recrystallized from 95% methanol (350 mL) to give 82 g (76%) of **36**: mp 148–150 °C; ^{13}C NMR of the aromatic region ($CDCl_3$) δ 170.97 (CONH), 159.29 (C-2, shift changes −8.6 Hz upon addition of D_2O), 158.78 (C-6, no shift), 137.84 (C-4), 103.79 (C-5), 102.71 (C-1 and C-3). Anal. ($C_8H_7BrO_4$) C, H, Br, O.

***O*-Acetyl-3-bromo-6-methoxysalicyclic Acid (39).** 3-Bromo-2-hydroxy-6-methoxybenzoic acid (**36**; 24.7 g, 0.10 mol) was dissolved in acetic anhydride (50 mL). Concentrated H_2SO_4 (0.2 mL) was added, and the mixture was stirred at 60 °C for 20 h. After cooling, the reaction mixture was poured into an ice–water mixture. The solvent was removed in vacuo. The residue was crystallized from diisopropyl ether: yield 24.5 g (85%); mp 146–147 °C. Anal. ($C_{10}H_9BrO_5$) C, H, Br.

Pharmacology. [^{3}H]Spiperone Binding. The assays were performed essentially as described earlier.[4] Rats were killed by decapitation, and the striatum was rapidly dissected out on ice. After homogenisation in Tris-HCl buffer (0.05 M, pH 7.6) the homogenate was centrifuged twice for 10 min at 48000*g*, resuspended, and recentrifuged. The final pellet was resuspended in Tris-HCl buffer (0.05 M, pH 7.6) containing 0.1% ascorbic acid and various salts to a final concentration of 5 mg/mL. The incubations were performed at 37 °C for 10 min in plastic trays and were terminated by filtration and subsequent washing on glass fiber paper (Whatman GF/B). (+)-Butaclamol (1 μM) was used for the determination of unspecific binding. The radioactivity of the filters was determined by scintillation spectroscopy. The IC_{50} values were calculated by using log-logit regression analysis.

Apomorphine-Induced Behavior. Male Sprague-Dawley rats, (275–325 g), were used. The behavior was scored 5, 20, 40, and 60 min after injection of apomorphine (1 mg/kg), given subcutaneously into the neck. The scoring was performed as described previously.[2] The test compounds were dissolved in saline or acetic acid and distilled water and injected ip 60 min prior to apomorphine. The ED_{50}'s for stereotypies are the doses that reduce the strength of apomorphine-induced stereotypies by 50% over the total observation period of 60 min. The ED_{50}'s for hyperactivity are the doses that reduce the hyperactivity response by 50% over the observation period of 60 min. The ED_{50} values, based on at least six dose levels with six to eight animals per dose level, were calculated by Theil's method[18] and correlated for ties following Sen's procedure[17] based on Kendell's τ. A slightly modified version of Sen's procedure was used to determine the 90% confidence interval.

Registry No. **1**, 96947-76-1; **2**, 96897-87-9; **3**, 96897-88-0; **4**, 96897-89-1; **4**·HCl, 96898-03-2; **5**, 96897-90-4; **6**, 84226-00-6; **7**, 96947-77-2; **8**, 84226-04-0; **8**·$^1/_2$HCl, 96947-81-8; **9**, 84226-05-1; **9**·$^1/_2$HCl, 96947-82-9; **10**, 96947-78-3; **10**·$^1/_2$HCl, 96947-83-0; **11**, 84226-14-2; **12**, 84226-06-2; **13**, 96897-91-5; **13**·$^1/_2$HCl, 96898-04-3; **14**, 96897-92-6; **14**·HCl, 96898-05-4; **15**, 84226-07-3; **15**·HCl, 96393-01-0; **16**, 84226-08-4; **16**·HCl, 96947-84-1; **17**, 96897-93-7; **17**·$C_4H_6O_6$, 96898-06-5; (±)-**18**, 96897-94-8; (±)-**18**·HCl, 84225-89-8; **19**, 84225-93-4; **20**, 82977-52-4; **20**·HCl, 82977-51-3; **21**, 96947-79-4; **21**·HCl, 84225-96-7; **22**, 96947-80-7; **22**·HCl, 84225-99-0; **23**, 84226-16-4; **24**, 96393-00-9; **25**, 96897-95-9; (±)-**26**, 84225-83-2; **27**, 38064-90-3; **28**, 19672-03-8; **29**, 36680-47-4; **30**, 54459-33-5; **31**, 96897-96-0; **32**, 96897-97-1; **33**, 96897-98-2; **34**, 96897-99-3; **35**, 82935-47-5; **35**·HCl, 82935-30-6; **36**, 84225-86-5; **37**, 63604-94-4; **38**, 96898-00-9; **39**, 84225-87-6; (*E*)-**40**, 96898-01-0; (*Z*)-**40**, 96898-02-1; I (X = H), 1466-76-8; I (X = F), 52189-67-0; I (X = I), 90347-70-9; I (X = NO_2), 55776-17-5; I (X = Br), 73219-89-3; $Ph_3P^+C_3H_7I^-$, 14350-50-6; 2,4-dimethoxybenzaldehyde, 613-45-6; 2′,4′-dimethoxyacetophenone, 829-20-9; 2′,4′-dimethoxypropiophenone, 831-00-5; (*S*)-*N*-ethyl-2-(aminomethyl)pyrrolidine, 22795-99-9; (*S*)-*N*-propyl-2-(aminomethyl)pyrrolidine, 84225-92-3; remoxipride, 80125-14-0.

