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CATECHOLAMINE UPTAKE BY ISOLATED CORONARY ARTERIES AND ATRIA OF THE KITTEN

E.J. CORNISH, R.G. GOLDIE & R.C. MILLER

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

1 Kitten coronary arteries and atria incubated with [^{3}H]-(−)-noradrenaline or [^{3}H]-(±)-isoprenaline showed concentration- and temperature-dependent accumulation of amines (unchanged and/or as metabolites). In addition, coronary arteries incubated with [^{3}H]-(±)-isoprenaline showed temperature-insensitive amine accumulation due to binding to connective tissue fibres.

2 With low concentrations of [^{3}H]-(−)-noradrenaline (20 ng/ml) accumulation of radioactivity in both tissues was neuronal since it was reduced by desipramine, metaraminol or cocaine but not by the extraneuronal inhibitor, cortisol.

3 In coronary arteries incubated with [^{3}H]-(±)-isoprenaline (5 μg/ml) for 1 or 10 min, uptake was extraneuronal since it was inhibited by cortisol, 17β-oestradiol or phenoxybenzamine.

4 Atria incubated with (±)-isoprenaline (5 to 1000 μg/ml) showed non-neuronal, biphasic accumulation of amine. There was a high capacity, low affinity initial uptake process (apparent K_m 136 μM) which was resistant to steroidal extraneuronal uptake inhibitors but which could be abolished by phenoxybenzamine. A slower uptake occurred after 2 min which was sensitive to steroidal and other extraneuronal uptake inhibitors.

5 Inhibition of uptake processes did not alter sensitivity of coronary arteries to dilator effects of catecholamines. However, experiments with extraneuronal uptake inhibitors were limited by the relaxant effects of cortisol and 17β-oestradiol themselves.

6 In atria inhibition of neuronal uptake by desipramine or cocaine led to supersensitivity to (−)-noradrenaline but not to (−)-adrenaline or (±)-isoprenaline. Chronotropic responses to (−)-noradrenaline were increased five fold and inotropic responses three fold.

7 Inhibition of extraneuronal uptake led to selective supersensitivity to (±)-isoprenaline. Cortisol increased chronotropic responses ten fold and inotropic responses three fold, while 17β-oestradiol produced less than two fold increases in sensitivity to isoprenaline.

Introduction

In perfused rat and guinea-pig hearts noradrenaline, in low concentrations, is mainly inactivated by neuronal uptake (Iversen, 1963) while isoprenaline is removed into two extraneuronal compartments (Bönisch & Trendelenburg, 1974). The first, rapidly effluxing extraneuronal compartment, is the major *O*-methylating site and is able to accumulate unchanged or *O*-methylated amines. The second compartment is of similar size but can only store unchanged amines (Bönisch, Uhlig & Trendelenburg, 1974; Uhlig, Fiebig & Trendelenburg, 1976). It has been suggested that the *O*-methylating compartment may reside in the coronary vasculature (Bönisch *et al.*, 1974). This idea was based on the observation that in rat ventricle slices, accumulation of isoprenaline was one quarter, and total formation of 3-methoxyisoprenaline was one fiftieth that shown by perfused hearts. However, little is known about catecholamine inactivation by the coronary vessels. Studies employing fluorescence histochemistry indicate that neuronal uptake can occur in the adrenergic plexus of the adventitial-medial border and that, in addition, there is extraneuronal uptake and temperature-insensitive connective tissue binding of amines (Gillespie & Muir, 1970; De la Lande, Harvey & Holt, 1974). On the other hand, responses to noradrenaline in pig, rabbit or ox coronary arteries are not affected by the neuronal uptake inhibitor, cocaine (Nishioka, 1971; De la Lande *et al.*, 1974; Kalsner, 1974) and, in rabbit coronary arteries, are not changed by extraneuronal inhibitors (De la Lande *et al.*, 1974). In contrast, in ox coronary arteries, extraneuronal in-

hibitors increase sensitivity to noradrenaline two to five fold and also inhibit [^{3}H]-(−)-noradrenaline accumulation (Kalsner, Frew & Smith, 1975).

The aim of the present study was to find out more about uptake processes in coronary arteries and to compare them with those in the myocardium of the same species. Isolated coronary arteries and atria of the kitten were used since these have been extensively studied in our laboratories (Cornish, Miller & Tolmer, 1974; Cornish & Miller, 1975; Goldie, 1976).

Methods

Kittens of either sex, weighing 500 to 1000 g, were anaesthetized with ether and the heart removed and continuously perfused by Langendorff's method with cold McEwen solution (1956) gassed with 5% CO_2 in O_2. The descending branches of the coronary arteries were dissected free from myocardial tissue. The atria were then removed.

Tracer studies

Left and right atria were cut in halves and these randomly allotted to control or treated groups. Arteries were cut open longitudinally to produce flat sheets and were then pooled to give samples weighing not less than 6 milligrams. Four or more atrial preparations or pooled arterial samples were used for each test group. Atria were placed under a resting tension of 2 g in organ baths containing 2 ml oxygenated McEwen solution at 37°C. Arteries were tied to a silver wire hook suspended in McEwen solution at 32°C and held upright by a stream of oxygen bubbles. Both types of preparation were allowed to equilibrate for 1 hour. In some experiments, they were then exposed to uptake inhibitors for 30 minutes. After this time tissues were incubated with [1-^{3}H]-(+)-sorbitol, [7-^{3}H]-(−)-noradrenaline or [7-^{3}H]-(±)-isoprenaline for periods of 1 to 40 minutes. The final sorbitol concentration for atria was 6 μg/ml (3.3×10^{-5} mol/l) (100 nCi/ml) and for arteries was 30 μg/ml (1.65×10^{-4} mol/l) (500 nCi/ml). Final bath concentrations were, noradrenaline 5 to 3200 ng/ml (0.03 to 18.9×10^{-6} mol/l), isoprenaline 0.5 to 1000 μg/ml (2.02×10^{-6} to 4.04×10^{-3} mol/l), equivalent to 100 nCi/ml for atria and 500 nCi/ml for arteries. Following incubation with tracer, atria were blotted dry and weighed while arteries were rinsed for 1 to 2 s with McEwen solution before drying and weighing. Tissues were placed in glass vials and digested for 2 h in 1 ml of NaOH 1 mol/l at 75°C. Fifteen ml of a 2:1 toluene:Triton X-100 mixture containing 0.4% PPO (Madsen, 1969) was added to each vial, followed by the addition of 100 μl of HCl (12 mol/litre). After this solution had clarified the radioactivity was determined in a Packard Liquid Scintillation Spectrometer model 3390. A 1 ml aliquot of each bath solution was also taken for estimation of radioactivity. Quench correction was employed using the automatic external standardization (A.E.S.) facility.

