Syntheses and Adrenergic Activities of Ring-Fluorinated Epinephrines

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The study of chemical and biological effects of fluorine substitution on the aromatic ring of catecholamines has now been extended to epinephrine (Epi). 2- and 6-fluoroepinephrines (2-FEpi and 6-FEpi) have been synthesized. Fluorine substitution on the 2- or 6-carbon of the aromatic ring alters the selectivity of epinephrine toward α- and β-adrenergic receptors, similar in manner to the change in selectivity seen with norepinephrine (NE). Thus, 2-FEpi is a relatively selective β-adrenergic ligand, while 6-FEpi is a relatively selective α-adrenergic ligand. Fluorine substitution of Epi also can markedly increase potency at either α- or β-adrenergic receptors.

In 1979, we reported that a fluorine substituent on the 2-position or 6-position of norepinephrine (NE) led to selectivity for β- or α-adrenergic receptors, respectively. This effect was due to a marked decrease in affinity of 2-fluoronorepinephrine (2-FNE) for α-adrenergic receptors and a marked decrease in affinity of 6-fluoronorepinephrine (6-FNE) for β-adrenergic receptors.[1,2] 2-FNE and 6-FNE remain about as potent as norepinephrine (NE) at β-adrenergic and α_1-adrenergic receptors, respectively. In contrast, 5-fluoronorepinephrine (5-FNE) did not display selectivity for α- or β-adrenergic receptors and was slightly more potent than the parent compound NE at the β-receptor. These analogues have proven to be useful pharmacological tools in both peripheral and central systems, in vitro and in vivo.[3]

The study of effects of ring-fluorine substitution on adrenergic activity have been extended to the α-adrenergic agonist phenylephrine (PE)[4] and to the β-adrenergic agonist isoproterenol (ISO).[5] One goal of these studies was to use the phenomenon of fluorine-induced adrenergic agonist selectivities to derive information regarding the structural and electronic requirements for interaction of phenethanolamines with α- and β-adrenergic receptors. 6-FISO had greatly reduced β-adrenergic potency compared to ISO, while 2-FISO was essentially identical in potency with ISO as a β-adrenergic agonist. None of the FISO's exhibited significant α-adrenergic activity. 6-Fluorophenylephrine (6-FPE) was more potent at α-adrenergic receptors and less potent at β-adrenergic receptors than PE. This combination of effects renders 6-FPE much more selective than PE for α-adrenergic receptors. 2-Fluorophenylephrine (2-FPE) was more potent at β-adrenergic receptors and less potent at α-adrenergic receptors than PE. Thus, for the FPE analogues, not only did fluorine alter adrenergic selectivity, it also caused an increase in potency. This marked increase in potency was not observed for the FNE and FISO analogues.

We have now extended our studies on the effects of ring-fluorine substitution on the physical and biological activities of norepinephrine[1] and dopamine,[6,7] to include the third naturally occurring catecholamine, epinephrine (Epi). The syntheses of 2-fluoroepinephrine (2-FEpi, **7a**) and 6-fluoroepinephrine (6-FEpi, **7b**), along with their α- and β-adrenergic properties, are described and contrasted to effects in the FNE series.

Chemistry

Previously reported intermediates **4a,b** were prepared by a slightly modified route.[1,5] 2-Fluoroveratraldehyde **2a** was obtained in 74% yield by regioselective formylation[8] of 3-fluoroveratrole **1a**, this procedure providing an improved route to this aldehyde. Similar formylation of 4-fluoroveratrole **1b** gave the aldehyde **2b** in 85% yield, higher than previously reported.[8] For each of the aldehydes, demethylation and benzylation were carried out to change protecting groups.[3] Elaboration of the side chain was accomplished as before,[1] by formation of the silylated cyanohydrin followed by in situ reduction. The amines **4a** and **4b** were obtained in 88% and 82% yields, respectively. N-Formylation of amines **4** with ethyl formate followed by reduction gave the corresponding *N*-methylamines.[4] The catechols were obtained by removal of the protecting groups by hydrogenation and isolated as oxalate salts.

Biology

The affinities of fluorine-substituted Epis for α_1-, α_2-, and β_1-adrenergic receptors were determined through inhibition of binding of appropriate ligands for these receptors (Figures 1–3; see the Experimental Section). The agonist activities of Epis on adrenergic receptors were determined as follows: for α_1-adrenergic activity, the effects of FEpis on stimulation of phosphoinositide breakdown in guinea pig cerebral cortical synaptoneurosomes (Figure 4); for α_2-adrenergic activity, the effects of the FEpis on inhibition of forskolin-stimulated adenylate cyclase activity in human platelet membranes (Figure 5); for β-adrenergic activity, the effects of FEpis on stimulation of isolated guinea pig atrial preparations (Figure 6), and stimulation of cyclic AMP accumulation of C6 glioma cells (Figure 7). The effects on atrial preparations are mediated through β_1-adrenergic receptors. For details on these procedures see the Experimental Section.

