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Uptake and metabolism of catecholamines in the perfused hearts of different species

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Summary

1. The uptake of (±)-^{3}H-noradrenaline was studied in isolated perfused hearts of rat, mouse, guinea-pig, pigeon and toad (*Bufo marinus*), and the IC50 (concentration causing 50% inhibition) values for inhibition of uptake of (±)-^{3}H-noradrenaline by (−)-noradrenaline were calculated. IC50 values ranging from 0·28 μM (rat heart) to 2·34 μM (toad heart) were found.

2. In all species except the toad, (−)-noradrenaline showed a higher affinity than (−)-adrenaline for the uptake process, but the reverse was found for the toad heart.

3. Mouse and pigeon hearts contained increasing amounts of metabolites of noradrenaline with increasing perfusion concentrations of noradrenaline, but the guinea-pig and toad hearts did not. The *in vitro* activities of noradrenaline catabolizing enzymes in heart homogenates were measured but did not explain the differences in the pattern of catabolism of noradrenaline found in the intact hearts of the different species.

4. In all hearts except the toad, cocaine was an effective blocking agent for the uptake of (±)-^{3}H-noradrenaline and led to an increase in ^{3}H-normetanephrine in these hearts. In the pigeon heart, cocaine plus phenoxybenzamine in the perfusate resulted in an inhibition of both ^{3}H-noradrenaline uptake and ^{3}H-normetanephrine formation.

5. In guinea-pig and pigeon perfused hearts, the uptake of ^{3}H-noradrenaline into atria and ventricles reflected the relative concentrations of endogenous catecholamines in these regions, but this was not found for rat, mouse and toad hearts.

6. It was concluded that species differences exist for both the accumulation and metabolism of catecholamines in isolated perfused hearts.

Introduction

There is now considerable evidence that the tissue uptake of catecholamines is an important mechanism for the removal of both exogenous and locally released catecholamines (see Iversen, 1967, 1968). The properties of this uptake process are best studied in an isolated tissue preparation in controlled conditions and the perfused heart provides a convenient intact organ preparation for such studies.

Kopin and co-workers first investigated the uptake and metabolism of a low concentration of (±)-^{3}H-noradrenaline in isolated perfused rat hearts and found that uptake was the major mechanism for the removal of infused noradrenaline,

while tissue metabolism played only a minor role (Kopin, Hertting & Gordon, 1962; Hertting, Kopin & Gordon, 1962). Iversen (1963, 1965a, b, c) also used the isolated perfused rat heart in an extensive analysis of the uptake of catecholamines over a wide range of concentrations. Because these studies were performed exclusively on the rat heart, the present investigation was undertaken to compare the uptake and metabolism of catecholamines in the isolated perfused hearts of some other species.

Methods

Perfusion techniques

The perfusion medium and technique for studying ^{3}H-noradrenaline uptake by rat, mouse, guinea-pig and pigeon hearts was essentially the same as used by Iversen (1963) for the rat heart, with the exception that mouse hearts were perfused at a perfusion pressure of 50 cm of water and a flow rate of 2–3 ml min^{-1}. Toad hearts (obtained in autumn from *Bufo marinus*) were perfused at room temperature as described for the frog heart in Burn (1952) using frog Ringer solution containing EDTA (disodium salt) 10 mg/l. and ascorbic acid 20 mg/l. at a flow rate of 5–6 ml min^{-1}.

Extraction of catecholamines and metabolites

At the end of the perfusion, hearts were blotted, weighed and extracted by the procedure of Iversen (1963), with the exception that pigeon hearts were treated with twice as much 0·4 N perchloric acid because of their larger weight.

