Acknowledgment. We are grateful to Dr. Garland Marshall and to Tripos Associates (St. Louis, MO) for a grant of the SYBYL software system and for helpful discussions. Research support from the Kansas Advanced Technology Commission made possible some of the hardware purchases for the University of Kansas Molecular Graphics and Modeling Laboratory in the Department of Medicinal Chemistry. Research Grants HL 34193 and GM 22988 from the Public Health Service, grants-in-aid from the American Heart Association and its Kansas Affiliate, and financial support from the University of Kansas General Research Fund made this work possible.

Synthesis and Adrenergic Activity of Ring-Fluorinated Phenylephrines

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2-Fluoro-, 4-fluoro-, and 6-fluorophenylephrine (6-FPE) were synthesized from the corresponding fluorinated 3-hydroxybenzaldehydes. New routes to 2-fluoro- and 6-fluoro-3-hydroxybenzaldehydes were developed based on regioselective lithiation of 2- and 4-[(dimethyl-*tert*-butylsilyl)oxy]fluorobenzene ortho to fluorine. As with norepinephrine and isoproterenol analogues, the adrenergic properties of phenylephrine were markedly altered by ring fluorination. The order of potency of the fluoro analogues as α_1-adrenergic agonists in the stimulation of contraction of aortic strips and of phosphatidylinositol turnover and potentiation of cyclic AMP accumulation in guinea pig synaptoneurosomes was 6-FPE > PE > 4-FPE > 2-FPE. The same pattern was observed for the displacement of radioligands specific for α_1- and α_2-adrenergic receptors on brain membranes. The order of potency for the displacement of [^{3}H]dihydroalprenolol, a β-specific adrenergic ligand from brain membranes, was 2-FPE > 4-FPE = PE ≫ 6-FPE. 6-FPE was much more selective for α-adrenergic receptors compared to β-receptors than was phenylephrine. A rationale for the observed fluorine-induced alterations in potency and selectivity of the FPEs for α- and β-adrenergic systems is presented based on fluorine-induced conformations due to electrostatic repulsion of fluorine and the benzyl hydroxyl group.

In 1979, we reported that ring-fluorinated analogues of norepinephrine display striking adrenergic agonist specificities that are dependent on the site of the fluorine substituent.[1] Thus, 2-fluoronorepinephrine (2-FNE) exhibits nearly pure β-adrenergic agonist properties, while 6-fluoronorepinephrine (6-FNE) exhibits predominantly α-adrenergic properties in several systems.[1-4] The adrenergic agonist properties of 5-fluoronorepinephrine (5-FNE) are quite similar to those of norepinephrine (NE), although a 2-7-fold greater potency relative to NE has been observed in some β-adrenergic systems. Several studies in both peripheral and central systems, in vivo and in vitro, have demonstrated the usefulness of these analogues as pharmacological tools.[3]

This remarkable and unexpected "fluorine effect" provides potential information regarding the structural and electronic requirements of α- vs. β-adrenergic receptor interactions with NE. Extension of investigation of the fluorine effect to isoproterenol (ISO), a specific and potent β-adrenergic agonist, provided further insights.[5] The 6-fluoro substituent in 6-FISO reduced β-adrenergic potency compared to ISO, while 2-FISO and 5-FISO were essentially identical in activity as β-adrenergic agonists to ISO itself. None of the FISOs exhibited significant α-adrenergic potency, suggesting that the effect of 6-fluorine substitution was to inhibit interaction with the β-adrenergic receptor, i.e., that the effect is "negative". Such an interpretation could also be used to explain the activity of the FNEs in that the substitution of fluorine in the 6-position in this series does ablate interaction with the β-adrenergic receptor. Conversely, a 2-fluoro substituent has a "negative" effect on interactions of NE with the α-adrenergic receptor.

We have now extended this study to the classic α-adrenergic agonist phenylephrine (PE).[3,6] This also represents the first noncatechol system we have investigated. While our primary interest concerns the adrenergic agonist properties of 2-FPE and 6-FPE (numbering such that the phenolic group is assigned the 3-position), the absence of the 4-hydroxyl group permits a new pattern of fluorine substitution, i.e., with fluorine in the 4-position. In this paper, we describe the synthesis of 2-FPE (**2a**), 4-FPE (**2b**), and 6-FPE (**2c**) and their α-adrenergic agonist properties in the aortic strip and brain preparations. We have compared the agonist properties with binding efficacies to α_1-, α_2-, and β-adrenergic receptors.

