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METABOLISM OF [^{3}H]-(±)-ISOPRENALINE BY ISOLATED ATRIA AND CORONARY ARTERIES OF THE KITTEN

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1 Isolated coronary arteries of the kitten accumulated more unchanged isoprenaline and metabolized more amine than atria following incubation for 1 to 20 min with [^{3}H]-(±)-isoprenaline (25 ng/ml or 5 μg/ml).

2 Cortisol (10 or 80 μM), U-0521 (120 μM) and oxytetracycline (100 μM) all reduced metabolite formation.

3 Cortisol inhibited 'Iso $Influx_{Min}$' (cellular isoprenaline accumulation plus total metabolite production). In contrast, it increased, decreased or did not alter accumulation of unmetabolized isoprenaline, depending upon the experimental conditions.

4 Isoprenaline accumulation was increased in atria and reduced in coronary arteries by U-0521, while oxytetracycline reduced accumulation in coronary arteries at the high amine concentration.

5 It is concluded that in atria, cortisol inhibits metabolism and has differential effects on a number of extraneuronal compartments which accumulate isoprenaline. Both cortisol and U-0521 appear to be extraneuronal uptake inhibitors and inhibitors of catechol-*O*-methyltransferase in coronary arteries. Oxytetracycline may have effects additional to inhibition of isoprenaline binding to connective tissue fibres.

Introduction

Kitten atria and coronary arteries accumulate isoprenaline extraneuronally (Cornish, Goldie & Miller, 1978). The aim of the present study was to examine the metabolism of isoprenaline following its uptake in these tissues.

It has previously been suggested from experiments on the rat heart, that coronary arteries have a higher capacity for *O*-methylation than do the myocardial cells of the ventricles and that they may be an important site of *O*-methylation when the heart is perfused with isoprenaline (Bönisch, Uhlig & Trendelenburg, 1974). Such an idea seems reasonable since other arteries show high catechol-*O*-methyltransferase (COMT) activity (Levin, 1976; Osswald, Garrett & Guimarães, 1976) and in experiments when [^{3}H]-noradrenaline (1.2 μM) was incubated with the rabbit ear artery, normetanephrine was the main metabolite formed (Head, Johnson, Berry & de la Lande, 1975b).

A further objective of this study was to determine how compounds previously shown to alter tissue radioactivity in kitten atria and coronary arteries following incubation with [^{3}H]-isoprenaline affected isoprenaline disposition and metabolism.

Methods

Kittens of either sex, weighing 700 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. Coronary arteries were dissected free from myocardial tissue and opened longitudinally to produce flat sheets. Atria were removed and halved. Coronary arteries from 5 hearts and atrial halves from 3 hearts were pooled to provide samples weighing at least 50 and 300 mg respectively. Samples were then incubated in 3 ml McEwen solution containing [^{3}H]-(±)-isoprenaline, 25 ng/ml or 5 μg/ml (0.12 or 24 μM) (10 μCi/ml) for 1, 10 or 20 min. Arteries were incubated at 32°C while atria were incubated at 37°C.

In some experiments tissues were exposed to cortisol (10 or 80 μM), U-0521 (120 μM) or oxytetracycline (100 μM) for 30 min prior to 1 or 20 min incubations with the tritiated catecholamine.

Extraction of radioactivity

Following incubation with tracer, arteries were rinsed for 1 to 2 s in ice-cold McEwen solution. Arteries and atria were then quickly blotted to remove surface moisture, frozen on a block of dry ice and weighed.

Arteries and atria were homogenized in 2 ml or 9 volumes respectively of ice cold HCl (0.1 M) containing ascorbic acid (0.57 mM). Homogenates were centrifuged at 5000 *g* for 30 min at 5°C in a Sorvall high speed centrifuge (model RC2-B). The total radioac-

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tivity of 0.5 ml aliquots of the supernatants or of the original incubation fluid was then estimated as previously described (Cornish *et al.*, 1978). Additional 1 ml aliquots of these fluids were frozen in acetone at −75°C and freeze-dried overnight. The radioactivity in the freeze-dried samples was extracted into 1 ml of an ice cold acetone-ethanol-HCl 0.1 M mixture (40:40:10) containing ascorbic acid (0.57 mM). Both isoprenaline and 3-methoxyisoprenaline are readily soluble in this solvent mixture. Aliquots (200 µl) of these final solutions were taken for estimation of total radioactivity (Cornish *et al.*, 1978) and determination of % recovery values or were taken for chromatographic studies.

