α,α′- and β,β′-Deuterium-Labeled Dopamine. Synthesis and Pharmacologic Actions†

J. M. Perel,* D. K. Dawson, P. G. Dayton, and L. I. Goldberg

Clinical Pharmacology Program, Departments of Medicine, Chemistry, and Pharmacology, Emory University, Atlanta, Georgia.
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The specificity of the pharmacological action of dopamine was investigated by substituting the alkyl side-chain hydrogens with deuterium. The α,α′-dideuterio compound was synthesized *via* reduction of 3,4-dimethoxyphenylacetonitrile with lithium aluminum deuteride. The β,β′-dideuteriodopamine was prepared from homoveratric acid by incorporating deuterium into the side chain with exchange procedures. Purity and extent of deuterium labeling (at least 90 atom %) were determined by several analytical criteria. No difference in pharmacologic activity was found between the deuterated dopamine and the normal compound; this suggests that this catecholamine acts directly on a dopaminergic receptor without the mediation of intermediates.

Previous studies in these laboratories have demonstrated that dopamine causes renal vasodilatation by action on a specific dopamine receptor.[1] Structure-activity studies have demonstrated that of a large series of analogs, only the *N*-methyl compound was active.[2] To extend these investigations, experiments were carried out with dopamine specifically labeled with deuterium in the α and β positions of the side chain.

There is a substantial body of information indicating that cleavage of the carbon-hydrogen bond is rate limiting in the oxidation of alkyl side chains *in vivo* and *in vitro*. For example, the biological half-life and pharmacological activity of butethal, deuterated at the penultimate carbon atom, have been found to be approximately double those obtained with the unlabeled barbiturate.[3] The *in vitro* hydroxylation showed a k_H/k_D of 1.6. Similar results have been reported with 3′-dideuteriopentobarbital,[4] *O*-nitroanisole-*methyl-d*$_3$,[5] morphine-*methyl-d*$_3$,[6] and side-chain-deuterated amphetamines.[7,8] Furthermore, the oxidation of tyrosine, phenylalanine, and tyramine by monoamine oxidase give isotope effects ranging from 1.4 to 2.3, indicating that the removal of an α-hydrogen is rate limiting.[9]

It has been shown that the side chain of dopamine is oxidized by dopamine-β-hydroxylase[10] and monoamine oxidase.[11] Therefore, on the basis of the above-cited studies, it is reasonable to expect that the metabolism of dopamine would exhibit deuterium isotope effects. The present investigation was undertaken to determine whether dopamine acts directly on a specific renal receptor or *via* an oxidized metabolite produced *in situ;* in the latter case an isotope effect would be observed *in vivo*.

Experimental Section‡,§

Mass spectrometric measurements were made by Morgan-Schaffer Corp., Montreal, Canada. Nmr were detd on A-60A spectrometer (Varian Associates) operating at 38° as a courtesy of Dr. J. H. Goldstein, Department of Chemistry, Emory University, Atlanta, Ga. D_2 was detd by combustion to D_2O and subsequent mass spectrum detd; performed by Mr. J. C. Cook, Suffern, N. Y. Capillary mp was detd in a Thomas Hoover Uni-Melt.

α,α′-Dideuterio-3,4-dimethoxyphenylethylamine (1). The compd was prepd from 3,4-dimethoxyphenylacetonitrile according to modifications of general procedures.[12-14] To 0.63 g (0.015 mole) of LAD# in 30 ml of anhyd Et_2O, a soln of 1.33 g (0.01 mole) of $AlCl_3$ in 15 ml of anhyd Et_2O was added rapidly with stirring. After 5 min a soln of 1.77 g (0.01 mole) of the nitrile in 20 ml of anhyd Et_2O was added dropwise while stirring; 1 hr later 15 ml of H_2O was added at 0°, followed by the addn of 15 ml of 6 *N* H_2SO_4. After sepg the Et_2O phase and discarding it, the aqueous phase was extd with four 10-ml portions of Et_2O to remove undesired products. After adjusting this phase to pH 11 with KOH (the vol was brought to 100 ml with H_2O) and extg with four 10-ml portions of Et_2O, drying over Na_2SO_4 and evapn, a yellow oil **(1)** was obtained; yield, 450 mg (24%).