Pharmacological studies

Atrial preparations were placed under a resting tension of 2 g and contractions recorded with a Grass Force-Displacement Transducer (FTO3C) coupled to a Grass Model 79 Polygraph. Chronotropic activity was measured using a Grass tachograph model 7P4C. Spontaneously beating right atria were used to study the chronotropic effects while left atria driven at 4 Hz with a pulse width of 2 ms and supramaximal voltage were used to study inotropic effects. In control experiments, constant cumulative concentration-response curves to noradrenaline, adrenaline and isoprenaline were obtained. In experiments where uptake inhibitors were used, curves to noradrenaline were obtained before addition of the inhibitor and thereafter, curves to all three amines were established.

The anterior descending coronary artery was used to study dilator responses to amines. All side branches were ligated with fine strands teased from 2 metric Mersilk and the artery was then cannulated with a 23 gauge needle from which the tip had been removed. When perfused intraluminally the artery developed a resistance to perfusion (30 to 90 mmHg) (Cornish *et al.*, 1974). When this became constant, amines were either injected intraluminally in a dose volume of 0.05 ml or were added to the surrounding fluid i.e. were applied extraluminally. Changes in perfusion pressure were measured on a smoked drum with a mercury manometer.

Drugs

The drugs used were noradrenaline hydrochloride, adrenaline bitartrate, isoprenaline hydrochloride, normetanephrine hydrochloride, 17β-oestradiol, oxytetracycline hydrochloride, caffeine (Sigma Chem. Co.); cortisol sodium succinate (Glaxo); phenoxybenzamine hydrochloride (SKF); cocaine hydrochloride (M & B); desipramine hydrochloride (Ciba); metaraminol bitartrate (MSD); U0521 (Upjohn); practolol hydrochloride (ICI); 3-methoxyisoprenaline hydrochloride (synthesized Vic. College of Pharmacy); [7-^{3}H]-(−)-noradrenaline, [7-^{3}H]-(±)-isoprenaline hydrochloride and [1-^{3}H]-(+)-sorbitol (Radiochemical Centre, Amersham).

All radioactive amine concentrations are expressed in terms of the base.

Results

[^3H]-(+)-*sorbitol experiments*

Atria The rates of diffusion of [^{3}H]-sorbitol into left and right atria were similar (Table 1). Equilibrium with the extracellular fluid (ECF) at 37°C was assumed to be complete at 40 min giving an estimated ECF volume equal to 43% of the tissue mass and hence a derived cellular content of 0.57 g/g wet wt. atrial tissue. At shorter time intervals, [^{3}H]-sorbitol values were used to estimate the volume of the ECF into which [^{3}H]-noradrenaline or [^{3}H]-isoprenaline might be expected to diffuse and so to enable their mean concentration in the ECF at any time to be calculated. The catecholamines did not themselves alter [^{3}H]-sorbitol distribution. At 7°C, [^{3}H]-sorbitol diffused into the atria more slowly or into a smaller space than at 37°C.

Coronary arteries The thin walled coronary arteries equilibrated with [^{3}H]-sorbitol more rapidly than the atria (Table 1). The mean ECF volume estimate, based on the 2, 10 and 20 min [^{3}H]-sorbitol values at 32°C was 33% giving a derived cellular content of 0.67 g/g wet wt. vascular tissue. As with atria, [^{3}H]-sorbitol values were lower in arteries incubated at 7°C.

[^3H]-(−)-*noradrenaline experiments*

Atria Right atria incubated with [^{3}H]-noradrenaline, 5 to 1600 ng/ml, for 2 min or 20 ng/ml for 10 min accumulated 1.4 to 2.6 times more radioactivity than left atria (Figures 1 & 2). In both atria, accumulation at 2 min increased biphasically with increasing amine concentration in the incubation fluid (Figure 1) suggesting the existence of two separate uptake processes. Cellular: mean extracellular tritium concentrations >1 were found in right atria incubated with [^{3}H]-noradrenaline 5 to 800 ng/ml, and for left atria incubated with 5 ng/ml, indicating that there was active uptake of amine. This observation was supported by the finding that accumulation was

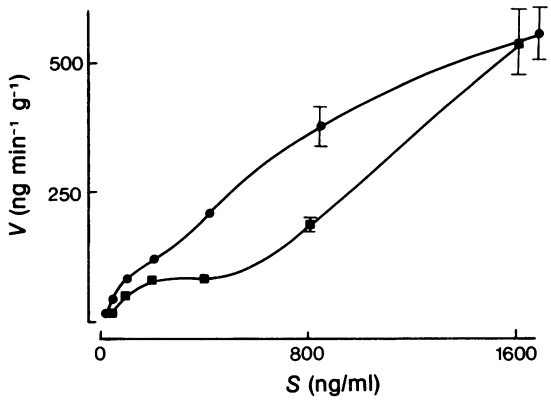

Figure 1 Relationship between the mean extracellular [^{3}H]-(−)-noradrenaline concentration (*S*) and the cellular uptake velocity (*V*) in kitten left (■) and right (●) atria following 2 min incubation with the amine. Each point is the mean of four experiments. The vertical bars show s.e. means.

