Biochemical Pharmacology, Vol. 16, pp. 793–802. Pergamon Press Ltd. 1967. Printed in Great Britain

THE INFLUENCE OF NOREPINEPHRINE ON THE DISTRIBUTION OF RADIOACTIVITY FROM SOME OF ITS ^{14}C-LABELED ANALOGUES AND PRECURSORS*

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(*Received* 28 *July* 1966; *accepted* 3 *November* 1966)

Abstract—The influence of slowly infused norepinephrine on the distribution of radioactivity from a group of structurally different ^{14}C-labeled compounds was examined. In short-term experiments it was shown that small amounts of norepinephrine stimulate the uptake of radioactivity in heart and brain from those compounds that are structurally analogous or biosynthetically related to norepinephrine. No such stimulation was observed in liver or kidney, nor was the stimulation apparent in any organ, including brain and heart, with compounds not bearing either of these relationships to norepinephrine. The effects of norepinephrine on the uptake of radioactivity from its analogues in heart and/or brain persisted even when the circulatory impact of norepinephrine was minimized by pretreatment with chlorpromazine. When large circulatory changes were deliberately produced, norepinephrine still had no specific effect on the distribution of radioactivity from unrelated compounds. Some possible relations between a norepinephrine-sensitive transport system and the regulation *in vivo* of extracellular norepinephrine and its analogues are discussed.

THIS laboratory has reported that intraperitoneal injections of either epinephrine or norepinephrine (NE) enhance the accumulation of amphetamine in the central nervous system of the rat.[1] Under the same conditions, the accumulation of α-aminoisobutyric acid (AIBA) in the brain is depressed by these catecholamines.[2, 3] The slow intravenous infusion of NE also appeared to stimulate the uptake of radioactivity from ^{14}C-amphetamine into brain.[4]

The investigation of the possible influence of catecholamines on transport was continued and is reported here. There are two important procedural differences between this study and the ones previously reported. First, NE and ^{14}C-labeled compounds were administered intravenously. Second, NE was infused slowly throughout the course of the experiment. The compounds studied include the analogues of amphetamine and NE, 2-phenylcyclopropylamine (PCP) and tyramine; the precursors of NE; 5-hydroxytryptamine and its precursors; and several amino acids which have no direct relation to the biosynthesis of the catecholamines.

MATERIALS AND METHODS

All experiments were performed on fasted male albino rats weighing 200 $\pm$ 25 g. The animals derive from a Wistar strain carried in this laboratory for some 15 yr.

* This investigation was supported in part by National Institutes of Health Grants NB-2800 and MH-5471. We wish to thank Miss Lee J. Toomajian and Mrs. Lorraine K. Jaffe for expert technical assistance.

The experiments were carried out on the conscious animal restrained in a plastic holder. Drugs were administered through one of the lateral tail veins.

Experiments in which the effects of chlorpromazine (CPZ) were studied utilized animals which were pretreated with an i.p. injection of 10 mg CPZ/kg 1 hr before the i.v. administration of drugs. These animals were kept in a 37° incubator to eliminate changes that might occur as a consequence of hypothermia. Rectal temperature was monitored with a Tele-thermometer probe (Yellow Springs Instruments Co.). No significant changes in rectal temperature were noted in the absence of CPZ, and these experiments were carried out at room temperature.

An Aminco motor-driven compensator was used to infuse animals with 0·14 M NaCl at a rate of 50 μl/min for a 7-min period prior to the administration of drugs, except in experiments with NE. Here, infusion was carried out at identical rates and times, except that the infusion medium contained 10 μg NE/ml (free base). This procedure ensured that the cannula, attached to a fine plastic tubing, was filled with NE solution prior to the injection of other drugs. The 7-min preinfusion of saline served as a control for the NE experiments. After preinfusion, 200 μl of a solution of drug, usually containing 2·5 μmoles/200 μl, was injected as rapidly as possible into a cannula inserted into a lateral tail vein. The slow infusion of either saline or NE was continued for 5 min after injection of the drug. Animals were killed by decapitation, the organs of interest rapidly removed, washed free of adherent blood in ice-cold saline, and homogenized in 4 vols of ice-cold 0·3 M perchloric acid, except in the case of heart where homogenization was in 0·01 N HCl prior to the addition of perchloric acid. Blood was collected in tubes coated with ethylenediamine tetra-acetate. After centrifugation, the supernatant fluid was assayed for radioactivity with a Packard Tri-Carb liquid scintillation spectrometer. The disintegrations/min (dpm) were routinely calculated by the channels ratio method, although periodic checks on this method were made with an internal standard.

