(1 mg/kg) before receiving carbidopa-^{14}C (20 mg/kg), the solvent extraction properties of the radioactive urinary metabolites remained unchanged. It has been found that acidic solutions of carbidopa may decompose in air to yield 3,4-dihydroxyphenylacetone. Thus, the presence of the latter in urine may well result during manipulation of the urine samples.

It would be expected that the flora of the lower intestine assume significance in the metabolism of carbidopa since incomplete absorption of the compound occurs.[15] Evidence was obtained for bacterial decarboxylation of the racemate of carbidopa in rat feces.[16] Dehydroxylation of catecholic acids following their ingestion is well known and *m*-hydroxyphenylacetic acids have been identified in urine. Although it is reported[17] that mammalian tissues do have the capacity to dehydroxylate catecholamino acids (in the meta position), it is suggested that metabolite VII, having a *m*-hydroxyl group, is a product of bacterial dehydroxylation. The amino acid Dopa or one of its metabolites is subject to bacterial dehydroxylation.[18,19] It seems the same conclusion applies to carbidopa, the hydrazino analog of Dopa.

Acknowledgments. The authors wish to thank Mr. J. L. Smith and Mr. W. A. Ocker for their advice on computer applications and Dr. R. L. Ellsworth for the synthesis of compound VII. The labeled carbidopa was synthesized by Mr. H. T. Meriwether and Mr. G. J. Gatto. The able assistance of Mr. S. D. White is also gratefully acknowledged. Thanks are also due to Dr. J. E. Baer for his assistance in the preparation of this manuscript.

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Norepinephrine Uptake Sites in Cardiac Tissue. Lack of Affinity of 6-Hydroxynorepinephrine and Related Compounds

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Received August 12, 1974

The effects of a series of phenethylamines and the corresponding phenethanolamines on (i) rate of uptake of radioactive norepinephrine into cardiac tissue *in vivo* and (ii) the rate of efflux of radioactive norepinephrine from prelabeled cardiac storage sites have been determined. The results indicate that *m*- and *p*-hydroxyphenethylamines and the corresponding phenethanolamines have high affinities for uptake into cytoplasm and storage vesicles of noradrenergic terminals in the heart. *o*-Hydroxyphenethylamines such as 2-hydroxyphenethylamine and 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine) also have moderate to high activity as inhibitors of norepinephrine uptake and as releasing agents for norepinephrine, but *o*-hydroxyphenethanolamines such as 2-hydroxyphenethanolamine, 2,5-dihydroxyphenethanolamine, and 2,4,5-trihydroxyphenethanolamine (6-hydroxynorepinephrine) have little or no activity as inhibitors of uptake or as releasing agents. 2,6-Dihydroxyphenethylamines have little or no activity as inhibitors of uptake or as releasing agents. The results are consonant with significant binding of the *gauche* conformers of 2-hydroxyphenethylamines to uptake sites. Such conformers would be preferred because of stabilization by hydrogen bonding between nitrogen and phenolic oxygen. Apparently a hydrophobic region of the site prevents binding of such stabilized gauche conformers of 2-hydroxyphenethanolamines and 2,6-dihydroxyphenethylamines.

Extensive investigations on the effects of various compounds on the uptake and release of radioactive norepinephrine from cardiac tissues *in vivo* have been reported.[1-20] Such studies provide the following information relevant to the design of drugs and pharmacologically active research tools: (i) structure-activity correlations with respect to inhibition of norepinephrine uptake at plasma membrane, a process important to the termination of the action of this neurotransmitter; (ii) structure-activity correlations with respect to displacement of norepinephrine from storage sites, a phenomenon which results in a lowered availability of norepinephrine as a neurotransmitter and/or replacement of the norepinephrine with "false neurotransmitters" of greater or lesser physiological efficacy. With suitable modifications the basic *in vivo* test system has provided data on (i) long-term effects on uptake and storage of norepinephrine by neurotoxic agents such as 6-hydroxydopamine (2,4,5-trihydroxyphenethylamine) and 5,7-dihydroxytryptamine;[19,20] (ii) efficacy of compounds as *in vivo* inhibitors of the decarboxylation of Dopa;[11] and (iii) the relative importance of monoamine oxidase to the *in vivo* metabolism of phenethylamines and tryptamines.[20]

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An early screening of various phenethylamines in a murine *in vivo* test system[9] provided results indicative of significant release of radioactive norepinephrine from cardiac sites by 6-hydroxynorepinephrine (2,4,5-trihydroxyphenethanolamine) and 6-hydroxyepinephrine. However, a recent report of the lack of significant effects of 6-hydroxynorepinephrine on endogenous levels of norepinephrine or on noradrenergic terminals in rat heart[21] provided the impetus for a reevaluation of our own earlier results.

