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Dihydroxytryptamines: Effects on Noradrenergic Function in Mouse Heart *in Vivo*

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SUMMARY

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A variety of tryptamines, including the four isomeric hydroxytryptamines and four isomeric dihydroxytryptamines, inhibit uptake of [^{3}H]norepinephrine into mouse heart *in vivo* and elicit release of [^{3}H]norepinephrine from cardiac storage sites. α-Methyl congeners are more potent releasing agents, except in monoamine oxidase-inhibited mice, where in most cases the parent amines become almost as efficacious. Only 5,7-dihydroxytryptamine and, to a lesser extent, 6,7-dihydroxytryptamine have long-term cytotoxic effects on noradrenergic terminals in heart, as evident in a marked reduction in uptake of [^{3}H]norepinephrine for 5-20 days after exposure to the amines and, for the 5,7-hydroxy-analog, in degenerative changes in the ultrastructure of atrial nerves. Cocaine prevents both [^{3}H]norepinephrine release and the long-term effects of 5,7-dihydroxytryptamine. α-Methyl-5,7dihydroxytryptamine does not elicit any long-term effects, nor does the parent amine in animals in which monoamine oxidase has been inhibited. Dihydroxytryptamines undergo autoxidation much more slowly than cytotoxic phenethylamines, such as 6-hydroxy-dopamine, which are autoxidized rapidly. The mechanism involved in the cytotoxic effects of 5,7-dihydroxytryptamine in heart tissue thus contrasts in many aspects with that involved in the action of 6-hydroxy-dopamine.

INTRODUCTION

A variety of evidence indicates that uptake of 5,6- and 5,7-dihydroxytryptamines into serotonin-containing neurons of the central nervous system elicits cytotoxic effects leading to degeneration of both axons and neuronal terminals (1-15). Such cytotoxic effects are not limited to serotonin-containing structures, but also occur to a lesser degree in both central and peripheral catecholamine-containing neurons (1, 5, 12, 14, 15). Selectivity of action of the dihydroxytryptamines appears to result from a higher affinity for uptake processes at serotonin-containing terminals than at catecholamine terminals (16-18). Cytotoxic effects of the dihydroxytryptamines are generally believed to be similar to the neurodegenerative effects of 6-hydroxydopamine on catecholamine-containing nerve terminals. With 6-hydroxy-dopamine and analogous phenethylamines, the cytotoxic effect appears to be dependent upon the active uptake of a readily autoxidizable amine (19). The present communication reports an evaluation of 5,6-, 5,7-, 6,7-, and

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4,7-dihydroxytryptamines, their α-methyl congeners, and other related tryptamines, such as serotonin (5-hydroxytryptamine), with regard to efficacy in inhibiting [^{3}H]norepinephrine uptake into cardiac tissue *in vivo* and in eliciting release of labeled stores of [^{3}H]norepinephrine from mouse heart *in vivo*, the capacity to induce cytological changes in neuronal structures as measured by long-term effects on [^{3}H]norepinephrine uptake and ultrastructural changes in cardiac sympathetic neurons, and rates of autoxidation *in vitro*. The results of these studies, some of which were carried out in cocaine-treated or monoamine oxidase-inhibited animals, are discussed in terms of mechanisms of chemically induced neurodegeneration by trihydroxyphenethylamines and dihydroxytryptamines.

MATERIALS AND METHODS

The following compounds were obtained from Regis Chemical Company under Research Contract SA 43-pH-3021 and provided by Dr. A. A. Manian, Psychopharmacology Service Center, National Institute of Mental Health: 4-hydroxy-, 6-hydroxy-, 7-hydroxy-, 5,6-dihydroxy-, 5,7-dihydroxy-, 6,7-dihydroxy-, and 5,6,7-trihydroxytryptamines as the creatinine sulfates; α-methyl-5,7-dihydroxy-, α-methyl-5,6-dihydroxy, *N,N*-dimethyl-5,6-dihydroxy-, and *N,N*-dimethyltryptamines as the oxalates; *N,N*-dimethyl-5-hydroxy-, 5-amino-, and 5-hydroxy-7-chlorotryptamines and α-methyl-2,4,5-trihydroxy- and 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine) as the hydrochlorides; and 3-β-aminoethyl-5-hydroxybenzothiophene. Additional samples of α-methyl-5,6-DHT[2] were prepared by hydrogenolysis of α-methyl-5,6-dibenzyloxytryptamine oxalate in aqueous methanol containing the theoretical amount of creatinine hydrogen sulfate with a palladium-on-charcoal catalyst. α-Methyl-5-hydroxytryptamine was obtained from the Upjohn Company. α-Methyl-6-chlorotryptamine HCl was obtained from Dr. A. Verbiscar of the Institute of Drug Design, Sierra Madre, Calif. 5-Fluoro- and 6-fluorotryptamines and their α-methyl analogues, as hydrochlorides, and 5-hydroxytryptamine creatinine sulfate were Calbiochem products. Tryptamine HCl was obtained from Sigma Chemical Company; tyramine HCl, from Matheson, Coleman, and Bell; α-methyltyramine and pargyline, from Abbott Pharmaceuticals, Inc.; hordenine sulfate, from Aldrich Chemical Company; iproniazid, from Hoffmann-La Roche; and cocaine HCl, from Nederlandsche Cocainefabriek, Amsterdam. 4,7-Dihydroxy- and α-methyl-4,7-dihydroxytryptamine were prepared by the following sequence. Nitration of 2,5-dibenzyloxybenzaldehyde yielded the 6-nitroderivative, and condensation with nitromethane and KOH afforded the corresponding nitrostyrene, which on reduction with iron–acetic acid yielded 4,7-dibenzyloxyindole. Reaction with dimethylformamide and phosphorus oxychloride afforded 4,7-dibenzyloxyindole-3-carboxaldehyde. Condensation with nitromethane or nitroethane, followed by reduction with lithium aluminum hydride and catalytic debenzylation with hydrogen and palladium on charcoal in ethanol, afforded 4,7-dihydroxy- and α-methyl-4,7-dihydroxytryptamine, respectively. Synthetic details will be published elsewhere.