In some experiments with tyramine, which is metabolized quite rapidly, aliquots of the supernatant fluid were passed through a Dowex-50 column in the H^+ form. Elution was discontinuous: first with water, then with 0·6 N NH_3, and finally with 2·0 N NH_3. This procedure divided the radioactivity into three portions. When a known mixture of *p*-hydroxyphenylacetic acid, octopamine, and tyramine was treated this way, the acid was removed first (H_2O), octopamine next (0·6 N NH_3), and finally tyramine (2·0 N NH_3).

Paper chromatography of the eluates was carried out in butanol:acetic acid:water (12:5:3) and in isopropanol:ammonia:water (20:1:2). R_f's of the chromatographed compounds were compared with those of known samples of *p*-hydroxyphenylacetic acid, octopamine, and tyramine. Aliquots of the 0·6 N NH_3 eluates from liver were refluxed for 2 hr with 2·0 N HCl prior to chromatography.

In some cases, tissue extracts were tested for the presence of peptides by the method of Rydon and Smith.[5]

Radioactive compounds used in this investigation were, per m-mole: 3,4-dihydroxyphenylethylamine-1-^{14}C hydrobromide, 6·4 mc; *p*-hydroxyphenylethylamine-1-^{14}C hydrobromide, 5·75 mc; *dl*-tyrosine-3-^{14}C, 6·85 mc; *l*-leucine-^{14}C, 231 mc; *l*-lysine-^{14}C, 40 mc; *dl*-tryptophan-3-^{14}C, 4·73 mc; *dl*-5-hydroxytryptophan-3-^{14}C, 4·46 mc; 5-hydroxytryptamine-2-^{14}C, 0·93 mc (New England Nuclear); *l*-phenylalanine-^{14}C, 142 mc (Schwartz BioResearch); *dl*-3(3,4-dihydroxyphenyl)alanine-2-^{14}C, 31·2

mc (Nuclear-Chicago); 2-phenylcyclopropylamine-1,2-^{14}C hydrochloride, 0·6 mc; *dl*-amphetamine (1-phenyl-1-^{14}C-2-aminopropane), 0·99 mc (gifts from Smith, Kline and French); and α-aminoisobutyric acid-1-^{14}C, 10·03 mc (Calbiochem).

RESULTS

The depression in the rate of AIBA accumulation in the central nervous system which is observed after intraperitoneal injection[2, 3] does not occur after intravenous injection of ^{14}C-AIBA. No significant effect of NE on the accumulation of AIBA is evident in blood, brain, heart, kidney, or liver even when NE is infused for 80 min after the intravenous injection of ^{14}C-AIBA. The effect of the concentration of NE used during the infusion on the circulation and selective perfusion rate through the organs examined is small. The results obtained with the intraperitoneal injection route suggest that the depression in the rate of AIBA accumulation is due to changes in blood flow through the peritoneum caused by the injection of catecholamines. Therefore, the enhanced uptake of amphetamine by brain after intraperitoneal injection[1] may have occurred despite its slower entry into blood, since amphetamine augments rather than diminishes the physiological effects of catecholamines.[6, 7]

Further investigations were concerned with the disposition of radioactivity from ^{14}C-PCP. This compound is like amphetamine in many of its physiological effects.[8] With NE, a sharp increase in radioactivity in the central nervous system is noted, while the radioactivity in other tissues remains essentially unchanged (Table 1). The increase is observed without a concomitant increase in blood radioactivity levels, although the data do not exclude the possibility that an increment occurred at some time earlier than the 5-min period chosen for the termination of the experiment. The fact that PCP inhibits monamine oxidase (MAO) activity[10] makes the change in its distribution as a consequence of added NE a complicating factor. Tyramine, which does not inhibit MAO[11] but is thought to mobilize NE,[6] was therefore used. The same pattern of response is found with tyramine. However, there is a significant increase in the amount of tyramine taken up by heart in the presence of NE (Table 1).