Evidence is now reported that demonstrates that *o*-hydroxyphenethanolamines such as 6-hydroxynorepinephrine have no affinity for norepinephrine uptake sites in heart and, therefore, *cannot* elicit release of norepinephrine from the storage sites. The corresponding *o*-hydroxyphenethylamines such as 6-hydroxydopamine have significant activity as inhibitors of uptake of radioactive norepinephrine and displace radioactive norepinephrine from cardiac storage sites, but *o*-dihydroxyphenethylamines are inactive. These results provide new insights into the structural requirements of uptake sites for dopamine and norepinephrine.

Results

The results of Table I clearly establish that the presence of *m*- or *p*-hydroxy substituents in phenethylamines or phenethanolamines confers high affinity as *in vivo* inhibitors of the uptake of norepinephrine into cardiac sites. In contrast, the phenethylamines containing an *o*-hydroxy substituent are markedly less active inhibitors and *o*-hydroxyphenethanolamines have virtually no activity. For example, 3-hydroxyphenethylamine (*m*-tyramine) and 3-hydroxyphenethanolamine (*m*-octopamine) are nearly equipotent and among the most active amines as inhibitors of uptake of norepinephrine. Addition of an *o*-hydroxy group to these compounds yields 2,5-dihydroxyphenethylamine and 2,5-dihydroxyphenethanolamine, respectively. The former compound is only sevenfold less active as an inhibitor of uptake than *m*-tyramine, while the latter compound is virtually inactive (300-fold less active than *m*-octopamine). Phenethylamines with two *o*-hydroxy substituents either had no effect on uptake of norepinephrine or were weak inhibitors. Two phenethanolamines with a polar substituent other than a phenolic group in the ortho position were tested as inhibitors of uptake of norepinephrine. Neither 2-methoxy- or 2-methoxy-5-hydroxyphenethanolamine inhibited uptake of norepinephrine at dosages as high as 100 μmol/kg.

The phenethylamines and selected phenethanolamines were also tested for activity as releasors of radioactive norepinephrine from cardiac sites (Table II). Compounds that were not effective inhibitors toward uptake of norepinephrine had little or no activity as releasing agents. The presence of *m*- and *p*-hydroxy substituents conferred greater releasing activity than the presence of an *o*-hydroxy group. The presence of an *o*-hydroxy group in the phenethanolamine series resulted in virtual abolishment of releasing activity, while two *o*-hydroxy groups were required to abolish activity in the phenethylamine series.

No long-term reduction in the ability of mouse heart to take up norepinephrine resulted from the administration of either 6-hydroxynorepinephrine or 6-hydroxyepinephrine in marked contrast to the prolonged reduction observed following the administration of 6-hydroxydopamine (Table III).

Table I. Inhibition of [^{3}H]Norepinephrine Uptake into Murine Heart by Phenethylamines and Phenethanolamines *in Vivo*[a]

Aromatic substn	ED_{50}, μmol/kg[b] Phenethylamine	Phenethanolamine
None	3.8	9.2
2-Hydroxy	13.8	137.2
3-Hydroxy	0.35	0.40
4-Hydroxy	0.69	0.82
2,3-Dihydroxy	1.16	
2,4-Dihydroxy	1.69	
2,5-Dihydroxy	2.48	116.8
2,6-Dihydroxy	n.i.[c]	
3,4-Dihydroxy	0.32	0.52
3,5-Dihydroxy	0.61	4.3
2,3,4-Trihydroxy	0.63	
2,3,5-Trihydroxy	1.20	
2,3,6-Trihydroxy	21.9	
2,4,5-Trihydroxy	0.73	n.i.[d]
2,4,6-Trihydroxy	n.i.[c]	
3,4,5-Trihydroxy	0.49	
2,3,4,5-Tetrahydroxy	5.83	
2,3,4,6-Tetrahydroxy	n.i.[c]	
2,3,5,6-Tetrahydroxy	n.i.[c]	
3,5-Dihydroxy-4-methoxy	1.55	5.52
2,4-Dihydroxy-5-methoxy	n.i.[c]	