Estimation of catecholamines and their metabolites

One ml portions of the perchloric acid extracts were taken for determination of total radioactivity and dried down in counting vials in a vacuum desiccator. Two ml of absolute ethanol was added, followed by 10 ml of a phosphor containing 0·01% (1,4-*bis*-2-(5-phenyloxazolyl)-benzene and 0·4% 2,5-diphenyloxazole in toluene. This procedure produced a yellow solution that was highly quenching, but on addition of 25 μl of ammonia (33% v/w) the colour and the quenching disappeared. The remainder of the perchloric acid extract was treated according to the ion-exchange chromatographic procedure of Iversen (1963), with the modification that Zeokarb 225 (mesh size 200, 8% DVB, Na^+ form) was used as the cation exchange resin. In a series of twelve recovery experiments, ^{3}H-noradrenaline and ^{3}H-normetanephrine added to heart extracts and carried through the purification stage gave a recovery of $82 \pm 1{\cdot}8\%$ (mean $\pm$ S.E.M.) for noradrenaline and $85{\cdot}2 \pm 0{\cdot}8\%$ (mean $\pm$ S.E.M.) for normetanephrine. All results have been corrected for these recoveries. Two ml portions of the eluates from the resin column were placed in counting vials and evaporated to dryness in a vacuum desiccator. The residue was taken up in 1 ml of absolute ethanol and 10 ml of phosphor added. All samples were counted for two 10 min periods in a Nuclear-Chicago Scintillation counter, Model 720. Counts per minute were converted to disintegrations per minute by means of the Channels Ratio Method (Bush, 1963). Deaminated metabolites were estimated by subtracting the sum of the corrected noradrenaline and normetanephrine radioactivity from the total radioactivity.

Fluorometric assay of noradrenaline and adrenaline

Noradrenaline and adrenaline in resin column eluates were measured by the trihydroxyindole method (von Euler & Lishajko, 1961) in a Farrand spectrophotofluorimeter. Mixtures of these two amines were estimated by the differential method of Cohen & Goldenberg (1957). In one experiment, the noradrenaline content of mouse atrial and ventricular tissue was measured by a semi-micro modification of the normal fluorometric technique (Simmonds, 1969).

Monoamine oxidase and catechol-O-methyl transferase estimations

Hearts were perfused with Krebs solution to wash out blood and the atria and ventricles were carefully dissected. These tissues were then blotted, weighed and homogenized in 10 volumes of 0·005 M phosphate buffer, pH 7·0. Monoamine oxidase (MAO) activity of the homogenate was assayed by a modification procedure of McCaman, McCaman, Hunt & Smith (1965) using ^{3}H-tyramine and ^{3}H-5-hydroxytryptamine as substrates (Jarrott & Iversen, in preparation). Catechol-O-methyl transferase (COMT) activity was determined by the modified method of McCaman (1965), using 3,4-dihydroxybenzoic acid and ^{3}H-methyl-S-adenosyl-methionine as substrates (Jarrott & Iversen, in preparation).

Protein estimation

The protein content of the homogenates was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Determination of IC50 values

A control group of at least four hearts was perfused with (±)-^{3}H-noradrenaline in a concentration of 10 ng/ml for 5 or 10 min, followed by a 2 min wash-out perfusion with drug-free medium. Then, groups of four hearts were perfused with the same concentration of (±)-^{3}H-noradrenaline in the presence of increasing concentrations of either (−)-noradrenaline, (−)-adrenaline or cocaine and the uptake of (±)-^{3}H-noradrenaline determined. The uptake of ^{3}H-noradrenaline as a percentage of that in control hearts was converted to a probit and the equation for the regression line of these values against log dose of added drug was calculated, using the method of least squares. IC50 (drug concentration causing 50% inhibition of uptake of (±)-^{3}H-noradrenaline) and its 95% confidence limits, and a plot of the regression line with standard errors of mean for each point was computed (Finney, 1964).

Materials

(±)-7-^{3}H-noradrenaline (1·07 Ci/mmol; The Radiochemical Centre, Amersham, Buckinghamshire), (±)-^{3}H-normetanephrine (generally labelled; 450 mCi/mmol; Volk Laboratories, California); S-adenosyl-methionine (^{3}H-methyl, 4·15 Ci/mmol; The Radiochemical Centre); 5-hydroxytryptamine creatinine sulphate (^{3}H, generally labelled; 300 mCi/mmol; The Radiochemical Centre); ^{3}H-tyramine hydrochloride (generally labelled; 2·1 Ci/mmol, New England Nuclear Corp., Boston, Massachusetts); (−)-noradrenaline bitartrate (Koch-Light); (−)-adrenaline base (Koch-Light); phenoxybenzamine hydrochloride (generously

donated by Smith, Kline and French Laboratories); reserpine base (Koch-Light); 5-hydroxytryptamine creatinine sulphate (B.D.H. Ltd.); tyramine hydrochloride (B.D.H. Ltd.); 3,4-dihydroxybenzoic acid (Koch-Light) and S-adenosyl-methionine chloride (Koch-Light).

