The Uptake of Isoprenaline and Noradrenaline by the Perfused Rat Heart

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SUMMARY

Isoprenaline is accumulated by the isolated heart when perfused through the coronary circulation. It is poorly bound by intracellular particles and readily removed by post-perfusion.

Uptake of isoprenaline is inhibited by normetanephrine but not by cocaine or metaraminol and corresponds to the $Uptake_2$ of Iversen.

The presence of isoprenaline does not convert the noradrenaline uptake process from $Uptake_1$ to $Uptake_2$, and it is therefore possible for these processes to coexist.

The results can be explained if there is a mosaic of $Uptake_1$ and $Uptake_2$ sites on the plasma membrane of the sympathetic neuron.

INTRODUCTION

Iversen (1) has presented evidence that in the rat heart there are two mechanisms responsible for the uptake of noradrenaline (norepinephrine) from the extracellular fluid. At low concentrations the first system operates, but at concentrations of noradrenaline greater than 6 to 9×10^{-6} M (1–1.5 μg/ml) this is replaced by a second uptake process. The dual mechanism also operates on adrenaline (epinephrine), but with a changeover at about 3×10^{-6} M (0.5 μg/ml). The distinction between these two processes, which may be called $Uptake_1$ and $Uptake_2$, rests on their susceptibility to inhibitors and on the relative affinities for noradrenaline and adrenaline (1, 2). Since the uptake at any ambient catecholamine concentration does not appear to be a simple sum of the uptake predicted from kinetic considerations for the two components, it appeared necessary to postulate a process switching off the first uptake and switching on the second uptake. The objective of this paper is to investigate further the mechanism determining which of these uptake systems operates.

METHODS

The methods of perfusing rat hearts and estimating the uptake of catecholamines have been described previously (3). 7-^{3}H-(±)-Isoprenaline was obtained from New England Nuclear Corporation, Boston, and 7-^{3}H-(±)-Noradrenaline from the Radiochemical Centre, Amersham, Bucks. In all cases a correction for extracellular amine was applied using the figure for extracellular space of 0.33 ml/g tissue (2).

Subcellular distributions were performed on hearts that had been perfused with isoprenaline 2.4×10^{-5} M (specific activity 3.4 mC/mmole). Each heart was homogenized in 5 ml of 0.3 M sucrose at 0° in a hand all-glass homogenizer. The homogenate was centrifuged at 1800 g for 10 min at 0° to remove nuclei and unbroken cells (P_1 fraction). The supernatant was then centrifuged at 86,000 g for 60 min at 0°. The radioactivity in the supernatant (S_2), pellet (P_2), and the first pellet were all measured.

In experiments with inhibitors the results are expressed as a percentage of the uptake in the absence of the inhibitor.

RESULTS

Uptake of (±)-Isoprenaline

Perfusion of the heart with (±)-isoprenaline (isopropylnoradrenaline) at a concentration of 2.4×10^{-5} M (5 μg/ml) led to a rapid uptake which reached a steady level after 20 min (Fig. 1). The concentration in the tissue at this time was about twice that in the medium. If the perfusing medium was changed over to plain Krebs solution after 10 min there was an initial rapid loss of isoprenaline from the heart, but this loss became slower and after 60 min the residual amount was 2.9 nmoles/g (0.62 μg/g). Uptake curves of similar form with half-times of 3–4 min and tissue/medium concentrations of 1–3 were found when perfusion concentrations of 1.2×10^{-7} M to 9.5×10^{-5} M (0.025–20 μg/ml) were employed.

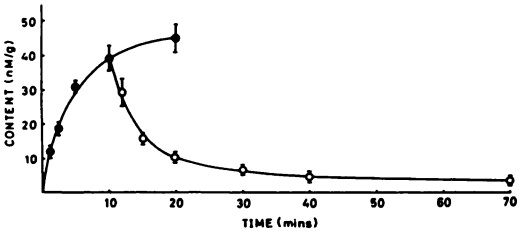

FIG. 1. *Uptake and loss of isoprenaline in the heart*

Hearts were perfused with Krebs solution containing (±)-isoprenaline at a concentration of 2.4×10^{-5} M (5 μg/ml). After varying periods of perfusion the hearts were removed and analyzed for content of (±)-isoprenaline (nmoles/g). In some experiments after perfusion with isoprenaline for 10 min the perfusion fluid was changed to plain Krebs solution. After varying periods of this post-perfusion hearts were also removed for determination of (±)-isoprenaline. Perfusion with isoprenaline ●——●. Post-perfusion with Krebs solution ○——○. Mean values from groups of six hearts ± standard error are expressed as nanomoles per gram.