[a]Compound administered intravenously with [^{3}H]norepinephrine and radioactivity in heart measured 20 min later (see methods). [b]For comparison the ED_{50} (μmol/kg) values for cocaine, imipramine, and α-methyltyramine were 1.8, 11.2, and 0.31, respectively. [c]n.i. = no inhibition of uptake at dosages up to 100 μmol/kg. [d]Neither 6-hydroxynorepinephrine nor 6-hydroxyepinephrine had any effect on the uptake at dosages up to 100 μmol/kg.

Table II. Release of [^{3}H]Norepinephrine from Murine Heart *in Vivo* by Phenethylamines and Phenethanolamines[a]

Aromatic substn	ED_{50}, μmol/kg[b] Phenethylamine	Phenethanolamine
None	510	565
2-Hydroxy	620	>900
3-Hydroxy	23.1	3.8
4-Hydroxy	38[c]	35.0
2,3-Dihydroxy	59	
2,4-Dihydroxy	65	
2,5-Dihydroxy	200	1000
2,6-Dihydroxy	n.r.[d]	
3,4-Dihydroxy	19	
3,5-Dihydroxy	11.5	6.6
2,3,4-Trihydroxy	74	
2,3,5-Trihydroxy	63	
2,3,6-Trihydroxy	n.r.[d]	
2,4,5-Trihydroxy	7	n.r.[d]
2,4,6-Trihydroxy	n.r.[d]	
3,4,5-Trihydroxy	10	
2,3,4,5-Tetrahydroxy	82	
2,3,4,6-Tetrahydroxy	n.r.[d]	
2,3,5,6-Tetrahydroxy	n.r.[d]	
3,5-Dihydroxy-4-methoxy	1.8	7.1

[a]Compounds were administered subcutaneously 1 hr after intravenous administration of [^{3}H]norepinephrine and radioactivity measured in heart 2 hr later (see Methods). [b]For comparison, the ED_{50} (μmol/kg) values for reserpine and α-methyltyramine were 0.3 and 12.9, respectively. [c]An ED_{50} for 4-hydroxyphenethylamine (tyramine) is difficult to estimate since a maximal release of only slightly greater than 50% of the norepinephrine is attained even at concentrations as high as 100 μmol/kg (*cf.* ref 19). [d]n.r. = no release at dosages of up to 100 μmol/kg.

Table III. Lack of Long Term Effects of 6-Hydroxynorepinephrine and 6-Hydroxyepinephrine on the Uptake of [^{3}H]Norepinephrine by Murine Heart *in Vivo*[a]

Compound	Dose, μmol/kg	Uptake at 5 days (% control)[b]
6-Hydroxynorepinephrine	120	99.7 ± 5.5
	240	105.2 ± 6.3
6-Hydroxyepinephrine	120	98.5 ± 4.8
	240	96.8 ± 5.7
6-Hydroxydopamine	75	74.0 ± 3.5
	150	39.3 ± 8.3

[a] Compound administered subcutaneously 5 days before measurement of uptake. [b] Uptake expressed as per cent ± S.E.M. of the radioactivity, 36,900 ± 1,100 cpm/heart found untreated mice 20 min after the intravenous administration of [^{3}H]norepinephrine (see Methods).

