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Differences in the uptake, storage and metabolism of (+)- and (−)-noradrenaline

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Summary

1. The rate of uptake of (+)- and (−)-noradrenaline was measured in isolated perfused hearts of reserpine treated rats, mice and guinea-pigs by fluorimetric analysis of the removal of catecholamine from the perfusion medium. In rat and mouse heart (−)-noradrenaline was taken up significantly more rapidly than (+)-noradrenaline, but no stereochemical specificity was found for noradrenaline uptake in guinea-pig hearts.

2. Using radioactively labelled (+)-^{14}C-noradrenaline and (−)-^{3}H-noradrenaline, the kinetic constants for uptake into noradrenaline-containing and dopamine-containing synaptosomes from rat brain were determined. The uptake by noradrenaline terminals in the hypothalamus had a higher affinity for (−)-noradrenaline than for the (+)-isomer, but no differences in affinity were found for uptake into dopamine terminals.

3. When equal amounts of labelled (+)- and (−)-noradrenaline were injected *in vivo* in double isotope experiments, the (+)-isomer disappeared more rapidly than the (−)-isomer from rat heart and spleen, but no significant differences were found between the rates of disappearance of the two isomers from rat brain or in the whole mouse.

4. Analysis of the radioactive metabolites of the two isomers of noradrenaline after administration of mixed doses of the labelled substances showed that a significantly higher proportion of (+)-noradrenaline was metabolized to normetanephrine than of (−)-noradrenaline, in the whole mouse, rat heart and rat brain.

Introduction

Most previous studies with radioactively labelled noradrenaline have made use of a racemic mixture of isotopically labelled material. There is, however, considerable evidence that the biochemical mechanisms responsible for uptake, storage and metabolism of noradrenaline are stereospecific for the naturally occurring (−)-isomer. Iversen (1963), Maickel, Beaven & Brodie (1963) and Malmfors (1965) reported that the uptake of noradrenaline by sympathetically innervated tissues favoured the (−)-isomer ; Maickel *et al.* (1963) and Kopin & Bridgers (1962) demonstrated a more rapid disappearance of (+)- than (−)-noradrenaline after accumulation of the two isomers in rat tissues ; Stjärne & von Euler (1965) found that noradrenaline storage vesicles from bovine splenic nerve had an affinity for (−)-noradrenaline some 5 times greater than for (+)-noradrenaline ; and Giachetti & Shore (1966a) showed that (−)-noradrenaline was degraded more rapidly by monoamine

oxidase than (+)-noradrenaline. In the isolated perfused rabbit heart, however, Draskoćzy & Trendelenburg (1968) found no difference in the rate of uptake of (+)- and (−)-noradrenaline when the tissue was perfused with a low concentration of catecholamine. These authors estimated the rate of uptake of noradrenaline by measuring the arteriovenous difference in noradrenaline concentration in the perfused heart, and they suggested that previous reports of the stereospecificity of noradrenaline uptake might have reflected differences in the intraneuronal storage and retention of accumulated amine rather than differences in the initial rate of uptake. The cogency of this argument, together with the recent availability of radioactively labelled (+)- and (−)-noradrenaline prompted us to re-examine the differences in the uptake, storage and metabolism of (+)- and (−)-noradrenaline in various tissues and in different mammalian species.

Methods

Isotopically labelled noradrenaline

Samples of (+)-noradrenaline (methylene-^{14}C) (specific activity=21·2 mCi/mmol) and (−)-noradrenaline-7-^{3}H (specific activity=2·18 Ci/mmol) were kindly supplied by Dr. M. Barnes, Radiochemical Centre, Amersham, England. These are now commercially available. The chemical purity of the labelled substances was confirmed by paper chromatography and by the finding that the recoveries of ^{14}C and ^{3}H-noradrenaline were similar when a mixture of the compounds was submitted to ion-exchange chromatography on Amberlite-CG-120 as described by Iversen (1963). Optical purity was investigated by a bioassay of the labelled catecholamines in which their ability to antagonize acetylcholine-induced contractions of the isolated rat colon was compared with authentic samples of non-radioactive (+)- and (−)-noradrenaline (Sterling-Winthrop Laboratories). The results of this experiment indicated that both (−)-^{3}H-noradrenaline and (+)-^{14}C-noradrenaline were more than 95% optically pure. Fluorimetric assay of samples of the two labelled materials confirmed the specific activities stated by the manufacturers.