exceptionally sensitive to temperature changes. After 10 min incubation with [^{3}H]-noradrenaline at 7°C, uptake was only about 5% of that at 37°C (Figure 2). There appeared to be little binding of amine to connective tissue fibres since oxytetracycline (100 μM), which inhibits such binding (Powis, 1973), did not reduce accumulation of radioactivity (Figure 2). Active uptake appeared to be neuronal since it was reduced 40 to 60% by desipramine (1 μM), metaraminol (1 μM) or cocaine (10 μM) (Figure 2). In contrast, incubation with the extraneuronal uptake inhibitors 3-methoxyisoprenaline (200 μM) or cortisol (80 μM) produced no inhibition of uptake. In fact, cortisol increased uptake in right atria by 77% ($P < 0.001$). Another extraneuronal uptake inhibitor, 17β-oestradiol (80 μM), did reduce uptake by 23% but this effect was only statistically significant in right atria ($0.05 > P > 0.02$) and was probably due to its weak inhibitory effect on neuronal rather than extraneuronal uptake (Iversen & Salt, 1970). Phenoxybenzamine, which inhibits both neuronal and extraneuronal uptake (Iversen, 1965), inhibited left atrial uptake by

Table 1 Mean sorbitol spaces (±s.e., $n = 4$) for atria and coronary arteries incubated at different temperatures

	Sorbitol spaces (μl/g)					
	Atria				*Coronary arteries*	
Time	*37°*		*7°*			
(min)	*left*	*right*	*left*	*right*	*32°*	*7°*
1	167 ± 11	186 ± 19	116 ± 3	118 ± 4	258 ± 28	183 ± 10
2	217 ± 3	227 ± 7	193 ± 3	191 ± 4	320 ± 11	—
10	395 ± 17	403 ± 7	323 ± 27	354 ± 11	319 ± 19	211 ± 1
20	417 ± 7	425 ± 4	394 ± 6	387 ± 5	343 ± 25	—
40	431 ± 4	429 ± 6	—	—	—	—

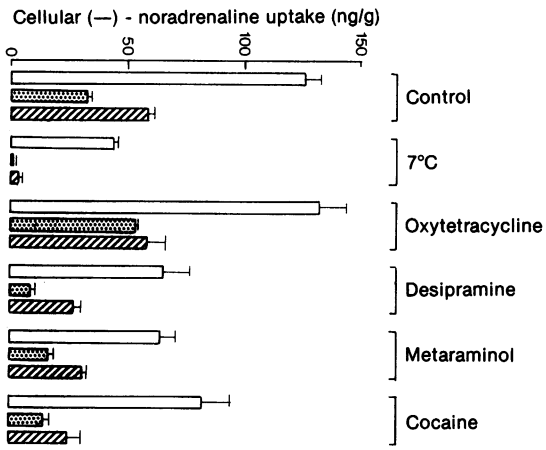

Figure 2 Effect of drugs and temperature reduction on cellular uptake of [^{3}H]-(−)-noradrenaline in kitten coronary arteries (open columns), left atria (stippled columns) and right atria (hatched columns) following 10 min incubation with amine. Each column with its horizontal bar indicates the mean ± s.e. mean of at least four experiments.

56% at a concentration of 10 μM and by 65% at 80 μM ($P < 0.001$). The corresponding inhibitions for right atria were 68 and 83% ($P < 0.001$).

Coronary arteries [^{3}H]-Noradrenaline 20 ng/ml was the only concentration of the amine studied in coronary arteries. After 10 min incubation with the amine, the concentration of radioactivity in the arterial cells was 6 times that in the extracellular fluid. Reduction of the temperature from 32° to 7°C resulted in a 65% ($P < 0.001$) reduction in tritium accumulation (Figure 2). As in atria, this active uptake seemed to be mainly neuronal since 60 min incubation with desipramine (1 μM) or 30 min incubation with metaraminol (1 μM) or cocaine (10 μM) reduced uptake by 48, 49 and 35% respectively ($P < 0.02$) (Figure 2) (30 min incubation with desipramine was ineffective). The extraneuronal uptake inhibitor cortisol (80 μM) was without effect while the mixed uptake inhibitor phenoxybenzamine (80 μM) reduced uptake by 47%. Binding of amine to connective tissue fibres seemed unimportant since oxytetracycline (100 μM) failed to inhibit uptake (Figure 2).

[^{3}H]-(±)-isoprenaline experiments

Atria Left and right atria incubated with [^{3}H]-isoprenaline 0.5 μg to 1 mg/ml showed a time-dependent increase in radioactivity. From these data, the initial uptake velocity (V) was calculated for each amine concentration. Using the 1 min mean extracellular

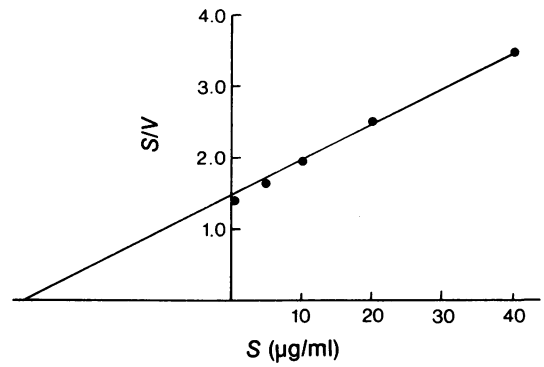

Figure 3 Linear transformation of the Michaelis-Menten equation as applied to an analysis of the uptake of [^{3}H]-(±)-isoprenaline in kitten atria. S = mean extracellular [^{3}H]-(±)-isoprenaline concentration after 1 min incubation with amine; V = initial uptake velocity as determined from uptake versus time curves.

isoprenaline concentration (S), a plot of S/V versus S (Figure 3) indicated that initial uptake obeyed Michaelis-Menten kinetics. The apparent K_m was 136 μM and the V_{max} 93 nmol min^{-1} g^{-1}. Uphill accumulation of amine was suggested by cellular:extracellular concentration ratios >1 for low amine concentrations following 20 (Figure 4) or 40 min incubations. Saturation of accumulation appeared to take place with increasing extracellular amine concentrations up to 100 μg/ml (Figure 4). Inspection of the results suggested that more than one uptake process might be operating. In order to examine this possibility, the net uptake of radioactivity was studied at various time increments. Initially, cellular accumulation versus mean extracellular concentration was plotted for 1, 2, 10 and 20 min data. From these curves which were based on 160 atrial preparations, the actual cellular uptakes corresponding to mean extracelllular amine concentrations of 5 and 500 μg/ml were found. Figure 5 shows the values obtained for the net accumulation of radioactivity by the atrial cells during the 1st, 2nd, 2nd to 10th and 10th to 20th min incubation periods. In both left and right atria biphasic cellular accumulation of radioactivity was observed with mean extracellular [^{3}H]-isoprenaline concentrations of 5 or 500 μg/ml. Following a rapid initial uptake which took place in the first minute there was little or no further accumulation during the second minute in left atria while in right atria there was, in fact, a net efflux of radioactivity. A second slower uptake began thereafter in both left and right atria. The initial and late uptake processes at these two amine concentrations were completely abolished by reduction of the experimental temperature from 37° to 7°C (Figure 6). All uptake thus appeared active