Thin layer chromatography

Silica-gel plates impregnated with sodium tetraborate (Head, Irvine & Kennedy, 1976b) were divided into 30 mm wide strips by scoring parallel grooves into their surfaces. Ice cold aliquots (200 µl) of the organic solvent extracts were applied under nitrogen as streaks close to the base of individual strips. These streaks were over-spotted with a mixture of non-radioactive isoprenaline and 3-methoxyisoprenaline in an acetone-ethanol-HCl 0.1 M mixture (40:40:10). The plates were developed in toluene:ethanol (1:1) for 4 h in an atmosphere of nitrogen at 6°C. Chromatograms were then dried at room temperature and dipped in acetone-pyridine (100:1) containing 1% ninhydrin. After further drying at room temperature, the plates were heated for 15 min at 100°C and the amines visualized as horizontal red streaks. Their location was checked by counting the radioactivity in consecutive 5 × 30 mm sections of each silica-gel strip. (In order to soften the silica-gel and enable it to be scraped off the plastic backing, 1 ml HCl (0.1 M) was applied to the surface of each strip.)

Preliminary experiments showed that the recovery of both [^{3}H]-methoxyisoprenaline and [^{3}H]-isoprenaline was 85% in each case so this correction factor was applied to results from subsequent experiments. Recovery of total radioactivity from silica-gel plates was estimated in individual experiments and invariably fell within the range of 80 to 100%. Estimates of cellular accumulation of isoprenaline were obtained by correcting tissue isoprenaline measurements for amine in the extracellular fluid (ECF) as previously described (Cornish *et al.*, 1978). Only single experiments were carried out under any stated condition because each experiment required tissues from 5 kittens.

Drugs

The drugs used were isoprenaline hydrochloride, oxytetracycline hydrochloride (Sigma Chemical Co.); cortisol sodium succinate (Glaxo); U-0521 (Upjohn); 3-methoxyisoprenaline (synthesized Victorian College of Pharmacy); [7-^{3}H]-(±)-isoprenaline hydrochloride (Radiochemical Centre Amersham); [3-methoxy-^{14}C]-isoprenaline hydrochloride (synthesized using S-adenosyl-(−)-[methyl-^{14}C]-methionine (Radiochemical Centre Amersham) according to the method of McCaman (1965) as modified by Jarrott (1971)).

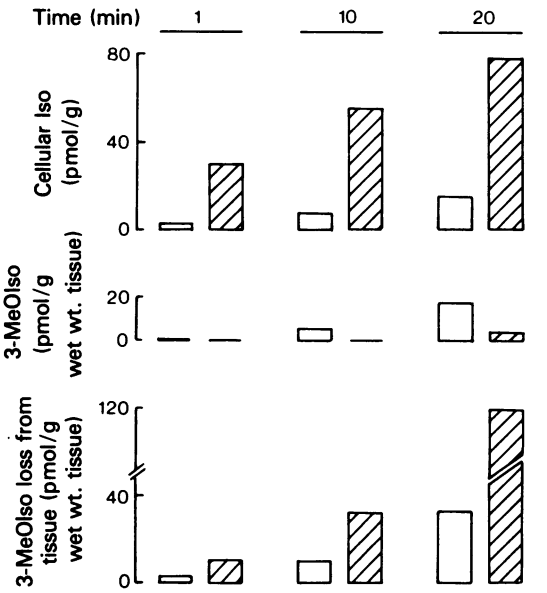

Figure 1 Cellular uptake of isoprenaline (Iso), tissue concentration of 3-methoxyisoprenaline (3-MeO Iso) and diffusional loss of 3-MeO Iso into incubation fluid following incubation of kitten atria (open columns) and coronary arteries (hatched columns) with [^{3}H]-(±)-isoprenaline 25 ng/ml.

Results

Atria and coronary arteries incubated for 1, 10 and 20 min with [^{3}H]-isoprenaline at low (25 ng/ml) or high (5 µg/ml) concentrations showed a time-dependent cellular accumulation of unchanged amine. Accumulation in left and right atria, when calculated on a wet tissue weight basis, were very similar and results were therefore pooled (Figures 1 and 2). Coronary arteries accumulated more isoprenaline than atria. At low and high amine concentrations, arterial values were 5 to 9 fold and 3 to 4 fold greater than atrial values respectively.