Uptake of (+)- and (−)-noradrenaline by isolated perfused hearts

Adult rats, mice or guinea-pigs were pretreated with reserpine (3 mg/kg i.p.) 18 h before removal of hearts for perfusion as described by Iversen (1963) and Jarrott (1970). The hearts were perfused with Krebs bicarbonate solution and the flow rate was adjusted by varying the height of the gravity feed reservoir to values of approximately 5 ml/min for guinea-pig and rat hearts and 1 ml/min for mouse hearts. After a preliminary perfusion with amine-free solution, the hearts were perfused with media containing a low concentration of non-radioactive (+)- or (−)-noradrenaline.

After perfusion for 1–2 min, two 5 min samples of the perfusate outflow were collected in graduated vessels containing 1 ml of 1 N hydrochloric acid (0·1 ml for mouse hearts) and approximately 10 mg of sodium metabisulphite. The collection vessels were surrounded by ice to chill the perfusate samples. A tracer amount of ^{3}H-noradrenaline was added to each sample as an internal recovery standard, and noradrenaline was then isolated by absorption and elution from a column (5·0 × 0·6 cm) of Amberlite CG-120 resin as described by Iversen (1963). Samples of the column eluate were counted to determine the recovery of noradrenaline (which was

usually in the range of 70–80%) and other samples were used for the fluorimetric assay of noradrenaline by the method of von Euler & Lishajko (1961); noradrenaline values were corrected for recovery. Samples of the perfusion medium before passage through the tissue were processed and assayed similarly. The flow rate and weight of each heart at the end of perfusion were also recorded and used to calculate the rate of uptake of noradrenaline from the difference in noradrenaline concentrations between the perfusion input and outflow.

Uptake of (+)- and (−)-noradrenaline by brain homogenates

The uptake of radioactively labelled noradrenaline was measured as described by Snyder & Coyle (1968). Homogenates of rat hypothalamus and striatum in 0·3 M sucrose were centrifuged at 1,000 *g* for 5 min to remove large particles, and samples of the supernatant were incubated with various concentrations of (+)-^{14}C-noradrenaline of (−)-^{3}H-noradrenaline for 5 min at 37° C in Krebs phosphate solution containing ascorbic acid, EDTA and pargyline to inhibit the spontaneous and enzymic breakdown of catecholamine (Snyder & Coyle, 1968). Each uptake sample was run in parallel with a blank in which the same amount of labelled catecholamine was added at the end of the incubation period. Samples and blanks were centrifuged at 100,000 *g* for 20 min and the radioactivity in the pellets was extracted by homogenization in 2 ml ethanol and counted. The radioactivity of blank samples was subtracted from that in experimental samples to obtain the estimates of noradrenaline uptake. The results were analysed by Michaelis-Menten kinetics as described by Snyder & Coyle (1968).

Isolation and separation of radioactively labelled noradrenaline and normetanephrine

Radioactive noradrenaline and normetanephrine were isolated from extracts of whole mouse, rat heart and spleen and rat brain and separated by ion exchange chromatography as described by Iversen (1963). The total radioactivity of such extracts was also determined by liquid scintillation counting of samples of the material used for the extraction of radioactive noradrenaline and normetanephrine.

Results

Uptake of (+)- and (−)-noradrenaline by isolated perfused hearts

To determine the rates of uptake of (+)- and (−)-noradrenaline animals were treated with reserpine 18 h before the experiment, in order to eliminate any differences due to stereospecificity of intraneuronal storage mechanisms (Draskoćzy & Trendelenburg, 1968). Hearts were then perfused with a concentration of (+)- or (−)-noradrenaline below that required to saturate the uptake mechanism (Jarrott, 1970). The rate of noradrenaline uptake under such conditions can be estimated by measuring the rate of removal of catecholamine from the perfusion medium during passage through the tissue. (Draskoćzy & Trendelenburg, 1968: Iversen, Glowinski & Axelrod, 1965).