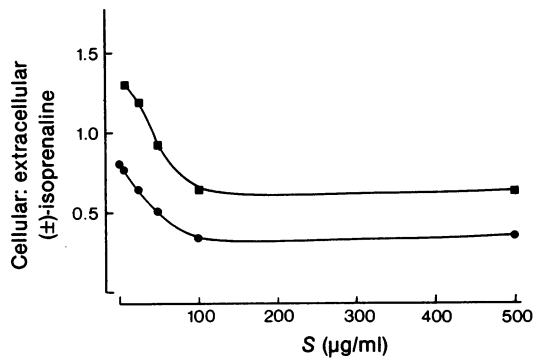

Figure 4 Relationship between the mean extracellular [^{3}H]-(±)-isoprenaline concentration (S) and the cellular:extracellular [^{3}H]-(±)-isoprenaline concentration ratio in kitten left atria incubated with amine for 10 min (●) and 20 min (■).

with no temperature-insensitive binding to connective tissue. In support of this observation oxytetracycline (100 μM) failed to reduce 1 min uptake in left or right atria or 10 min uptake in left atria incubated with [^{3}H]-isoprenaline 5 μg/ml. On the other hand it did unexpectedly decrease ^{3}H accumulation at 10 min by 38% in right atria ($0.01 > P > 0.001$) (Figure 6).

Experiments were next carried out to see if the initial and late uptake processes could be differentiated pharmacologically. With a standard concentration of [^{3}H]-isoprenaline, 5 μg/ml, in the incubation fluid, a variety of compounds were examined for inhibitory effects on uptake of radioactivity at 1 min, i.e. initial uptake, and on uptake at 10 min, i.e. net uptake resulting from initial and late uptake processes. The neuronal uptake inhibitors desipramine (1 μM), metaraminol (1 μM) and cocaine (10 μM) did not affect either of these uptake processes. Incubation with the extraneuronal uptake inhibitors cortisol (80 μM), 17β-oestradiol (10 to 80 μM), caffeine (80 μM) and 3-methoxyisoprenaline (50 μM) did not inhibit initial uptake in right atria. In left atria these compounds were also inactive with the exception of 3-methoxyisoprenaline which produced a 24% decrease in initial uptake ($0.05 > P > 0.02$). In contrast, all the extraneuronal inhibitors decreased accumulation of radioactivity at 10 min by amounts varying from 39 to 60% (Figure 6). This indicates that they were selective inhibitors of the late uptake process. In order to test this possibility further the effects of cortisol on cellular accumulation of radioactivity at various time increments was studied in 57 atria. The cellular values obtained corresponding to mean extracellular isoprenaline concentrations of 5 and 500 μg/ml are shown in Figure 5. In both left and right atria, initial uptake was clearly resistant to inhibition by the

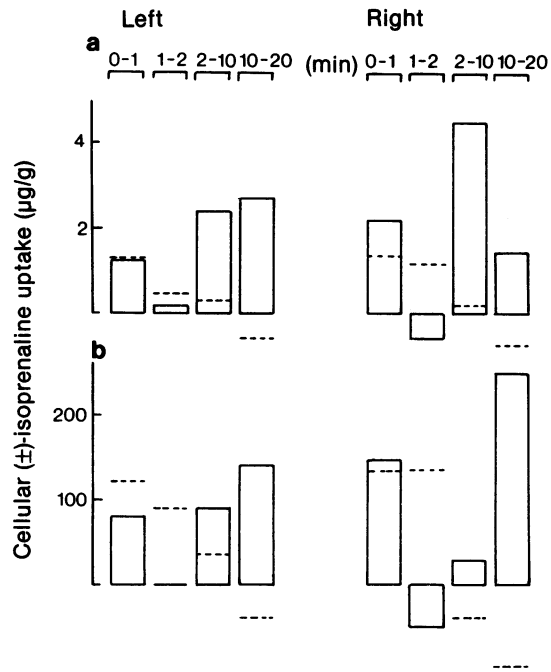

Figure 5 Net incremental accumulation of [^{3}H]-(±)-isoprenaline by left and right atrial cells. The mean extracellular concentration of isoprenaline was 5 μg/ml (a) or 500 μg/ml (b). Solid lines represent untreated atria and dotted lines represent atria pretreated with cortisol 80 μM.

steroid and in fact, with the higher external amine concentration it was increased. In sharp contrast, cortisol produced a very marked inhibition of the late uptake process.

In an attempt to find a selective inhibitor of the steroid-resistant initial uptake process, other compounds were tested. A high dose of cocaine (30 μM) which would be expected to inhibit extraneuronal as well as neuronal uptake (Iversen, 1965) did not inhibit initial or late uptake in right atria. However, it decreased initial uptake by 34% ($P < 0.001$) and uptake at 10 min by 39% ($P < 0.001$) in left atria. Right atrial uptake was also insensitive to phenoxybenzamine (10 μM) while initial uptake in left atria was reduced by 30% ($0.02 > P > 0.01$) and 10 min uptake by 27% ($0.05 > P > 0.02$). The results with cocaine and phenoxybenzamine on left atria thus indicate an inhibitory effect on initial rather than late uptake. A higher dose of phenoxybenzamine (80 μM) abolished initial uptake in both atria while only reducing uptake at 10 min by 57% in right ($P < 0.001$) and 58% in left atria ($P < 0.001$) (Figure 6). This result again suggests some selectivity of phenoxybenzamine in blocking initial uptake.