DL-[^{3}H]Norepinephrine (specific activity, 5–10 Ci/mmole) was purchased from New England Nuclear Corporation and Sigma Chemical Company. The [^{3}H]norepinephrine was prepared in 0.9% NaCl solution at a final concentration of tritium of 50 μCi/ml and stored at −20°. [^{3}H]Tyramine (5–10 Ci/mmole) was purchased from New England Nuclear Corporation. All experiments were carried out using National Institutes of Health general-purpose male albino mice weighing 17–20 g.

Inhibition of uptake of [^{3}H]norepinephrine by mouse heart *in vivo* was estimated by measuring the tritium content of cardiac tissue following the intravenous administration of [^{3}H]norepinephrine, compared to the amount retained following the simultaneous administration of the same dose of [^{3}H]norepinephrine and varying

[2] The abbreviations used are: DHT, dihydroxytryptamine; HT, hydroxytryptamine.

doses of a test compound. The stock solution of [^{3}H]norepinephrine (0.5 ml) was diluted with 0.9% NaCl containing 0.2–400 μg of the test compound, and 0.1 ml of this solution containing 2.5 μCi of [^{3}H]norepinephrine, alone or in the presence of test compound, was administered via the tail vein. Animals were killed after 20 min, and their hearts were removed immediately and stored on an aluminum plate cooled with Dry Ice. After weighing, the hearts were digested at 70° for 4 hr in screw-capped counting vials containing 0.6 ml of 2 parts perchloric acid (60%) and 1 part hydrogen peroxide (30%). After cooling, 10 ml of a phosphor solution (Aquasol, New England Nuclear Corporation) was added, and radioactivity was measured by scintillation spectrometry with an efficiency of 21.5%. Five to ten animals were used at each dose, and the results were expressed as percentages of control values ± standard errors of the mean. ED_{50} values were obtained from the linear portion of the dose-response curve. Release of [^{3}H]norepinephrine was estimated as described (19, 20), except that the total tritium remaining in the heart was measured in individual hearts as described above. The uptake of [^{3}H]norepinephrine in long-term experiments was measured as described (19). Monoamine oxidase activity in heart and liver was measured with radioactive tyramine (21). The rate of autoxidation of amines was measured with a Clark electrode (19). Results are presented as means ± standard deviations.

Mouse tissues were prepared for electron microscopic examination as follows. At autopsy tissues were fixed in a mixture containing 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 25° for 1 hr. After fixation blocks were washed in buffer, cut into 1-mm pieces, postfixed in 1% OsO_4 in cacodylate buffer, dehydrated in ethanol, and embedded in Araldite. Thin sections, stained with uranyl acetate and lead citrate, were examined in a Philips 200 electron microscope.

RESULTS

Inhibition of [^{3}H]norepinephrine uptake. All the tested tryptamine derivatives, with the exception of 5,6,7-trihydroxytryptamine, inhibited the uptake of [^{3}H]norepinephrine. However, with many derivatives the inhibition was evident only at high concentrations (Table 1). The inhibition was dose-dependent, and ED_{50} values were obtained from the linear portions of the dose-response curves except in the case of 6,7-DHT, in which acute toxic effects intervened. In general the range of efficacy of the tryptamines was at least an order of magnitude less than for various phenethylamines, such as 6-hydroxydopamine, tyramine, or phenethylamine. The most effective tryptamines were 6-HT and 7-HT. 5-HT was much less effective, although its α-methyl congener was very active. The dihydroxytryptamines and their α-methyl congeners were relatively inactive.