In contrast, when 3-methoxyisoprenaline was detected in the tissues, levels were always higher in atria than in arteries. Rapid diffusional loss of 3-methoxyisoprenaline from the tissues was evident from

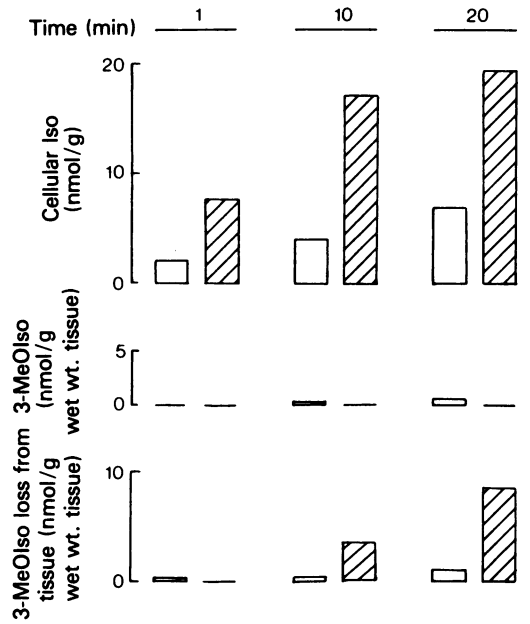

Figure 2 Cellular uptake of isoprenaline (Iso), tissue concentration of 3-methoxyisoprenaline (3-MeO Iso) and diffusional loss of 3-MeO Iso into incubation fluid following incubation of kitten atria (open columns) and coronary arteries (hatched columns) with [^{3}H]-(±)-isoprenaline 5 μg/ml.

measurements of the amount of metabolite in the incubation fluid. In fact, more metabolite was always recovered from this fluid than from the tissues. Total production of 3-methoxyisoprenaline on a unit wet weight basis was greater in arteries than in atria. Arteries metabolized 2 to 3 fold and 5 to 6 fold more isoprenaline than atria at low and high amine concentrations respectively.

The minimum amount of isoprenaline which must have entered the tissue cells (Iso $Influx_{Min}$), equals the cellular content of isoprenaline, plus total metabolite produced. This influx measurement was, overall, 3 to 5 fold higher in arteries than in atria.

Cortisol, which is considered to be an inhibitor of extraneuronal uptake (Graefe & Trendelenburg, 1974; Graefe, 1975) had complex effects on the accumulation of unchanged isoprenaline in both arteries and atria (Tables 1 and 2). However, it consistently reduced the formation of 3-methoxyisoprenaline. This effect was particularly marked in arteries where *O*-methylation was reduced by 84 to 94% with the two concentrations of cortisol tested (10 and 80 μM).

Accumulation of unmetabolized isoprenaline was increased in both arteries and atria by the lower concentration of cortisol used (10 μM). In contrast, storage of isoprenaline was consistently reduced in arteries by cortisol (80 μM) and in atria it was either unchanged or reduced by this higher steroid concentration. In atria, reductions were found following 1 min incubation with isoprenaline (25 ng/ml) and following 20 min incubation with isoprenaline (5 μg/ml).

In order to obtain a more precise assessment of the effects of cortisol on the extraneuronal uptake of isoprenaline, its effects on Iso $Influx_{Min}$ were calculated. When low concentrations of steroid and amine were tested, Iso $Influx_{Min}$ was reduced by 31 and 20% in atria and arteries respectively even though storage of unchanged isoprenaline was increased. Similarly, although accumulation of unchanged isoprenaline was not affected by cortisol (80 μM) following 20 min incubation with isoprenaline (25 ng/ml) or 1 min incubation with isoprenaline (5 μg/ml), Iso $Influx_{Min}$ was reduced by 61 and 12% respectively.

In order to see whether the increased storage of isoprenaline produced by cortisol (10 μM) was secondary to inhibition of COMT, experiments were carried out with the proven COMT inhibitor U-0521 (120 μM) (Guldberg & Marsden, 1975). As expected U-0521

Table 1 Effects of cortisol and U-0521 on the storage and metabolism of [^{3}H]-(±)-isoprenaline (25 ng/ml) by isolated atria and coronary arteries (C Art) of the kitten

Incubation time (min)		*1*	*20*	*20*	*20*
Treatment		*Cortisol (80 μM)*	*Cortisol (10 μM)*	*Cortisol (80 μM)*	*U-0521 (120 μM)*
			% control value		
Cellular Iso	Atria	42	145	107	408
	C Art	—	180	53	59
Total 3-Meo Iso produced	Atria	30	45	18	0
	C Art	—	16	11	0
Iso $Influx^{*}_{Min}$	Atria	35	69	39	97
	C Art	—	80	28	23

* Iso $Influx_{Min}$ = cellular isoprenaline (Iso) + total 3-methoxyisoprenaline (3-MeO Iso) produced.

markedly reduced or abolished *O*-methylation in atria and coronary arteries (Tables 1 and 2). This was associated with an increase in the accumulation of unchanged isoprenaline in atria, which did not appear to be due to an increased influx of isoprenaline *per se*, since U-0521 did not influence measurements of the Iso $Influx_{Min}$. At the low amine concentration, Iso $Influx_{Min}$ expressed as pmol/g wet weight tissue was 64 for controls and 62 in the presence of U-0521 while the corresponding values at high amine concentration were 8.8 and 8.5 nmol/g wet weight tissue respectively.