(17) Sen, P. K. *J. Am. Stat. Assoc.* **1968**, *63*, 1379.
(18) Theil, H. *Ned. Akad. Wetensch. Proc., Ser A* **1950**, *53*, 386.

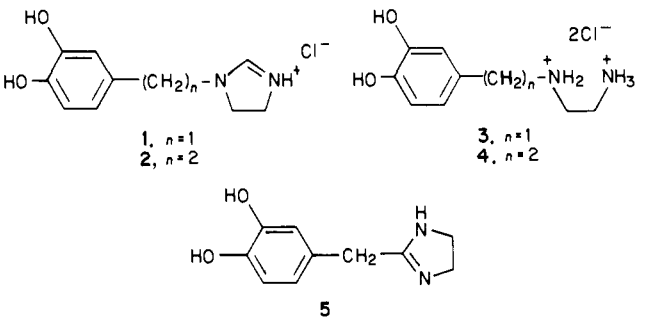
N-Substituted Imidazolines and Ethylenediamines and Their Action on α- and β-Adrenergic Receptors

Akihiko Hamada,† Emily L. Yaden,‡ J. S. Horng,‡ Robert R. Ruffolo, Jr.,‡§ Popat N. Patil,† and Duane D. Miller*†

Division of Medicinal Chemistry and Pharmacognosy and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, and the Department of Cardiovascular Pharmacology, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. *Received November 7, 1984*

A series of N-substituted imidazolines and ethylenediamines were synthesized and examined for their activity in α- and β-adrenergic systems. The length of the intermediate side chain between the catechol and imidazoline ring or the amine of the ethylenediamine segment was shown to affect the adrenergic activity. *N*-[2-(3,4-Dihydroxyphenyl)ethyl]imidazoline hydrochloride (**2**) and *N*-[2-(3,4-dihydroxyphenyl)ethyl]ethylenediamine dihydrochloride (**4**), both with two methylene groups between the catechol and amine segment, were found to be somewhat selective for α_2-adrenergic receptors while 1-(3,4-dihydroxybenzyl)imidazoline hydrochloride (**1**) and *N*-2-(3,4-dihydroxybenzyl)ethylenediamine dihydrochloride (**3**), both with one methylene group between the catechol and amine segment, were more selective for α_1-adrenergic receptors in a pithed rat model. Of the four compounds examined, only compound **2** showed significant direct activity on β_1- and β_2-adrenergic receptors.

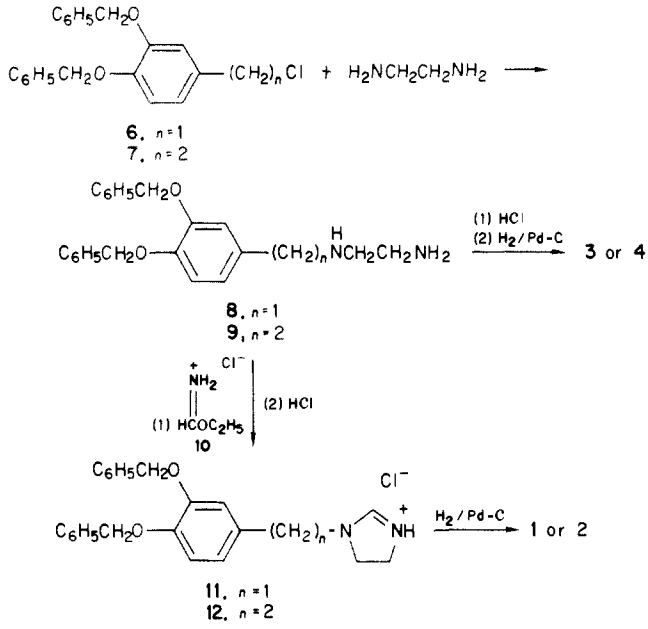
We report the α-adrenergic activity of imidazolines **1** and **2** and their respective open-chain analogues **3** and **4**. The imidazoline **1** represents one of the positional isomers of the potent α-adrenergic agonist 2-(3,4-dihydroxybenzyl)-imidazoline (**5**).[1] Little work has been reported on N-substituted imidazolines,[1-5] and no investigation has appeared in adrenergic systems with the catechol segments substituted on the nitrogen atom of an imidazoline as shown with **1** and **2**. There have been extensive reports on the structure–activity relationships of 2-substituted imidazolines. Some of the results indicate the following:



† The Ohio State University.
‡ Eli Lilly and Co.
§ Present address: Director of Cardiovascular Pharmacology, Smith Kline and French Laboratories, Philadelphia, PA 19101.