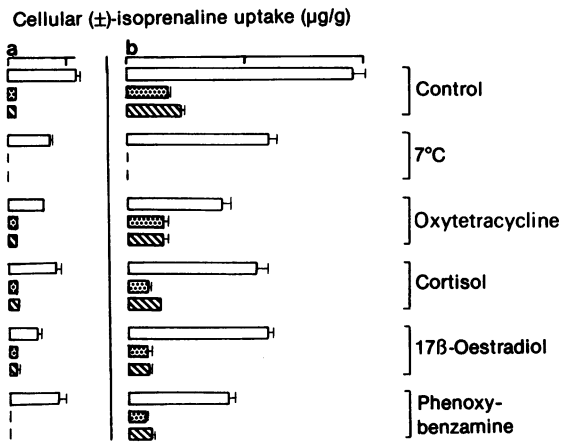

Figure 6 Effects of drugs and temperature reduction on cellular uptake of [^{3}H]-(±)-isoprenaline in kitten coronary arteries (open columns), left atria (stippled columns) and right atria (hatched columns) following (a) 1 min and (b) 10 min incubation with amine. Each column with its horizontal bar indicates the mean ± s.e. mean of at least four experiments.

Coronary arteries Arteries incubated with [^{3}H]-isoprenaline 5 and 50 μg/ml showed time-dependent accumulation of amine (Figure 7). There was no evidence of more than one uptake process operating. By 20 min the arterial cells contained 4.5 and 2.2 times more radioactivity than the incubation fluid at the low and high amine concentrations respectively. Only part of accumulation was temperature-sensitive. Thus reduction of the temperature from 32° to 7°C decreased uptake at 1 min by 40% ($P < 0.001$) and at 10 min by 37% ($P < 0.001$) (Figure 6). The remainder of the radioactivity in the cells could be attributed to amine binding by connective tissue fibres since oxytetracycline (100 μM) reduced radioactive accumulation at 1 min by 50% ($P < 0.001$) and at 10 min by 58% ($P < 0.001$) (Figure 6). The results with oxytetracycline together with those at reduced temperature indicate that at 1 min, 40 to 50% of observed radioactive accumulation was due to active uptake of amine while at 10 min the range was 37 to 42%. Active uptake at both 1 and 10 min could be reduced by a number of extraneuronal uptake inhibitors. Thus at 1 min, cortisol (80 μM), 17β-oestradiol (80 μM) and phenoxybenzamine (80 μM) inhibited total radioactive accumulation by 30, 59 and 29% respectively ($P < 0.05$). The corresponding inhibitions at 10 min were 42, 38 and 55% ($P < 0.001$) (Figure 6). A dose of cocaine (30 μM) which inhibits extraneuronal uptake (Iversen, 1965) reduced radioactive accumulation by 22% at 1 min ($0.1 > P > 0.05$) by 47% at 10 min ($P < 0.001$).

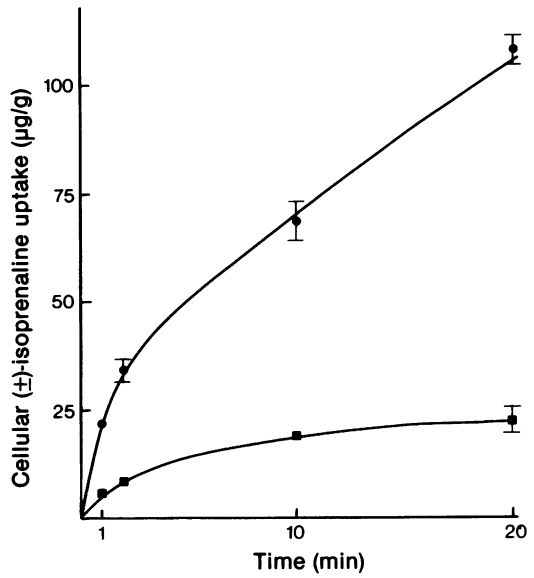

Figure 7 Relationship between the cellular uptake of [^{3}H]-(±)-isoprenaline and incubation time in coronary arteries. Concentrations of amine in the incubation medium were 5 μg/ml (■) and 50 μg/ml (●). Points and vertical bars represent means ± s.e. means of 4 experiments.

Inhibition of catechol-O-methyl transferase by U0521

Catechol-*O*-methyl transferase (COMT) is the main enzyme which metabolizes isoprenaline following extraneuronal uptake (Guldberg & Marsden, 1975). In the above experiments the radioactivity accumulated by cells must include both unchanged and *O*-methylated amine. In order to see if inhibition of COMT altered tritium accumulation, the effect of U0521 (120 μM) was tested; 30 min incubation with the COMT inhibitor failed to alter either the initial or late accumulation of radioactivity in atria exposed to [^{3}H]-isoprenaline, 5 μg/ml. However, it decreased accumulation of radioactivity in the arteries at 32°C by 43% at 1 min ($P < 0.001$) and by 53% at 10 min ($P < 0.001$), but was inactive at 7°C. These inhibitions are thus equivalent to total inhibition of active arterial uptake as outlined earlier.

Another consequence of *O*-methylation is the production of metabolites which might diffuse from tissues treated with catecholamines and inhibit further extraneuronal uptake (Mireyless & Foster, 1973). However, these were only active in high concentrations. As described above, 3-methoxyisoprenaline (50 μM) reduced uptake at 10 min by 46% in left ($P < 0.001$) and 51% in right atria ($0.05 > P > 0.02$). With a higher concentration (200 μM) the corresponding reductions were 77% and 71% ($P < 0.001$). Nor-