Results

Inhibition of uptake of (±)-^{3}H-noradrenaline by (−)-noradrenaline and (−)-adrenaline in rat, mouse, guinea-pig, pigeon and toad hearts

The control uptake from perfusates containing 10 ng/ml (±)-^{3}H-noradrenaline was first established to be linear with time over 2·5, 5 and 10 min intervals for each species. After 5 min, the mean uptake of (±)-^{3}H-noradrenaline in ng/g was found to be 42·6±0·91 (rat), 36·0±2·10 (mouse), 34·4±3·52 (guinea-pig), 12·8±1·37 (pigeon) and 31·9±2·40 (toad). The uptake of ^{3}H-noradrenaline was then measured after a 5 min perfusion in the presence of non-radioactive (−)-noradrenaline or (−)-adrenaline in concentrations ranging from 0·15 to 7·5 μM. Figure 1 shows the effect of (−)-noradrenaline on the uptake of (±)-^{3}H-noradrenaline by the hearts of different species. When the slopes of the lines were analysed statistically by the procedure of Finney (1964) only the slope of the regression line for the mouse heart deviated significantly from parellelism. Table 1 summarizes the IC50 values obtained. The IC50 for (−)-adrenaline was lower than that for (−)-noradrenaline in the toad heart; in all the other species the reverse was the case.

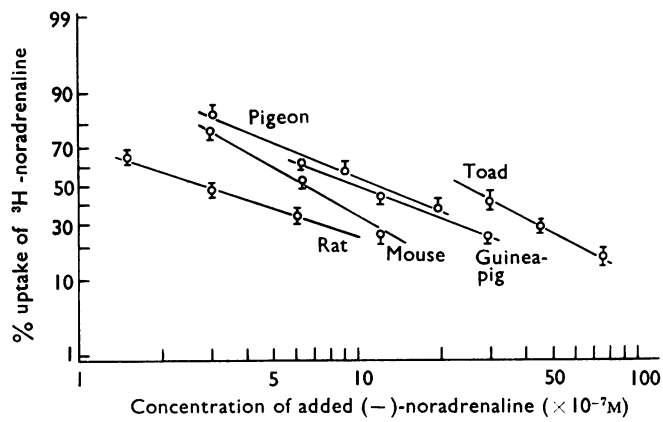

FIG. 1. Calculated regression lines for the inhibition of uptake of ^{3}H-noradrenaline by added (−)-noradrenaline. Each point represents the mean±S.E. obtained on four hearts.

TABLE 1. *Inhibition of uptake of ^{3}H-noradrenaline by non-radioactive (−)-noradrenaline and (−)-adrenaline*

Species	IC50(μM) (−)-Noradrenaline	(−)-Adrenaline
Rat	0·28 (0·22–0·35)	1·02 (0·86–1·23)
Mouse	0·65 (0·55–0·75)	1·08 (0·84–1·39)
Guinea-pig	0·98 (0·77–1·26)	2·72 (2·25–3·29)
Pigeon	1·15 (0·86–1·53)	2·96 (2·44–3·59)
Toad	2·34 (1·95–2·82)	0·96 (0·62–1·47)

The IC50 for (−)-noradrenaline and (−)-adrenaline was measured as described in the text. The figures in parentheses are the 95% confidence limits for the IC50.

Metabolism of (±)-^{3}H-noradrenaline in the presence of increasing quantities of (−)-noradrenaline

For each concentration of added (−)-noradrenaline used in the determination of the IC50 values, metabolites of (±)-^{3}H-noradrenaline were also determined The percentage of total tissue radioactivity represented by noradrenaline, normetanephrine, and deaminated metabolites at each concentration of (−)-noradrenaline is shown in Fig. 2. It can be seen that a species difference in the metabolism of noradrenaline exists: O-methylation was prominent in the mouse and pigeon hearts at high perfusion concentrations of noradrenaline whereas only minor amounts of metabolites were found at all concentrations of noradrenaline in guinea-pig and toad hearts. In all species, the amount of deaminated metabolites was very low at all concentrations of noradrenaline.