0022-2623/85/1828-1269$01.50/0

Scheme I



(1) Substitution on the imidazoline ring in addition to the 2-position causes a decrease in α-agonist activity.[1-3] (2) Increasing the bridge length between the imidazoline ring and the phenyl ring at the 2-position decreases α-agonist activity.[6] (3) Opening the imidazoline ring is associated with a loss of binding to α-adrenergic receptors.[4] The general overview of the structural requirements for the potent imidazoline derivatives substituted with a benzyl group at the 2-position have been recently reviewed.[5] Consequently, we were interested in studying the structure–activity relationships among imidazolines that have the catechol ring moiety substituted on a nitrogen of the imidazoline ring. We prepared compounds **1** and **2**, which provided an opportunity to investigate the effect of a catechol group as an N-substituent of imidazolines on α- and β-adrenoreceptors. Since no prior study had been carried out with imidazolines of this nature we also felt that the open-chain analogues **3** and **4** should also be investigated.

Chemistry. The preparation of **1–4** was carried out as shown in Scheme I. The N-substituted ethylenedimaines **8** and **9**, the key precursors of catechol diamine salts **3** and **4** and catechol imidazoline salts **1** and **2**, were prepared from halides **6** and **7** and ethylenediamine. The contamination of **8** and **9** with N,N′-disubstituted diamines was successfully prevented by using a large excess of ethylenediamine. The halide **7** (n = 2) was synthesized from [bis(benzyloxy)phenyl]acetic acid[7] by treatment with diborane followed by thionyl chloride. The first attempt at cyclizing diamines **8** and **9** with ethyl formate[8] gave a mixture of products that was difficult to purify. The employment of ethylformamidinium chloride **10**[9] provided for a much easier purification and isolation of the desired imidazolines **11** and **12** in good yield. The N-substituted imidazoline salts **11** and **12** were then catalytically hydrogenated to give the desired catechol imidazoline salts **1** and **2** as illustrated in Scheme I.

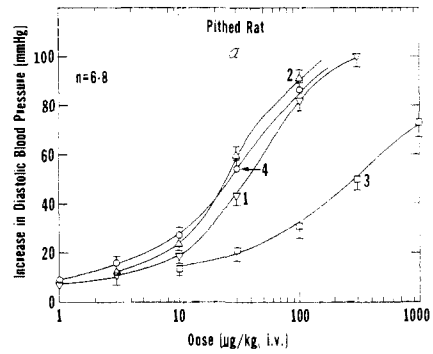


Figure 1. Pressor responses of **1–4** in pithed rats. Responses were evaluated in control rats and in rats pretreated with propranolol (3 mg/kg, iv, 15 min) plus reserpine (5 mg/kg, ip, 24 h).

Table I. Characterization of α-Adrenoceptor-Mediated Pressor Response in Pithed Rats

compd	ED_{50},[a] μg/kg, iv	dose ratio[b] prazosin (0.1 mg/kg, iv)	dose ratio[b] yohimbine (1 mg/kg, iv)	selectivity α_1/α_2
cirazoline	0.6 ± 0.1	12.3 ± 0.9	1.0 ± 0.2	12.3
B-HT 933	26 ± 3.2	1.1 ± 0.1	9.4 ± 0.8	0.1
1	35 ± 3.3	5.3 ± 0.4	2.4 ± 0.2	2.2
2	24 ± 4.2	1.3 ± 0.1	2.1 ± 0.3	0.7
3	305 ± 1.7	5.5 ± 0.2	4.4 ± 0.3	1.3
4	28 ± 2.4	1.7 ± 0.2	5.7 ± 0.4	0.3
5[c]	0.35	0.8 ± 0.2	0.4 ± 0.1	2.0

[a] Dose required to increase diastolic blood pressure by 50 mmHg. [b] ED_{50} of agonist following pretreatment with antagonist divided by control ED_{50}. [c] Data taken from ref 14.

Biological Results and Discussions. The pressor responses of compounds **1–4** at a dose of a 1 μg/kg, iv, in the pithed rat are shown in Figure 1. It is apparent from the figure that compounds **1**, **2**, and **4** show similar effects while **3** is significantly weaker.

It is known that the pressor responses elicited by drugs in pithed rats may be due to a mixed population of postsynaptic vascular α_1- and/or α_2-adrenergic receptors.[10-18] The pressor activities of **1–4** were examined after pretreatment with the α_1-adrenoceptor-selective antagonist prazosin (0.1 mg/kg, iv) or the α_2-adrenoceptor-selective