It was of interest to determine whether the increased radioactivity with ^{14}C-tyramine in heart and brain under the influence of NE reflected a change in the metabolic pattern. Table 2 shows the distribution of radioactivity in an acid or neutral fraction (H_2O eluate), a basic fraction (0·6 N NH_3), and a very basic fraction (2·0 N N H_3). The radioactivity is differently distributed in heart under the influence of added NE. The acid or neutral fraction represents about the same percentage of the total radioactivity under both experimental and control conditions, but the absolute amount is much greater in the NE-treated animals. The percentage of basic material eluted with 2·0 N NH_3 is almost doubled. The radioactivity eluted from brain and heart chromatographs, on paper, as *p*-hydroxyphenylacetic acid (H_2O), as octopamine (0·6 N NH_3) and as tyramine (2·0 N NH_3) in the butanol–acetic acid–water and the isopropanol–ammonia–water systems.

The very different distribution of radioactivity found in liver was investigated. The fraction eluted at 0·6 N NH_3 was chromatographed in both solvent systems; the radioactivity found did not correspond to any of the expected products. Presumptive evidence for the presence of peptides[5] in this fraction was obtained. Refluxing with 2·0 N HCl for 2 hr yielded products that were negative for peptides and

TABLE 1. THE EFFECT OF NOREPINEPHRINE ON THE DISTRIBUTION OF RADIOACTIVITY FROM ^{14}C-PCP* AND ^{14}C-TYRAMINE*

	No. of animals	Brain (% dose)†	Brain (T/B)‡	Heart (% dose)	Heart (T/B)	Liver (% dose)	Liver (T/B)	Kidney (% dose)	Kidney (T/B)	Blood (% dose)
PCP										
Control§	6	1·14	0·37	3·14	1·04	10·5	3·5	16·8	5·6	3·02
NE§	7	1·96	0·74	3·24	1·22	10·4	3·9	16·1	6·1	2·66
Σ Ranks‖		21		37		38		46		48
P Values¶		⩽0·01		N.S.**		N.S.		N.S.		N.S.
Tyramine		(% dose ± S.D.)		(% dose ± S.D.)		(% dose ± S.D.)		(% dose ± S.D.)		(% dose ± S.D.)
Control§	22	0·07 ± 0·008	0·78	2·93 ± 0·074	32·5	0·99 ± 0·087	11·0	5·13 ± 0·509	57·2	0·09 ± 0·014
NE§	22	0·10 ± 0·018	1·00	4·82 ± 0·215	48·2	1·04 ± 0·124	10·4	4·96 ± 0·686	49·6	0·10 ± 0·017
P Values		⩽0·01		⩽0·001		N.S.		N.S.		N.S.

* Radioactive compound, 2·5 μmoles/200 g animal, injected as described in text.
† Results reported as % of administered dose/g wet tissue/200 g animal.
‡ Tissue/blood ratio.
§ Control (saline) or NE-infused as described in text.
‖ Probability of difference calculated by a nonparametric method[9] because of the relatively few cases examined. Σ Ranks of controls given; 24 is 1% critical point; 27 is 5% critical point for 6 × 7 table.
¶ Determined by parametric methods.
** N.S. No significant difference.

