STEREOSELECTIVITY OF NOREPINEPHRINE STORAGE SITES IN HEART

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In recent years, the uptake, storage, and metabolism of norepinephrine has been the subject of intensive study in this and other laboratories (Whitby, et al., 1961; Crout, et al., 1961; Montanari, et al., 1963). The investigations have been facilitated by the availability of d,l-norepinephrine-7-H^3 of extremely high specific activity. By using extremely small quantities of the tritiated amine to label body stores, it has been possible to determine turnover rates, pool sizes and rate of biosynthesis (Montanari, et al., 1963; Brodie and Beaven, 1963) and too examine the metabolic products released normally, as well as under the influence of various drugs (Hertting, et al., 1962; Kopin and Gordon, 1962; Nash, et al., 1963).

The labeled amine is a racemic mixture of the d- and l-isomers, but in calculations of turnover rates and pool sizes, the assumption was made that the uptake and storage at sympathetic nerve endings is stereospecific for the l-isomer. Thus, the interpretation of studies carried out with the d,l-norepinephrine-7-H^3 was open to question since there was no direct proof that the d-amine was not being taken up, stored, released, and metabolized, but at a rate faster than the l-isomer (Brodie and Beaven, 1963). In fact, Kopin and Bridgers (1963) reported that after the subcutaneous administration of d,l-norepinephrine-7-H^3 both the d- and l-isomers are taken up in equal quantities but the d-isomer leaves the tissue at a faster rate.

The present paper indicates that very little of the d-isomer is taken up by the heart after administration of d,l-norepinephrine-7-H^3 to rats. In addition, the d-isomer leaves the tissue at a rapid rate. Less than 3% of the norepinephrine-H^3 present in the heart after three hours is the d-isomer and after 24 hours, all of the norepinephrine-H^3 in the heart is the l-isomer.

METHODS

Radioactivity was measured in the Packard Tri-Carb liquid scintillation counter, using 15 ml. of a solution containing 13 g. of PPO (2,5-diphenyloxazole) and 500 mg. of POPOP (1,4-bis-2-(5-phenyloxazolyl)-benzene) in 1500 ml. toluene and 2500 ml. of ethylene glycol monomethyl ether. This mixture permits H^3 counting at approximately 8% efficiency even in the presence of 10% water. All samples were re-counted after addition of a standard amount of H^3 toluene, and values were corrected to dpm.

RESULTS

Male, Sprague-Dawley rats (180 g.), given 20 μC of d,l-norepinephrine-7-H^3 (New England Nuclear Corp., specific activity 2.67 C/m mole) by injection in the tail vein, were killed at various times thereafter. The hearts were removed and homogenized in 4 volumes of cold 0.4N $HClO_4$. After centrifuging, the supernatant containing the norepinephrine was removed, adjusted to pH 7.0 with solid K_2CO_3, and recentrifuged to remove the precipitate of $KClO_4$.

An aliquot of the supernatant liquid (1.5 ml.) was added to a solution containing 2.5 g. of d,l-norepinephrine hydrochloride in 15.0 ml. of distilled water. After the addition of $NaHSO_3$ (200 mg.), the solution was chilled to 8-10°C. in an ice bath. The free base, d,-l-norepinephrine, was then precipitated by dropwise addition of 5.0 ml. of 8.0 N NH_4OH with vigorous stirring. After the solution had been cooled at 4°C. for 15 min., the crystalline d,l-norepinephrine was collected by vacuum filtration and washed twice with ice-cold distilled water, twice

with cold methanol and three times with ether. The crystals were air-dried for 15 min., then dried overnight at 25° in a vacuum desiccator. The yield of d,l-norepinephrine (m.p. 215-218°C., dec.) was 80-85%.