In these experiments there were no significant differences in heart weight or in perfusion flow rate between hearts perfused with (+)- or (−)-noradrenaline. The rate of uptake during the first collection period (1–6 min) was similar to that measured between 6 and 11 min; these values have, therefore, been pooled. There was

a highly significant difference between the rate of uptake of the isomers of noradrenaline in rat and mouse hearts, but no difference in the guinea-pig heart (Table 1). As expected, (−)-noradrenaline was taken up more rapidly than (+)-noradrenaline by rat and mouse hearts.

Uptake of (+)- and (−)-noradrenaline by homogenates of rat brain

In previous studies Snyder & Coyle (1968) and Coyle & Snyder (1969) have described the properties of catecholamine uptake by homogenates of rat brain. They showed that the uptake occurs largely into synaptosomes in such homogenates, and have described various differences in the uptake systems in noradrenaline-rich areas of brain (such as the hypothalamus) and in the dopamine-rich striatal area. By measuring the inhibition of (±)-^{3}H-noradrenaline uptake by non-radioactive (+)- and (−)-noradrenaline Coyle & Snyder (1969) showed that the uptake system in noradrenaline terminals was stereochemically specific, with a higher affinity for the (−)-isomer, whereas the uptake system in dopamine terminals did not discriminate between the two isomers of noradrenaline. The results described here confirm these findings. By using radioactively labelled (+)- and (−)-noradrenaline it was possible to determine directly the *Km* values for the uptake of the two isomers by noradrenaline and dopamine terminals (Fig. 1, Table 2). The values obtained are in good agreement with the *Ki* values for (+)- and (−)-noradrenaline reported by Coyle & Snyder (1969).

TABLE 1. *Rate of uptake of (+)- and (−)-noradrenaline by isolated perfused hearts of different species*

Species and isomer	NA concentration inflow (ng/ml)	Rate of flow (ml/min)	NA concentration outflow (ng/ml)	Rate of uptake ((ng/min)/g heart)
Rat-(−)-NA	20	4·7±0·33	11·3±0·25	53·8±3·51
Rat-(+)-NA	20	4·8±0·45	15·0±0·09	32·0±3·77‡
Mouse-(−)-NA	40	1·1±0·07	27·3±0·22	108·5±1204
Mouse-(+)-NA	40	0·9±0·10	31·8±1·15	67·7± 5·99†
G. pig-(−)-NA	20	6·0±0·56	12·2±0·16	31·4±1·84
G. pig-(+)-NA	20	5·4±0·50	12·3±0·32	32·0±1·30

Animals were treated with reserpine (3 mg/kg, i.p.) 18 h beforehand. Hearts were perfused with a medium containing non-radioactive (+)- or (−)-noradrenaline (NA) at the concentration indicated. After 1 min perfusion the perfusate outflow was collected and NA was isolated and assayed as described in **Methods**. The rate of uptake was calculated from the input-output difference, the heart weight and the flow rate. Results are means ± S.E.M. for $n=9$ (rat), $n=7$ (mouse) and $n=6$ (guinea-pig). ‡ = $P<0.001$. † = $P<0.02$ when compared with corresponding value for (−)-NA.

TABLE 2. *Kinetic constants for uptake of (+)- and (−)-noradrenaline homogenates of rat hypothalamus and striatum*

	^{3}H-(−)-NA		^{14}C-(+)-NA	
	Km (μM)	*Vmax* ((nmol/g)/min)	*Km* (μM)	*Vmax* ((nmol/g)/min)
Hypothalamus	0·20±0·005	0·65±0·05	0·79±0·09*	1·08±0·09*
Striatum	2·00±0·06	2·34±0·18	1·93±0·16	2·80±0·15

Values are means ± S.E.M. for four experiments of the type illustrated in Fig. 1 for each brain region, and each isomer. * = $P<0.01$ when compared with corresponding values for (−)-NA.