(1) Miller, D. D.; Hamada, A.; Craig, E. C.; Christoch, G. G.; Galluci, J. D.; Rice, P. J.; Banning, J. W.; Patil, P. N. *J. Med. Chem.* **1983**, *26*, 957.
(2) Mujic, M.; Van Rossum, J. M. *Arch. Int. Pharmacodyn. Ther.* **1965**, *155*, 432.
(3) De Jong, A. P.; Soudijn, W. *Eur. J. Pharmacol.* **1981**, *69*, 175.
(4) Jarrott, B.; Summers, R. J.; Culvenor, A. J.; Lewis, W. J. *Circ. Res. Suppl.* **1980**, *46*, 1.
(5) Ruffolo, R. R., Jr. "Adrenoceptors and Catecholamine Action"; Kunos, G., Ed.; Wiley: New York, 1983; p 1.
(6) Ruffolo, R. R., Jr.; Yaden, E. L.; Waddel, J. E.; Dillard, R. D. *J. Pharmacol. Exp. Therm.* **1980**, *214*, 535.
(7) Carlson, H.; Lindquist, M. *Helv. Chim. Acta* **1962**, *45*, 270.
(8) Pandit, U. K.; Bieraugal, H. *J. Chem. Soc., Chem. Commun.* **1979**, 117.
(9) Hechelhammer, W. *Chem. Abstr.* **1959**, *53*, P6088e.
(10) Docherty, J. R.; McGrath, J. C. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *312*, 107.
(11) Timmermans, P. B. M. W. M.; Zwieten, P. A. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *312*, 17.
(12) Kobinger, W.; Pichler, L. *Eur. J. Pharmacol.* **1980**, *65*, 393.
(13) Yamaguchi, I.; Kopin, I. J. *J. Pharmacol. Exp. Ther.* **1980**, *214*, 275.
(14) Ruffolo, R. R.; Patil, P. N.; Miller, D. D. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1983**, *323*, 221.
(15) Timmermans, P. B. M. W. M.; Kwa, H. Y.; van Zwieten, P. A. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1979**, *310*, 189–193.
(16) Drew, G. M. *Eur. J. Pharmacol.* **1980**, *65*, 85.
(17) Langer, S. Z.; Massingham, R.; Shepperson, N. *Clin. Sci.* **1980**, *59*, 225s.
(18) Ruffolo, R. R., Jr.; Nelson, W. L.; Yaden, E. L. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1983**, *322*, 93–97.

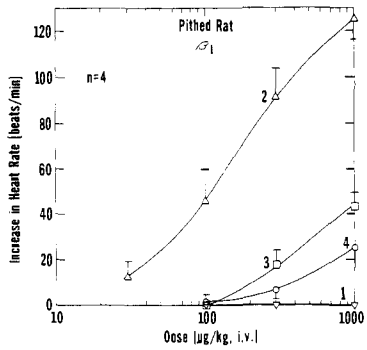


Figure 2. Positive chronotropic effects of 1–4 in phenoxybenzamine-pretreated pithed rats.

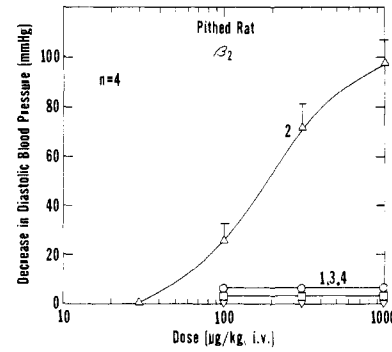


Figure 3. Vasodepressor effects of 1–4 in phenoxybenzamine-pretreated pithed rats with vascular tone induced and maintained by constant angiotensin II infusion.

antagonist yohimbine (1 mg/kg, iv), in order to determine the contribution of each α-adrenoceptor subtype to the pressor activity. The dosages of prazosin and yohimbine used provide selective α_1- and α_2-adrenergic blockade and little or no crossover between the α-adrenoceptor subtypes. The dose ratios produced by prazosin and yohimbine in antagonizing the pressor responses of 1–4 are summarized in Table I. Larger dose ratios are associated with greater antagonism. In Table I are the reference antagonists cirazoline[19] and B-HT 933,[20] which are selective agonists respectively of α_1- and α_2-adrenoceptors. The pressor response of B-HT 933 is selectively antagonized by yohimbine and relatively unaffected by prazosin, while the converse is true for cirazoline, which is consistent with earlier work.[14,19] Although the four analogues are less active than **5**[14] in producing a pressor response in the pithed rat, it is important to note that both **1** and **3** produce their effects to a larger extent through α_1-receptors while **2** and **4** are working primarily through α_2-adrenergic receptors. The α_1/α_2 selectively is summarized in Table I.

It is known that myocardial β-adrenergic receptors are not a homogeneous population but are rather a mixture of β_1- and β_2-adrenergic receptor populations in a number of mammalian species.[21,22] The pithed rat has been a very useful model to study β_1-adrenergic mediated chronotropic effects of adrenergic receptors.[14] The β_1-adrenergic mediated increase in heart rate was determined for 1–4 in the pithed rat following pretreatment with reserpine (5 mg/kg, ip, 24 h) and the irreversible α-adrenergic antagonist phenoxybenzamine (3 mg/kg, iv). The actions of the four compounds are shown in Figure 2. The β_1-adrenergic mediated positive chronotropic effects of these compounds displayed the following order of potency: **2** $\gg$ **3** > **4** > **1**.

Docherty and McGrath[10] and Wilffert[23] have shown β-adrenoceptor mediation decreases vascular tone and is predominately mediated through β_2-adrenergic receptors. To find the relative β_2-adrenoceptor activity mediated by the different agents 1–4, we examined their abilities to reduce blood pressure in reserpine- (5 mg/kg, ip, 24 h) and phenoxybenzamine- (3 mg/kg, iv, 15 min) pretreated pithed rats whose blood pressure was elevated by a constant intravaneous infusion of angiotensin II (150 ng/kg per min). The results are shown in Figure 3, and only compound **2** showed β_2-agonist activity.