Table I. Ultraviolet Spectral Data[a]

compd (solvent)[b]	λ_{max}, nm	ϵ	compd (solvent)[b]	λ_{max}, nm	ϵ
2a (A)	270.5	1200	**2c** (A)	279.5	2830
2a (B)	286.5	2100	**2c** (B)	300.5	3600
2b (A)	270.5	1750	1 (A)	272.5	1700
2b (B)	286.5	3460	1 (B)	291.0	3100

[a] No attempts were made to account for possible effects of side-chain amine protonation. [b] Solvents: A, H_2O; B, 0.1 N NaOH.

Chemistry. The synthetic approach to **2a-c** (UV spectral data in Table I) was based on the corresponding benzyloxyfluorobenzaldehydes (**3a-c**) as key intermediates. Of these, **3b** and **3c** were prepared by introduction of the hydroxyl group into commercially available fluorobenz-

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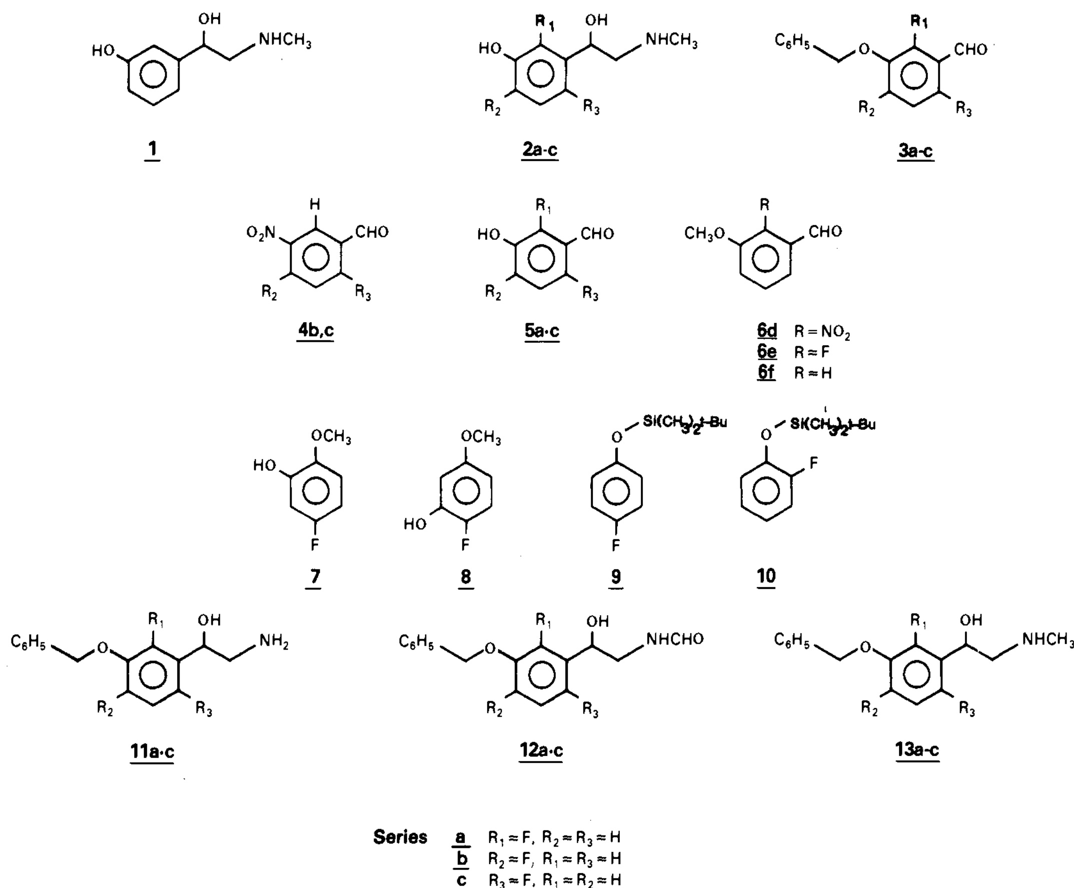

Figure 1. Structures of compounds mentioned in this paper.

aldehydes. Nitration of 4-fluorobenzaldehyde gave 3-nitro-4-fluorobenzaldehyde (**4b**).[7] After protection of the aldehyde by acetal formation, the nitro group was hydrogenated. The intermediate amino acetal was diazotized, and the diazonium salt was decomposed by the procedure of Cohen et al.[8] to give 4-fluoro-3-hydroxybenzaldehyde (**5b**), benzylation of which gave the desired **3b**. A parallel procedure, starting with 2-fluorobenzaldehyde,[9] was used to prepare **3c** through the intermediates **4c** and **5c** (see Figure 1).