α,α′-Dideuteriodopamine Hydrobromide (2). Hydrolysis of **1** was carried out by the procedure of Stoermer.[15] To 450 mg (0.0019 mole) of **1** a mixt of 1 ml of 47% HBr and 5 ml of glacial AcOH was added and refluxed 3 hr and then evapd to dryness *in vacuo* below 60°. The light brown residue **2** was washed with Me_2CO until white. It was then crystd from abs EtOH–Me_2CO–*n*-C_7H_{16} with prior charcoal treatment; yield, 152 mg (26%); mp 212–214° (lit.[16,17] 210–214°). *Anal.* ($C_8H_{14}BrNO_2$) N.

Purity was established by the following criteria: tlc on 0.5-mm thick MN-Cellulose 300G plates, particle size <10 μ, in a solvent system: MeOH–*n*-BuOH–C_6H_6–H_2O (4:3:2:1) with 0.01% Na_2EDTA added;[18] visualization with $K_3Fe(CN)_6$–ethylenediamine in EtOH–H_2O followed by heating at about 60° for 3.5 min; observation under uv light, R_f of **2** was 0.48 (green fluorescent spot), in agreement with lit. In the same system **1** gave no fluorescent spot; 3-methoxydopamine and 4-methoxydopamine had R_f values of 0.65 (blue spot) and 0.65 (green spot), respectively. Nmr analysis in Me_2CO-d_6–D_2O indicated that the α-methylene protons at δ 1.22 (multiplet) with solvent (quintet) set at 0, had vanished. Detn of D_2 (combustion) indicated 89.7% of theoretical incorporation.

α,α′-Dideuteriohomoveratric Acid (3). The compd was prepd from homoveratric acid by modification of the procedure of Ives.[19] To 19.6 g (0.1 mole) of the acid, 20 ml (0.1 mole) of a 40% soln of NaOD** (98% isotopic purity) in D_2O and 200 ml of D_2O** (99.8% isotopic purity) were added. The mixt was heated at 60° with *rapid stirring* for 3–4 hr and then the solvent was removed *in vacuo*. The addn of 200 ml of D_2O, heating at 60° for 3–4 hr while stirring rapidly, and removal of solvent *in vacuo* was repeated three more times. The mixt was then dissolved in about 250 ml of D_2O, acidified with 6 *N* HCl, and extd four times with 100 ml of $CHCl_3$; yield after drying over Na_2SO_4 and evapn of solvent, 18.4 g (94%); mp 95–98°. Nmr data was consistent with incorporation in the α,α′ position. Mass spectral analysis indicated 65% incorporation. Further exchange was carried out on a sample made up of 7 g from the above yield and 12.6 g of unlabeled homoveratric acid; the same conditions were used except that reaction time was 8 hr. After four passes with D_2O, mass spectral analysis indicated only 70% incorporation. Therefore, the procedure was again modified as follows: 0.6 mole of NaOD was added to the resulting product and the temp was raised to 100°. Three additional passes increased incorporation to 85%. Three more additional passes under these conditions were carried out which in view of the subsequent analysis of the eventually synthesized **5**, must have had an incorporation of at least 95%; recrystallized ($CHCl_3$); yield, 19 g (97%); mp 95-98°.

α,α′-Dideuteriohomoveratramide (4). The compd was prepd according to a published general procedure.[20] To 10 g (0.05 mole)

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‡Where analyses were indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of theoretical values.

§Compounds **2** and **6** can also be referred to as 3′,4′-dihydroxyphenylethyl-*1*-^2H-amine hydrobromide and 3′,4′-dihydroxyphenylethyl-*2*-^2H-amine hydrobromide, respectively.

#Ventron Corp., Beverly, Mass. (Alfa).

**Diaprep, Inc., Atlanta, Ga.

of 3 50 ml (0.42 mole) of $SOCl_2$ was added; the mixt was refluxed 1 hr and then poured onto 150 ml of concd NH_4OH at 0°; recrystallized (H_2O); yield, 3.36 g (34%); mp 141–142°; (lit.[21] 145–147°).