The present studies indicate that appropriately N-substituted imidazolines possess significant α_1-, α_2-, β_1-, and β_2-adrenergic activities. The intermediate side chain between the catechol group and the amine portion of an imidazoline or ethylenediamine segment has a tremendous effect upon the adrenergic activity observed. Compounds **1** and **3** with a 1-carbon unit (CH_2 group) separating the catechol from the amine of either the imidazoline **1** or the amine of the ethylenediamine group **3** show selective α_1-adrenergic activity. However, compounds **2** and **4** with a 2-carbon unit ($-CH_2CH_2-$, ethylene group) between the catechol and the imidazoline as in **2** or the ethylenedimaine segment as in **4** show selective α_2-adrenergic agonist activity. It is interesting to note that only compound **2** showed significant β-adrenergic activity. This work indicates that the α- and β-adrenergic activity of N-substituted imidazolines and ethylenediamines is very sensitive to the number of carbon atoms separating the catechol and amine segments of molecules such as 1–4. This difference in activity represents a novel finding and could provide valuable information in approaches to make very selective adrenergic stimulants.

Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover melting point apparatus. IR spectral data were obtained with a Beckman 4230 infrared spectrophotometer, and NMR spectral data were obtained with a Bruker HX-90E NMR spectrometer (90 MHz) in the pulse mode. Mass spectra were obtained with a Du Pont Model 21-491 double-focusing mass spectrometer. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Analytical results for elements indicated were within ±0.4% of the theoretical values.

***N*-[3,4-Bis(benzyloxy)benzyl]ethylenediamine (8).** To a solution of ethylenediamine (30 g, 0.50 mol) in toluene (40 mL) was added dropwise a solution of 3,4-bis(benzyloxy)benzyl chloride[7] (0.01 mol) in toluene (10 mL) over a period of 15 min at room temperature. The resulting mixture was refluxed for 4 h followed by addition of NaOH (0.5 g) in H_2O (10 mL), and the solvent and excess diamine were removed in vacuo to give a residue. The residue was taken up in CH_2Cl_2 (100 mL), washed with H_2O (3 × 100 mL), and dried on anhydrous Na_2CO_3. The green oil obtained was taken up in a mixture of CH_2Cl_2 (40 mL) and EtOH (10 mL), and to the resulting solution was added anhydrous HCl gas. Removal of the solvent gave a viscous residue, which was taken up in H_2O (50 mL), alkalized with NaOH, extracted with CH_2Cl_2 (100 mL), and dried over anhydrous Na_2CO_3. The solvent was evaporated in vacuo to afford a viscous oil, which was dried under vacuum to yield 2.3 g of a viscous pale yellow liquid (64%). NMR ($CDCl_3$): δ 7.50–7.25 (m, 10 H), 6.94–6.85 (m, 3 H), 5.16 (s, 2 H), 5.13 (s, 2 H), 3.68 (s, 2 H), 2.82–2.53 (m, 4 H). To a solution of free diamine (500 mg) in MeOH (50 mL) was added HCl gas until the solution was saturated at room temperature. The solvent was removed, and crystallization from MeOH/CH_2Cl_2 yielded 490 mg (82%) of solid dihydrochloride salt, mp 211–213

(19) Ruffolo, R. R., Jr.; Waddell, J. E. *J. Pharmacol. Exp. Ther.* **1982**, *222*, 29.
(20) Van Meel, J. C. A.; deJonge, A.; Timmermans, P. B. M. W. M.; Zwieten, P. A. *J. Pharmacol. Exp. Ther.* **1981**, *219*, 760.
(21) Carlsson, E.; Ablad, B.; Brandstrom, A.; Carlsson, B. *Life Sci.* **1972**, *11*, 953.
(22) Broadley, K. J. *J. Auton. Pharmacol.* **1982**, *2*, 119.
(23) Wilffert, B.; Timmermans, P. B. M. W. M.; van Zwieten, P. A. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 762.

°C. Recrystallization from MeOH gave a solid, mp 215–216 °C. Anal. ($C_{23}H_{28}Cl_2N_2O_2$) C, H, N.

***N*-(3,4-Dihydroxybenzyl)ethylenediamine (3).** The diamine dihydrochloride salt (**8**) (2.0 g, 4.6 mmol) was treated with Pd–C (10%, 300 mg) and hydrogen (45 psi) in MeOH (75 mL) for 2.5 h at room temperature. Filtration and removal of the solvent gave a white solid, which was recrystallized from MeOH/EtOH to yield 1.04 g (89%) of catechol diamine dihydrochloride salt **3**, mp 184.5–185.0 °C. Anal. ($C_9H_{16}Cl_2N_2O_2$) C, H, N.

1-[3,4-Bis(benzyloxy)benzyl]imidazoline Hydrochloride (11). To a solution of ethylenediamine **8** (3.7 g, 10.3 mmol) in dry CH_2Cl_2 (50 mL) was added ethylformimidate hydrochloride **11** (2.3 g, 20.9 mmol) with cooling in an ice bath under an argon atomosphere. The resulting mixture was stirred at room temperature under argon for 20 h, washed with 10% NaOH (50 mL), and dried over anhydrous Na_2CO_3. The solution was evaporated in vacuo, and the oil was taken up in EtOH (10 mL), saturated with HCl gas, and evaporated to give a viscous residue, which solidified when taken up in a mixture of methyl ethyl ketone and ethyl acetate. The solid was recrystallized from CH_2Cl_2–ethyl acetate twice to afford 3.2 g (77%) of colorless crystals, mp 153–154 °C. Anal. ($C_{24}H_{25}ClN_2O_2$) C, H, N.