Release of [^{3}H]norepinephrine. Many of the hydroxytryptamines showed a dose-dependent release of [^{3}H]norepinephrine from mouse heart (Table 1). The relative efficacy of the monohydroxytryptamines was 7-HT = 6-HT > 5-HT > 4-HT. Whereas the efficacy of 7-HT and 6-HT was virtually unaffected by inhibition of monoamine oxidase, the efficacy of both 4-HT and 5-HT was increased about 10-fold. The efficacy of the dihydroxy derivatives was 5,6 > 5,7 > 6,7 > 4,7-DHT. The efficacy of 5,6-DHT was not greatly increased in monoamine oxidase-inhibited animals, while that of 5,7-, 6,7- and 4,7-DHT was increased approximately 2-fold. Except in the case of α-methyl-5,6-DHT, the α-methyl congeners were more potent releasing agents than their parent compounds.

Long-term effects on [^{3}H]norepinephrine uptake. Of the tryptamines tested, only 5,7-DHT and, to a lesser extent, 6,7-DHT induced any significant decrease in the uptake of [^{3}H]norepinephrine measured 5 days after a single exposure to the amine (Table 2). No alteration in [^{3}H]norepinephrine uptake was observed for α-methyl-5-HT, α-methyl-5,7-DHT, or 5,6-DHT, even though at the highest dose administered these compounds effectively displaced norepinephrine from cardiac stores (see Table 1). Inhibition of monoam-

TABLE 1

Effect of tryptamines on uptake and release of [^{3}H]norepinephrine in mouse heart in vivo

For measurement of inhibition of [^{3}H]norepinephrine uptake, various amounts of test compounds were administered with a constant amount of [^{3}H]norepinephrine (2.5 μCi) intravenously (tail vein) to mice, and the total retention of tritium by heart was determined after 20 min (see MATERIALS AND METHODS). For measurement of release, [^{3}H]norepinephrine (5 μCi) was administered intravenously 60 min prior to the subcutaneous injection of various doses of the test compound. Aminals were killed after another 2 hr. Mean tritium content per heart was determined (see MATERIALS AND METHODS), and ED_{50} values were derived from dose-response curves (percentage of control values vs. log dose).

Compound	ED_{50}		
	Inhibition of [^{3}H]norepinephrine uptake	[^{3}H]Norepinephrine release	
		Control	Monoamine oxidase inhibited[a]
	μmoles/kg	*μmoles/kg*	
4-HT	35	220	21
5-HT	22	160	16
6-HT	3.5	79	65
7-HT	4.3	80	75
4,7-DHT		150	71
5,6-DHT	9.8	32	43
5,7-DHT	15	60	26
5,7-DHT[b]		NR[c]	
6,7-DHT	>>30[d]	95	40
5,6,7-Trihydroxytryptamine	NI[e]	NR[c]	
α-Methyl-5-HT	2.5	10	13
α-Methyl-4,7,-DHT		72	
α-Methyl-5,6-DHT	23	NR[c]	
α-Methyl-5,7-DHT	24	6.1	
N,N-Dimethyl-5-HT	90	>13,000	
N,N-Dimethyl-5,6-DHT	770	120	
N,N-Dimethyl-5,7-DHT	12	>1,000	
N,N-Dimethyltryptamine	14	900	240
Tryptamine	23	2,100	290
4-Aminotryptamine	110	220	
5-Fluorotryptamine		1,300	320
α-Methyl-5-fluorotryptamine		340	
6-Fluorotryptamine		>1,300	380
α-Methyl-6-fluorotryptamine		230	
α-Methyl-6-chlorotryptamine		1,200	
7-Chloro-5-HT	110	120	
5-Hydroxybenzothiophene-3-β-ethylamine	5.1	120	58
Tyramine	0.69	56[f]	9.5
α-Methyltyramine	0.34	13	
N,N-Dimethyltyramine		>400	>80
6-Hydroxydopamine	0.73	6.8	4.3
α-Methyl-6-hydroxydopamine	0.60	4.2	
Cocaine	1.8	NR[c]	

[a] Iproniazid (150 mg/kg, intraperitoneally) was given 18 hr prior to labeling with [^{3}H]norepinephrine.

[b] Cocaine (4.5 mg/kg, intraperitoneally) was given 10 min before 5,7-DHT (100 μmoles/kg).

[c] No release at 100 μmoles/kg.

[d] LD_{75} = 30 μmoles/kg.

[e] No inhibition at 100 μmoles/kg.

[f] Variable from 18 to 56 μmoles/kg (see ref. 19).