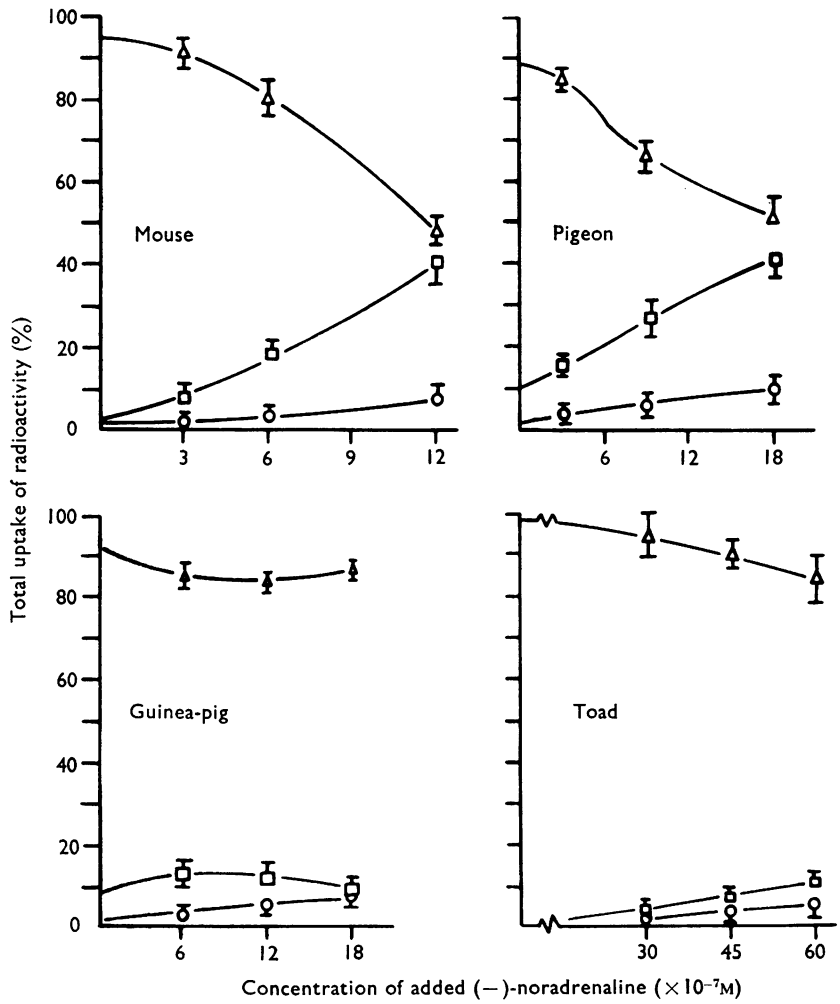

FIG. 2. Radioactive metabolites present in hearts after perfusion with (±)-^{3}H-noradrenaline in the presence of increasing amounts of non-radioactive (−)-noradrenaline. Each point is the mean ± S.E. for four hearts; values are expressed as percentages of the total radioactivity present in the tissue. △, ^{3}H-noradrenaline; □, ^{3}H-normetanephrine; ○, ^{3}H-deaminated metabolite.

TABLE 2. *Protein, MAO and COMT activities of atrial and ventricle homogenates of hearts from different species*

	Rat		Mouse		Guinea-pig		Pigeon		Toad	
Species	Atria	Ventricle	Atria	Ventricle	Atria	Ventricle	Atria	Ventricle	Atria	Ventricle
Protein—mg/g	75 ±3·3	96 ±2·1	62 ±12·8	99 ±3·9	47 ±1·1	64 ±2·6	68 ±3·0	77 ±5·0	64 ±6·1	74 ±9·0
COMT—μmol product formed/h per g protein	7·5±0·72	5·1±0·23	9·6±0·13 *	13·4±0·66	4·3±0·21	4·3±0·27	5·9±0·42	5·5±0·14	1·1±0·22 ***	3·8±0·09
MAO—μmol product formed/h per g protein										
5-HT subst.	45·5±1·62 ***	91·1±5·21	16·7±1·38 ***	3·3±0·30	13·2±1·67	10·8±2·38	10·9±1·53 *	5·5±0·49	2·7±0·22 ***	6·9±0·10
TM subst.	104·0±6·06 ***	242·2±2·60	98·7±6·15	34·8±1·92	79·9±6·92	80·5±2·91	24·5±2·60 *	13·1±1·85	48·7±3·45	55·8±1·85

Each value is the mean±S.E. for four homogenates.

*** $P<0·001$ when compared with ventricle. ** $P<0·01$ when compared with ventricle. * $P<0·05$ when compared with ventricle.

The radioactivity present in the control toad hearts after perfusion with 100 nCi/ml of (±)-^{3}H-noradrenaline was examined chromatographically by the method described in Iversen (1963). More than 85% of the radioactivity was found to be noradrenaline and approximately 10% had an R_F similar to that of adrenaline.