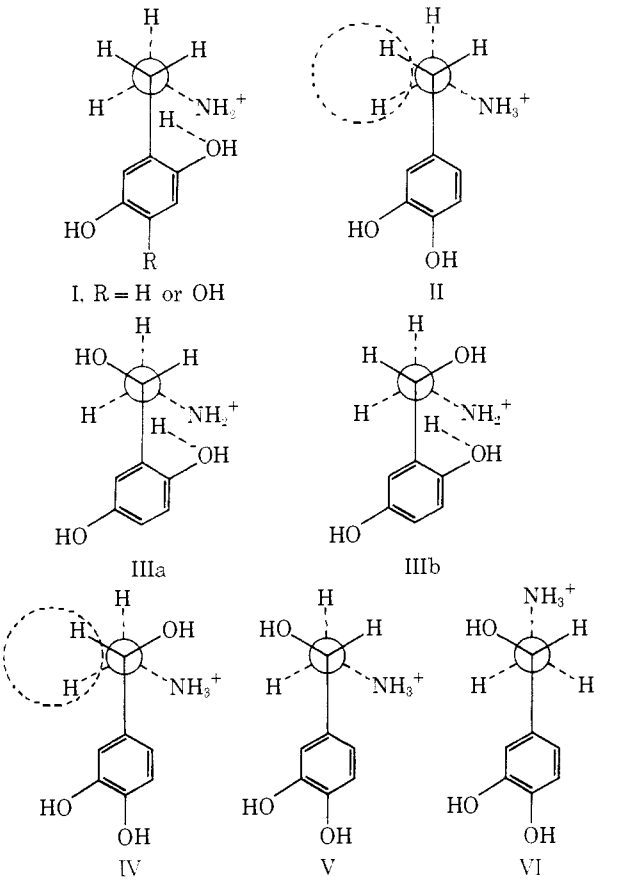
Discussion

The present study was initiated to confirm the recent report[21] that 6-hydroxynorepinephrine had no long-term effects on parameters related to noradrenergic function, such as norepinephrine levels and uptake mechanisms in cardiac tissue. After many experiments, it was concluded that neither 6-hydroxynorepinephrine nor 6-hydroxyepinephrine has any significant effects on uptake of radioactive norepinephrine measured 5 days after the administration of the 6-hydroxy derivative (Table III). Dosages as high as 100 μmol/kg were employed. Under this protocol, 6-hydroxydopamine at 50 μmol/kg caused a 50% reduction in uptake of radioactive norepinephrine.[19] In addition, 6-hydroxynorepinephrine and 6-hydroxyepinephrine had no effect on *in vivo* uptake of radioactive norepinephrine into the heart (Table I) and did not cause significant release of radioactive norepinephrine from cardiac sites at dosages as high as 100 μmol/kg (Table II). These latter negative results contrast with our earlier data which indicated that both 6-hydroxynorepinephrine and 6-hydroxyepinephrine had significant releasing activity.[9] One of the inherent problems with 6-hydroxynorepinephrine is its great instability and the resultant problems with adequate characterization of the material used in biological studies. The most tenable explanation for the discrepancy between the present negative results, in which some effort to characterize and stabilize the 6-hydroxy derivatives was made, and the earlier positive results, obtained with uncharacterized products, is that in the earlier studies hydrogenolysis of the benzylic alcohol group, perhaps due to the presence of acid and/or a highly active catalysts, might have occurred, resulting in formation of 6-hydroxydopamine or *N*-methyl-6-hydroxydopamine, respectively. The release would then have been due to these compounds.

In view of the difficulties inherent in carrying out experiments with the highly labile 6-hydroxynorepinephrine, attention was now turned to a model set of compounds, 2,5-dihydroxyphenethylamine and 2,5-dihydroxyphenethanolamine. As in the 2,4,5-trihydroxy compounds, the phenethylamine had significant activity as both an inhibitor of uptake of norepinephrine and as a releasing agent (Tables I and II), while the phenethanolamine was 47- and 5-fold less active in these assays. Similarly, in the 2-hydroxy compounds, the phenethanolamine was about tenfold less active than the phenethylamine in both assays. Reduced activities for phenethanolamines were not apparent when phenethylamines and phenethanolamines with phenolic groups only in meta and para positions are compared. Obviously, 2-hydroxyphenethanolamines have little or no affinity for norepinephrine uptake sites in cardiac tissue and, hence, are not actively concentrated into the tissue to any great extent. This explains their lack of releasing activity (Table II) and the lack of long-term neurodegenerative effects[21] that had been expected for 6-hydroxynorepinephrine. Such neurodegenerative activity for trihydroxyphenethylamines has been shown to depend on both affinity for active uptake sites and ease of autoxidation.[19]

Since active uptake into the neuronal terminal is a prerequisite for release of norepinephrine from storage sites, it was not unexpected that the compounds with no affinity for the uptake site did not have significant activity toward release of norepinephrine (Table II). Obviously, however, additional factors are important in determining efficacy as releasing agents. Thus, 2,3,4-trihydroxyphenethylamine and the 2,4,5-trihydroxy isomer are nearly equipotent as inhibitors of uptake of norepinephrine, but the latter compound is about tenfold more active as a releasing agent. Release of norepinephrine, of course, requires not only that the amine have affinity for the uptake site, but that it be transported into the cell after binding to the uptake site. Certain compounds may bind, but not be transported, and this aspect is presently under investigation. Differences in intracellular disposition of amines by monoamine oxidase and differences in affinity for vesicular uptake mechanisms will also be important as determinants of the efficacy of the amines as releasing agents.