TABLE 2

Long-term effect of hydroxytryptamines on uptake of [^{3}H]norepinephrine in mouse heart

Test compounds were administered subcutaneously. Five days later [^{3}H]norepinephrine was given by intravenous injection, and the mean tritium content per heart was determined 20 min later (see MATERIALS AND METHODS).[a]

Compound	Dose	Uptake at 5 days	
		Normal	Monoamine oxidase-inhibited[b]
	μmoles/kg	*% control*	*% control*
4,7-DHT	200	82.2 ± 7.6	106.6 ± 10.2
5,6-DHT	200	104.7 ± 5.8	107.6 ± 4.3
	400	96.0 ± 4.2	102.0 ± 5.6
5,7-DHT	100	70.2 ± 7.5	100 ± 6.7
	150	54.0 ± 5.2	105.8 ± 6.6
	200	42.6 ± 5.4	92.8 ± 10.3
	300	18.0 ± 5.8	102.2 ± 5.3
5,7-DHT[c]	150	101.0 ± 5.2	
6,7-DHT	30	84.4 ± 5.5	
	70	60.1 ± 6.4	51.2 ± 6.6
5,6,7-Trihydroxytryptamine	400	84.0 ± 10.2	
α-Methyl-4,7-DHT	20	87.6 ± 5.5	83.6 ± 4.7
α-Methyl-5,6-DHT	200	102.0 ± 5.4	
	500	89.6 ± 6.2	
α-Methyl-5,7,-DHT	200	90.6 ± 8.5	
	400	81.7 ± 4.8	
6-Hydroxydopamine	30	75.7 ± 5.0	41.3 ± 3.8

[a] The monohydroxytryptamines 4-HT, 5-HT, α-methyl-5-HT, 6-HT, and 7-HT had no significant long-term effect at a dose of 400 μmoles/kg.

[b] Either iproniazid (150 mg/kg) or pargyline (50 mg/kg) was given intraperitoneally 18 hr prior to administration of the test compound. Results were identical with either inhibitor. Uptake at 5 days in animals treated with monoamine oxidase inhibitor alone was identical with that of untreated controls.

[c] Cocaine (45 mg/kg, intraperitoneally) was given 10 min before 5,7-DHT.

ine oxidase by iproniazid or pargyline prior to the administration of 5,7-DHT completely blocked the long-term effect of this amine on [^{3}H]norepinephrine uptake, while similar treatment potentiated the long-term effect of 6-hydroxydopamine. Inhibition of monoamine oxidase did not significantly alter the long-term effect of 6,7-DHT on [^{3}H]norepinephrine uptake, but it did result in a decrease in the toxicity of 6,7-DHT.[3] There was no inhibition of [^{3}H]norepinephrine uptake by iproniazid or pargyline under the conditions employed, and the activity of monoamine oxidase in homogenates of liver and heart made at the time of the administration of 5,7-DHT was reduced by 86.9 ± 5.7% and 95.6 ± 7.8, respectively, as compared to control animals. There was a 2-fold increase in the ability of 5,7-DHT to displace [^{3}H]norepinephrine from heart under these conditions (Table 1). Treatment of animals with cocaine, 10 min prior to the administration of 5,7-DHT, markedly reduced the long-term effects of this amine. Cocaine also reduced the ability of 5,7-DHT to displace [^{3}H]norepinephrine from heart (Table 1).

The long-term effects of 5,7-DHT on uptake of [^{3}H]norepinephrine was dose-dependent, as shown by the dose-response curves measured 1–20 days after administration of 5,7-DHT (Fig. 1). The decrease in uptake by mouse heart reached a limiting value of approximately 20% of control

[3] Unpublished observations.

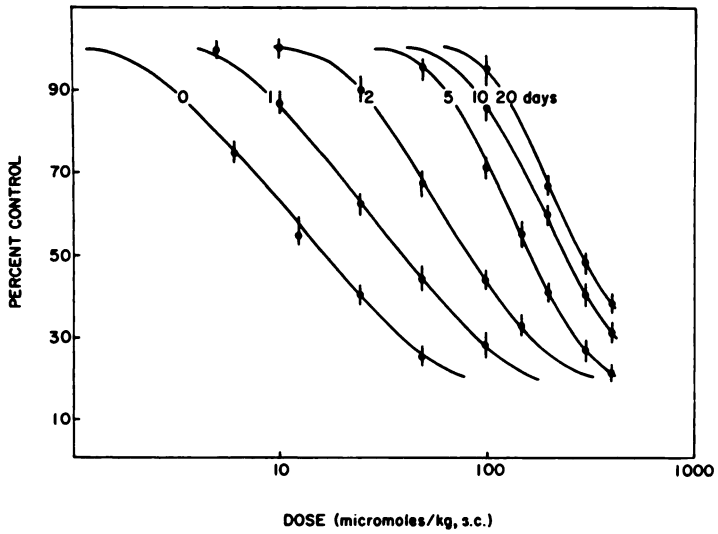


FIG. 1. *Dose-response curves for effect of prior administration of 5,7-dihydroxytryptamine on uptake of [³H]norepinephrine by mouse heart in vivo*

5,7-DHT was administered either intravenously simultaneous with [³H]norepinephrine or subcutaneously 1-20 days prior to the [³H]norepinephrine. The mean tritium content of hearts was determined 20 min after administration of [³H]norepinephrine.