TABLE 2. EFFECT OF NOREPINEPHRINE ON UPTAKE AND METABOLISM OF ^{14}C-TYRAMINE* IN 5 MIN

	(dpm × 10^3/g tissue)									
	Heart		Kidney		Liver		Brain		Blood	
	Saline†	NE†	Saline	NE	Saline	NE	Saline	NE	Saline	NE
Total	610	992	1120	995	223	229	16·8	23·4	15·3	18·6
Dowex-50 H^+ eluates										
H_2O	495	712	554	459	113	134	12·7	18·3	7·0	8·5
% Recovered	81·7	70·6	52·5	48·6	41·1	57·5	75·6	77·2	46·3	47·2
0·6 N NH_3	6	11	21	19	122	94	1·2	1·6	1·1	1·3
% Recovered	1·0	1·1	2·0	2·0	51·9	40·3	7·1	6·8	7·3	7·2
2·0 N NH_3	105	286	480	467	neg.	5	2·9	3·8	7·0	8·2
% Recovered	17·3	28·3	45·5	49·4		2·1	17·3	16·0	46·3	45·6
Total recovered dpm	606	1009	1055	945	235	233	16·8	23·7	15·1	18·0
% Recovered	99·3	101·7	94·2	95·0	105·3	101·7	100·0	101·3	98·7	96·8

* ^{14}C-Tyramine-HBr, 9·42 μc, neutralized and injected in 0·2 ml saline.
† Saline or NE infusion begun 7 min before injection of tyramine and continued for 5 min.

chromatographed as a mixture of octopamine and tyramine. The percentage of octopamine in liver is not much higher than that found in brain.

The effect of NE on the accumulation of 5-hydroxytryptamine and its precursors was examined. No difference in the accumulation of radioactivity was noted in any organ with any member of this series. In the NE precursor series, only phenylalanine failed to demonstrate enhanced uptake of radioactivity (Table 3).

TABLE 3. EFFECT OF NOREPINEPHRINE ON THE DISTRIBUTION OF RADIOACTIVITY FROM SOME ^{14}C-LABELED COMPOUNDS

Compound*	No. of animals	(% injected dose/g wet weight)†				
		Brain	Heart	Liver	Kidney	Blood
A. 5-Hydroxytryptophan and its precursors						
Tryptophan						
Saline	6	0·32	0·67	1·24	2·12	1·39
NE	6	0·32	0·66	1·28	2·61	1·15
Σ Ranks‡		37	39	36	42	33
5-Hydroxytryptophan						
Saline	6	0·08§	0·27	3·80	6·00	6·08
NE	6	0·10	0·30	4·12	5·48	6·28
Σ Ranks			37	42	32	40
5-Hydroxytryptamine						
Saline	6	0·03§	1·10	1·74	0·68	1·07
NE	6	0·02	1·18	1·60	1·27	0·96
Σ Ranks			34	41	25	30
B. 3,4-Dihydroxyphenylethylamine and its precursors						
Phenylalanine						
Saline	6	0·18	0·37	1·88	4·72	0·40
NE	6	0·17	0·33	2·00	4·44	0·38
Σ Ranks		40	36	33	39	38
Tyrosine						
Saline	6	0·32	0·86	6·60	5·80	0·50
NE	6	0·37	0·99	6·52	6·00	0·56
Σ Ranks		29	30	34	39	35
3,4-Dihydroxyphenylalanine						
Saline	6	0·14	0·56	1·92	10·08	1·10
NE	6	0·18	0·69	2·04	9·96	1·13
Σ Ranks		24	26	32	41	37
3-4-Dihydroxyphenylethylamine						
Saline	6	0·02§	0·18	4·32	6·84	2·04
NE	6	0·02	0·30	3·94	6·54	2·20
Σ Ranks			23	41	39	34
C. Amino acids unrelated to NE						
Lysine						
Saline	6	0·69	0·23	2·10	3·23	0·26
NE	6	0·74	0·20	2·37	3·15	0·24
Σ Ranks		30	37	33	41	39
Leucine						
Saline	6	0·30	0·96	1·09	1·04	0·29
NE	6	0·32	1·06	0·99	1·28	0·29
Σ Ranks		39	34	34	40	39

* All compounds, 2·5 μmoles, administered 7 min after infusion of either saline or NE begun. All animals killed 5 min after injection of indicated compound.