Retention of (+)- and (−)-noradrenaline by rat and mouse tissues

To examine the possibility that differences might exist in the intraneuronal storage of (+)- and (−)-noradrenaline, the retention of radioactively labelled (+)-^{14}C-noradrenaline and (−)-^{3}H-noradrenaline was examined in the following experiments. (−)-^{3}H-noradrenaline was diluted to a specific activity equal to that of (+)-^{14}C-noradrenaline by the addition of non-radioactive (−)-noradrenaline. The following total doses containing equal amounts of each isomer were then administered: 8 μg by intravenous injection in the tail vein of mice; 40 μg by intravenous injection in the tail vein of rats and 1·6 μg by injection into the lateral ventricle of the rat brain. At various times after injection radioactive noradrenaline was isolated from extracts of the whole mouse (Iversen & Whitby, 1962), rat heart and spleen or rat brain. (+)-^{14}C-Noradrenaline and (−)-^{3}H-noradrenaline were discriminated by liquid scintillation counting using a channels ratio method (Okita, Kabara, Richardson & LeRoy, 1957).

There were no significant differences in the rate of disappearance of (+)- and (−)-noradrenaline from the whole mouse or from the whole rat brain (Figs. 2, 3): the initial accumulation of each isomer was also similar in these experiments. In the rat heart and spleen, however, there was a slightly higher initial accumulation of (+)-noradrenaline followed by a somewhat more rapid disappearance of the (+)-isomer

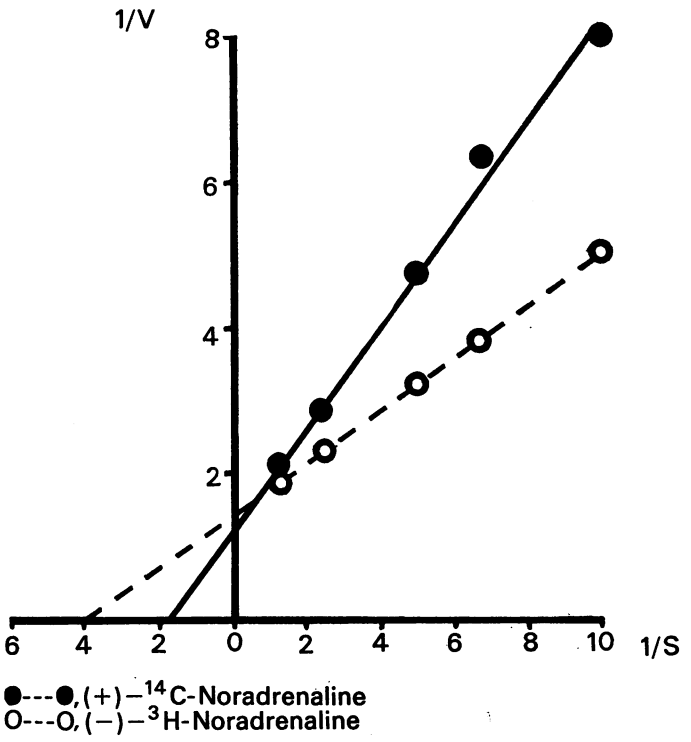

FIG. 1. Kinetic analysis of uptake of (+)-^{14}C-noradrenaline and (−)-^{3}H-noradrenaline by homogenates of rat hypothalamus. V=rate of uptake ((nmol/min)/g wet weight original) and S=concentration of noradrenaline in incubation medium (−μM). Results of a single experiment with each isomer are illustrated; the kinetic constants derived from four similar experiments are summarized in Table 2.

(Fig. 4). In the spleen the half times for disappearance of (+)- and (−)-noradrenaline between 1 h and 6 h after injection were approximately 4 h and 8 h respectively. In the rat heart the disappearance of labelled noradrenaline was multiphasic, with half times for an initial phase (0–1 h) of approximately 0·75 h for (+)-noradrenaline and 2·5 h for (−)-noradrenaline and for a subsequent phase (1–6 h) of approximately 3·75 h for (+) and 9·0 h for (−)-noradrenaline.