Since there were no readily apparent routes to **3a** from commercially available fluorinated precursors, we chose to introduce fluorine into a protected 3-hydroxybenzaldehyde using our previously described photochemical variant of the Schiemann reaction. 3-Methoxy-2-nitrobenzaldehyde (**6d**) was converted to the acetal. Hydrogenation gave the amino acetal, which was diazotized in fluoroboric acid and irradiated in situ by the usual procedure. In this case, the desired fluoroanisaldehyde **6e** was accompanied by significant amounts of *m*-anisaldehyde (**6f**). Unfortunately, the solubilities and chromatographic behavior of **6e** and **6f** were quite similar, and pure **6e** could be obtained only after tedious chromatographic procedures. Demethylation of **6e** followed by benzylation of the phenol gave **3a**, but the synthetic sequence clearly suffered from the inconvenience of the initial separation procedure as well as a small overall yield.

In another project, we required 5-fluoroguaiacol (**7**). Lithiation of 4-fluoroanisole followed by reaction with trimethyl borate and oxidation was expected to proceed cleanly ortho to the methoxyl group to give **7**.[10] To our surprise, the product was contaminated with significant amounts of another product tentatively identified as the regioisomer **8**, suggesting to us that lithiation was occurring to some extent ortho to fluorine. From this we have developed a new strategy for the synthesis of **3a** as well as **3c**. Replacement of the methyl ether of 2- and 4-fluoroanisole with the bulky *tert*-butyldimethylsilyl ether permits regioselective lithiation ortho to fluorine. Thus, lithiation of **9** and **10** with *sec*-butyllithium at −78 °C, followed by quenching of the carbanion with dimethylformamide (DMF) and fluoride desilylation, gives excellent yields of the fluorobenzaldehydes **5c** and **5a**, respectively. The structures of these aldehydes are readily secured by their identities to the products obtained by the alternate synthetic routes. In addition, coupling constants derived from first-order analysis of the NMR spectrum of **5a** (and of **5b**) are completely consistent with the assigned structure. Because H-4 and H-5 have similar chemical shifts, the spectrum of **5c** is not first order (see Experimental Section for NMR data).

Zinc iodide catalyzed addition of trimethylsilyl cyanide to the fluorobenzaldehydes **3a–c** followed by in situ lithium aluminum hydride reduction gave the amino alcohols **11a–c**. N-Formylation was achieved by refluxing solutions of **11a–c** in ethyl formate to give **12a–c**, lithium aluminum hydride reduction of which produced the *O*-benzylphenylephrines **13a–c**. Hydrogenolysis over Pd on carbon gave **2a–c** isolated as either the oxalate or free base (see Experimental Section).

The presence of fluorine ortho to the phenolic group in **2a** and **2b** causes the expected increase in phenol acidity. The spectrophotometrically determined pK_a values are as follows: **1**, 9.63; **2a**, 8.31; **2b**, 8.17; **2c**, 9.56 (see Experi-

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Table II. Effect of Fluorine Substitution on the Affinity of Phenylephrine in Cerebral Cortical Membranes

compd	displacement of receptor-specific ligands (K_i),[a] μM			
	α_1 [^{3}H]WB 4101	α_1 [^{3}H]prazosin	α_2 [^{3}H]clonidine	β [^{3}H]dihydroalprenolol
(−)-PE	2.5 ± 0.2	6.1 ± 0.8	0.39 ± 0.10	13
(±)-2-FPE[b]	6.6, 6.8	17 ± 3	1.2 ± 0.02	4.0
(±)-4-FPE[b]	3.7	10 ± 0.6	1.0	19
(±)-6-FPE[b]	1.3 ± 0.12	2.1 ± 0.2	0.23 ± 0.01	180

[a] K_i values were calculated from IC_{50} values using the Cheng–Prusoff equation and are either means ± SEM for 3–5 experiments or are from a single experiment each point determined in triplicate. [b] In the case of the FPEs, the IC_{50} values for the active *R* enantiomer should be half the value for the racemate.