† Results reported as % of administered dose/g wet tissue/200 g animal.

‡ For 6 × 6 table, 5% critical point is 26; 1% critical point is 23.

§ Significance not calculated if organ radioactivity is 2% or less of blood.

That this effect is characteristic only of amino acids immediately related to NE is indicated by the negative findings with phenylalanine and tryptophan and further with lysine, a basic, and leucine, a neutral amino acid (Table 3).

The absence of important changes in radioactive compound in blood under the influence of added NE suggested that the procedures employed did not significantly influence the blood volume or circulation. Nevertheless, a check was performed under conditions with maximal likelihood of such effects. Mixtures of nonradioactive tyramine and ^{14}C-leucine or ^{14}C-AIBA were injected into animals in the presence and absence of NE. Under these conditions, large increases in radioactivity were observed in blood, brain, heart, kidney, and liver. This effect was eliminated by CPZ, a drug that abolishes or reverses NE effects on circulation.[12] This contrasts with the continued effects of NE on PCP accumulation in brain and tyramine accumulation in heart in the presence of CPZ. These differences are clearly evident when a plot is made of the tissue/blood ratio under the influence of NE (Fig. 1).

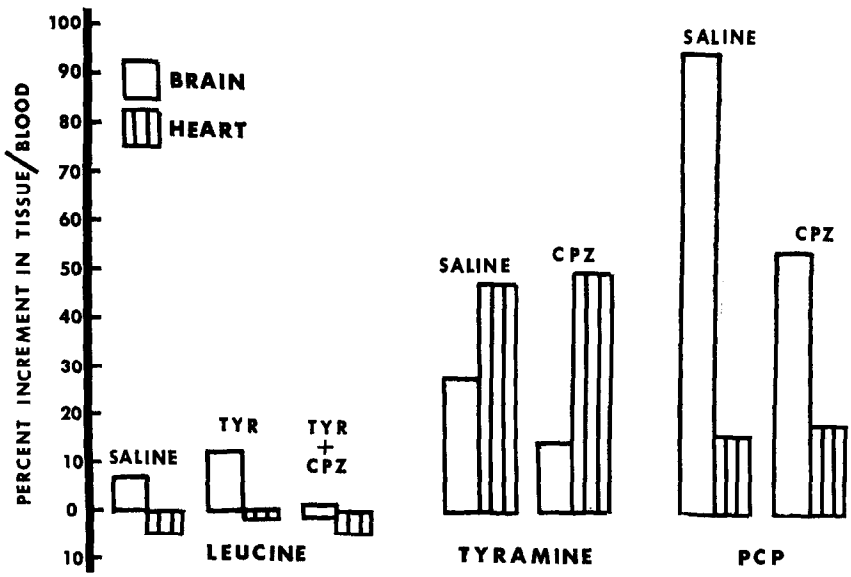

FIG. 1. Average per cent increment in tissue-blood ratios of radioactivity

$$\left[\frac{\text{tissue}}{\text{blood}}\,(\text{NEP})\Big/\frac{\text{tissue}}{\text{blood}}\,(\text{saline}) \times 100 - 100\right]$$

produced by NEP in brain and heart from ^{14}C-leucine, ^{14}C-tyramine, and ^{14}C-PCP.

DISCUSSION

In these experiments NE appeared to stimulate specifically the accumulation of radioactivity in heart and brain, but only from its ^{14}C-labeled analogues and precursors. Enhanced uptake is apparently independent of catecholamine-induced changes in circulation even in brain, a tissue into which NE does not enter.[13]