All Epis fully displaced the α_1- and α_2-adrenergic ligands [^{3}H]WB4101 and [^{3}H]clonidine from brain membranes. As noted with other adrenergic agonists, the affinity of the FEpis for α_2-adrenergic receptors is higher than the affinity for α_1-adrenergic receptors. The order of potency of the FEpis for α-adrenergic receptor binding was 6-FEpi > Epi

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Table I. Effect of Fluorine Substitution on the α- and β-Adrenergic Properties of Epinephrine

	affinity:[a] K_i, μM				agonist potency:[a] EC_{50}, μM			
amine	α_1[b]	α_2[c]	β_1[d]	α_1/α_2[e]	α_1[f]	α_2[h]	β_1[g]	β[i]
Epi	2.0	0.005	3.0	400	0.4	0.3	0.26	0.23
2-FEpi	16.5	0.066	1.8	250	3.3	>5	0.04	0.04
6-FEpi	0.7	0.009	63	78	0.8	0.1	>1.5	>1.5
NE[j]	1.5	0.03[k]	0.4	50			0.35	
2-FNE[j]	>50	0.70[k]	0.35	71			0.3	
6-FNE[j]	1.5	0.012[k]	>3	125			36.	

[a] Activities have been adjusted (see Figures 1–7) for the presence of the inactive (+) isomer in racemates of FEpis. [b] Adjusted K_i values versus displacement of [^{3}H]WB4101. [c] [^{3}H]Clonidine. [d] Dihydro[^{3}H]alprenolol. [e] Ratios of K_i values for α_1- and α_2-adrenergic receptors. [f] Adjusted EC_{50} values were from dose–response curves of the amine-induced increase of phosphatidylinositol breakdown in synaptoneurosomes from guinea pig cerebral cortex. [g] Adjusted EC_{50} values were from dose–response curves for the percent maximal increase in contraction rate of isolated guinea pig atria. [h] Adjusted EC_{50} values were from dose–response curves for the inhibition of forskolin-elicited stimulation of adenylate cyclase in human platelet membranes. [i] Adjusted EC_{50} values were from dose–response curves for the stimulation of accumulation of cyclic AMP in cultured C6 glioma cells. For details, see the Experimental Section. [j] Values for NE and FNEs are taken from Nimit et al.[25] and are adjusted for the presence of inactive (+) isomers. [k] K_i values for inhibition of [^{3}H]clonidine binding by NE and FNEs (see ref 25) were redetermined under present assay conditions.

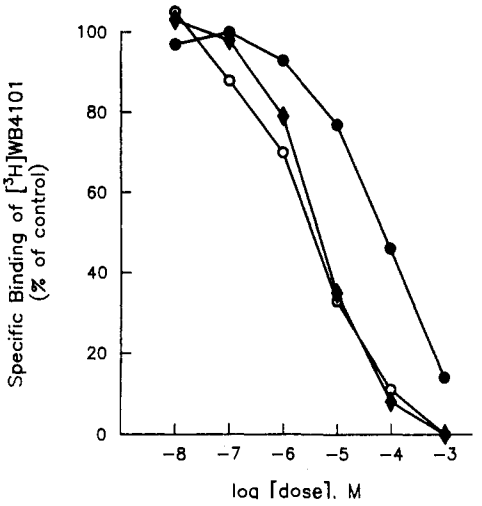


Figure 1. Inhibition of the specific binding of [^{3}H]WB4101 to α_1-adrenergic receptors in guinea pig cerebral cortical membranes by (−)-Epi (◆), (±)-2-FEpi (●), and (±)-6-FEpi, (○). Results, expressed as percent of control with each point performed in triplicate, are from a representative experiment, repeated three times with similar results.

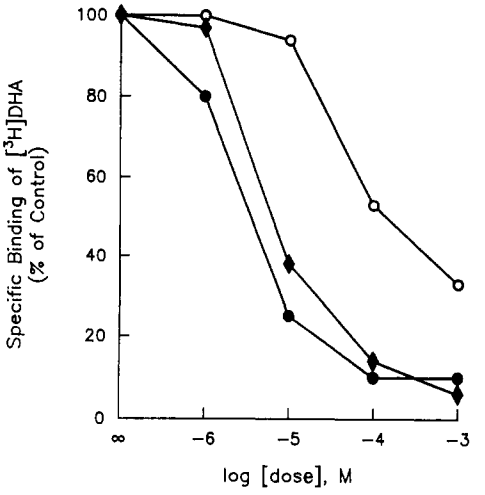


Figure 3. Inhibition of the specific binding of dihydro[^{3}H]alprenolol to β_1-adrenoceptors in rat cerebral cortical membranes by (−)-Epi (◆), (±)-2-FEpi (●), and by (±)-6-FEpi (○). Results, expressed as percent of control with each point performed in triplicate, are from a representative experiment, repeated three times with similar results.

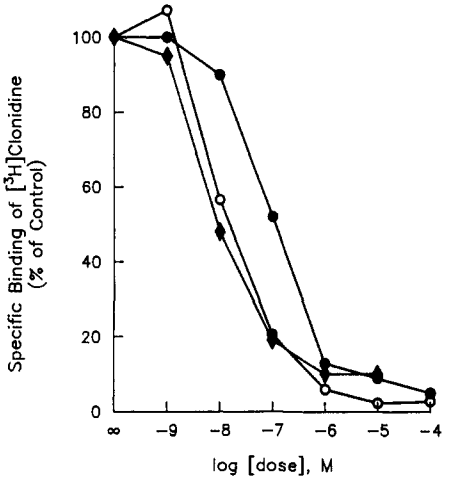


Figure 2. Inhibition of the specific binding of [^{3}H]clonidine to α_2-adrenoceptors in guinea pig cerebral cortical membranes by (−)-Epi (◆), (±)-2-FEpi (●), and (±)-6-FEpi (○). Results, expressed as percent control with each point performed in triplicate, are from a representative experiment, repeated three times with similar results.