In coronary arteries, U-0521 approximately halved the accumulation of unchanged isoprenaline and reduced Iso $Influx_{Min}$ by 77% and 61% at the low and high amine concentrations respectively.

The capacity of untreated coronary arteries to accumulate more unchanged isoprenaline than atria could be partly due to temperature-independent binding of amine to connective tissue fibres in these vessels (Cornish *et al.*, 1978). The effects of oxytetracycline (100 μM) which has been reported to inhibit such binding (Powis, 1973; 1976) was therefore tested on coronary arteries. At the low amine concentration, it did not affect the cellular isoprenaline accumulation following a 20 min incubation period, but reduced total metabolite production by 64% (Table 3). In contrast, at high amine concentration it reduced both parameters. Accumulation of unchanged isoprenaline was inhibited by 64% and *O*-methylation by 80%.

Discussion

Kitten atria and coronary arteries showed a high capacity for accumulation of unchanged isoprenaline. Other tissues which have also been found to retain isoprenaline include the rat heart, cat nictitating membrane, rabbit ear artery and dog mesenteric artery (Bönisch *et al.*, 1974; Graefe & Trendelenburg, 1974; Head, Irvine & Johnson, 1975a; Osswald *et al.*, 1976). At the high amine concentration studied (5 μg/ml) both kitten atria and arteries in fact, retained more unchanged isoprenaline than metabolite and this also holds true for arteries at the low amine concentration tested (25 ng/ml). Diffusional loss of 3-methoxyisoprenaline from coronary arteries into the incubation fluid was much more rapid than has been previously observed in the rabbit ear artery or dog mesenteric artery, but in each of these vascular preparations more metabolite was always found to have effluxed than was retained (Head *et al.*, 1975a; Osswald *et al.*, 1976). The greater capacity of isolated

Table 2. Effects of cortisol and U-0521 on the storage and metabolism of [^{3}H]-(±)-isoprenaline (5 μg/ml) by isolated atria and coronary arteries (C Art) of the kitten

Incubation time (min)		*1*	*20*	*20*
Treatment		*Cortisol (80 μM)*	*Cortisol (80 μM)*	*U-0521 (120 μM)*
		% control value		
Cellular Iso	Atria	94	33	119
	C Art	—	66	52
Total 3-MeO Iso produced	Atria	58	75	11
	C Art	—	6	10
Iso $Influx^{*}_{Min}$	Atria	88	41	97
	C Art	—	48	39

* Iso $Influx_{Min}$ = cellular isoprenaline (Iso) + total 3-methoxyisoprenaline (3-MeO Iso) produced.

Table 3 Effects of oxytetracycline (100 μM) on the storage and metabolism of [^{3}H]-(±)-isoprenaline by isolated coronary arteries of the kitten following 20 min incubation

Iso concentration	*25 ng/ml*	*5 μg/ml*
	% control value	
Cellular Iso	106	36
Total 3-MeO Iso produced	36	20
Iso $Influx^{*}_{Min}$	64	31

* Iso $Influx_{Min}$ = cellular isoprenaline (Iso) + total 3-methoxyisoprenaline (3-MeO Iso) produced.

coronary arteries to produce 3-methoxyisoprenaline compared to atria, is in accord with the suggestion that the vasculature may be an important metabolic site for isoprenaline in the perfused heart (Bönisch *et al.*, 1974).

The higher cellular isoprenaline concentration found in coronary arteries relative to atria is not due to the lower temperature used for arterial experiments since temperature reduction decreases rather than increases isoprenaline uptake (Cornish *et al.*, 1978). It could represent a greater isoprenaline storage by vascular smooth muscle cells compared to cardiac cells or alternatively might be due to amine binding to connective tissue fibres in the arteries. However, oxytetracycline, which inhibits such binding (Powis, 1973) only inhibited isoprenaline accumulation at high amine concentration. This result confirmed a previous observation that it reduced the total cellular radioactivity when arteries were incubated with a high concentration of isoprenaline but not with a low concentration of noradrenaline (Cornish *et al.*, 1978). The result also indicates that at low amine concentration, the difference in isoprenaline accumulation between coronary arteries and atria is unrelated to amine binding.