These results are surprising. Tyramine, for example, competes with NE for transport through the plasma membrane,[14] for vesicular binding sites,[15] and for enzymes found in the presynaptic terminal.[16] If competition at these sites were the primary factor determining the results, a decrement or qualitative change in organ radioactivity,

rather than an increment, should have been observed. Despite the large excess of tyramine employed in these experiments (of the order of 1000 to 1), competition between these two compounds cannot be excluded on theoretical grounds. Many instances are known in which inhibitors are less or more effective than the substrate in forming the enzyme–substrate complex. Sulfanilamide, for example, must be used at 1000 times the concentration of *p*-aminobenzoic acid in order to inhibit folic acid synthesis,[17] whereas succinic dehydrogenase activity is significantly inhibited even when the concentration of succinate is 1000 times greater than that of oxaloacetate.[18]

While the increased concentration of *p*-hydroxyphenylacetic acid in the experimental animals is evidence for facilitation of tyramine transport by NE, it is not conclusive. If intracellular reactions for the conversion of tyramine were stimulated by NE, the greater concentration gradient between intracellular and extracellular tyramine so formed might enhance transport despite some inhibition of the carrier system. But it is not likely that changes in the disposition of radioactivity following transport can account for the increments. The relative concentration of tyramine and *p*-hydroxyphenylacetic acid is only slightly disturbed by the administration of NE, whereas octopamine concentration undergoes no relative change. Further, the absolute increase in tyramine in the presence of NE is evidence against its stimulated cellular conversion. It is probable, therefore, that the transport system for tyramine is implicated in the NE effect, and that this effect involves a facilitation of tyramine transport.

The assumption of facilitated transport may explain the different results with PCP and tyramine in brain. Since transport through the blood–brain barrier is highly selective,[19] stimulation of this system may augment transport, but not modify its specificity. The suggestion that NE may stimulate transport through the blood–brain barrier, without itself entering brain, is similar to that made for several other hormones which selectively change the permeability of cell membranes.[20]

The sympathomimetic properties of tyramine are thought to arise, at least in part[1] from competition between tyramine and NE for plasma membrane transport sites.[2], While kinetic studies are required to determine if the same carrier transports both compounds, the assumption of a common carrier accounts for the inhibition of NE uptake by tyramine.[22]

The stimulation of this postulated common carrier by NE does not preclude the possibility of demonstrating competitive effects. Enzymes are known which are activated by their substrates, and activation sites are separable from the reaction sites. A fully activated enzyme of this class shows the normal competitive phenomena at its reaction site.[23] With the large excess of tyramine usually employed, it is not surprising that tyramine inhibition of NE uptake can be demonstrated.[14]

While there are contradictory reports about the concentration of tyramine in the central nervous system,[24, 25] its presence in the normal animal is indicated by its identification in urine.[26] If tyramine is a normal constituent, it could enhance adrenergic activity by competing with NE for transport. Facilitated removal of tyramine by NE would then limit its action. MAO is well suited for an auxiliary role in this homeostatic process. It has been shown that tyramine has a greater affinity for this enzyme than has NE,[16] and it may be inferred from the rate of *p*-hydroxyphenylacetic acid formation reported here that oxidation occurs rapidly *in vivo*. These processes—plasma membrane competition with NE, NE facilitation of transport, and rapid

oxidation—are all consistent with the postulate that a physiological role for the naturally occurring analogues of NE is the regulated potentiation of NE.

Implicit in this discussion is the suggestion that NE may facilitate its own uptake. There is evidence for a slow nonstimulated release of NE.[27] This release may excite the continuous miniature postsynaptic potentials characteristic of sympathetically innervated tissues[28] and comparable to those described in cholinergic receptors.[29] The assumption of a NE-facilitated removal of NE suggests a sensitive mechanism for maintaining concentrations of NE in the synaptic space sufficient for the excitation of miniature postsynaptic potentials but insufficient for fully activating the receptors. Evidence that re-incorporation is predominant over catechol-O-methyltransferase activity in the disposition of NE[30] is consistent with this view. Similar mechanisms for the regulation of other compounds have been described. Glucose 6-phosphate activates glycogen synthetase,[31] inorganic phosphate activates phosphofructokinase,[32] and citrate stimulates fatty acid synthesis,[33] leading in each case to a fall in the concentration of the activator.

Further investigation of these implications is being conducted on isolated tissue preparations.

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