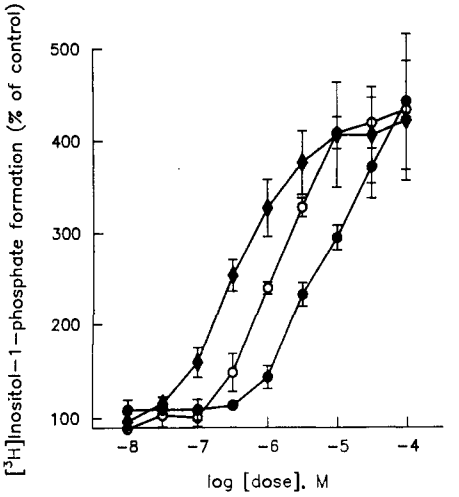


Figure 4. α_1-Adrenergic agonist activity: Stimulation of phosphatidylinositol turnover in guinea pig cerebral cortical synaptoneurosomes by (−)-Epi (◆), (±)-2-FEpi (●), and (±)-6-FEpi (○). Results, expressed as percent of control (±SEM) with each point performed in triplicate, are from a representative experiment, repeated three times with similar results.

≫ 2-FEpi for α_1- and Epi > 6-FEpi > 2-FEpi for α_2-adrenergic receptors (Table I). The order of potencies of FEpis for stimulation of α_1-adrenergic receptor-mediated phosphoinositide breakdown in synaptoneurosomes was Epi > 6-FEpi > 2-FEpi and for α_2-adrenergic receptor-mediated inhibition of adenylate cyclase in human platelet

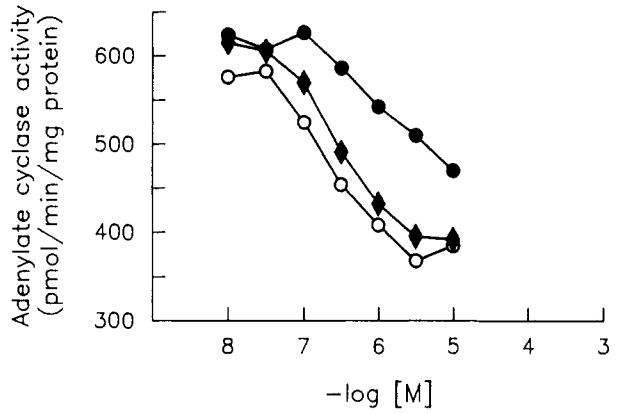


Figure 5. α_2-Adrenergic agonist activity: Inhibition of adenylate cyclase activity in human platelet membranes in the presence of forskolin (10 μM) by (−)-Epi (◆), (±)-2-FEpi (●), and (±)-6-FEpi (○). Results, expressed as adenylate cyclase activity in picomoles of cyclic AMP formed per minute per milligram of membrane protein with each point performed in triplicate, are from a representative experiment, repeated three times with similar results. Activity with forskolin alone was 630 pmol/min per mg of protein.

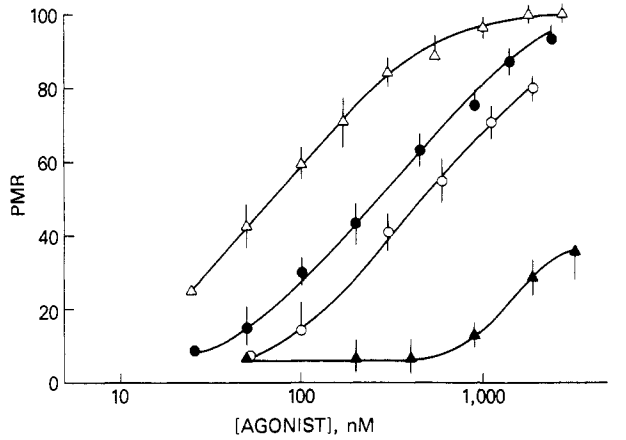


Figure 6. β_1-Adrenergic agonist activity: Dose–response curves for the stimulation of contraction rate of isolated guinea pig atrial preparations by (−)-Epi (●), (±)-Epi (○), (±)-2-FEpi (△), and (±)-6-FEpi (▲). Results are presented as percent of maximal rate increase (PMR) ± SEM for four individual preparations. Maximal rates were determined in the presence of 3–5 μM (−)-Epi.

membranes the order was 6-FEpi > Epi ≫ 2-FEpi (Table I).

All Epis displaced the β-adrenergic ligand [^{3}H]dihydroalprenolol from rat brain membranes. The order of potencies for β-adrenergic receptor binding was 2-FEpi > Epi ≫ 6-FEpi (Table I). The same order of potencies was obtained for both β-adrenergic receptor-mediated accumulation of cyclic AMP in C6 glioma cells and for in vitro cardiotonic activity on guinea pig atrial preparations, namely 2-FEpi > Epi ≫ 6-FEpi. It should be noted that 6-FEpi appears to be a partial agonist for stimulation of atria.