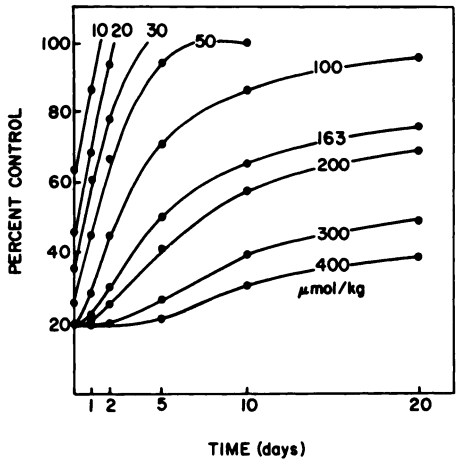


FIG. 2. *Rate of recovery of uptake of [³H]norepinephrine in mouse heart in vivo after treatment with 5,7-dihydroxytryptamine*

The uptake of [³H]norepinephrine in hearts of animals treated with 5,7-DHT (20-400 μmoles/kg) 1-20 days prior to the measurement of uptake is expressed as a percent of the uptake in controls. Zero-time values were obtained with simultaneous intravenous administration of [³H]norepinephrine and 5,7-DHT. The mean tritium content of hearts was determined 20 min after intravenous administration of [³H]norepinephrine.

at all doses of 5,7-DHT. It would appear that this represents a nonspecific component of [³H]norepinephrine uptake. A gradual increase in the slope of the linear portion of the dose-response curve and a displacement to the right occurred as the time interval between exposure to 5,7-DHT and measurement of uptake was increased. That the rate of return of [³H]norepinephrine uptake to control values was indeed much more rapid after lower doses of 5,7-DHT is shown in Fig. 2. At higher doses a lag phase in the recovery of [³H]norepinephrine uptake appeared, during which time neuronal uptake was virtually absent. At the maximum dose of 5,7-DHT (400 μmoles/kg) this lag phase persisted for 5 days, and recovery was minimal even after 20 days. The results depicted in Figs. 1 and 2 are consonant with a reversible impairment of function of noradrenergic terminals at lower concentrations of 5,7-DHT, while at higher concentrations more and more terminals appear to have undergone irreversible impairment of function.

Rate of autoxidation. The rates of autox-

idation of 5,6-DHT, 5,7-DHT, and their α-methyl congeners were virtually the same. At an amine concentration of 0.2 mM the oxygen consumption was approximately 10–16 nmoles/min (Table 3). The rate observed for 5,7-DHT was thus only about 1/6 that of 6-hydroxydopamine. The rates observed for 6,7- and 4,7-DHT and 5,6,7-trihydroxytryptamine were nearly equivalent, and about 4-fold higher than for 5,6- and 5,7-DHT. The rate of autoxidation of 5-HT was quite slow, and approximately equivalent to that of dopamine.

Morphological alterations. The ultrastructure of mouse atria was examined 2 and 20 days after exposure to 5,7-DHT (400 μmoles/kg), at which time the uptake of [^{3}H]norepinephrine was 20% and 38% of control values. The ultrastructure of heart muscle fibers was unaltered as compared to atrial preparations from untreated control animals. However, many degenerating axons were seen in small unmyelinated nerves, found primarily in subepicardial locations and adjacent to coronary arteries (see ref. 22). The injured axons contained many vesicles of variable size, with a highly electron-dense content (compare III and IV with I and II in Fig. 3). These axons often had a moderate but distinct electron-dense axoplasm. They were sometimes found in close proximity to terminal cholinergic axons with electron-lucent synaptic vesicles (Fig. 3, III). The latter appeared to be completely normal. Increased cytoplasmic volume and increased numbers of ribosomes were frequently observed in Schwann cells surrounding the injured axons (Fig. 3, IV). Structural evidence of axon injury and the presence of electron-dense vesicles was still clear, although to a lesser degree, 20 days after exposure to 5,7-DHT. Similar ultrastructural changes were observed in nerve fibers in other sympathetically innervated organs. For comparative purposes atria were examined after a single injection of 6-hydroxydopamine (50 μmoles/kg). The ultrastructure and localization of injured axons were similar to those described in rat atria after 6-hydroxydopamine (23).