In vitro *enzyme activities of homogenates of rat, mouse, guinea-pig, pigeon and toad hearts*

Monoamine oxidase and COMT activities of homogenates of atria and ventricles of each species were determined as described in **Methods**. There were differences in MAO activity between atria and ventricles except in the guinea-pig as well as differences between species (Table 2). Tyramine in all species was a much better substrate for monoamine oxidase than was 5-hydroxytryptamine. The average ratio of activities with tyramine and 5-HT as substrate was 6·6 for all species. Catechol-O-methyl transferase activity was more evenly distributed between atria and ventricles, although there was a small but significant difference between atria and ventricles of mouse and toad hearts.

Inhibition of uptake of (±)-^{3}H-noradrenaline by cocaine and phenoxybenzamine

Cocaine was a potent inhibitor of ^{3}H-noradrenaline uptake in the mouse, guinea-pig, and pigeon heart (Table 3). ^{3}H-noradrenaline uptake in the toad heart, however, was only inhibited by 20% in the presence of a high concentration of cocaine (1×10^{-5}M). In the mouse, guinea-pig and pigeon heart there was an increased formation of ^{3}H-normetanephrine when high concentrations of cocaine were present, and this phenomenon was particularly obvious in the pigeon heart. An additional experiment was performed in which groups of pigeon hearts were perfused with a

TABLE 3. *Inhibition of uptake of ^{3}H-noradrenaline by cocaine*

Species	IC50 (μM)
Mouse	2·0
Guinea-pig	5·0
Pigeon	4·5

Groups of hearts (four) were perfused with (±)-^{3}H-noradrenaline (10 ng/ml) for 5 min and then with the same quantity of noradrenaline in the presence of two concentrations of cocaine. The IC50 was estimated graphically.

TABLE 4. *Effect of cocaine and phenoxybenzamine on uptake and metabolism of noradrenaline in the pigeon heart*

	Deaminated metabolites	^{3}H-noradrenaline	^{3}H-normetanephrine
Radioactivity recovered in control hearts after 5 min perfusion (nCi/g)	0·4±0·05	45·8±4·49	4·2±0·57
	% values in control		
Control	100±13·4	100±7·0	100±14·2
Control+10^{-5}M cocaine	58± 9·2	5±0·7	376±25·5
Control+10^{-5}M cocaine+$1 \times 10_{-4}$M phenoxybenzamine	48± 7·1 NS	1·5±0·7 *	153±20·4 *

NS=$P>0·05$. *=$P<0·01$ when compared with cocaine alone.

Groups of pigeon hearts were perfused with (*a*) 10 ng/ml (±)-^{3}H-noradrenaline for 5 min not washed out with drug-free Krebs at the conclusion, (*b*) 10 ng/ml (±)-^{3}H-noradrenaline in the presence of cocaine (1×10^{-5}M) and (*c*) 10 ng/ml (±)-^{3}H-noradrenaline in the presence of cocaine (1×10^{-5}M) and phenoxybenzamine (1×10^{-4}M). Uptake was corrected for ^{3}H-noradrenaline in the extracellular space at the end of the perfusion.

TABLE 5. *Relation between endogenous catecholamine concentration and (±)-^{3}H-noradrenaline uptake in the atria and ventricles of different species*

	Rat		Mouse		Guinea-pig		Pigeon		Toad	
	Atria	Ventricle	Atria	Ventricle	Atria	Ventricle	Atria	Ventricle	Atria	Ventricle
Endogenous catecholamine μg/g	1·70±0·11	0·87±0·34	1·10±0·06	1·18±0·30	3·44±0·20	1·68±0·93	1·84±0·03	0·95±0·03	3·65±0·23 (Adrenaline)	5·18±0·25
Uptake (±)-^{3}H-noradrenaline nCi/g	63·1±2·46	41·7±0·86	53·0±0·99	62·0±0·49	51·1±2·30	24·9±3·40	40·1±4·49	23·2±3·22	61·3±1·75	62·5±1·14
Specific activity (nCi/μg)	37·1±1·29	47·9±0·99 *	48·2±0·91	52·5±0·41 *	14·8±0·45	14·8±1·35	21·7±2·44	24·4±3·39	16·8±0·48	12·1±0·22 *

Hearts were perfused with 20 nCi/ml of ^{3}H-noradrenaline for 5 min followed by perfusion with drug-free Krebs solution for 2 min. Groups of four to six hearts were used for each determination.