Table III. Effect of Fluorine Substitution on the Agonist Potency of Phenylephrines (PE)

PE	potency of PE (EC_{50}), μM		stimulation of [^{3}H]inositol phosphate accumulation[b] (% control at 100 μM PE)
	aortic contraction[a]	augmentation of [^{3}H]cyclic AMP accumulation	
(−)-PE	27 ± 2	26	161 ± 5
(±)-2-FPE[c]	105 ± 22	40	134 ± 8
(±)-4-FPE[c]	39 ± 11	35	136 ± 2
(±)-6-FPE[c]	11 ± 2	4.0	162 ± 4

[a] Values are means ± SEM for three experiments or are from a single experiment each point determined in triplicate. [b] Efficacy measured at single concentration of 100 μM for PE or FPE. [c] In the case of the FPEs, the EC_{50} values for the active *R* enantiomer should be half the value for the racemate.

mental Section). The NMR data for **2a–c** are given in the Experimental Section and are completely consistent with the assigned structures, although analyses of the spectra in the aromatic region were not carried out due to complexity resulting from overlapping chemical shifts in these four-spin systems.

Biology. The effects of fluorine substitution on the adrenergic activities of phenylephrines were examined in several systems. Direct interactions of **2a–c** and **1** with α_1-, α_2-, and β-adrenergic receptors were measured by using specific radioligand displacement techniques with brain membranes (see Experimental Section). The α_1-adrenergic agonist potencies were compared by measurement of contraction of guinea pig aortic strips and stimulation of phosphatidylinositol turnover and potentiation of 2-chloroadenosine stimulation of cyclic AMP accumulation in guinea pig cerebral cortical synaptoneurosomal preparations (see Experimental Section).

All the FPEs displaced the α_1-adrenergic specific ligands [^{3}H]WB 4101 and [^{3}H]prazosin and the α_2-specific ligand [^{3}H]clonidine from receptor sites in guinea pig cerebral cortical membranes (Table II). While the affinity of the FPEs was 5–10-fold greater for α_2-adrenergic receptors compared to α_1-adrenergic receptors, the order of potency for both α_1- and α_2-adrenergic receptors was the same: 6-FPE > (−)-PE > 4-FPE > 2-FPE.

The EC_{50} values obtained for the FPEs in the isolated guinea pig aortic preparation are shown in Table III. All of the FPEs proved to be full α_1-adrenergic agonists in this system, each eliciting the same maximal contraction of the aortic preparation as does NE. The response to FPEs was insensitive to propranolol (5 μM) and completely blocked by phentolamine (5 μM). The order of potency was 6-FPE > (−)-PE $\simeq$ 4-FPE > 2-FPE.

The results obtained for the augmentation by the FPEs of the accumulation of [^{3}H]cyclic AMP elicited by 10 μM 2-chloroadenosine in synaptoneurosomes from guinea pig cerebral cortex are shown in Table III. The potencies of the FPEs in eliciting this α_1-adrenergic response[11] were 6-FPE > (−)-PE > 4-FPE $\simeq$ 2-FPE. The stimulation of [^{3}H]phosphatidylinositol turnover in synaptoneurosomes by FPEs was ascertained at a single concentration of 100 μM (Table III). 6-FPE and (−)-PE caused the largest stimulation for this α_1-adrenergic response[11] with 2- and 4-FPE less effective.

All the PEs displaced the β-adrenergic specific ligand, [^{3}H]dihydroalprenolol, from receptor sites in rat cerebral cortical membranes (Table II). The majority of such binding sites have characteristics of β_1-adrenergic receptors.[4] The order of potency was 2-FPE > (−)-PE > 4-FPE ≫ 6-FPE. It should be noted that (±)-2-FPE (K_i = 4 μM) shows increased affinity, while (±)-6-FPE (K_i = 180 μM) shows greatly reduced affinity compared to (−)-PE (K_i = 13 μM) for the β_1-adrenergic receptor.