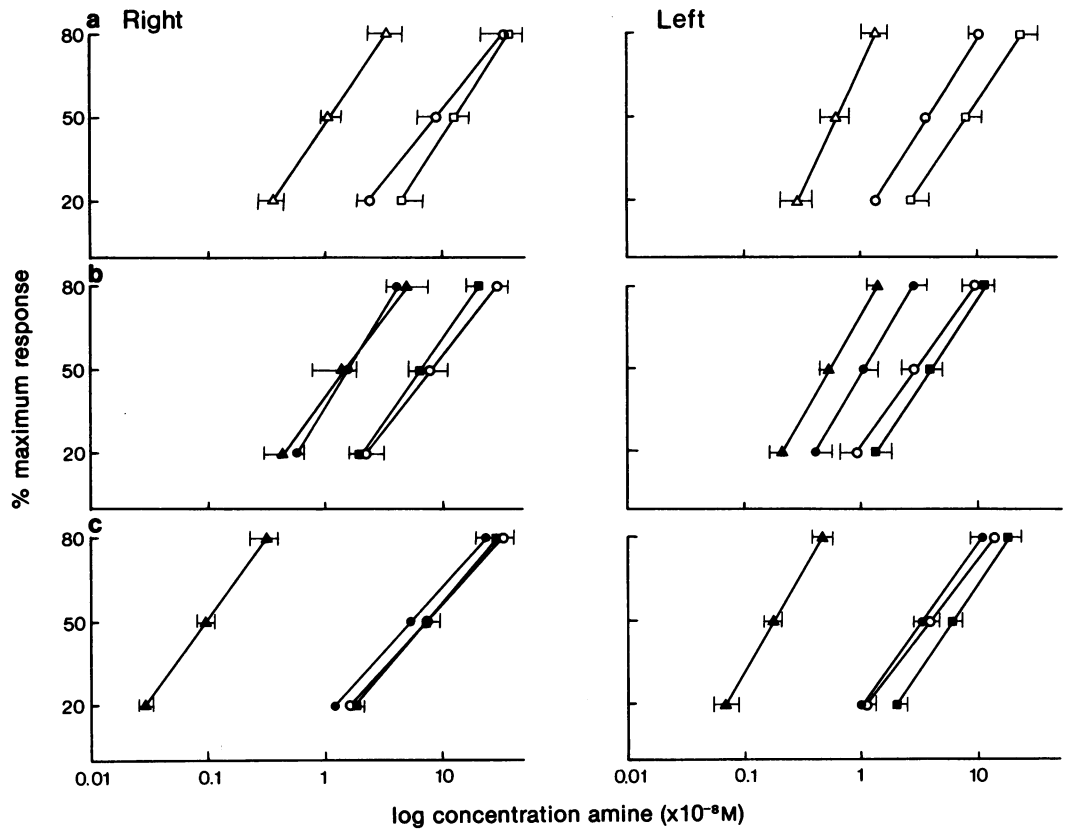

Figure 8 Log_{10} concentration-response curves for (−)-noradrenaline (○), (−)-adrenaline (□) and (±)-isoprenaline (△) in untreated right and driven left kitten atria (a) and in atria treated with cocaine 10 μM (b) and cortisol 80 μM (c) (solid symbols). Each point is the mean of 4 or 5 experiments. The horizontal lines show s.e. means.

metanephrine was even less potent being inactive at 50 μM while 200 μM produced 65% inhibition of tritium accumulation in left atria ($P < 0.001$) and 56% inhibition in right atria ($0.01 > P > 0.001$).

Supersensitivity to catecholamines following uptake inhibition

Atria Cocaine (10 μM), desipramine (1 μM), cortisol (80 μM) and 17β-oestradiol (20 μM) had no apparent effects on resting atrial rate or force. In the presence of a higher dose of cocaine (30 μM) left atria did not follow stimulation at a frequency of 4 Hz and right atria became arrhythmic when exposed to catecholamines.

The mean concentration-response curves obtained for the chronotropic and inotropic effects of (−)-noradrenaline, (−)-adrenaline and (±)-isoprenaline in the absence of uptake inhibitors are shown in Figure 8. The slopes of the curves were not significantly different ($P > 0.05$, paired t test). The molar potency ratios for (−)-adrenaline and (±)-isoprenaline relative to (−)-noradrenaline (=1) were not significantly different ($P > 0.05$, Student's nonpaired t test) in right and left atria (Table 2). The two neuronal uptake inhibitors cocaine (10 μM) and desipramine (1 μM) produced similar parallel shifts to the left in the concentration-response curve for (−)-noradrenaline (Figure 8). There were five and three fold increases in sensitivity to the chronotropic and inotropic effects of this amine respectively. However, the relative molar potency ratios for (−)-adrenaline and (±)-isoprenaline were comparable to those in control experiments (Table 2). The two extraneuronal inhibitors cortisol (80 μM) and 17β-oestradiol (20 μM) failed to alter significantly sensitivity to (−)-noradrenaline or (−)-adrenaline (Figure 8, Table 2). Cortisol increased the molar potency ratio of (±)-isoprenaline relative to (−)-noradrenaline ten fold in right and three fold in left atria ($P < 0.05$, Student's nonpaired t test).

However, 17β-oestradiol increased this ratio less than two fold and the effect was only significant in right atria. Maximal responses to amines and slopes of the concentration-response curves were similar in the absence and presence of uptake inhibitors (Student's nonpaired *t* test).

A number of effective uptake inhibitors in tracer experiments were unsuitable for examining changes in tissue sensitivity to amines because of other pharmacological effects. For example, both phenoxybenzamine (10 μM) and metaraminol (1 μM) released endogenous catecholamines. The highest nonstimulatory concentration of metaraminol (0.25 μM) did not increase sensitivity to catecholamines. The β-adrenoceptor blocking action of 3-methoxyisoprenaline overrode any potentiation of amine responses it might have produced due to uptake inhibition (similarly the COMT inhibitor, U0521, was such a potent β-agonist that it could not be meaningfully tested).

Coronary arteries Molar potency ratios for (−)-adrenaline or (±)-isoprenaline relative to (−)-noradrenaline (=1) were not significantly different for intraluminal or extraluminal addition of amines (Table 3). Uptake inhibitors were therefore only tested on responses to intraluminal application of amines.

The neuronal uptake inhibitors cocaine (10 μM) and desipramine (1 μM) did not alter the sensitivity of arteries to (−)-noradrenaline (Table 4). A higher dose of cocaine (30 μM) was tested in 3 preparations and found to depress rather than potentiate (−)-noradrenaline responses. The extraneuronal inhibitor cortisol (40 μM) reduced spontaneous tone by more than 50% in 7 of 15 preparations making it impossible to study dilator responses to catecholamines themselves. In 5 of the remainder, responses to (−)-noradrenaline were unaffected by the steroid (Table 4). In a further 3, where (±)-isoprenaline was tested before and after cortisol, there was no change in the dilator activity of this amine.

17β-Oestradiol (20 μM) and a higher concentration of cortisol (80 μM) caused total loss of arterial tone. These dilator responses were unaffected by practolol (2 μM) but could be reversed by drug-free perfusion.