The present results, in addition to providing an explanation to the lack of neurodegenerative effects for 6-hydroxynorepinephrine, are relevant to the conformation of dopamine and norepinephrine that interacts with uptake sites at catecholamine terminals. Thus, the fact that 6-



hydroxydopamine, 2,5-dihydroxyphenethylamine, and related compounds, which are stabilized in the gauche conformation by strong hydrogen bonding between nitrogen and phenolic oxygen (I, *cf.* ref 22), have a high affinity for the uptake process suggests that uptake of the gauche conformer of dopamine (II) will be a permitted if not preferred process. Conversely, the observations that 6-hydroxynorepinephrine and related *o*-hydroxyphenethanolamines, which would also be stabilized in the gauche conformation by the strong hydrogen bonding between nitrogen and phenolic oxygen (IIIa,b, *cf.* ref 23), have little affinity for the uptake process suggests that the uptake of the gauche conformer of norepinephrine (IV or V) is not a preferred process. Instead, the results might be interpreted as indicative of preferred binding and transport of the transoid form of norepinephrine (VI). Other conformers of 2-hydroxyphenethanolamines with hydrogen bonding between the benzylic oxygen and nitrogen or between oxygens are of course possible but would not appear likely to be major contributors (*cf.* ref 23). It is important to note that in the 2-hydroxyphenethanolamine series, two gauche conformers with hydrogen bonding between phenolic oxygen and nitrogen are possible, only one (IIIb) of which corresponds sterically to the preferred gauche conformer of norepinephrine (IV, *cf.* ref 24). Since IIIa might be expected to be somewhat more stable than IIIb, an alternative more plausible interpretation of the present data is, therefore, possible. Norepinephrine in the gauche conformation does, like dopamine, have affinity for the uptake site, but the general area indicated by the dotted circle in II and IV will not accommodate a polar grouping. This explanation would account for lack of affinity for uptake sites of 2-hydroxyphenethanolamines and for 2,6-dihydroxyphenethylamines. The results of molecular orbital calculations on various substituted phenethylamines and phenethanolamines[22-24] and nuclear magnetic resonance spectroscopy on catecholamines[25] are consonant with these interpretations of hydrogen bonding.

The two 2-methoxyphenethanolamines tested did not inhibit uptake of norepinephrine and such compounds would be expected to have a gauche conformer stabilized by hydrogen bonding between nitrogen and oxygen. No 2-methoxyphenethylamines were investigated in the present study. 2-Methoxy- and 6-methoxynorepinephrine were virtually inactive as releasing agents for cardiac norepinephrine, while the corresponding 2-methyl- and 6-methylnorepinephrine were active releasing agents.[15] Thus, the presence of an ortho substituent such as methyl is not of itself sufficient to limit affinity of a phenethanolamine to the uptake site. Instead, the ortho substituent must be capable of hydrogen bonding to the side-chain nitrogen.

The present investigation extends our knowledge of the structural requirements for interaction of amines to proposed uptake sites in neurogenic membranes. However, in spite of extensive investigation of such uptake phenomenon in many laboratories, virtually nothing is known of the basic mechanisms involved in the transport of biogenic amines across biomembranes. Development of specific radiolabeled inhibitors with high affinity for uptake sites might permit the assay and isolation of the relevant macromolecules. Further studies on amines, cocaine analogs, and phenothiazines are being directed toward the development of such specific inhibitors.

Experimental Section

Materials. All synthetic intermediates and the final products were characterized as authentic by thin-layer chromatography, nuclear magnetic resonance spectroscopy, and electron impact and chemical ionization mass spectrometry.