A more surprising observation was that oxytetracycline markedly reduced metabolite formation at both amine concentrations tested. Whether this is due to direct inhibition of COMT or to reduced amine access to COMT is not known. The degree of inhibition of metabolite production was less than was found with the known COMT inhibitor, U-0521. Unfortunately this latter compound at the high concentration tested (120 μM) inhibited arterial uptake of unmetabolized isoprenaline by about 50% at both amine concentrations. A similar inhibitory effect of U-0521 on extraneuronal uptake has been reported in the rat heart (Bönisch *et al.*, 1974). However, in rat heart the K_i value (230 μM) was almost twice the concentration used in the kitten coronary arteries. This concentration difference led us to speculate previously that reduced total cellular radioactivity following U-0521 was more probably due to COMT inhibition than block of uptake, and that arteries might normally store mainly 3-methoxyisoprenaline (Cornish *et al.*, 1978). Clearly this is not the case.

In other arteries, inhibition of COMT by U-0521 (10 or 100 μM) has been associated with an increased tissue accumulation of unchanged isoprenaline (Head *et al.*, 1975a; Osswald *et al.*, 1976). A similar increase was found after U-0521 treatment in kitten atria and has been reported to occur in the rat heart (Bönisch & Trendelenburg, 1974). It has been suggested that in the mesenteric artery, increased accumulation of isoprenaline following U-0521 might be due to lack of extra-neuronal inhibition by 3-methoxyisoprenaline (Osswald *et al.*, 1976). However, this is a less likely explanation for results in the perfused rat heart where metabolites are rapidly flushed from the tissue and in the kitten atria where 3-methoxyisoprenaline is only a weak inhibitor of extra-neuronal uptake (Cornish *et al.*, 1978). The increase may simply reflect storage of isoprenaline in lieu of metabolite since Iso $Influx_{Min}$ is not changed by U-0521 in atria.

It was interesting that cortisol (10 μM) also markedly inhibited *O*-methylation following a 20 min incubation of arteries and atria with the low isoprenaline concentration and caused an associated increase in isoprenaline storage in both atria and arteries. The increase in atria was less than with U-0521 and this is probably due to the opposing weak inhibition of extraneuronal uptake produced by this concentration of cortisol as revealed by a small decrease in Iso $Influx_{Min}$.

At a higher concentration, cortisol (80 μM) also reduced *O*-methylation and produced a greater reduction in Iso $Influx_{Min}$ at 20 min than was observed with the lower concentration. The results suggest that cortisol may inhibit both COMT and extraneuronal uptake. If this is true, then the two opposing actions of cortisol on isoprenaline storage might account for the fact that no net change in isoprenaline accumulation was observed following 20 min incubation with the low amine concentration.

The possible dual activity of cortisol does not simply explain the results obtained with the higher isoprenaline concentration (5 μg/ml). Although cortisol (80 μM) was clearly an inhibitor of metabolite production and reduced cellular isoprenaline accumulation at 20 min, in line with the hypothesis, it produced minimal effects on storage of unchanged amine and Iso $Influx_{Min}$ at 1 min. This particular result is, on the other hand, substantiated by earlier work where total cellular radioactivity was measured (Cornish *et al.*, 1978). As in the earlier study, the temporal selectivity of cortisol's action may be explained by the observation that at high isoprenaline concentrations (5 to 1000 μg/ml) uptake is biphasic. An initial high capacity, low affinity uptake (apparent K_m 136 μM) being resistant to steroidal extra-neuronal uptake inhibitors (Cornish *et al.*, 1978). The present results show that both this initial amine uptake and also the second uptake process are associated with COMT containing compartments which also store isoprenaline (Figure 2). Multicompartmental extraneuronal storage and metabolism of isoprenaline has been described in several other tissues. Steroids have also been found to produce differential effects on the influx and efflux of isoprenaline and 3-methoxyisoprenaline to and from such compartments (Bönisch *et al.*, 1974; Graefe & Trendelenburg, 1974; Uhlig, Bönisch & Trendelenburg, 1974; Head, Irvine & de la Lande, 1976a).

The general inhibitory effect of cortisol on isoprenaline uptake and metabolism with the low con-

centration of isoprenaline (25 ng/ml) explains why cortisol causes supersensitivity to the chronotropic and inotropic effects of this amine in kitten atria (Kaumann, 1972; Goldie, 1976; Cornish *et al.*, 1978).

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