Caffeine (80 μM) was tested on 3 arteries since it

Table 2 Effect of uptake inhibitors on inotropic (I) and chronotropic (C) responses to catecholamines

		Mean molar potency ratios relative to noradrenaline (=1) before uptake inhibition		
Inhibitor		*(−)-NA*	*(−)-Ad*	*(±)-Iso*
None (5)	I	1.0	0.6 ± 0.1	6.9 ± 0.9
	C	1.0	0.9 ± 0.2	7.7 ± 0.9
Cocaine (4)	I	3.4* ± 0.8	0.8 ± 0.1	5.8 ± 1.3
10 μM	C	5.0* ± 0.3	1.1 ± 0.2	6.3 ± 0.7
Desipramine (6)	I	3.5* ± 0.5	0.7 ± 0.1	6.6 ± 0.7
1 μM	C	4.7* ± 0.5	1.2 ± 0.2	8.8 ± 1.4
Cortisol (5)	I	1.2 ± 0.1	0.8 ± 0.2	22.9† ± 2.8
80 μM	C	1.3 ± 0.3	1.1 ± 0.3	76.6† ± 27.2
17β-Oestradiol (4)	I	2.0 ± 0.4	0.6 ± 0.2	13.0 ± 3.3
20 μM	C	1.3 ± 0.2	1.0 ± 0.2	13.7† ± 2.4

Values are given ± s.e.mean. Figures in parentheses indicate number of atria tested.
* EC_{50} for noradrenaline after inhibitor significantly less than before inhibitor, $P < 0.05$, (paired *t* test).
† Relative potency of isoprenaline significantly higher in presence of inhibitor, $P < 0.05$, (Student's non-paired *t* test).

Table 3 Relative molar potencies of amines as dilators in kitten coronary arteries

	Mean molar potency ratios relative to noradrenaline (=1)		
Application	*(−)-NA*	*(−)-Ad*	*(±)-Iso*
Intraluminal (5)	1.0	0.35 ± 0.06	8.8 ± 0.6
Extraluminal (6)	1.0	0.45 ± 0.05	11.3 ± 2.4

Values are given ± s.e. mean. Numbers in parentheses represent the number of preparations used.

is an extraneuronal uptake inhibitor in ox coronary arteries (Kalsner *et al.*, 1975). In two of these preparations it increased tone but did not alter the sensitivity of the arteries to (±)-isoprenaline.

Discussion

The present study indicates that neuronal and extraneuronal removal of amines by the coronary arteries accounts for part of the amine uptake measured in the perfused cat heart (Graefe, Bönisch, Fiebig & Trendelenburg, 1975). It remains to be determined whether or not most amine *O*-methylation also occurs at this vascular site as has been proposed by Bönisch *et al.*, (1974). The COMT inhibitor U0521, at a concentration of 120 μM, reduced total arterial radioactivity to an extent accounted for by inhibition at the extraneuronal uptake compartment. This concentration is only half the K_i for extraneuronal uptake in rat heart (Bönisch *et al.*, 1974) but has been reported to abolish cardiac *O*-methylation (Bönisch & Trendelenburg, 1974; Bönisch *et al.*, 1974). It would thus appear that the observed effect of U0521 in coronary arteries is due to enzymatic inhibition and not simply due to block of extraneuronal uptake. If this is the case, the results also indicate that coronary arteries store mainly 3-methoxyisoprenaline and cannot store unchanged isoprenaline in lieu of its metabolite when COMT is inhibited. However, such a substitution has been reported in guinea-pig hearts and our results do not rule out the possibility that this could occur in kitten atria treated with U0521 as it would not necessarily lead to a marked change in total radioactivity accumulated. Certainly kitten atria seem to be able to store unchanged isoprenaline even when COMT activity is intact. For example, with a mean extracellular isoprenaline concentration of 500 μg/ml, by 1 min at least 81 μg (383 nmol) and 147 μg (696 nmol) of isoprenaline had been transported into each g wet wt. left and right atrial cells respectively (Figure 5). (This uptake was active since it was virtually abolished at 7°C). This amount of isoprenaline would saturate extraneuronal COMT since the K_m for this enzyme in rat heart is only 2.9 μM (Bönisch *et al.*, 1974).

The discovery of both an initial and late uptake of isoprenaline in kitten atria is not surprising in view of reports of two extraneuronal uptake compartments with different kinetics in rat and guinea-pig heart (Bönisch *et al.*, 1974), cat nictitating membrane (Graefe & Trendelenburg, 1974), rabbit aorta (Henseling, Eckert & Trendelenburg, 1976; Levin, 1976) and rabbit ear artery (Head, Irvine & De la Lande, 1976). Meaningful kinetics could only be established for initial uptake for which an apparent K_m 136 μM, V_{max} 93 nmol min^{-1} g^{-1} was calculated (Figure 3). These values are higher than those reported for rat heart by Bönisch *et al.* (1974) (K_m 21 μM, V_{max} 38 nmol min^{-1} g^{-1}), or by Graefe *et al.* (1975) (K_m 70 μM, V_{max} 45 nmol min^{-1} g^{-1}). There are not only kinetic but also pharmacological differences between initial uptakes in the two species. In the kitten heart, initial uptake was insensitive to the steroidal inhibitors, cortisol and 17β-oestradiol, was weakly inhibited by 3-methoxyisoprenaline in left but not right atria and was virtually abolished by phenoxybenzamine in both atria. All four compounds inhibited late uptake to varying degrees in left and right atria (Figure 6). In contrast, in the rat, influx of isoprenaline into two extraneuronal uptake compartments was steroid-sensitive (Bönisch *et al.*, 1974). It is tempting to speculate that initial uptake in kitten atria takes place into fibroblasts since these connective tissue cells have been proposed by Jacobowitz & Brus (1971) to be a major extraneuronal uptake compartment in guinea-pig heart. They found that fibroblasts and blood vessels showed much greater fluorescence following perfusion of hearts with noradrenaline than did cardiac muscle cells. Uptake into fibroblasts was

Table 4 Relative molar potencies of amines in the presence of uptake inhibitors as dilators in kitten coronary arteries