* $P = <0·005$ when compared with atrial value.

high concentration of cocaine (10^{-5}M) in the presence and absence of phenoxybenzamine (10^{-4}M). In the presence of cocaine alone there was more than a three-fold increase in normetanephrine formation, a decrease in noradrenaline uptake, and a decrease in deamination when compared with a drug-free control. Phenoxybenzamine together with cocaine, however, inhibited the increase in O-methylation and resulted in a further inhibition of uptake of noradrenaline. The further decrease in deamination which was observed was not statistically significant (Table 4).

Uptake of (±)-^{3}H-noradrenaline into atria and ventricles and its relation to endogenous noradrenaline content

Hearts were perfused with (±)-^{3}H-noradrenaline (10 ng/ml) for 5 min, dissected into atria and ventricles and the uptake of (±)-^{3}H-noradrenaline (nCi/g wet weight) measured (Table 5). At the same time the endogenous content of catecholamines was measured in atria and ventricles using animals of the same strain. In mouse, guinea-pig, pigeon and rat hearts the catecholamine exhibited the same excitation and emission peak wavelengths as authentic noradrenaline and in these species differential fluorimetry failed to show an adrenaline concentration greater than 0·02 μg/g. The catecholamine in the toad heart exhibited the same fluorescence spectra as adrenaline and differential fluorimetry indicated that approximately 0·04 μg/g noradrenaline was present in the atria, and less than this amount in the ventricle. (In the case of the toad, " atria " includes sinus venosus tissue, while " ventricle " includes truncus arteriosus). When the uptake of ^{3}H-noradrenaline was expressed as nCi/μg endogenous catecholamine there were significant differences between atria and ventricles in rat, mouse and toad, but not in guinea-pig or pigeon hearts.

In an additional experiment, groups of rat hearts were perfused with the same amount of (±)-^{3}H-noradrenaline in the presence or absence of cocaine (10^{-6}M). This resulted in a similar percentage inhibition of ^{3}H-noradrenaline uptake in atria (55·8 ± 6·85%) and ventricles (61·8 ± 2·74%) (mean ± S.E.M.).

Discussion

The present study has shown that noradrenaline is accumulated by the isolated hearts of various species. In previous studies of catecholamine uptake by adrenergic neurones, Iversen (1965a) and Green & Miller (1966) have shown that adrenaline and noradrenaline competed for a common uptake site and that the concentration of (±)-noradrenaline necessary to produce 50% inhibition of (±)-^{3}H-noradrenaline uptake was approximately equal to the *Km* value for (±)-noradrenaline determined by standard kinetic methods. In the present study, it was found that the IC50 for (−)-noradrenaline in the rat heart was 0·28 μM, which is in good agreement with the affinity constant for uptake (*Km*) for (−)-noradrenaline published by Iversen (0·27 μM). The IC50 measured for the other species (Fig. 2), therefore, probably approximates the *Km* value.

The absolute values for IC50 ranged from 0·28 μM for (−)-noradrenaline in the rat to 2·34 μM for (−)-noradrenaline in the toad. In all species except the toad, the results indicate that (−)-adrenaline has a lower affinity for the uptake site than (−)-noradrenaline, the naturally occurring catecholamine. The reverse situation

observed in the toad heart is particularly interesting as adrenaline and not noradrenaline has been shown, both in this and other studies (Falck, Haggendal & Owman, 1963 ; Angelakos, Glassman, Millard & King, 1965), to be the endogenous catecholamine in the cardiac sympathetic innervation of this species.

There was a marked difference between species in the pattern of metabolism of (±)-^{3}H-noradrenaline as the concentration of added (−)-noradrenaline was increased. Both toad and guinea-pig hearts showed no significant increase in the concentration of ^{3}H-normetanephrine, with increases in noradrenaline concentration, whereas in mouse and pigeon hearts there was a rise in ^{3}H-normetanephrine under these conditions. There was also a considerable increase in ^{3}H-normetanephrine formation at high perfusion concentrations of noradrenaline in the rat heart (Iversen, 1963) and this is thought to be due to the uptake and metabolism of noradrenaline in cardiac muscle cells (Lightman & Iversen, 1969). In all species, very small amounts of deaminated metabolites were present in the tissue after 5 min perfusion at all concentrations. It is likely that these amounts underestimate the extent of deamination of ^{3}H-noradrenaline during the perfusion, as deaminated products are probably washed out of the tissue soon after their formation (Landsberg & Axelrod, 1968).