1-(3,4-Dihydroxybenzyl)imidazoline Hydrochloride (1). Imidazoline **11** (1.0 g, 2.4 mmol) in EtOH (30 mL) was hydrogenated on 10% Pd/C (200 mg) with hydrogen (45 psi) at room temperature for 2.5 h. Filtration and removal of the solvent gave a viscous oil, which was taken up in acetone and solidified upon standing. Two recrystallizations of the solid from EtOH–acetone yielded 0.33 g (59%) of catechol imidazoline (**1**), mp 158–159 °C. Further recrystallization from EtOH gave colorless crystals, mp 159–159.5 °C. Anal. ($C_{10}H_{13}ClN_2O_2$) C, H, N.

2-[3,4-Bis(benzyloxy)phenyl]-1-chloroethane (7). To a solution of [bis(benzyloxy)phenyl]acetic acid (**13**) (13.0 g, 37 mmol) in dry THF (30 mL) was added dropwise diborane solution (45 mL, 0.97 M in THF) over 30 min with cooling in an ice bath followed by stirring for 15 h at room temperature. After adding MeOH (20 mL) dropwise with cooling in an ice bath, the solvent was evaporated to give an oily residue, which was taken up in ether (150 mL), washed with saturated Na_2CO_3 (100 mL), and dried over anhydrous $MgSO_4$. Removal of the solvent afforded an oily residue, which solidified upon standing, and recrystallization from ether–*n*-hexane gave 11.05 g (89%) of 2-[3,4-bis(benzyloxy)-phenyl]ethanol (**14**), mp 54.5–55.5 °C. NMR ($CDCl_3$): δ 7.40–7.26 (m, 10 H), 6.94–6.65 (m, 3 H), 5.15 (s, 2 H), 5.13 (s, 2 H), 3.77 (br q, 2 H), 2.75 (t, J = 6.358 Hz, 2 H), 1.25 (br t, 1 H). Anal. ($C_{22}H_{22}O_3$) C, H, N.

To a solution of alcohol **14** (11.0 g) in CCl_4 (80 mL) was added $SOCl_2$ (15 g) dropwise over 15 min at room temperature, and the resulting mixture was refluxed for 24 h. Removal of the solvent gave an oily residue, which was treated by flash column chromatography on silica gel (petroleum ether–ethyl acetate (5%)) to afford 9.7 g (84%) of the chloride (**6**). Crystallization from pentane in the cold and recrystallization from ether–*n*-hexane gave colorless crystals, mp 24–26 °C. NMR ($CDCl_3$): δ 7.49–7.28 (m, 10 H), 6.96–6.71 (m, 3 H), 5.17 (s, 4 H), 3.66 (t, J = 7.31 Hz, 2 H), 2.97 (t, J = 7.63 Hz, 2 H). Anal. ($C_{22}H_{21}ClO_2$) C, H, N.

***N*-[2-(3,4-Dihydroxyphenyl)ethyl]ethylenediamine Dihydrochloride Salt (4).** To ethylenediamine (56 g, 935 mmol) was added **7** (6.6 g, 18.7 mmol) in toluene (50 mL) dropwise over 15 min at room temperature. The resulting mixture was refluxed for 5 h followed by addition of NaOH (1 g) in H_2O (20 mL). Removal of the solvent and excess diamine in vacuo gave a residue, which was taken up in CH_2Cl_2 (250 mL), washed with H_2O (3 × 100 mL), and dried on anhydrous Na_2CO_3. To the dried CH_2Cl_2 solution was added EtOH (50 mL) saturated with HCl gas to afford a precipitate that was taken up in H_2O (100 mL), alkalized by 10% NaOH, extracted with CH_2Cl_2 (100 mL), and dried on anhydrous Na_2CO_3. Evaporation of the solvent gave 5.0 g (71%) of *N*-[2-[3,4-bis(benzyloxy)phenyl]ethyl]ethylenediamine (**9**). The oily diamine obtained could be converted to the dihydrochloride salt by treatment with HCl gas in CH_2Cl_2 and recrystallization from MeOH to give crystals, mp 144–146 °C. Anal. ($C_{24}H_{30}Cl_2N_2O_2$) C, H, N.

A mixture of the diamine dihydrochloride salt **9** (0.8 g), 10% Pd–C (0.2 g), EtOH (30 mL), and H_2O (20 mL) was shaken for 2 h at room temperature under a H_2 pressure of 45 psi. Filtration and removal of the solvent gave a solid, which was recrystallized from MeOH twice to yield 0.42 g of catechol **4** (88%), mp 242–243 °C. Anal. ($C_{10}H_{18}Cl_2N_2O_2$) C, H, N.