Discussion

The effects of fluorine substitution on the adrenergic properties of Epi demonstrate again the ability of fluorine to alter interactions of phenethanolamines with adrenergic receptors. In all series of fluorinated phenethanolamines, namely the FNEs, FISOs, FPEs, and the FEpis, a 2-fluoro substituent increases selectivity for β-adrenergic receptors, while a 6-fluoro substituent increases selectivity for α-adrenergic receptors. Moreover, the increased potency of 2-FEpi relative to Epi at β-adrenergic receptors is the first instance of such enhanced activity of a 2-fluoro-substituted

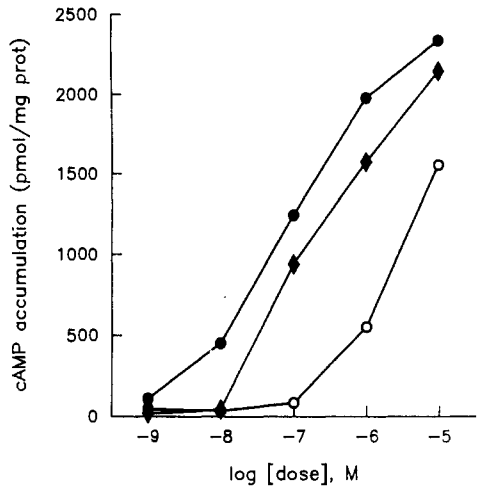


Figure 7. β-Adrenergic agonist activity: Stimulation of cyclic AMP accumulation in C6 glioma cells by (−)-Epi (♦), (±)-2-FEpi (●), and (±)-6-FEpi (○). Results, expressed as accumulation of cyclic AMP in picomoles of cyclic AMP formed per 10 minutes per milligram of protein with each point performed in triplicate, are from a representative experiment, performed three times with similar results.

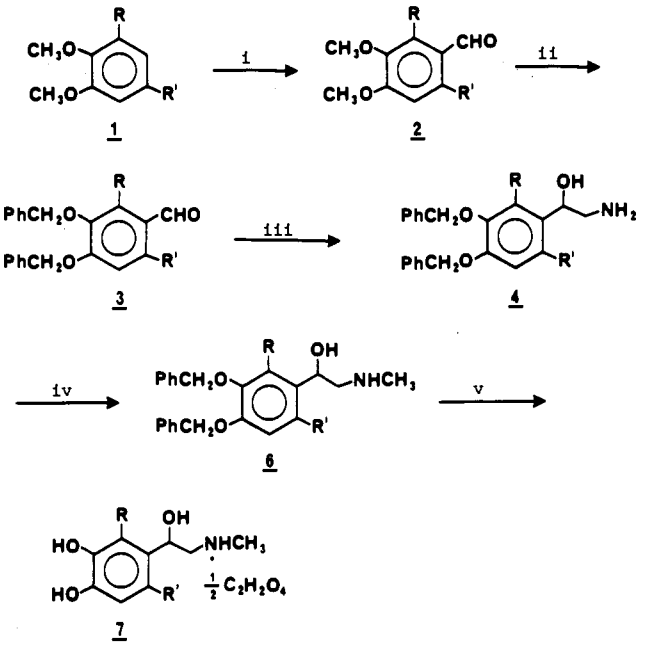
catecholamine for β-adrenergic receptors. The increased potency of 6-FEpi relative to Epi at α_1-adrenergic receptors had a precedent in the increased potency of 6-FPE relative to PE at α_1- (and α_2-) adrenergic receptors. Although, 6-FNE had appeared to be similar in potency to NE at α_1- and α_2-adrenergic receptors,[25] present data using 1 nM [^{3}H]clonidine to define α_2-adrenergic binding sites indicate that 6-FNE is about 3-fold more potent than NE (Table I).

The effects of fluorine substitution on the adrenergic activity of dopamine should be noted. At α_1- and β_1-adrenergic receptors, dopamine has low potency, which is not markedly affected by fluorine substitution.[3,7] However, at the α_2-adrenergic receptor, dopamine is relatively potent, and this potency is unaffected by a fluorine substituent at either the 2-, 5-, or 6-position.[3]

Effect of 2-Fluoro Substituent. In the case of NE, a 2-fluoro substituent caused a great decrease in potency at both α_1- and α_2-adrenergic receptors.[3] A similar decrease was manifest in the Epi series (Table I). The 2-fluoro substituent in the NE series causes no change in potency at the β-adrenergic receptor. In the Epi series a 2-fluoro substituent significantly increases potency at the β-adrenergic receptor. The reason underlying no significant effect of a 2-fluoro substitutent on potency at β-adrenergic receptors in the NE series and an increase in potency in the Epi series is not known. It should be noted that in the PE series, a 2-fluoro substituent also increases β-adrenergic potency.[4] Both Epi and PE, unlike NE, have an *N*-methyl substitutent. However, in the ISO series, with an *N*-isopropyl substituent, the 2-FISO is less potent than ISO at β_1-adrenergic receptors.[5] In both the NE and Epi series, the 2-fluorocatecholamine is a highly selective β-adrenergic agonist.