2-Hydroxyphenethanolamine,[26] 2,5-dihydroxyphenethanolamine,[27] 2-methoxy-5-hydroxyphenethanolamine, and 2-methoxyphenethanolamine[28] were prepared as follows. 2-Hydroxy- and 2,5-dihydroxybenzaldehydes were converted to *O*-benzyl derivatives by reaction at 110° for 2 hr with 1 or 2 equiv of benzyl chloride–K_2CO_3 in DMF, followed by cooling, dilution with H_2O, and, respectively, either extraction with Et_2O to provide the 2-benzyloxybenzaldehyde as a oil after distillation at 190° and 5 mm (yield 80%) or by filtration to collect the water-insoluble precipitate, followed by recrystallization from EtOH to afford 2,5-dibenzyloxybenzaldehyde (mp 87–88°, yield 85%). With 1 equiv of benzyl chloride, 2,5-dihydroxybenzaldehyde afforded 2-hydroxy-5-benzyloxybenzaldehyde which was obtained homogeneous after silica gel chromatography with CH_2Cl_2 (*cf.* ref 29). Conversion to 2-methyl-5-benzyloxybenzaldehyde was then accomplished in refluxing acetone for 5 hr with an excess of Me_2SO_4 and K_2CO_3 and a catalytic amount of $NaOCH_3$. After reaction an excess of H_2O was added. Stirring was continued for 20 min, followed by filtration and evaporation *in vacuo*. The residual oil was purified by silica gel chromatography with CH_2Cl_2 (overall yield 30%). 2-Methoxybenzaldehyde was prepared in the same manner from 2-hydroxybenzaldehyde (yield 50%).

Aldehydes were then condensed with CH_3NO_2 to afford the corresponding phenylnitroethanols (*cf.* ref 30). The condensation of 2-benzyloxybenzaldehyde (1.05 g) with CH_3NO_2 (1 ml) was effected in 3 ml of THF to which was added 1.2 ml of 10% NaOH. After stirring for 30 min at room temperature, 3 ml of 2% AcOH was added. An Et_2O extract was dried (Na_2SO_4) and evaporated to afford a residue which was recrystallized from 95% EtOH (mp 65–70°, yield 75%). 2-Methoxy-5-benzyloxybenzaldehyde and 2-methoxybenzaldehyde were condensed with CH_3NO_2 in the same manner except the reaction time was 36 hr (yield 40–50%). The condensation of 2,5-dibenzyloxybenzaldehyde (230 mg) with CH_3NO_2 (150 mg) was effected in 5 ml of a 1:1 mixture of dioxane–H_2O at 3° to which was slowly added 1 equiv of 10% NaOH. After stirring the resultant mixture for less than 5 min, an excess of 2% AcOH was added, followed by stirring at 1 hr from 0 to 5°. An Et_2O extract of the resultant brown oil was prepared, dried (Na_2SO_4), and evaporated. The residual oil was purified by silica gel chromatography (CH_2Cl_2) to yield 50 mg of the desired phenylnitroethanol (yield 20%).

Reduction of the phenylnitroethanols to phenethanolamines (*cf.* ref 31) was effected by addition of the nitroethanol in THF to a stirred suspension of $LiAlH_4$ in THF, followed by reflux and stirring for 24 hr. Excess $LiAlH_4$ was decomposed with H_2O and the mixture filtered through Celite. After evaporation of the filtrate, the residue was dissolved in Et_2O and the HCl salt of the amine precipitated by addition of Et_2O saturated with HCl. Yields for this step were 30–50%.

Removal of the protecting benzyl groups was effected by hydrogenolysis in EtOH with 30 psi of H_2 for 4 hr with 10% Pd/C as catalyst (yields >80%). The final products were homogeneous as judged by thin-layer chromatography on silica gel with *n*-BuOH–AcOH–H_2O (4:1:1) and gave satisfactory nuclear magnetic resonance and mass spectra.

6-Hydroxyepinephrine hydrochloride was prepared from the tetrabenzyl derivative[22] by hydrogenolysis in EtOH with 30 psi of H_2 for 4 hr with 10% Pd/C as catalyst. Efforts to prepare a crystalline salt of 6-hydroxyepinephrine for analysis were unsuccessful. The material, however, showed essentially one spot (R_f ~0.3) on silica gel chromatography with *n*-BuOH–AcOH–H_2O (4:1:1). For comparison, authentic 6-hydroxydopamine has R_f of ~0.5 in this solvent system. Chemical ionization mass spectral analysis of 6-hydroxyepinephrine revealed the expected protonated molecular ion at m/e 200. An nmr spectrum in D_2O was consonant with that expected of 6-hydroxyepinephrine. Solutions of 6-hydroxyepinephrine hydrochloride were significantly less stable than those of 6-hydroxydopamine as evidenced by rate of color formation and the rapid appearance of materials with low R_f values on thin-layer chromatographic analysis. Solutions were, therefore, stabilized with 1% ascorbic acid[19] and used immediately after preparation. The 6-hydroxynorepinephrine hydrochloride was kindly provided by Dr. C. Sachs, Karolinska Institute, Stockholm, Sweden. Satisfactory mass spectra were not, however, obtained with this material.