***N*-[2-[3,4-Bis(benzyloxy)phenyl]ethyl]imidazoline Hydrochloride Salt (12).** A mixture of diamine **9** (1.0 g, 2.66 mmol), ethyl formamidate **10** (1.0 g, 9.1 mmol), and dry CH_2Cl_2 (32 mL) was stirred at 0 °C for 30 min and at room temperature for 20 h under argon atomosphere. The mixture was then washed with 10% NaOH (30 mL) and dried over anhydrous Na_2CO_3. To the CH_2Cl_2 solution was added EtOH (10 mL), which had been saturated with HCl gas. The solvent was removed to give a viscous oily product. The product was taken up in 50 mL of CH_2Cl_2, washed with H_2O (50 mL), and dried over Na_2SO_4. The solvent was evaporated to afford a viscous residue that was solidified when taken up in methyl ethyl ketone and recrystallized from CH_2Cl_2–ethyl acetate twice to yield 0.65 g (58%) of imidazoline salt **12**, mp 108–109 °C. NMR ($CDCl_3$): δ 8.782 (s, 1 H), 7.55–7.27 (m, 10 H), 6.93–6.63 (m, 3 H), 5.198 (s, 2 H), 5.122 (s, 2 H), 3.89–3.46 (m, 6 H), 2.824 (t, J = 6.65 Hz, 2 H). Anal. ($C_{25}H_{27}ClN_2O_2$) C, H, N.

***N*-[2-(3,4-Dihydroxyphenyl)ethyl]imidazoline Hydrochloride Salt (2).** Imidazoline **12** (0.7 g) in EtOH (50 mL) was hydrogenated with 10% Pd–C (0.15 g) with hydrogen (45 psi) at room temperature for 3 h. Filtration and removal of the solvent afforded a white solid, which was recrystallized from EtOH–ethyl acetate twice to yield 0.283 g (70.6%) of catechol **2**, mp 206–208 °C. Anal. ($C_{11}H_{15}ClN_2O_2$) C, H, N.

Pressor Activity in Pithed Rats. Normotensive male albino rats (270–310 g, Sprague-Dawley, Harlan Industries, Indianapolis, IN) were anesthetized with methoxyflurane. The tracheas were cannulated, and the rats were then pithed by inserting a steel rod (1.5 mm in diameter) through the orbit and foramen magnum down into the spinal canal. Immediately after pithing, the tracheal cannula was attached to a Harvard Apparatus Model 680 rodent respirator, and the rat was artificially ventilated with room air at a frequency of 60 cycles/min with a volume of 2 mL/100 g of body weight. Systemic arterial blood pressure was measured from the right carotid artery via a Statham P23 ID pressure transducer and recorded on a Beckman R411 Dynograph recorder. Heart rate was recorded with a Beckman 98758 cardiotachometer triggered by the pulse pressure. The right femoral vein was cannulated for iv administration of drugs in a volume of 1 mL/kg. The preparation was allowed to equilibrate for at least 30 min before drug administration. All pressor responses are expressed as increases in diastolic blood pressure in millimeters of mercury (mmHg).

It is now known that pressor responses elicited by α-adrenoceptor agonists in pithed rats may result from activation of a mixed population of postsynaptic vascular α_1- and/or α_2-adrenoceptors.[15-18] The pressor responses of the test compounds were therefore evaluated after pretreatment with the α_1-adrenoceptor-selective antagonist prazosin (0.1 mg/kg, iv) or the α_2-adrenoceptor-selective antagonist yohimbine (1 mg/kg, iv), in order to establish the contribution made by each α-adrenoceptor subtype to the pressor activity observed. In our hands, these doses of prazosin and yohimbine produce selective antagonism of postsynaptic vascular α_1- and α_2-adrenoceptors, respectively, with no crossover between the α-adrenoceptor subtypes.[18] This is further demonstrated in Table I where the pressor response of the α_1-adrenoceptor-selective agonist cirazoline[19] is antagonized by prazosin and relatively unaffected by yohimbine, whereas the converse is true for the pressor response elicited by the α_2-adrenoceptor-selective agonist B-HT 933.[20]

Vasodepressor Activity in Pithed Rats. Rats were anesthetized and pithed as described above, and the left carotid artery and left femoral vein were cannulated for recording blood pressure and administration of drugs, respectively. The right femoral vein was cannulated for constant infusion of angiotensin II with a Sage Instruments Model 352 infusion pump. Phenoxybenzamine (3 mg/kg, iv) was administered, followed 15 min later by yohimbine (1 mg/kg, iv). This procedure is necessary to antagonize completely vascular α_1- and α_2-adrenoceptors that mediate vasoconstriction and complicate the analysis of β_2-adrenoceptor-mediated vasodepressor effects. Following an additional 15-min equilibration period, angiotensin II elevated diastolic blood pressure in pithed rats from the basal value of 39

± 5 mmHg to 108 ± 4 mmHg. Once the angiotensin II pressor response had stabilized (within 10 min), the β_2-adrenoceptor-mediated vasodepressor effect of each agonist was investigated by iv adminstration, and drug-induced decreases in diastolic blood pressure (mmHg) were recorded. Each rat received only one test compound.

Statistical Evaluation. Results are expressed as the mean ±SEM. Statistical differences between two means ($P < 0.05$) were determined by the student t-test for unpaired observations or by testing of overlap of 95% confidence limits.[24] All straight lines were drawn by linear regression[25] and tested, wherever possible, for deviations from linearity by analysis of variance in regression.[24]

Acknowledgment. We are grateful for support of this research by the National Institutes of Health (Grant GM29358).