Effect of 6-Fluoro Substitutent. In the NE series, a 6-fluoro substituent strikingly reduces potency at β-adrenergic receptors.[3] A similar decrease was manifest in the Epi series. The 6-substituent in the NE series has little effect on potency at an α_1-adrenergic receptor but does increase potency at an α_2-adrenergic receptor (Table I). However, in the Epi series, the 6-fluoro substituent can either increase or decrease potency at α_1-adrenergic and α_2-adrenergic receptors (Table I). It should be noted that, in the PE series, the 6-fluoro substituent increases potency

Scheme I[a]



SERIES: a R = F, R' = H
b R = H, R' = F

[a] (i) $TiCl_4$, Cl_2CHOMe; (ii) 1. BBr_3, 2. $PhCH_2Br$; (iii) 1. TMSCN, 2. LAH; (iv) 1. HCO_2Et, 2. LAH; (v) $H_2C_2O_4$, H_2, Pd/C.

at both α_1- and α_2-adrenergic receptors.[4] The underlying reasons for different effects of 6-fluoro substituents on potency of amines of α-adrenergic receptors are the subject of current research.

In both NE and Epi series the 6-fluoro catecholamine is a highly selective α-adrenergic agonist. Further, in both series, as for NE itself, the amines are more potent at α_2- than at α_1-adrenergic receptors.

The basis for the fluorine-induced potency and selectivity of catecholamine adrenergic receptor ligands is as yet unknown. Several explanations are under consideration.[2,4,9-12] To account for increased potency resulting from fluorine substitution in the PE series, we previously proposed[4] dipole–dipole repulsive interactions, which could promote conformations favorable for interactions with α- or β-adrenergic receptors. A similar scheme was proposed independently by DeBernardis et al.[9] to account for dramatically increased α_2- vs α_1-adrenergic potencies of certain conformationally defined analogues—electrostatic repulsion based conformational prototypes, or "ERBCOPs". While the semirigid analogues studied by DeBernardis clearly define conformational requirements for α_2-selectivity, we have noted that 6-fluoro analogues of NE, PE, and Epi all show comparable α_1/α_2 ratios as the parent compounds. Therefore, it would appear that the increased α_2 potencies observed by Debernardis for certain conformationally defined analogues and our fluorine-induced adrenergic selectivities presumably have different mechanistic origins.[10] In fact, in view of the remarkably enhanced α_2-selectivity that certain conformationally defined analogues have shown,[9] our results suggest that the dipole–dipole repulsion model may not be valid for the fluoro analogues of PE, NE, and Epi.

An alternative explanation for fluorine-induced adrenergic agonist selectivities is that alterations in the electron distribution of the aromatic ring, due to the strong electron withdrawing influence of the fluoro substituent, causes the changes in affinity at α- vs β-adrenergic receptors. Thus, we proposed that the decrease in electron density on the carbon to which fluorine is bound could result in reduction of an electrostatic binding interaction with a positive center on the receptor.[5] Our later discovery of the enhanced potency of 6-FPE at α-adrenergic receptors initially seemed inconsistent to what we had described as a purely negative interactive model. Nonetheless, we feel that electronic factors, as yet unrecognized, still may be important.[10] In this regard, it is interesting to note that Scichida et al.[13] have found evidence for an electrostatic interaction between the retinylidene chromophore of a fluorinated retinal analogue and rhodopsin. They postulate that this may involve interaction of the high electron density associated with fluorine and either an ionized or unionized glutamic acid residue. Further, Dixon et al.[14] have proposed that binding of ligands to the β-adrenergic receptor is analogous to the interaction of retinal with rhodopsin, and have presented evidence that an aspartic acid and an asparagine residue are important for agonist binding.[15] We are exploring further the possible implications of these reports with respect to our fluorine-induced adrenergic selectivities.

In analyzing various proposals, it becomes clear that a central issue concerns whether or not the presence of an interaction of the ethanolamine side chain with an ortho-situated fluorine is necessary to induce adrenergic selectivity. The lack of a clear pattern of adrenergic selectivity shown by fluorinated analogues of dopamine suggested that such an interaction is necessary. To explore this issue further, we have initiated studies with other fluorinated analogues of potent adrenergic ligands wherein such direct interaction also is unlikely.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were obtained with a Beckman spectrometer IR 420 and NMR spectra were obtained with a Varian Model XL 300-MHz spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc. (Atlanta, GA) and results obtained were within ±0.4% of the theoretical values. Purity and identity of all compounds and intermediates were confirmed by mass spectrometry and thin-layer chromatography (TLC). Chemical-ionization mass spectra were obtained using a Finnigan 1015 mass spectrometer. TLC was performed on silica gel GF254 (Analtech), and spots were visualized by a UV lamp. Flash chromatography was performed with use of flash chromatography silica gel 60, 40–240 mesh from EM Reagents.