DISCUSSION

A variety of tryptamine derivatives inhibit the uptake of [^{3}H]norepinephrine into cardiac tissue and displace it from

TABLE 3

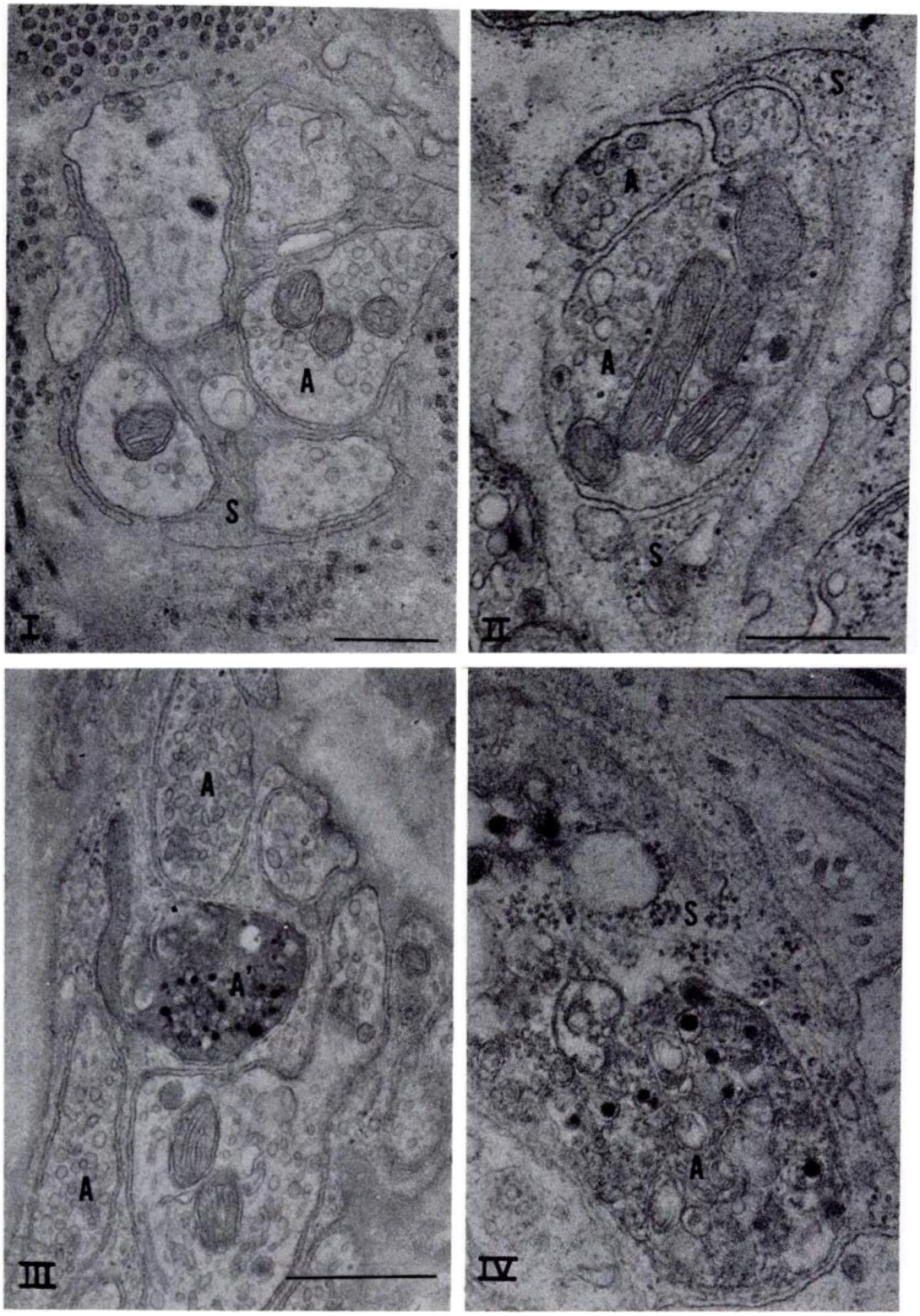
Autoxidation rates of hydroxytryptamines

Initial rates of oxygen consumption were measured with a Clark oxygen electrode in a closed cell equipped with a magnetic stirrer. The total cell volume was 1.7 ml; temperature was maintained at 37°; the solvent was 0.2 M phosphate buffer, pH 7.0. Test compounds were dissolved in 0.01–0.07 ml of deoxygenated water immediately before injection into the cell via a sealed port.

Tryptamine	Oxidation rate		
	0.2 mM[a]	0.5 mM	1.0 mM
	nmoles/min	*nmoles/min*	*nmoles/min*
5-HT		<2.0	3.7
4,7-DHT	20	38	56.0
5,6-DHT	5.5	11.0	22.0
5,7-DHT	8.2	16.5	36.2
6,7-DHT	22.0	40.5	59.0
5,6,7-Trihydroxytryptamine	19	36	53.0
α-Methyl-5,6-DHT	5.4	10.9	
α-Methyl-5,7-DHT	8.0	13.0	
6-Hydroxydopamine[b]	66.3 ± 3.3	276 ± 13.1	
5-Hydroxydopamine[b]	8.5 ± 3.9	20.4 ± 3.3	41.3 ± 2.6
Dopamine[b]			<2.0

[a] Final concentration of amine.