Registry No. 1, 96826-13-0; 1·HCl, 96826-02-7; 2, 96826-14-1; 2·HCl, 96826-03-8; 3, 96826-15-2; 3·2HCl, 96826-04-9; 4, 96826-16-3; 4·2HCl, 96826-05-0; 5, 72143-18-1; 6, 1699-59-8; 7, 96826-06-1; 8·2HCl, 96826-07-2; 9, 96826-08-3; 9·2HCl, 96826-12-9; 10·HCl, 16694-46-5; 11·HCl, 96826-09-4; 12·HCl, 96826-10-7; 13, 1699-61-2; 14, 96826-11-8; ethylenediamine, 107-15-3.

(24) Sokal, R. A.; Rohlf, F. J. "Regression in Biometry"; Freeman: San Francisco, 1969; pp 404–493.

(25) Woolf, C. M. "Principles of Biometry: Statistics for Biologists"; van Nostrand: Princeton, NJ, 1968.

Molecular Mechanism of Action of 5,6-Dihydroxytryptamine. Synthesis and Biological Evaluation of 4-Methyl-, 7-Methyl-, and 4,7-Dimethyl-5,6-dihydroxytryptamines[1]

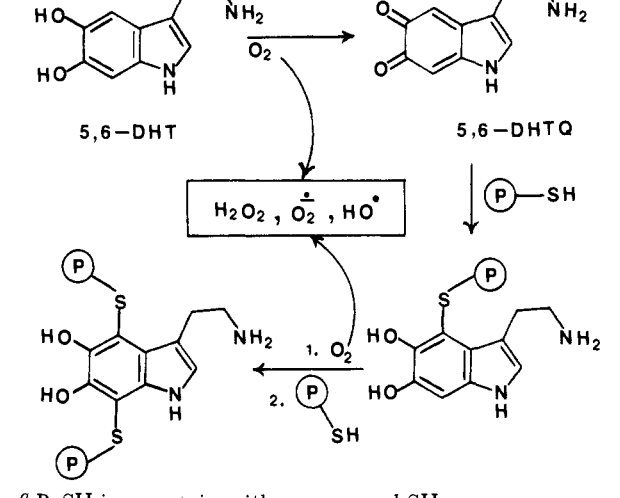
Achintya K. Sinhababu,[†] Anil K. Ghosh,[‡] and Ronald T. Borchardt*[,†]

Departments of Medicinal Chemistry and Pharmaceutical Chemistry, The University of Kansas, Lawrence, Kansas 66045.
Received December 21, 1984

The major mechanism by which the serotonin neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) expresses its neurodegenerative action may involve alkylation of biological nucleophiles by the electrophilic quinoid autoxidation products. To determine the relative importance of various sites on these autoxidation products toward alkylation we have rationally designed and synthesized 4-Me-5,6-DHT (**16a**), 7-Me-5,6-DHT (**16b**), and 4,7-Me_2-5,6-DHT (**16c**). The indole nucleus of these analogues was constructed by the reductive cyclization of the corresponding 2,β-dinitrostyrenes, and the aminoethyl side chain was introduced via gramine methiodides. Redox data showed that all the analogues are more readily oxidized compared to 5,6-DHT. The biological activity was evaluated in differentiated neuroblastoma N-2a cells in culture. The order of inhibitory potency, as determined by measuring the inhibition of incorporation of [^{3}H]thymidine into DNA, was **16c** ≫ **16a** > 5,6-DHT ≃ **16b**. The order of affinity (expressed as IC_{50} values in μM) for serotonergic uptake as determined by measuring their inhibition of [^{3}H]-5-HT uptake was 5,6-DHT (4) > **16c** (20) > **16a** (23) > **16b** (52). The results of these studies established that these rationally designed C-methylated analogues of 5,6-DHT are suitable probes for elucidating the molecular mechanism of action of 5,6-DHT.

5,6-Dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT) have become widely used pharmacological tools because of their ability to produce selective destruction of 5-hydroxytryptamine- (5-HT) containing nerve terminals.[2-7] The molecular mechanisms whereby 5,6-DHT and 5,7-DHT exert their neurodegenerative effects are still in question; however, the specific transport of these agents into serotonergic neurons by the appropriate neuronal membrane pumps is a prerequisite to their neurodegenerative effects. The chemical events that occur intraneuronally and eventually produce the destruction of the nerve terminals are initiated by the intraneuronal autoxidation of the neurotoxins. For 5,6-DHT two molecular theories have been proposed to explain the resulting cytotoxic effects:[4-6,8-12] (a) The quinone-like compound(s) generated by nonenzymatic autoxidation of 5,6-DHT may act as alkylating agent(s). (b) The H_2O_2, $O_2^{-}\cdot$, and HO·, which are also generated during autoxidation, may act as oxidizing agents. Although the relative importance of these two mechanisms is not known, neuronal degeneration is believed to be the result of alkylation of neuronal proteins by the quinone(s) together with oxidation of lipids and proteins by the reduced oxygen species.[4-6,8-12] Because of the complexity of the autoxidation reaction, it has not yet been possible to characterize the DHT-derived product(s), which has been

Chart I[a]



[a] P-SH is a protein with an exposed SH group.

[†] Department of Medicinal Chemistry.
[‡] Department of Pharmaceutical Chemistry.

(1) Presented in part at the 184th National Meeting of the American Chemical Society, Kansas City, MO, Sept 12–17, 1982; Abstr. MEDI 26.

0022-2623/85/1828-1273$01.50/0