Fluoroveratraldehydes (2a,b). Titanium tetrachloride (15.6 g, 87 mmol) in 20 mL of anhydrous methylene chloride was added dropwise over 0.5 h to a solution of 7.79 g (49.88 mmol) of fluoroveratrole **1a**[8,16,17] in 50 mL of anhydrous methylene chloride

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under nitrogen and cooled with an ice–water bath. To the resulting bright orange solution was added dropwise over 15 min 5.0 mL (6.355 g, 55.28 mmol) of α,α-dichloromethyl methyl ether in 15 mL of anhydrous methylene chloride. The solution, which had turned red, was stirred at ice-bath temperature for 0.5 h, allowed to warm to room temperature, and stirred for an additional 4.5 h. The emerald-green solution was then poured into 200 g of cracked ice. The methylene chloride layer was separated, and the aqueous layer was extracted three times with ether. The combined organic fractions were washed three times with saturated sodium bicarbonate and once with brine. After drying over sodium sulfate, the solvent was removed and the residue was purified by flash chromatography with 15% ethyl acetate in petroleum ether as eluant. Crystallization from ethyl acetate/petroleum ether gave 6.75 g (74%) of aldehyde **2a** as a white crystalline solid: mp 52.5–53.5 °C (lit.[6] mp 52.5–53.5 °C). In a similar manner,[8] 10.42 g (66.73 mmol) of 6-fluoroveratrole **1b** was converted to 10.38 g of white crystalline 6-fluoroveratraldehyde **2b** (85% yield): mp 96–97.5 °C (lit.[8] 94–96 °C).

Preparation of Fluorinated *N*-Formyl-3,4-bis(benzyloxy)phenethanolamines (5a,b). A suspension of 444 mg (1.21 mmol) amine **4a**[5] in 10 mL of ethyl formate was refluxed for 4 h to give a homogeneous solution. TLC (silica gel, ethyl acetate) indicated that the reaction was complete. Ethyl formate was removed under vacuum, and the residue was crystallized from ethyl acetate/petroleum ether to give 395 mg (83%) of amide **5a** as a white crystalline solid: mp 96–97 °C; ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1 H, CHO), 7.41–7.26 (m, 10 H, Ar H), 7.14–7.08 (m, 1 H, Ar H), 6.78–6.75 (m, 1 H, Ar H), 5.83 (br, 1 H, NH), 5.12–5.02 (m, 5 H, 2 $ArCH_2$, ArCH), 3.76–3.68 (m, 1 H, OH), 3.50–3.39 (m, 2 H, CH_2N); IR (KBr) 3275 (OH), 1640 (C=O) cm^{-1}. Anal. ($C_{23}H_{22}NO_4F$) C, H, N. In a similar fashion, 317 mg of amine **4b**[1] was converted to 304 mg (89%) of white crystalline amide **5b**: mp 89–91 °C; ^{1}H NMR ($CDCl_3$) δ 8.09 (s, 1 H, CHO), 7.44–7.25 (m, 10 H, Ar H), 7.05 (d, 1 H, Ar H, J_{HF}^{meta} = 7.0 Hz), 6.65 (d, 1 H, Ar H, J_{HF}^{ortho} = 11.5 Hz), 5.76 (br m, 1 H, NH), 5.18–5.03 (m, 5 H, 2 $ArCH_2$, ArCH), 3.75–3.32 (m, 3 H, CH_2N, OH); IR (KBr) 3300 (OH), 1645 (C=O) cm^{-1}. Anal. ($C_{23}H_{22}NO_4F$) C, H, N.

Preparation of Fluorinated 3,4-Bis(benzyloxy)epinephrines (6a,b). A solution of 450 mg (1.14 mmol) of amide **5a** in 15 mL of anhydrous tetrahydrofuran was added dropwise to a stirred suspension of 250 mg of lithium aluminum hydride in 30 mL of anhydrous tetrahydrofuran while cooling with ice–water. The mixture was allowed to warm up to room temperature and then refluxed for 4 h. It was then cooled with ice–water, and the excess hydride was decomposed by the method of Fieser.[18] The inorganic salts were filtered and washed with hot ethyl acetate. The combined organic solution was dried over sodium sulfate and concentrated to give a white solid residue, which was crystallized from ethyl acetate/petroleum ether to give 198 mg (46%) of white crystalline compound **6a**: mp 90–92 °C; ^{1}H NMR ($CDCl_3$) 7.44–7.26 (m, 10 H, Ar H), 7.16–7.10 (m, 1 H, Ar H), 6.77–6.74 (m, 1 H, Ar H), 5.17–5.09 (m, 4 H, 2 $ArCH_2$), 4.97–4.93 (m, 1 H, ArCH) 2.87–2.81 (m, 1 H, OH), 2.71–2.64 (m, 2 H, CH_2N), 2.46 (s, 3 H, NCH_3); IR (KBr) 3280 (OH) cm^{-1}. ($C_{23}H_{24}NO_3F$) C, H, N. By the same procedure, 602 mg of **5b** was converted to 397 mg (68%) of white crystalline **6b**: mp 82–85 °C; ^{1}H NMR ($CDCl_3$) δ 7.44–7.25 (m, 10 H, Ar H), 7.11 (d, 1 H, Ar H, J_{HF}^{meta} = 7.1 Hz), 6.64 (d, 1 H, Ar H, J_{HF}^{ortho} = 11.5 Hz), 5.16–5.07 (m, 4 H, $ArCH_2$), 4.96–4.92 (m, 1 H, ArCH), 2.85–2.59 (m, 3 H, CH_2N, OH), 2.45 (s, 3 H, NCH_3); IR (KBr) 3280 (OH) cm^{-1}. Anal. ($C_{23}H_{24}NO_3F$) C, H, N.

