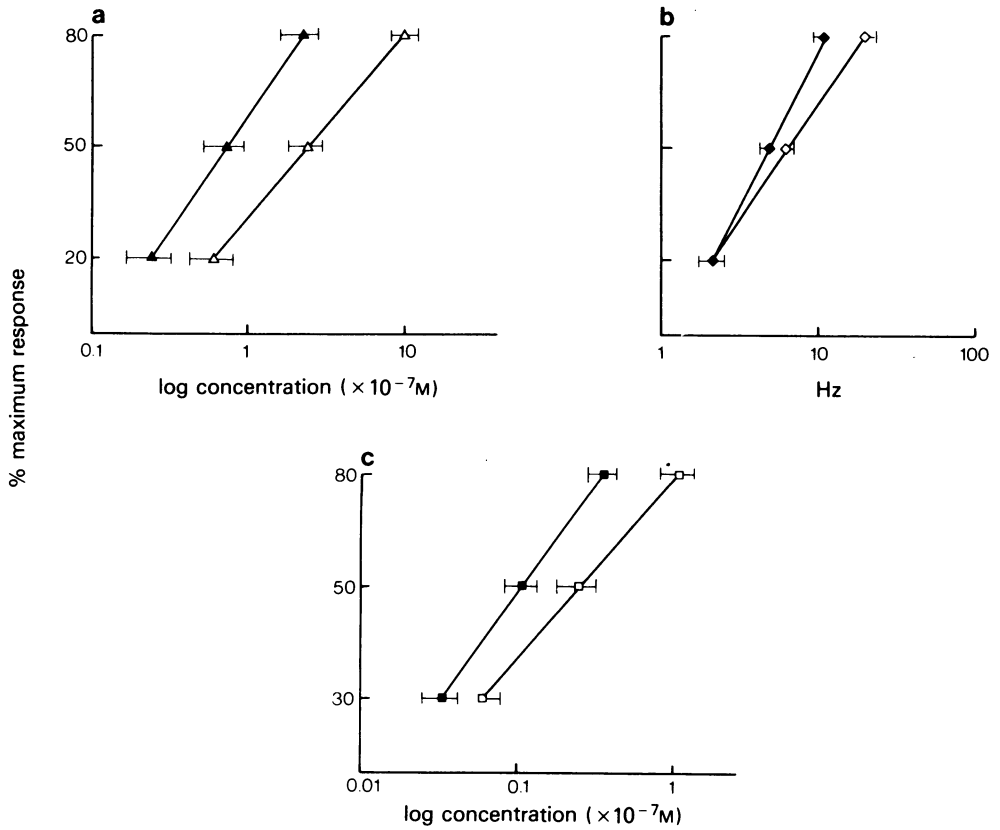

Figure 6 Concentration-response lines (a, c) and frequency-response lines (b) for dilator responses to noradrenaline (a), isoprenaline (c) and nerve stimulation (b) in untreated sheep coronary arteries (open symbols) and in arteries exposed to U0521 60 μM (c) or 120 μM (a, b) (closed symbols). Horizontal lines represent s.e. mean.

creased responses to isoprenaline. If caffeine's effects were due to phosphodiesterase inhibition it would have been expected to potentiate responses to nerve stimulation as well as to catecholamines because all three responses are mediated via β-adrenoceptors.

Since extraneuronal uptake is closely associated with *O*-methylation of amines in many tissues we examined the effects of the COMT inhibitor, U0521. It increased responses to isoprenaline and noradrenaline but only increased responses to high frequencies of nerve stimulation. The enhancement of catecholamine responses confirms similar observations made in cattle and rabbit coronary arteries (De la Lande *et al.*, 1974; Kalsner *et al.*, 1975), although in pig coronary arteries another COMT inhibitor, pyrogallol, was inactive (Nishioka, 1971).

U0521 is not only a COMT inhibitor; in the rat heart and kitten coronary artery it acts as an extraneuronal uptake inhibitor (Bönisch, Uhlig & Trendelenburg, 1974; Cornish & Goldie, 1978). However, its effects in the sheep coronary artery are clearly different from those of the selective extraneuronal uptake inhibitor cortisol. Several interpretations can be placed on the results obtained in sheep coronary arteries. Firstly *O*-methylation may only be important when relatively high concentrations of amine are present in the tissue. This could account for the selective effect of U0521 observed on responses produced by high frequency stimulation and on its effect on responses to the exogenous amines. Secondly in this artery, *O*-methylation and extraneuronal uptake may not be linked. Thirdly the effect of U0521 may not reflect its activity as an enzyme inhibitor or extraneuronal uptake inhibitor.

In addition to catecholamine inactivation by uptake processes and enzymatic metabolism, binding can occur to various tissues such as cell and basement membranes and connective tissue. Such binding is in-

sensitive to uptake inhibitors. However, it has been shown that catecholamine binding to pure samples of collagen and elastin can be inhibited by tetracyclines such as oxytetracycline (Powis, 1973). In the sheep coronary artery the failure of oxytetracycline to modify responses to nerve stimulation indicates that such binding does not limit transmitter activity.