In view of the species difference observed in the metabolism of noradrenaline by the intact heart, one might expect to observe similar differences in the activities of catechol-O-methyl transferase and monoamine oxidase in heart homogenates between species. However, this was not the case ; guinea-pig heart homogenates had only slightly less catechol-O-methyl transferase activity than the pigeon and mouse (Table 4), and in all species, monoamine oxidase activity in heart homogenates was considerably greater than catechol-O-methyl transferase activity. There are many possible explanations for the failure of studies of noradrenaline metabolism in tissue homogenates to reflect the pattern of noradrenaline metabolism in the intact organ. For example, the small amount of O-methylation in perfused guinea-pig hearts might be due to a low endogenous concentration of the methyl donor, S-adenosyl-methionine in this species. In the *in vitro* enzyme assays, exogenous S-adenosyl-methionine is added to the heart homogenates, and the catechol-O-methyl transferase activity becomes maximal.

There were differences in the distribution of monoamine oxidase activity between atria and ventricles, but since a large proportion of cardiac monoamine oxidase is extraneuronal (Potter, Cooper, Willman & Wolfe, 1965), the significance of this distribution is obscure. Potter *et al.* (1965) found a higher activity of monoamine oxidase in atria compared to ventricles in the dog heart and similar findings were obtained in the present study for mouse and pigeon hearts. However, the rat heart contained a higher activity in the ventricles compared to the atria. In all species, the ratio of monoamine oxidase activity in atria/ventricles was essentially the same whether measured with tyramine or 5-hydroxytryptamine as substrate. Thus it is unlikely that the enzyme in atria is different from the enzyme in ventricles. Catechol-O-methyl transferase activity in the two regions of all species was essentially the same ; similar findings have been reported for the dog heart (Potter *et al.*, 1965).

It has been shown in the rat heart perfused with ^{3}H-noradrenaline that in the presence of a large concentration of cocaine there is an increased concentration of ^{3}H-normetanephrine, together with a fall in the accumulation of ^{3}H-noradrenaline

(Eisenfeld, Krakoff, Iversen & Axelrod, 1967 ; Eisenfeld, Axelrod & Krakoff, 1967). A similar phenomenon was observed in the present studies in mouse, guinea-pig and pigeon hearts. This effect was particularly pronounced in the pigeon heart, where there was more than a three-fold increase in the accumulation of normetanephrine in the presence of cocaine. Furthermore, phenoxybenzamine prevented the cocaine-induced increase in ^{3}H-normetanephrine in the pigeon heart as reported in the rat heart by Eisenfeld *et al.* (1967). The uptake of ^{3}H-noradrenaline by the toad heart was relatively insensitive to inhibition by cocaine ; a similar phenomenon has been reported in the avian median eminence (Björklund, Falck & Ljujggren, 1968).

Kopin, Gordon & Horst (1965) have postulated that the ability of tissues to remove noradrenaline depends on the density of the sympathetic innervation, as reflected by the endogenous noradrenaline. Many workers have shown an uneven distribution of catecholamines in various regions of the heart (Shore, Cohn, Highman & Maling, 1958 ; Angelakos, Fuxe & Torchiana, 1963 ; Dahlström, Fuxe, Maya-Tu & Zetterström, 1965 ; Potter *et al.*, 1965) and if the endogenous noradrenaline concentration does reflect the density of adrenergic nerves then the uptake of ^{3}H-noradrenaline should parallel the distribution of endogenous noradrenaline. However, only in the guinea-pig and pigeon heart did the uptake of ^{3}H-noradrenaline into atria and ventricles parallel the endogenous distribution of noradrenaline. It is possible that an uneven perfusion of the heart regions by ^{3}H-noradrenaline is responsible for this discrepancy in the case of mouse, rat and toad heart. Another possibility is that the presence of " chromaffin-like " cells reported in atria of rats, mice, guinea-pig and cat (Jacobowitz, 1967) may obscure the relationship between endogenous noradrenaline concentration and the uptake of ^{3}H-noradrenaline.

The present study has revealed several species differences in the uptake and metabolism of noradrenaline, and these findings emphasize that caution should be exercised in extrapolating results on noradrenaline uptake determined in one organ of one species to other organs and other species.

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