Initial rates of uptake were calculated, and it was clear that as the concentration of isoprenaline increased the rate tended toward a maximum (Fig. 2). Plotting the substrate concentration divided by the rate of uptake against the substrate concentration (Fig. 3) gave a linear relationship typical of a process obeying adsorption kinetics. A straight line fitted by the method of least squares gave a value for the maximum rate (V_{max}) of 15.5 nmole/g/min and for the dissociation constant of

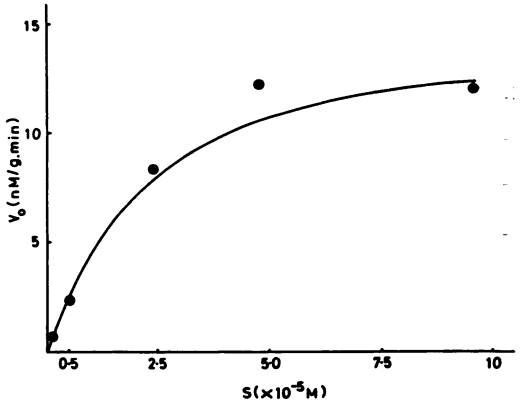

FIG. 2. *Initial rates of isoprenaline uptake*

The initial rates of uptake of isoprenaline were determined by measuring the uptake for several time periods, plotting uptake rate against time and extrapolating to zero time. Initial rates of uptake (ηmoles/g/min) are plotted against the perfusion concentration.

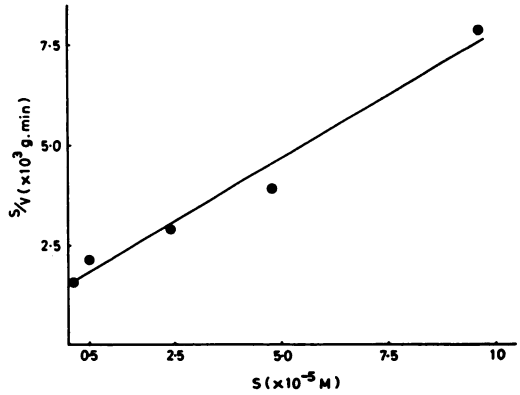

FIG. 3. *Kinetics of isoprenaline uptake*

The data of Fig. 2 are plotted in a manner suitable for the determination of the dissociation constant (K) of the transport process and of the maximum rate of transport. The line is fitted by the method of least squares. The values found were $K = 2.34 \times 10^{-5}$ M: $V_{max} = 15.5$ nmoles/g/min.

2.34×10^{-5} M. The maximum velocity was intermediate between that of noradrenaline accumulated by $Uptake_1 = 1.4$ nmole/g/min and by $Uptake_2 = 100$ nmoles/g/min. The dissociation constant of isoprenaline was also intermediate between the two values for noradrenaline.

Subcellular Distribution of Isoprenaline

Most of the isoprenaline in homogenates of heart tissue was found in the supernatant fraction (S_2) and only very little in the particulate fraction (P_2) (Table 1). After 15 min of post-perfusion the fraction in P_2 was greater than after 2 min, but nevertheless the total counts in P_2 had decreased by over half. This would indicate that the binding of isoprenaline on the particles is relatively weak. The behavior of noradrenaline under comparable conditions showed that after uptake from a concentration of 3×10^{-5} M and post-perfusion for 2 min about 10% of the cellular noradrenaline was in the particulate fraction (Iversen, personal communication). The binding of isoprenaline is therefore weaker than that of noradrenaline.

Effects of Inhibitors on the Accumulation of Isoprenaline

The data presented in Table 2 show that neither cocaine nor metaraminol in the concentrations used caused an inhibition of the uptake of isoprenaline. On the other hand 10^{-5} M normetanephrine inhibited uptake by 54%. Cocaine and metaraminol at these concentrations are effective inhibitors of noradrenaline accumulation by $Uptake_1$ (1, 2; see also Table 3). However, although cocaine and metaraminol are not effective inhibitors of $Uptake_2$, normetanephrine is one of the best inhibitors available for $Uptake_2$ (1, 2). The behavior of isoprenaline uptake toward these inhibitors is there-

TABLE 1
Subcellular distribution of ^{3}H-isoprenaline

All hearts were perfused with $2.4 \times 10^{-5}M$ ^{3}H-isoprenaline for 10 min followed by post-perfusion with amine-free Krebs solution for 2 or 15 min

	P_1[a]		P_2[a]		S_2		
Post-perfusion (min)	Cpm per gram heart	% total	Cpm per gram heart	% total	Cpm per gram heart	% total	P_2:S_2
2	3,211	19	668	4	13,140	77	0.05
15	557	17	288	9	2,484	74	0.12

[a] P_1 = pellet deposited after 1800 *g* for 10 min; P_2 = pellet deposited after 86,000 *g* for 60 min.