[b] Data from ref. 19.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previously labeled storage sites (Table 1) (20, 24). Inhibition of uptake in the present studies was measured at concentrations of [^{3}H]norepinephrine at which only uptake at the neuronal plasma membrane is significant (25). Phenolic groups at positions 6 and 7 of the indole ring conferred the highest activity (Table 1). α-Methyltryptamines in most cases were more efficacious than the parent compound, while the *N,N*-dimethyl tertiary amines were not as effective. α-Methyl-5,6-DHT was a remarkable exception, in that it apparently had very little activity as a releasing agent. An explanation for this anomalous result is not obvious. Similar structure-activity relationships pertained with phenethyl- and phenethanolamines, where phenolic groups at position 3 or 4 conferred high activity, and where tertiary amines were ineffective and α-methyl analogues more effective (Table 1) (20, 26). The isosteric analogue of serotonin, in which the nitrogen of the indole nucleus is replaced by a sulfur, had high potency both as an inhibitor of [^{3}H]norepinephrine uptake and as a releasing agent (Table 1).

The apparent affinity of dihydroxytryptamines for neuronal amine uptake and storage mechanisms in cardiac tissue, coupled with the ease with which alterations in adrenergic function can be studied in this tissue, make it an ideal test system for investigating the structure-activity relationships involved in the neurodegenerative effects of dihydroxytryptamines. The major disadvantage of this type of test system is that alterations in the apparent efficacy of various test compounds may be due to transport and metabolic factors. Such considerations, however, would not appear to affect the conclusions reached in the present paper. In a previous study of phenethylamines, only tri- and tetrahydroxyphenethylamines which were actively taken up into adrenergic structures and which were, in addition, very readily autoxidized were effective as neurodegenerative agents (19). In the present study only 5,7-DHT and, to a lesser extent, 6,7-DHT were found to have long-term effects on noradrenergic uptake mechanisms in mouse heart. The long-term cytotoxic effects of 5,7-DHT were dependent upon the active uptake into neuronal structures, as evidenced by the blockade of the long-term effect by prior treatment with the potent uptake inhibitor, cocaine (Table 2). Prior treatment with cocaine was also shown to block the ability of 5,7-DHT to release labeled stores of [^{3}H]norepinephrine (Table 1). Destruction of noradrenergic structures in various peripheral organs of mice after intraperitoneal injection of a high dose of 5,7-DHT were reported (27) during preparation of the present manuscript. The lack of long-term effects of 5,6-DHT on the cardiac uptake of [^{3}H]norepinephrine is somewhat surprising in view of its long-term effects on central noradrenergic neurons and its relatively high potency toward inhibition of uptake of [^{3}H]norepinephrine (Table 1). 5,6-DHT has been reported to be less effective than 5,7- and 6,7-DHT in eliciting destruction of central noradrenergic structures (14, 15, 28). Some long-term effects on noradrenergic terminals in rat heart in response to high doses of 5,6-DHT have been reported (11), but such effects were not evident in

FIG. 3. *Morphological alterations in mouse atria after 5,7-dihydroxtryptamine*

I. Unmyelinated nerve from untreated mouse atria. Several normal-appearing axons are seen embedded in Schwann cell cytoplasms. One of these (A) contains three mitochondrial profiles as well as synaptic vesicles. II. Unmyelinated nerve from untreated mouse atrium. The axons have only a sparse investment of Schwann cell cytoplasms. In two axons (A) numerous synaptic vesicles are present, suggesting that the section is near the axon terminal. III. Unmyelinated nerve from mouse atrium 2 days after administration of 5,7-DHT. Two normal-appearing axons (A) contain many synaptic vesicles, but in the center of the field may be seen a degenerating axon (A'), containing vesicles which vary in size, some of which have a highly electron-dense content. IV. A degenerating axon from mouse atrium 2 days after 5,7-DHT. The axon (A) contains many vesicular profiles, some of which have a highly electron-dense content. The enclosing Schwann cells (S) show some degree of cytoplasmic reaction, as evidenced by increased volume and increased numbers of ribosomes. The bars equal 0.5 microns.

the present study in mice. The reported studies in rats were carried out after treatment of the animals with Dibenamine. This *alpha* adrenergic antagonist may, by reducing 5,6-DHT-induced vasoconstriction, have allowed greater amounts of 5,6-DHT to reach the uptake sites in the heart. It is noteworthy that 5,6-DHT has recently been reported to be concentrated into storage granules of blood platelets without inducing any morphological damage (29). The low efficacy of 6,7-DHT in heart may reflect its lower affinity for uptake mechanisms as compared to 5,7-DHT. The only other dihydroxytryptamine tested, the 4,7-isomer, was relatively ineffective with respect to inhibition of cardiac uptake of [^{3}H]norepinephrine and had no long-term effect on noradrenergic uptake.