The 2,4-dihydroxy,[26] 2,5-dihydroxy-,[27] and 2,6-dihydroxyphenethylamines were prepared by demethylation of the corresponding dimethoxyphenethylamines with refluxing concentrated HBr for 20–40 min, followed by evaporation *in vacuo* and recrystallization of the hydrobromide salt from methanol–ether (yields 30–80%). The 2,6 isomer (mp 188–190°. *Anal.* C, H, N) has apparently not

been reported in the literature. With certain of the tri- and tetrahydroxyphenethylamines the corresponding tri- or tetramethoxyphenethylamine[19] was suspended in H_2CCl_2 and cooled to −60° and 6 equiv of BBr_3 in H_2CCl_2 was added. The mixture was allowed to warm to room temperature. After another 2 hr, excess water was added and the products were absorbed on a Dowex 50-X8 column and eluted with 1 *N* HCl. After evaporation, the amine hydrochlorides were recrystallized from methanol–ether (yields 20–60%). This method proved more satisfactory than the demethylation with HBr.[19]

All other compounds were from commercial sources or as previously acknowledged.[9,15,19,20]

[^{3}H]-DL-Norepinephrine (sp act. 5–10 Ci/mmol) was purchased from the New England Nuclear Corp. and from Sigma Chemical Co. It was prepared in normal saline at a final concentration of 50 μCi/ml and stored at −20° until use.

Methods. All animal experiments were with National Institutes of Health, general-purpose, male albino mice of 17–20 g. Inhibition of uptake of radioactive norepinephrine was assayed by measurement of tritium content of cardiac tissue 20 min after intraveneous administration of 2.5 μCi of [^{3}H]-DL-norepinephrine in 0.1 ml of saline either alone or in the presence of the test substance. Details of the methodology have been reported.[19,20] Release of radioactive norepinephrine was assessed by measurement of tritium content of cardiac tissue 3 hr after intravenous administration of 5 μCi of [^{3}H]-DL-norepinephrine in 0.1 ml of saline. Compounds tested for releasing activity were administered 1 hr after the norepinephrine. Details of the methodology have been reported.[9,20] Long-term effects of compounds on uptake of radioactive active norepinephrine were measured after subcutaneous administration of the test compound. Five days later, the tritium content of cardiac tissue was measured 20 min after intravenous administration of 5.0 μCi of [^{3}H]-DL-norepinephrine in 0.1 ml of saline.[19,20]

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Antiarrhythmic Agents. 2-, 3-, and 4-Substituted Benzylamines

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 Received August 30, 1974

The synthesis of a series of 2-, 3-, and 4-substituted benzylamine derivatives is described. These compounds were studied for their effect on experimental cardiac arrhythmias. Many of the derivatives, but in particular 2-(*p*-methoxyphenylethynyl)benzylamine (**3d**), α,α-dimethyl-4-(phenylethynyl)benzylamine (**7a**), and α,α-dimethyl-4-phenethylbenzylamine (**12g**), showed good antiarrhythmic activity.

α,α-Dimethyl-4-(α,α,β,β-tetrafluorophenethyl)benzylamine (1), a new orally effective antiarrhythmic compound, has been selected for clinical evaluation in man. The synthesis,[1] pharmacological properties,[2] and metabolic disposition[3] of 1 have been described. Concurrent with this work, ancillary explorations of the structure–activity relationships of a series of similar compounds were undertaken. Of particular interest was the extent to which the tetrafluoroethyl bridge of 1 and related structures contributed to its potent antiarrhythmic activity. In order to delineate this structure–activity relationship, a series of 2-, 3-, and 4-substituted benzylamine derivatives, having substituents other than the tetrafluroethyl moiety, were prepared and examined for antiarrhythmic activity.

Chemistry. Several possibilities exist for modifying the tetrafluoroethyl bridge of 1. Changes that have been made