Metabolism of (+)- and (−)-noradrenaline in vivo

The tissue extracts obtained from the experiments described above were also examined to determine their content of total radioactivity (noradrenaline + labelled

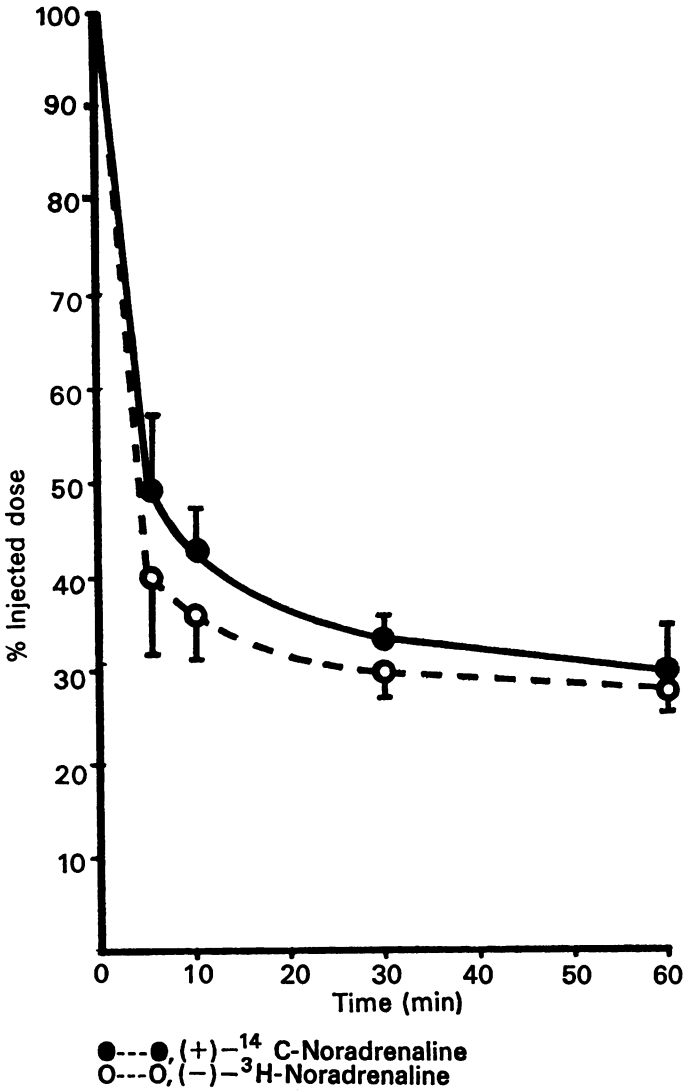

FIG. 2. Disappearance of unchanged (+)-^{14}C-noradrenaline and (−)-^{3}H-noradrenaline from whole mouse after intravenous injection of a mixed dose containing 4 μg of each substance at zero time. Results are expressed as percentages of total injected dose remaining, and are means ± S.E.M. of six animals at each time.

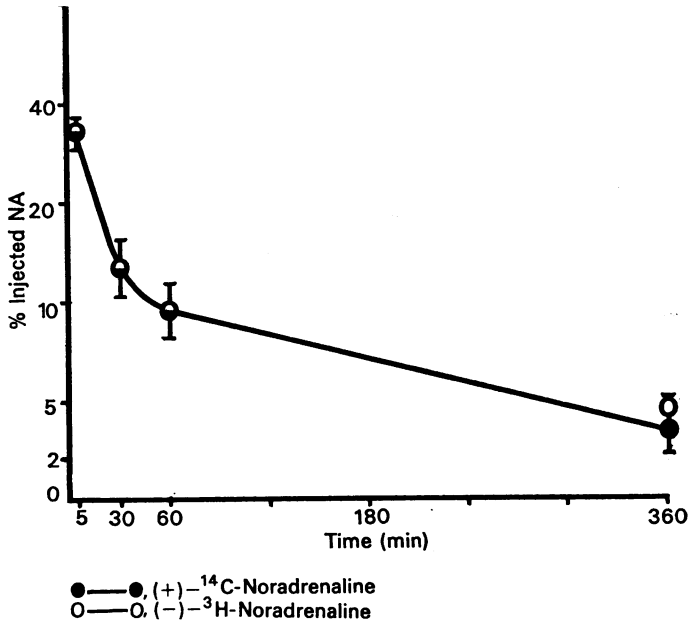

FIG. 3. Disappearance of unchanged (+)-^{14}C-noradrenaline and (−)-^{3}H-noradrenaline from rat brain after injection of 0·8 μg of each substance into the lateral ventricle of the brain at zero time. Results are means ± S.E.M. for six animals at each time.