The isolation of d-norepinephrine-d-bitartrate was accomplished by a semimicro modification of the procedure used by Tullar (1948) to resolve d,l-norepinephrine. A portion of the d,l-norepinephrine (1.69 g.) was dissolved with vigorous stirring on a steam bath, in a solution of 1.55 g. of d-tartaric acid in 1.0 ml. of distilled water. The solution was cooled to room temperature and diluted to 10 ml. by the dropwise addition of absolute methanol with vigorous stirring. Crystallization was induced by scratching the sides of the tube. After 3-4 hours at room temperature, the large mass of crystals were separated by centrifugation and washed once with 1.0 ml. of 90% methanol. After overnight drying in vacuo at 25°C. the product weighed 97 mg. (m.p. 160-164°C.). Two recrystallizations from 95% methanol yielded 61 mg. of d-norepinephrine-d-bitartrate (m.p. 163-166°C). Samples of the recrystallized preparation were assayed for tritium content. The results (in Table I) are corrected for the percentage yield of cold material.

For the isolation of l-norepinephrine-d-bitartrate monohydrate, 1.55 g. of d-tartaric acid and 1.69 g. of d,l-norepinephrine were dissolved in 3.0 ml. of distilled water. The solution was cooled to 3-5°C. for several hours and a voluminous precipitate was induced by stirring and scratching the sides of the tube. After centrifugation, the precipitate was washed, first with 0.5 ml. of ice-cold water, then with 1.0 ml. of cold 95% methanol. The crystals were air-dried overnight, then recrystallized twice by dissolving in 1.0 ml. of water at 50°C. and cooling to 0°C. The final yield of l-norepinephrine-d-bitartrate monohydrate was 67 mg. (m.p. 100-104°C). Samples of the purified preparation were assayed for tritium content. The results are shown in Table I after correction for the yield of cold material.

Table I

Radioactivity in d- and l-norepinephrine of rat heart at various times after administration of d,l-norepinephrine-7-H^3

Time after Injection	Total Radioactivity	d-norepinephrine[a]		l-norepinephrine[b]	
		H^3	% of Total	H^3	% of Total
	dpm./g.	dpm./g.	%	dpm./g.	%
STD[c]	8.94×10^7	4.01×10^7	44.9	3.88×10^7	43.4
5 min.	8.65×10^5	7.03×10^4	8.13	7.34×10^5	84.9
30 min.	7.01×10^5	5.90×10^4	8.42	--	--
3 hr.	5.28×10^5	1.32×10^4	2.50	--	--
24 hr.	1.63×10^5	0[d]	0.00	1.44×10^5	88.3
24 hr.	1.41×10^5	0[d]	0.00	1.16×10^5	82.4

[a] d-norepinephrine isolated as d-norepinephrine-d-bitartrate.

[b] l-norepinephrine isolated as l-norepinephrine-d-bitartrate monohydrate.

[c] $HClO_4$ extract of heart with d,l-norepinephrine-H^3 added during the homogenization.

[d] No significant counts over background on a total time of 300 min. with background of 43 counts per min.

In other experiments, larger quantities of d,l-norepinephrine were resolved and the optical rotation of the tartrate salts determined. The value obtained for d-norepinephrine-d-bitartrate was $[\alpha]_D^{23} = +37.2°$ and for l-norepinephrine-d-bitartrate $[\alpha]_D^{23} = -9.9°$. These values are in agreement with those reported by Tullar (1948).

The results indicate that less than 10% of the norepinephrine-H^3 found in the rat heart after administration of d,l-norepinephrine-7-H^3 is the d-isomer. Furthermore, the d-isomer is lost by the tissue at a much more rapid rate than the l-isomer. Twenty-four hours after administration of d,l-norepinephrine-H^3, the rat hearts contain 5×10^4 dpm./g. of l-norepinephrine-H^3 and no detectable d-norepinephrine-H^3. These results demonstrate an inherent stereospecificity in the uptake and binding of norepinephrine by peripheral sympathetic nerves. The pharmacological implications of these findings are discussed elsewhere (Maickel, et al., 1963).

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