In conclusion, in the sheep coronary artery responses to noradrenaline and nerve stimulation are not modified by neuronal or extraneuronal uptake or by binding of noradrenaline to connective tissue fibres while extraneuronal uptake reduces responses to isoprenaline.

The authors wish to thank Professor C. Raper for criticism of the manuscript and Glaxo for a gift of cortisol sodium succinate. The work was supported by a grant-in-aid from the National Heart Foundation of Australia. Reprint requests to E.J.C. please.

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.

BASSETT, J.R. (1971). β-Adrenoceptor antagonist activity of 3-methoxyisoprenaline. *Br. J. Pharmac.*, **41**, 113–121.

BÖNISCH, H., UHLIG, W. & TRENDELENBURG, U. (1974). Analysis of the compartments involved in the extraneuronal storage and metabolism of isoprenaline in the perfused heart. *Naunyn-Schmiedebergs Arch. Pharmac.*, **283**, 223–244.

BORDA L., SCHUCHLEIB, R. & HENRY P.D. (1977). Effects of potassium on isolated canine coronary arteries. Modulation of adrenergic responsiveness and release of norepinephrine. *Circulation Res.*, **41**, 778–786.

CALLINGHAM G.A. & BURGEN A.S. (1966). The uptake of isoprenaline and noradrenaline by the perfused rat heart. *Molec. Pharmac.*, **2**, 37–42.

CLARKE, D.E., JONES, C.J. & LINLEY, P.A. (1969). Histochemical fluorescence studies on noradrenaline accumulation by uptake$_2$ in the isolated rat heart. *Br. J. Pharmac.*, **37**, 1–9.

CORNISH, E.J. & GOLDIE R.G. (1978). Extraneuronal uptake and O-methylation by isolated kitten atria and coronary arteries. *Proc. 7th Int. Congr. Pharmac.*, (in press).

CORNISH, E.J., GOLDIE, R.G. & MILLER, R.C. (1978). Catecholamine uptake by isolated coronary arteries and atria of the kitten. *Br. J. Pharmac.*, **63**, 445–456.

CORNISH, E.J. & MILLER, R.C. (1974). Comparison of the β-adrenoceptors in the myocardium and coronary vasculature of the kitten heart. *J. Pharm. Pharmac.*, **28**, 23–30.

DE LA LANDE, I.S., HARVEY, J.A. & HOLT, S. (1974). Response of the rabbit coronary arteries to autonomic agents. *Blood Vessels*, **11**, 319–337.

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

DREW, G.M. & LEVY, G.P. (1972). Characterisation of the coronary vascular beta-adrenoceptor in the pig. *Br. J. Pharmac.*, **46**, 348–350.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines. Handb. exp. Pharmac.* N.S. Vol. 33, ed. Blaschko, H. & Muscholl, E. pp. 283–335. Berlin and Heidelberg: Springer-Verlag.

GILLESPIE, J.S. & MUIR, T.C. (1970). Species and tissue variation in extraneuronal and neuronal accumulation of noradrenaline. *J. Physiol.*, **206**, 591–604.

IVERSEN, L.L. & SALT, P.J. (1970). Inhibition of catecholamine uptake$_2$ by steroids in the isolated rat heart. *Br. J. Pharmac.*, **40**, 528–530.

KALSNER, S. (1974). Sensitisation 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.

KAUMANN, A.J. (1972). Potentiation of the effects of isoprenaline and noradrenaline by hydrocortisone in cat heart muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **273**, 134–153.

LEVER, J.D., MUMTAZUDDIN, A. & IRVINE, G.J. (1965). Neuromuscular and intercellular relationships in the coronary arterioles. A morphological and quantitative study by light and electron microscopy. *J. Anat.*, **99**, 829–840.

MCEWEN, L.M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol.*, **131**, 678–689.

MALOR, R., GRIFFIN, C.J. & TAYLOR, S. (1973). Innervation of the blood vessels in guinea-pig atria. *Cardiovascular Res.*, **7**, 95–104.

MYLECHARANE, E.J. & RAPER, C. (1973). Influence of N-alkyl substitution on antagonism at β_1- and β_2-receptor sites. *Eur. J. Pharmac.*, **21**, 375–378.

NISHIOKA, M. (1971). Pharmacological responses of the smooth muscle of the pig's excised coronary artery, especially in relation to Ca. *Kobe J. med. Sci.*, **17**, 129–159.

POWIS, G. (1973). Binding of catecholamines to connective tissue and the effect upon the response of blood vessels to noradrenaline and to nerve stimulation. *J. Physiol.*, **234**, 145–162.

TODA, N. (1975). Regional differences in the response to nicotine in isolated canine arteries. *Eur. J. Pharmac.*, **35**, 151–160.

VERITY, M.A. (1971). Morphologic studies of the vascular neuro-effector apparatus. In *Physiology and Pharmacology of Vascular Neuroeffector Systems.* ed. Bevan, J.A., Furchgott, R.F., Maxwell, R.A. & Somlyo, A.P. pp. 2–12. Basel: S. Karger.

(Received December 4, 1978.)