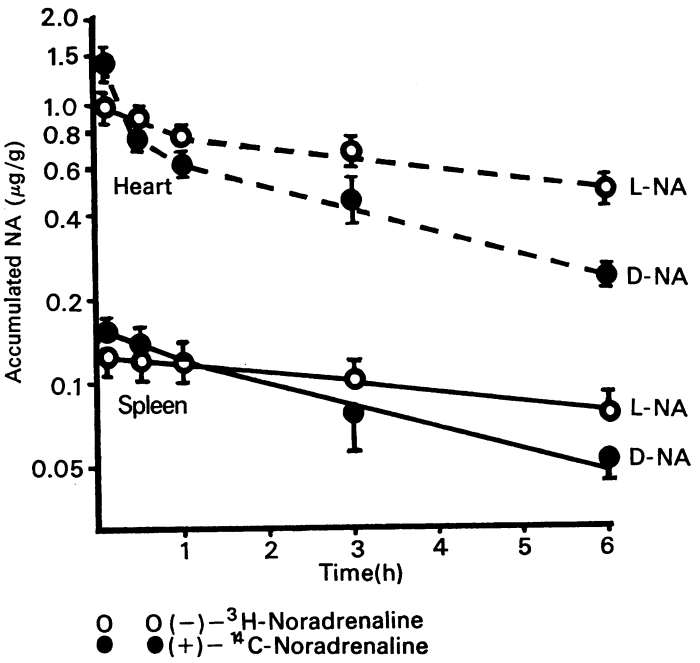

FIG. 4. Disappearance of unchanged (+)-^{14}C-noradrenaline and (−)-^{3}H-noradrenaline from rat heart and spleen after intravenous injection of a mixed dose containing 20 μg of each substance at zero time. Results are means ± S.E.M. for six animals at each time.

metabolites) and radioactive normetanephrine. These results indicated that there were significant differences in the pattern of metabolism of the isomers of noradrenaline *in vivo* (Tables 3, 4 & 5). After intravenous injection of a mixture of (+)-^{14}C-noradrenaline and (−)-^{3}H-noradrenaline in the mouse there was a striking difference in the pattern of metabolites derived from the two isomers. Whereas (+)-^{14}C-normetanephrine was the major metabolite (95% of total metabolites at 5 min) present in the animal at all stages after injection, (−)-^{3}H-normetanephrine represented only a minor component (about 10%) of the total metabolites of (−)-^{3}H-noradrenaline (Table 3). Similarly in the rat heart and brain there was a significantly greater accumulation of (+)-^{14}C-normetanephrine than of (−)-^{3}H-nor-

TABLE 3. *O-methylation of* (+)- *and* (−)-*noradrenaline* in vivo *after intravenous injection in the mouse*

Time after injection (min)	Amount of labelled normetanephrine as % of total injected dose	
	(−)-^{3}H-NMN	(+)^{14}C-NMN
5	6·3±0·96	50·6±6·9
10	4·3±0·34	50·0±4·9
30	3·8±0·40	35·0±6·0
60	3·6±0·50	31·0±2·3

Mice were injected with a mixture of ^{3}H-(−)-NA and ^{14}C-(+)-NA and the whole body content of labelled normetanephrine (NMN) was assayed at various times after injection (c.f. legend Fig. 2). Values are means ± S.E.M. for six animals. Values for (+)-NMN are all significantly greater than those for (−)-NMN at $P<0·001$.