TABLE 2
Effect of drugs on uptake of ^{3}H-isoprenaline[a]

Drug		Isoprenaline concentration ($\times 10^{-5}$M)						Average values for all concentrations of isoprenaline
		0.012	0.047	0.12	0.47	1.2	2.4	
Cocaine	2×10^{-6}M	104	112	96	112	99	98	104
Metaraminol	1×10^{-7}M	98	100	102	113	106	99	103
	1×10^{-6}M	103	—	111	—	—	95	103
Normetanephrine	1×10^{-5}M	51	34	47	54	51	37	46

[a] Uptake was measured after 5 min perfusion. Results are expressed as a percentage of uptake without inhibitor. Mean values for 4–8 hearts.

fore of the Uptake$_2$ pattern over the whole range of isoprenaline concentrations from 1.2×10^{-7} M to 9.5×10^{-5} M.

When isoprenaline was accumulated by the heart over a 10-min period and the heart was then post-perfused with Krebs solution containing normetanephrine the loss of isoprenaline was reduced. The reduction was 60% for 10^{-5} M and 75% for 10^{-4} M normetanephrine. Iversen (1) found that normetanephrine depressed the loss of noradrenaline accumulated by Uptake$_2$.

Effects of Inhibitors on Noradrenaline Uptake in the Presence of Isoprenaline

The effects of cocaine, metaraminol, and normetanephrine on the uptake of noradrenaline from a concentration of 3×10^{-8} M are shown in Table 3. The uptake was inhibited by cocaine and metaraminol, but not by normetanephrine. When isoprenaline was also present in the perfusing medium this pattern was unchanged.

TABLE 3
Effect of inhibitors of ^{3}H-noradrenaline uptake in the presence of isoprenaline[a]

Drug		Isoprenaline concentration ($\times 10^{-5}$M) 0	0.12	0.47	2.4	Average values
Cocaine	2×10^{-6}M	37	33	33	50	38
Metaraminol	1×10^{-7}M	72	60	82	78	73
	1×10^{-6}M	40	33	20	40	33
Normetanephrine	1×10^{-5}M	94	94	100	89	94

[a] Uptake was measured after 5 min perfusion with 3×10^{-8}M ^{3}H-noradrenaline. Results of perfusion in the presence of isoprenaline together with an inhibitor are expressed as percentage of the uptake of noradrenaline in the presence of the concentration of isoprenaline alone. Results are mean values for 4–8 hearts.

DISCUSSION

The experiments reported here have shown that the perfused rat heart can take up isoprenaline by a process which appears to be identical to that responsible for the uptake of noradrenaline and adrenaline from perfusion solutions containing high concentrations of these amines (i.e., by Uptake$_2$). The effects of inhibitors and of post-perfusion were similar for all three catecholamines. Only a small amount of the accumulated isoprenaline was bound to particles, and this distribution resembled that of noradrenaline taken up from a perfusion concentration of 3×10^{-5} M.

It can be seen in Table 4 that there is a regular progression in kinetic constants of uptake from noradrenaline to isoprenaline. The increased size of the *N*-alkyl substituent leads to an increased affinity for the uptake system and at the same time to a decrease in the transport maximum. Preliminary experiments with *N*-ethylnoradrenaline showed that its parameters of uptake are probably intermediate between those of adrenaline and isoprenaline. The reciprocal relationship between the affinity of an amine for the uptake process and its transport maximum may well be due to an inverse dependence between the dissociation rate of the amine-carrier complex and the strength of the complex formed. If the rate of dissociation of the complex at the inner face of the plasma membrane of the sympathetic neuron were the rate-limiting step in transport, the inverse relationship found would be expected.

TABLE 4
Kinetic constants for noradrenaline, adrenaline, and isoprenaline (Uptake$_2$)

Drug	K (M)	V_{max} (nmole/g/min)
Noradrenaline (1)	25.2×10^{-5}	100
Adrenaline (1)	5.2×10^{-5}	64.4
Isoprenaline	2.3×10^{-5}	15.5

No trace of accumulation of isoprenaline by Uptake$_1$ was found in the present study. However, we have previously found that isoprenaline will inhibit noradrenaline up-

take by $Uptake_1$ (2), and this has been confirmed in this study. It would seem that isoprenaline is a nontransported inhibitor of $Uptake_1$.