β,β′-Dideuterio-3,4-dimethoxyphenylethylamine (5). The reduction of **4** was carried out by the general method of Brown and Heim.[22] In 20 ml of THF (distd over LAH and dried over CaH_2), 0.05 g (0.0025 mole) of **4** was dissolved. This was added dropwise (15 min) at 0° to 0.32 g (0.023 mole) of 1 *M* B_2H_6 in THF under N_2, while stirring. The mixt was then refluxed 8 hr and, upon cooling, 10 ml of 6 *N* HCl was added. After removal of the solvent by distn, the cooled aqueous phase was made alk (pH 10) with NaOH pellets. The soln was extd two times with 50-ml portions of Et_2O, and, upon drying over Na_2SO_4, the solvent was removed by distn. To remove H_3BO_3, 10 ml of MeOH and enough AcOH were added for acidification, and the solvent was distd off. MeOH was added three more times and removed by distn; yield, 0.40 g (crude oil).

β,β′-Dideuteriodopamine Hydrobromide (6). The 0.40 g of **5** was treated by a modification of the procedure used for the hydrolysis of **1** to **2**. After 6 hr of refluxing, aliquots were analyzed by tlc (the same conditions as for **2**) until complete hydrolysis was indicated (total time 18–56 hr); yield, 0.21 g; mp 210–214° (lit.[16,17] 210–214°). Mass spectrometric analysis indicated 95% of theor incorporation. *Anal.* ($C_8H_{14}BrNO_2$) N. Nmr data using Me_2CO-d_6 indicated that the β-methylene protons at δ 3.12 (multiplet) with solvent (quintet) set at 0 has essentially vanished.

α,α′-Dideuterioveratrylamine (7). The compd was prepd from 3,4-dimethoxyphenylnitrile by redn with LAD# according to the same procedure used for the prepn of **1**. After ten redn reactions, each on a 0.01-mole scale, and work-up of products, **7** was obtained as a yellow oil; yield, 1.70 g (10% per run).

α,α′-Dideuterioveratryl Alcohol (8). The compd was prepd from **7** by a modification of the method of Whitmore and Langlois.[23] An individual reaction on each batch of **7** was carried out. In 10 ml of H_2O, 1.7 g of **7** was suspended (three-neck round-bottom flask fitted with a dropping funnel, trubore stirrer, and *outlet* for *gas evolution*) and 2 ml of 6 *N* HCl was added. Then the mixt was placed in an ice bath and a soln of 2.07 g (0.03 mole) of $NaNO_2$ in 15 ml of H_2O was added dropwise with continuous stirring and a slow rate of heating to 70–80° until evolution of N_2 *stopped* or *slowed down*. During the heating, a small amt 6 *N* HCl (about 1–2 ml) was added to the reaction to prevent the soln from becoming alk due to hydrolysis of $NaNO_2$. To the material, at 25°, NaCl was added to saturate the soln and 1–2 ml of 0.1 *N* HCl to convert any unreacted amine to its HCl salt. This aqueous soln was extd 3 times with 25 ml of Et_2O each time. The pooled Et_2O exts were dried over Na_2SO_4, filtered, and evapd *in vacuo;* yield, 1.74 g (70%, crude).