Fluoroepinephrines (7a,b). A mixture of 170 mg (0.446 mmol of **6a**, 30 mg (0.238 mmol) of oxalic acid dihydrate, and 85 mg of 10% Pd/C in 50 mL of methanol was hydrogenated at 40 psi for 6 h. Filtration to remove the catalyst was done under a constant stream of nitrogen, and the solution was concentrated and filtered over a glass wool plug. Crystallization from methanol/ether afforded 95 mg (87%) of the oxalate salt of 2-FEpi **7a** as off-white-brown solid: mp 185–187 °C; ^{1}H NMR (D_2O) δ 6.83 (q, ABX, 1 H, J_{HH} = 8.04, J_{HF}^{meta} = 8.27 Hz), 6.81 (q, ABX, 1 H, J_{HH} = 8.04, J_{HF}^{para} = −1.82 Hz), 5.24–5.20 (m, 1 H, ArCH), 3.38–3.28 (m, 4 H, CH_2N, OH, NH), 2.79–2.77 (d, 3 H, NCH_3); IR (KBr) 3450 (OH) cm^{-1}. Anal. ($C_9H_{12}NO_3F{\cdot}0.5C_2H_2O_4$) C, H, N. In a similar manner, 283 mg (0.742 mmol) of amine **6b** was hydrogenated to give 133 mg (73%) of the oxalate salt of 6-FEpi **7b** as a white crystalline solid: mp 175–177 °C; ^{1}H NMR (D_2O) δ 6.96 (d, 1 H, Ar H, J_{HF}^{meta} = 7.1 Hz), 6.75 (d, 1 H, Ar H, J_{HF}^{ortho} = 11.2 Hz), 5.22–5.18 (m, 1 H, ArCH), 3.39–3.28 (m, 3 H, CH_2N, OH), 2.79 (s, 3 H, CH_3); IR (KBr) 3420 (OH) cm^{-1}. Anal. ($C_9H_{12}NO_3F{\cdot}0.5C_2H_2O_4$) C, H, N. C: calcd, 48.78; found, 48.27.

Biological. Determination of Agonist Response in the Isolated Guinea Pig Atrium. Atria were obtained from male, Harley guinea pigs (250–350 g) and individually bathed in 20-mL organ chambers containing a balanced salt solution made up with the following components (mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 0.5; NaH_2PO_4, 0.4; $NaHCO_3$, 12; and glucose, 5.6; the solution was maintained at 35 °C and aerated with 95% O_2/5% CO_2. The tissues were adjusted to a basal tension of 1 g and washed repeatedly for at least 60 min before measurements were performed. Cocaine (10 μM) was added to the balanced salt solution where indicated. Tension and contraction rate were monitored with a Grass Model 79D polygraph and Grass FT/3C force-displacement transducers. Rate measurements were taken 60 s after addition of the drug to the bath. Concentration–response curves were obtained at cumulative concentrations from 2.5 to 3000 nM. Tissues were washed in drug-free solution until the contraction rate returned to base line values between each response curve. Results were expressed as the increase in the percent of maximal rate of atrial contraction (PMR). Maximal rate was obtained by exposing the atria to 3–5 μM (−)-EPI. ED_{50} values were estimated graphically from plots of PMR vs amine concentration (nM).

Determination of [^{3}H]WB4101 Binding to Guinea Pig Cerebral Cortical Membranes. Measurement of [^{3}H]WB4101 (2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxane; sp. act. = 19.8 Ci/mmol, New England Nuclear, Boston, MA) binding to α_1-adrenergic receptors and displacement by FEPI's was carried out by the method of U'Prichard et al.[19] Brain membranes were prepared as follows: Male Hartley guinea pigs (175–200 g) were killed by decapitation, and the brain was quickly removed and placed in ice-cold 50 mM Tris·HCl buffer, pH 7.4. The cerebral cortex was dissected, and the tissue was disrupted in a Polytron (20 s at maximal speed). The homogenate was centrifuged at 10000*g* for 20 min and washed two times in fresh buffer (1 g/20 mL). The final membrane preparation was suspended in 40 mL of buffer to give approximately 0.9–1.2 mg of protein/mL. Under the conditions described by U'Prichard et al.[19] (20 min, 25 °C), the K_d of WB4101 was 1.4 nM, and specific binding, defined as that blocked by 1 μM phentolamine, represented 50–60% of total binding. Inhibition curves were performed with 0.22 nM [^{3}H]WB4101.

Determination of [^{3}H]Clonidine Binding to Guinea Pig Cerebral Cortical Membranes. Measurements of [^{3}H]clonidine (sp. act. = 20.4 Ci/mmol, New England Nuclear, Boston, MA) binding to α_2-adrenergic receptors in guinea pig cerebral cortical membranes (see above) was carried out according to the procedure of U'Prichard et al.[19] Under these conditions (20 min, 25 °C) the K_d for [^{3}H]clonidine was 5 nM, and specific binding, defined as that blocked by 10 μM clonidine, was 50–70% of the total binding. Inhibition curves were performed with 1.1 nM [^{3}H]clonidine.