Although 5,6- and 6,7-DHT had either no or minimal-long term effects on cardiac noradrenergic terminals, both compounds were quite cardiotoxic. 5,6-DHT caused massive subepicardial lesions, and 6,7-DHT caused generalized vascular damage and subendocardial lesions.[3] 5,7-DHT showed no gross toxicity other than the long-term effects on noradrenergic neurons.

The mechanisms involved in the neurodegeneration elicited by 5,7-DHT appear to be unique and not clearly related to the mechanisms involved in polyhydroxyphenethylamine-induced neurodegeneration. For example, inhibition of monoamine oxidase apparently reduces the metabolism of 6-hydroxydopamine *in vivo* and results in potentiation both of its effects on the release of [^{3}H]norepinephrine and of its long-term effects on uptake of [^{3}H]norepinephrine (Tables 1 and 2). Furthermore, the potency of α-methyl-6-hydroxydopamine as a releasing agent is greater than that of the parent amine. With 4-HT, 5-HT, 5,7-DHT, and 6,7-DHT, the ED_{50} values for the release of [^{3}H]norepinephrine were decreased 2–10-fold in monoamine oxidase-inhibited animals (Table 1). Monoamine oxidase inhibition had little or no effect on the release of [^{3}H]norepinephrine by 6-HT, 7-HT, or 5,6-DHT. Presumably a shift in the dose-response curve to the left is more pronounced with tryptamines for which monoamine oxidase-catalyzed oxidation is a major extra- or intraneuronal metabolic route. Many of these tryptamines are apparently metabolized by monoamine oxidase (26, 30–32), but no quantitative, comparative studies on the hydroxy- and dihydroxytryptamines have been reported. By analogy to the results obtained with 6-hydroxydopamine, it was expected that inhibition of monoamine oxidase would also potentiate the long-term effects of 5,7-DHT on noradrenergic cardiac mechanisms. Instread, inhibition of monoamine oxidase completely abolished the long-term effects of 5,7-DHT both on the uptake of [^{3}H]norepinephrine and on the ultrastructure of cardiac noradrenergic neurons. This blockade of the long-term effects occurred concomitantly with a 2-fold decrease in the ED_{50} for release of [^{3}H]norepinephrine by 5,7-DHT in monoamine oxidase-inhibited animals. This result strongly suggests a dependency of the neurodegenerative effect on the monoamine oxidase-catalyzed oxidative metabolism of 5,7-DHT, perhaps to a cytotoxic metabolite. The finding that the more potent releasing agent, α-methyl-5,7-DHT, which should not be a substrate for monoamine oxidase, elicited virtually no long-term effects (Table 2) further supports this conclusion.

Another anomalous aspect of the neurodegeneration elicited by 5,7-dihydroxytryptamine became apparent upon measurement of the rates of autoxidation of various tryptamines (Table 3). With polyhydroxyphenethylamines only those compounds which are substrates for the uptake mechanism and undergo very rapid autoxidation elicited neurodegenerative effects (15). However, all the isomeric dihydroxytryptamines exhibited relatively slow rates of autoxidation, slower in fact than those of certain trihydroxyphenethylamines, such as 5-hydroxydopamine, which do not cause degeneration of cardiac noradrenergic nerve terminals. Rates of autoxidation of the dihydroxytryptamines are much greater than that of 5-HT. Thus, with the dihydroxytryptamines, there is no clear correlation between amine-elicited

neurodegenerative effects and either concentration in neuronal structures or rates of autoxidation. Studies are in progress with radioactive tryptamines to attempt to establish correlations between extent of covalent binding and neurodegeneration. Such studies may provide evidence to substantiate the role of monoamine oxidase in the neurodegenerative action of 5,7-DHT in cardiac tissue.

Whether the present results are applicable to central neurons is at present not known. 5,7-DHT appears to be more potent than 5,6-DHT in eliciting degeneration of central serotonin-containing neurons, in spite of the apparent lower affinity of 5,7-DHT for the serotonin uptake mechanism (16, 17), suggesting that this amine is inherently more neurotoxic. Prior treatment of rats with the monoamine oxidase inhibitor nialamide was reported to enhance greatly the toxic effects of intraventricularly administered 5,6-DHT (2), while pargyline slightly potentiated the 5,6-DHT-elicited reduction in brain serotonin (33). Long-term effects of 5,6-DTH on peripheral sympathetic neurons, studied principally in vas deferens, were still observed in rats which had been treated with both reserpine and the monoamine oxidase inhibitor nialamide prior to administration of 5,6-DHT (11). Studies on the effects of various dihydroxytryptamines and their α-methyl congeners on central serotonin and catecholamine neurons in normal and monoamine oxidase-inhibited animals should provide further insight into the role of this enzyme in the cytotoxic effects of dihydroxytryptamines in serotonin and catecholamine neurons. Indeed, subsequent to the present studies, a preliminary report appeared indicating that in monoamine oxidase-inhibited animals 5,7-DHT still depleted central serotonin but had no effect on norepinephrine (34).

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