Attempted Alternate Synthesis of β,β′-Dideuteriodopamine Hydrobromide. α,α′-Dideuterioveratryl chloride was prepd by the method of Decker and Pschorr.[24] To 2.96 g (0.017 mole) of **8** in a small round-bottom flask fitted with a $CaCl_2$ drying tube, $SOCl_2$ was added directly at room temp and allowed to stand until excess reagent had escaped. The resulting dark brown tar was exhaustively extd with petr ether and the pooled exts were dried over Na_2SO_4, filtered, and evapd *in vacuo*. The nitrile was obtained by reacting the crude chloride with KCN and DMF (containing 5% H_2O v/v) at 70° for 2 hr. After sepn of the ppt, distn of the solvent *in vacuo*, and extn of the residue with petr ether, the exts were dried over Na_2SO_4. The amine was prepd by the previously described procedures from the nitrile. Because of the possibility of autooxidation[25] the synthetic material was compared to 6-hydroxydopamine,†† the mono- and dimethoxydopamine analogs by tlc, under the same conditions as for **2**. In this system the R_f of 6-hydroxydopamine was 0.37 while the dopamine-like compd had R_f 0.48 (green fluorescent spot) which is identical with that of authentic dopamine; mp 210–212 . *Anal.* ($C_8H_{14}BrNO_2$) calcd; C, 40.69; H, 5.98; N, 5.93. Found: C, 37.37; H, 4.55; N, 6.50.

Pharmacology. The deuterated dopamine derivs were assayed for dopamine-like activity according to a technique described previously.[2] Mongrel dogs were anesthetized by iv injection of sodium pentobarbital, 30 mg/kg. Renal artery blood flow was measured continuously by means of a Medicon electromagnetic flowmeter. Arterial blood pressure was measured from a carotid artery by a Stathma P23-D transducer. The data were recorded on a Grass polygraph. Phenoxybenzamine was injected in a dose of 10 mg/kg into the artery under study in order to block potential α-adrenergic vasoconstricting action of dopamine and its derivs. The deuterated derivs then were compared with the effects of dopamine by injecting the amines in twofold dosage increments (geometric progression) into the renal artery. Expts were also carried out after ia infusion of propranolol, 2 mg/kg, to rule out β-adrenergic vasodilation. In two addnl expts the effects of dopamine and deuterated dopamine were detd on cardiac contractility (Walton–Brodie strain gauge arch) and blood pressure as previously described.[26]

††Regis Chemical Co., Chicago, Ill.

Results

α,α′-Dideuteriodopamine (2). The α,α′-dideuteriodopamine was compared with dopamine in 4 experiments in which the amines were injected into the renal artery. In each experiment the response to **2** was identical with that produced by dopamine. In one of these experiments, propranolol (in a dose of 2 mg/kg, which completely blocked the renal vasodilation produced by isoproterenol) affected neither **2** nor dopamine. In one experiment iv injections of α,α′-dideuteriodopamine and dopamine in doses of 2, 4, 8, 16, and 32 μg/kg produced similar effects on cardiac contractile force and blood pressure. With 2 μg/kg, the blood pressure was slightly decreased; with 4 μg/kg there was a slight pressure increase followed by a more prolonged depressor effect. There was no increase in contractile force with either of these doses. In doses of 16 and 32 μg/kg both **2** and dopamine produced comparable increments in contractile force and blood pressure.

β,β′-Dideuteriodopamine (6). The β,β′-dideuteriodopamine was found to produce similar effects as dopamine on the kidney in three experiments. In one of these, the renal vasodilation was not changed by a dose of 2 mg/kg of propranolol. In one experiment **6** and dopamine produced similar increments in cardiac contractile force in doses ranging from 4 to 20 μg/kg.‡‡

Discussion

Extreme specificity of dopamine in renal vasodilatation had been demonstrated; to extend these studies of structure–activity relationships of dopamine and to establish the maximal structural requirements for its activity, α,α′- and β,β′-dideuteriodopamine were synthesized§§ and tested for activity in the dog. Kier and coworkers have presented evidence that the conformation of dopamine is different from norepinephrine,[28,29] lending support to the concept of a specific dopamine receptor.

The C–D bond is both shorter and tighter than the C–H bond.[30,31] Therefore, by substituting the β hydrogens of the side chain of dopamine with deuterium, it was thought that the rate of the *in vivo* conversion to norepinephrine would be markedly reduced by the deuterium isotope effect, if the hydroxylation were the rate-determining step involved in the pharmacologic activity. In the unlikely event that dopamine activity was mediated through monoamine oxidase, a comparable effect would have been observed upon substitution in the α position. Furthermore, due to the shorter C–D and different hydrogen bonding properties, these changes might have resulted in a change in activity,

‡‡In a preliminary report[27] we indicated that α,α′-dideuteriodopamine was active and that β,β′-dideuteriodopamine as synthesized by the second procedure was inactive (see Methods). It appears that the latter compound, although having similar chemical properties as dopamine, is probably not dopamine. We thank Drs. Bryan B. Molloy and Ronald R. Tuttle of Eli Lilly, Indianopolis, Ind., for pointing out that β,β′-dideuteriodopamine synthesized by their procedure which differed from ours was pharmacologically equipotent to dopamine.