Determination of Dihydro[^{3}H]alprenolol Binding to Rat Cerebral Cortical Membranes. Measurement of dihydro-[^{3}H]alprenolol (sp. act. = 35.6 Ci/mmol, New England Nuclear, Boston, MA) to β_1-adrenergic receptors in rat cerebral cortical membranes was carried out as described previously.[4] The buffer was 50 mM Tris·HCl (pH 7.4) containing 10 mg $MgCl_2$ and 1 mM EDTA. Under these conditions (30 min, 30 °C) the K_d for dihydro[^{3}H]alprenolol was 1 nM, and specific binding, defined as that blocked by 1 μM propranolol, was 75–82% of total binding.

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Inhibition curves were performed with 1 nM dihydro[^{3}H]alprenolol.

Determination of Phosphoinositide Turnover of Synaptoneurosomes. The procedure is described in detail elsewhere.[20] In brief, the pellet obtained from the synaptoneurosome preparation from one guinea pig cerebral cortex was resuspended in 10–15 mL of fresh buffer containing 1 μM [^{3}H]inositol. Aliquots of 320 μL of the suspension (~1 mg of protein) were distributed in 5 mL of polypropylene tubes and incubated at 37 °C. After 60 min, 20 μL of 200 mM LiCl were added to each tube. Ten minutes later, FEPIs were added in 20 μL of H_2O. The final incubation volume was 400 μL. The tubes were gassed briefly with 95% CO_2/5% CO_2, capped, and incubated for 90 min at 37 °C. At the end of the incubation period the tubes were centrifuged, and the tissue was washed with fresh buffer to remove the free [^{3}H]inositol. Then, 1 mL of 6% trichloroacetic acid was added, and the tubes were vortexed and centrifuged. Inositol phosphates were analyzed in the supernatant according to the method of Berridge et al.[21] Anion exchange columns (AG 1-X8, formate form) were used to separate the inositol phosphates. The trichloroacetic acid supernatant was added to the column. After being washed four times with 3 mL of water to elute free [^{3}H]-inositol, [^{3}H]inositol 1-phosphate was eluted with 2 mL of 200 mM ammonium formate/100 mM formic acid. This eluate was collected in vials. Hydrofluor was added (8 mL), and radioactivity was determined by liquid scintillation counting. The trichloroacetic acid precipitate (see above) was resuspended in 0.5 mL of a mixture of aqueous 1 M KCl containing 10 mM inositol and methanol (1:1), and 0.5 mL of chloroform was added. The tubes were mechanically shaken for 5 min and then centrifuged in order to separate the two phases. Aliquots (200 μL) from the chloroform phase were placed in scintillation vials and evaporated at room temperature. Betafluor was added, and radioactivity was determined by liquid scintillation counting to provide an index of [^{3}H]inositol incorporation into [^{3}H]phosphoinositide lipids. Results are expressed as cpm of [^{3}H]inositol phosphates per 10 000 cpm of radioactivity incorporated into lipids or, alternatively, as percentage of control response.

Determination of Inhibition of Adenylate Cyclase in Platelet Membranes. α_2-Adrenergic inhibition of adenylate cyclase was determined in human platelet membranes as described.[22] In brief, adenylate cyclase was measured in a medium containing 0.1 mM [α-^{32}P]ATP (0.3–0.4 μCi/tube), 0.1 mM cyclic AMP, 1 μg/mL adenosine deaminase, 0.1 mM rolipram, 0.2 mM EGTA, 5 mM creatine phosphate as Tris salt, 0.4 mg/mL creatine kinase, 2 mg/mL bovine serum albumin, 1 μM GTP, 10 μM forskolin, 1 mM $MgCl_2$ and 50 mM Tris·HCl, pH 7.4, in total volume of 100 μL. Incubations were initiated by the addition of platelet membranes (10–15 μg of protein/tube) to reaction media that had been preincubated for 5 min at 37 °C. Reactions were stopped by the addition of 0.4 mL of 125 mM zinc acetate and 0.5 mL of 144 mM Na_2CO_3. Cyclic AMP was purified as described.[23]

Determination of Cyclic AMP Accumulation in Glioma Cells. The C6 glioma cells were cultured as described.[24] The day before the experiment the cells were subcultured in multidish trays. The day of the experiment the medium was removed, and cells were washed twice with buffer A (118 mM NaCl, 4.7 mM KCl, 3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 0.5 mM EDTA, 10 mM glucose, and 20 mM HEPES, pH 7.4). One milliliter of fresh buffer A plus 30 μM rolipram (a phosphodiesterase inhibitor) was added to each well. After 10 min agents were added in 10 μL. Incubations were stopped after 10 min by removing the buffer and adding 1 mL of 0.1 M HCl to each well. After 30 min, cyclic AMP levels were determined in the neutralized media with a commercially available kit (Amersham, Arlington Heights, IL).

Registry No. **1a**, 394-64-9; **1b**, 398-62-9; **2a**, 37686-68-3; **2b**, 71924-62-4; **3a**, 61338-96-3; **3b**, 71924-67-9; **4a**, 81441-11-4; **4b**, 71924-68-0; **5a**, 115562-22-6; **5b**, 115562-22-6; **6a**, 115562-23-7; **6b**, 115562-27-1; **7a**, 115562-24-8; **7a** (oxalate), 115562-25-9; **7b**, 115562-29-3; **7b** (oxalate), 115562-28-2; cAMP, 60-92-4; adenylate cyclase, 9012-42-4.

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