The findings presented here agree with those of Hertting (4), who found that after intravenous injection of isoprenaline small amounts were found in the heart after 10 min but most of this had disappeared by 2 hours. Both the total uptake and its retention were small compared with noradrenaline.

The fact that isoprenaline is taken up exclusively by $Uptake_2$, a process insensitive to inhibition by cocaine and desipramine, is consistent with the well known observation that these drugs do not sensitize tissues to the pharmacologic actions of isoprenaline, whereas they do sensitize to noradrenaline and adrenaline. It leaves unanswered, however, the question whether the tissue uptake by $Uptake_2$ significantly modifies pharmacologic responses to isoprenaline.

The most interesting finding in the present study is that accumulation of noradrenaline by $Uptake_1$ and of isoprenaline by $Uptake_2$ can proceed simultaneously. This finding is incompatible with the suggestion that only one of these mechanisms can operate at any time and that the effect of a raised concentration of noradrenaline or adrenaline is to "switch off" $Uptake_1$ and "switch on" $Uptake_2$. A simpler explanation compatible with the present findings is that the plasma membrane of the sympathetic endings has a mosaic of sites of the $Uptake_1$ and $Uptake_2$ types. If an amine can be taken up by either type of site but has a higher affinity for one of the two this site will act as a sink picking up the major part of the amine reaching the membrane by diffusion. In the case of noradrenaline the affinity for $Uptake_1$ is about 400 times that for $Uptake_2$ so that $Uptake_1$ sites will act as very efficient sinks. If the concentration of noradrenaline is raised and the adsorptive and transport capacity of $Uptake_1$ becomes saturated this will become a decreasingly effective sink and a greater proportion of noradrenaline will be taken up by $Uptake_2$. It would be expected therefore that the concentration at which $Uptake_2$ becomes prominent for a particular amine will depend on the relative affinity constants for the two systems and the transport maximum. Adrenaline with a lower affinity for $Uptake_1$ and a higher affinity for $Uptake_2$ than noradrenaline should show a transition at a lower concentration. Iversen (1) found that the noradrenaline transition occurred at 9×10^{-6} M and the adrenaline transition at 3×10^{-6} M.

A consequence of this arrangement of uptake sites would also be that when $Uptake_1$ was selectively inhibited the noradrenaline normally taken up by $Uptake_1$ would now become available for $Uptake_2$. It would therefore be expected that a selective inhibitor of $Uptake_1$ would not be able to completely inhibit noradrenaline uptake. This should be particularly evident under conditions where $Uptake_1$ is nearly saturated. This prediction was tested with metaraminol which is a highly selective inhibitor of $Uptake_1$ (2). Metaraminol 10^{-6}

TABLE 5

Effect of metaraminol and normetanephrine on noradrenaline uptake[a]

Noradrenaline (M)	Metaraminol (M)	Normetanephrine (M)	Noradrenaline uptake (nmoles/g)	% inhibition
3×10^{-6}	0	0	4.5	0
3×10^{-6}	10^{-7}	0	3.4	23
3×10^{-6}	10^{-6}	0	2.7	41
3×10^{-6}	10^{-5}	0	2.5	43
3×10^{-6}	10^{-4}	0	2.5	43
3×10^{-6}	10^{-4}	10^{-4}	1.6	65

[a] Uptake was measured after 5 min.

M produced a 41% inhibition of the uptake of noradrenaline from a perfusion concentration of 3×10^{-6} M (Table 5). This was hardly increased at all by raising the metaraminol concentration to 10^{-4} M. However the residual uptake could be substantially depressed by normetanephrine, an inhibitor of $Uptake_2$.

The results presented in this paper suggest that the processes concerned with catecholamine uptake are independent of each other and interact only to the extent that they may compete for the available catecholamine in the extracellular fluid.

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REFERENCES

1. L. L. Iversen, *Brit. J. Pharmacol.* **25**, 18–33 (1965).
2. A. S. V. Burgen and L. L. Iversen, *Brit. J. Pharmacol.* **25**, 34–49 (1965).
3. L. L. Iversen, *Brit. J. Pharmacol.* **21**, 523–537 (1963).
4. G. Hertting, *Biochem. Pharmacol.* **13**, 1119–1128 (1964).