§§The procedures described herein, could also be utilized for the preparation of specifically tritiated dopamines. The labeling occurs exclusively in the alkyl side chain since no deuterium incorporation was detected in the phenyl ring after exchange with D_2O(NaOD) at 100°.

which would reflect geometrical requirements or spatial requirements of the receptor. The absence of these changes in our study suggests the concept that dopamine acts directly on the dopamine receptor.

The substitution of two hydrogens by deuterium is one of the smallest possible changes that can be made on an organic molecule. Apparently, the substitution of deuterium for hydrogen does not affect the conformational requirements needed for dopamine action *in vivo*. The deuterium-labeled dopamine compounds described in this study should be valuable for studies of the enzymatic mechanism of the metabolism of dopamine.

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Synthesis and Biological Activity of 17-Esters of 6-Dehydro-16-methylene-17α-hydroxyprogesterones

E. L. Shapiro,* L. Weber, H. Harris,† C. Miskowicz,† R. Neri,‡ and H. L. Herzog

Natural Products Research Department, Process Research and Development Department, and Physiology and Biochemistry Department, Schering Corporation, Bloomfield, New Jersey 07003.

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The progestational and antiandrogenic activities of 6-dehydro-6-halo(fluoro, bromo, chloro)-16-methylene-17α-acetoxyprogesterones (**1**), as well as related activities of 17-esters of the 6-chloro compounds (**1**), are reported. A convenient synthesis for this class of compounds is also described.

We have long been interested in the chemistry and pharmacology of 16-alkyl and alkylidene derivatives of 17α-hydroxyprogesterone.[1,2] Our early work[1] showed that 16-methylene substitution enhances progestational activity far more than either 16α-methyl or 16β-methyl in this series. Investigations of 6-chloro-16-methylene-17α-hydroxy-4,6-pregnadiene 17-acetate (**1**, X = Cl; R = CH_3CO) reported by us§ and by others[6] indicated that introduction of the 6-chloro-6-dehydro function into the preferred parent structure appeared to lead to a compound of exceptional progestational and antiandrogenic activity. In this article we now describe a systematic study of the influence of the alteration of halogen at 6 and the acyl group at 17 on the progestational and antiandrogenic activities of the resulting structures (generic formula **1**) (Table I). We also present a convenient general method for the synthesis of all of the members of the generic family **1** from the precursors in common, **3** and **4a**.[7,#]

From the readily available 16-methyl-3β-hydroxy-5,16-pregnadien-20-one (**2**),[9] a five-step reaction sequence *via* **3**, **4a**, **5**, and **6** may be utilized to afford the members of the generic structure **1** (Scheme I). A less satisfactory alternative route **7** → **8**[10] → **4a** was abandoned because chloranil dehydrogenation of **7** to **8** was accompanied by objectionable amounts of simultaneous 16α,17α-oxide opening.[11] The presence of exocyclic methylene at 16 prior to epoxidation of the 6,7 double bond was not permissible since the 16-unsaturation represented an undesirable point of competitive attack by the epoxidizing agent.

†Process Research and Development Department.

‡Physiology and Biochemistry Department.

§ The antiandrogenic activity of **1** (X = Cl, R = CH_3CO) has been reported by Rocky and Neri,[3] Casmer, *et al.*,[4] and Neri.[5]

#Syhora and Mazac[8a] report the preparation of **1** (X = Cl, R = CH_3CO) by other routes. Syhora, *et al.*,[8b] report also **1**, X = Br and F, R = CH_3CO, without constants and **1**, X = Cl, R = caproyl (see Experimental Section).