TABLE 4. *Metabolites of* (+)- *and* (−)-*noradrenaline in rat heart after injection* in vivo

Time after injection (min)	^{3}H-(−)-NA		^{14}C-(+)-NA	
	Metabolites as % total radioactivity	NMN as % total radioactivity	Metabolites as % total radioactivity	NMN as % total radioactivity
5	33·2±8·3	7·1±1·1	40·5±8·4	14·4±2·7*
30	33·0±6·6	5·2±0·4	49·5±4·0*	13·6±1·1‡
60	39·0±3·2	5·3±0·3	58·7±2·9†	14·2±0·7‡
180	37·5±8·1	6·3±0·4	52·0±9·5	22·6±2·7‡
360	32·5±6·9	7·3±0·6	57·3±2·6†	31·0±3·5‡

Rats received an intravenous injection of a mixture of ^{3}H-(−)-NA and ^{14}C-(+)-NA as described in Fig. 3. At various times after injection the heart was assayed for total radioactivity, labelled noradrenaline (NA) and labelled normetanephrine (NMN). The results are mean values ± S.E.M. for six animals at each time. *=$P<0·05$; †=$P<0·01$; ‡=$P<0·001$, when compared with corresponding values for (−)-isomer.

TABLE 5. *O-Methylation of* (+)- *and* (−)-*noradrenaline in rat brain after intraventricular injection* in vivo

Time after injection (min)	Amount of labelled normetanephrine as % total injected dose	
	^{3}H-(−)-NMN	^{14}C-(+)-NMN
5	6·0±1·0	10·0±1·2
30	2·7±0·2	7·4±0·9
60	2·1±0·3	3·7±0·3
360	0·6±0·05	0·8±0·07

Labelled normetanephrine (NMN) was assayed in whole rat brain at various times after injection of a mixture of (+)- and (−)-noradrenaline as described in Fig. 4. Results are means ± S.E.M. for six animals at each time; all values for (+)-NMN are significantly higher than those for (−)-NMN at $P<0·05$.

metanephrine at all times after the administration of a mixed dose of the two isomers of noradrenaline (Tables 4 & 5). The rat heart contained relatively less unchanged (+)-^{14}C-noradrenaline and relatively more labelled metabolites of this isomer at most times after injection (Table 4).

Discussion

These results confirm that the initial uptake of noradrenaline by nerve terminals in the rat heart and brain is stereospecific for the (−)-isomer of noradrenaline. In the isolated rat heart the rates of uptake of (+)- and (−)-noradrenaline were measured as recommended by Draskoćzy & Trendelenburg (1968) by following the rate of removal of catecholamine from the perfusion medium. The animals were also pretreated with reserpine to eliminate intraneuronal storage mechanisms, so that our results reflected only differences in the initial rates of uptake of (+)- and (−)-noradrenaline across the neuronal membrane. Previous results have shown that the rate of uptake of noradrenaline measured in this way is the same in reserpine treated hearts as in untreated hearts (Lindmar & Muscholl, 1964; Iversen, *et al.*, 1965). The rates of uptake of (+)- and (−)-noradrenaline into the rat heart from a perfusion concentration of 20 ng/ml were 32 and 54 (ng/min)/g heart respectively. These values are in good agreement with those obtained previously when the initial rates of accumulation of (+)- and (−)-noradrenaline were 26 and 57 (ng/min)/g heart respectively (Iversen, 1963). The uptake of noradrenaline by the isolated mouse heart was similarly stereospecific, but no such specificity appeared to exist for the uptake of noradrenaline by the guinea-pig heart. Draskoćzy & Trendelenburg (1968) also found no stereospecificity for noradrenaline uptake in the rabbit heart, so it appears that considerable species differences may exist in this phenomenon (c.f. Jarrott, 1970).

Our results on noradrenaline uptake by nerve terminals in homogenates of the rat hypothalamus and striatum are in good agreement with the predictions made by Coyle & Snyder (1969). Although in our experiments normal rather than reserpine treated brain tissue was used, the accumulation of noradrenaline during a brief incubation (5 min) can be used as a reliable measure of uptake, since it is not significantly affected by reserpine treatment (Snyder & Coyle, 1968). The values of *Km* for the uptake of (+)- and (−)-noradrenaline into noradrenaline containing terminals in the hypothalamus are in agreement with the data of Coyle & Snyder (1969) and are also similar to the values previously reported for noradrenaline uptake in the sympathetic nerve terminals of the rat heart (Iversen, 1963). In the dopamine terminals of the striatum, however, there was no stereospecificity in the uptake of (+) and (−)-noradrenaline, in confirmation of the findings of Coyle & Snyder (1969).