	Mean molar potency ratios relative to noradrenaline (=1) before uptake inhibition		
Inhibitor	*(−)-NA*	*(−)-Ad*	*(±)-Iso*
None	1.0 (5)	0.35 ± 0.06 (5)	8.8 ± 0.6 (5)
Cocaine 10 μM	0.83 ± 0.17 (3)	—	—
Desipramine 1 μM	1.0 (9)	0.60 ± 0.11 (4)	7.2 ± 1.4 (4)
Cortisol 40 μM	0.80 ± 0.17 (5)	—	—

Values are given ± s.e. mean. Numbers in parentheses represent the number of preparations used.

markedly inhibited by phenoxybenzamine and *O*-methylated catecholamines while these drugs only reduced uptake into cardiac muscle cells to a small extent. In kitten atria the coronary vasculature can be ruled out as a likely site for initial uptake since 1 min uptake into coronary arteries was sensitive to steroidal inhibitors (Figure 6). Presumably, kitten atria accumulate noradrenaline as well as isoprenaline extraneuronally. Cellular accumulation of noradrenaline increased biphasically with increasing external amine concentration, left atria showing the greatest inflection in the curve (Figure 1). These curves bear a striking resemblance to those for noradrenaline and adrenaline accumulation in perfused rat heart which originally led Iversen (1965) to study extraneuronal uptake. Although we have not attempted to find out whether there are two types of extraneuronal uptake for noradrenaline as for isoprenaline, this seems likely (Trendelenburg, 1976).

Detailed kinetic analysis of extraneuronal uptake in coronary arteries was not attempted due to shortage of tissue. Binding of amine to connective tissue would have to be avoided in any such study. Adventitial stripping to remove most of the connective tissue (and neuronal uptake sites) is impractical in these small, fragile arteries. The only alternative would be to carry out all experiments in the presence of oxytetracycline to inhibit connective tissue binding (Powis, 1973). However, while initial and late uptake processes in atria could be differentiated, not only kinetically but also pharmacologically, no indication of two separate uptake processes were found when drugs were tested in coronary arteries as inhibitors of the accumulation of isoprenaline at 1 and 10 min (Figure 6). Other blood vessels do possess two extraneuronal uptake compartments (Henseling *et al.*, 1976; Levin, 1976; Head *et al.*, 1976).

In tracer experiments no attempt was made routinely to inhibit amine metabolizing enzymes since it was not feasible to use such inhibitors in comparable tissue sensitivity experiments. For example, it was found that the COMT inhibitor, U0521, had marked chronotropic effects due to its β-adrenoceptor agonist activity (Kaumann, Wittmann, Birnbaumer & Hoppe, 1977). As detailed earlier, even some uptake inhibitors had pharmacological effects on the heart or coronary arteries which excluded their use in sensitivity studies.

Supersensitivity to catecholamines was observed following uptake inhibition in atria but not in coronary arteries. In the doses tested in atria, cocaine and desipramine were approximately equiactive in inhibiting neuronal uptake and in inducing selective supersensitivity to noradrenaline (Figure 8; Table 2). Cortisol (80 μM) and 17β-oestradiol (20 μM) reduced isoprenaline uptake at 10 min in left atria by 48 and 67% respectively and in right atria by 39 and 50%. However, this was not reflected in comparable sensitivity changes to isoprenaline. Thus, cortisol increased sensitivity to the chronotropic effects of isoprenaline ten fold and to its inotropic effects three fold while 17β-oestradiol only produced a significant, two fold, increase in sensitivity to isoprenaline in right atria (Table 2). It should be noted that the increases in sensitivity produced by extraneuronal uptake inhibitors were selective for isoprenaline. The results with cortisol are in accord with those published by Kaumann (1972) and Goldie (1976). In addition, they show that cardiac responses to adrenaline are less dependent on changes in the activity of uptake processes than are responses to noradrenaline and isoprenaline.

Two types of experiments showed that neuronal uptake did not limit amine sensitivity in coronary arteries. The first involved intra- and extraluminal application of amines and the second employed neuronal uptake inhibitors. Studies in other arteries, especially in the rabbit ear artery, suggest that noradrenaline applied extraluminally is less active than that applied intraluminally. This difference in sensitivity arises because noradrenaline applied extraluminally has to traverse the medial-adventitial border, where it is susceptible to neuronal uptake, before it reaches the smooth muscle cells (De la Lande, Frewin & Waterson, 1967). In contrast, noradrenaline applied intraluminally is only exposed to extraneuronal uptake into the smooth muscle cells themselves (De la Lande, Hodge, Lazner, Jellett & Waterson, 1970). As in other arteries, the sympathetic nerves to the coronary arteries into which catecholamines may be accumulated, terminate in the medial-adventitial border (De la Lande *et al.*, 1974; Denn & Stone, 1976). However, the relative potencies of noradrenaline, adrenaline and isoprenaline (i.e. of good, intermediate and poor substrates for neuronal uptake) were not significantly different for intraluminal and extraluminal application in kitten coronary arteries (Table 3). It was thus not surprising to find that neuronal uptake inhibitors failed to induce supersensitivity to these amines (Table 4) and in fact this has previously been reported for cocaine in other species (Nishioka, 1971; De la Lande *et al.*, 1974; Kalsner, 1974). Finally, since cocaine-induced supersensitivity decreases markedly with increasing neuromuscular interval (Verity, 1971), very little effect of cocaine would be expected in coronary arteries where this interval is of the order of 4000 Å (Malor, Griffin & Taylor, 1973). The dilator activity of cortisol and 17β-oestradiol prevented an adequate assessment of the role of extraneuronal uptake in modifying amine responses. However, the few experiments which were possible with cortisol and also with caffeine did not reveal any changes in amine sensitivities. This is in accord with the lack of effect of other extraneuronal inhibi-

tors in rabbit coronary arteries (De la Lande *et al.*, 1974). In contrast, Kalsner *et al.* (1975) reported that extraneuronal inhibitors increased the sensitivity of ox coronary arteries to noradrenaline. This was in line with their findings that the inhibitors reduced uptake of [^{3}H]-(±)-noradrenaline at 10 minutes. However, it should be noted that in the present study cortisol did not produce any such inhibitory effect with [^{3}H]-(−)-noradrenaline (20 ng/ml).

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