We have also examined differences in the retention and metabolism of (+) and (−)-noradrenaline after the *in vivo* administration of mixed doses of these isomers, using double isotope techniques to distinguish (+)-^{14}C-noradrenaline and its metabolites from (−)-^{3}H-noradrenaline. The relatively low specific activity of the (+)-^{14}C-noradrenaline unfortunately necessitated the use of rather high doses in these experiments. Although the uptake mechanism for noradrenaline in sympathetic nerves is stereospecific, the (−)-isomer will only be taken up more rapidly if tissues are exposed to subsaturating concentrations of noradrenaline. At higher concentrations the two isomers may be taken up at equal rates, or the (+)-isomer may even

be taken up more rapidly than (−)-noradrenaline (Iversen, 1963). Since high doses of noradrenaline had to be used for the double isotope experiments this may explain the absence of any significant differences in the initial accumulation of unchanged (+)- and (−)-noradrenaline by tissues of the mouse or by the rat heart, spleen or brain (Figs. 2–4). After loading the tissues with (+)- and (−)-noradrenaline, however, there was a more rapid disappearance of (+)-noradrenaline from the rat heart and spleen (Fig. 3). This finding is consistent with the suggestion that intraneuronal storage mechanisms have a lower affinity for (+)- than for (−)-noradrenaline (Stjärne & von Euler, 1965). The differences in retention, however, were not very great. No differences could be detected in the rate of disappearance of the stereoisomers of noradrenaline in the whole mouse, or in the rat brain after the injection into the cerebrospinal fluid. The latter finding confirms that reported by Iversen & Glowinski (1966) who found no difference in the rate of disappearance of (−)-^{3}H-noradrenaline and (±)-^{3}H-noradrenaline from rat brain.

Examination of the pattern of metabolism of (+)- and (−)-noradrenaline *in vivo* suggested that these are important differences in the metabolic fate of these two isomers. In the whole mouse the major metabolites of (−)-noradrenaline were deaminated substances, with only a minor accumulation of (−)-normetanephrine (Table 3). In contrast (+)-noradrenaline was metabolized almost exclusively to (+)-normetanephrine shortly after injection, and (+)-normetanephrine remained the major metabolite present for up to 1 h after injection. Similarly in the rat heart and brain significantly greater amounts of (+)-normetanephrine than (−)-normetanephrine were found at all times after injection of the isomers of noradrenaline (Tables 4, 5). These findings may reflect in part a poorer uptake of (+)-noradrenaline and hence its greater availability for O-methylation at extraneuronal sites. However, since there were no important differences in the accumulation of unchanged (+)- and (−)-noradrenaline in the various tissues examined it seems more likely that the differences in metabolism reflect stereospecific handling of the two isomers by the metabolic enzymes. In particular, (−)-noradrenaline and (−)-normetanephrine may be deaminated more rapidly than the corresponding (+)-isomers of these substances by the enzyme monoamine oxidase (Giachetti & Shore, 1966b). There is no evidence for stereospecificity in the activity of catechol-O-methyl transferase towards noradrenaline (Axelrod & Tomchick, 1958).

In conclusion, we have confirmed that various differences exist in the uptake, storage and metabolism (+) and (−)-noradrenaline. These differences, however, are not very great. It would seem that the biochemical mechanisms involved in the handling of noradrenaline are less able to discriminate between the optical isomers than is the case for adrenoceptors, where differences in the potency of (+) and (−)-noradrenaline of 100-fold or more are frequently found. Nevertheless, in view of the differences which do exist between the optical isomers, and since radioactively labelled (−)-noradrenaline of high specific activity is now available at little extra cost it seems clear that future studies should, wherever possible, make use of this material.

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