Discussion

Our present results demonstrate further dramatic effects of fluorine substitution on the adrenergic receptor specificities of biogenic amines. However, the fluorine effects, for the first time, are reflected in a significant increase, rather than a decrease, in agonist potencies. Our initial results with isolated aortic preparations, in which responses are due to α_1-adrenergic activity, suggested that 2-FPE had the expected decrease in α-adrenergic potency and that 6-FPE was comparable to PE in α-adrenergic potency.[3] It is now apparent that while (±)-2-FPE is less potent than (−)-PE in aortic preparations, (±)-6-FPE is 2-fold more potent than (−)-PE (Table III). Indeed, since probably only the *R* enantiomer of the racemic FPEs is active, then (*R*)-6-FPE would be 4-fold more potent than (−)-(*R*)-PE at the α_1-adrenergic receptors mediating contraction of aortic strips. As measured directly by displacement of receptor specific radioligands, 2-FPE and PE are essentially equipotent at both α_1- and α_2-adrenergic receptors, while 6-FPE is 4–6 times more potent in the displacement of α_1- and α_2-specific ligands than is PE, based on the assumption that the activity of racemic FPEs is limited to the *R* enantiomer (Table II). It should be noted that while phenylephrine is often considered as selective for (postsynaptic) α_1-adrenergic receptors (see, however, ref 12 and references therein), both phenylephrine and the FPEs are actually more potent in displacing binding of [^{3}H]clonidine from α_2-adrenergic receptors than in displacing either [^{3}H]WB 4101 or [^{3}H]prazosin from α_1-ad-

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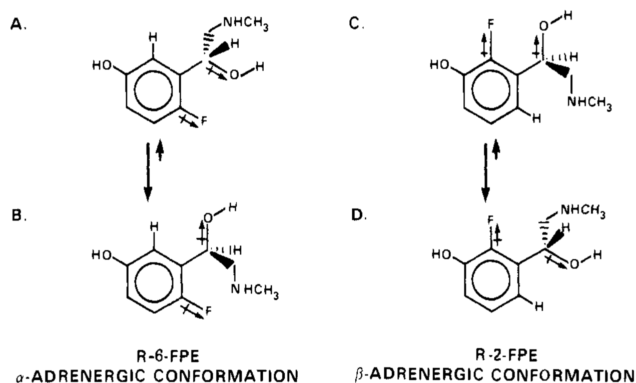

Figure 2. Conformations of **2a–c** (see text for details).

renergic receptors in brain membranes. The α_2-adrenergic agonist activity of phenylephrine, manifest in inhibition of adenylate cyclase, has been demonstrated.[13]

Fluorine substitution in PE exerts a negative effect on β-adrenergic potency as shown by the fact that 6-FPE is approximately 10 times less effective in displacing the β-adrenergic receptor ligand [^{3}H]dihydroalprenolol than is PE (Table II). Indeed, (±)-6-FPE is a much more selective α-adrenergic receptor agonist than is (–)-PE. Thus (±)-6-FPE shows an α_1/β selectivity of 86–140-fold and an α_2/β selectivity of 780-fold in the binding assays, while (–)-PE shows an α_1/β selectivity of only about 2–5-fold and an α_2/β selectivity of 33-fold.

There are theoretical, as well as practical, implications of these results. We have discussed in previous reports possible mechanisms for fluorine-induced alteration of agonist specificities.[3,4] Our recent suggestion[5] that fluorine inhibits specific electrophilic interactions of the receptor with the aromatic ring, thus causing the observed reduction in binding efficiency, is not consonant with the observed increase in agonist potencies observed in the PE series. In 1983, Buhler and Thakker et al.[14] reported direct measurement of fluorine-induced conformational effects in a polycyclic system, which effects they ascribed to electrostatic repulsion of fluorine and a benzylic hydroxyl group. It was pointed out to us that similar interactions and conformational changes in fluorinated amines might be responsible for our observed alterations in agonist specificities (D. Thakker, personal communication). Indeed, DeBernardis et al.[15] have recently described conformationally defined adrenergic agonists with an observed pattern of adrenergic receptor binding specificities consistent with the same electrostatic repulsion model and did propose such a model. The conformations of **2a-c** that would result from such repulsive interaction are shown in Figure 2. The preferred conformations, B and D, are shown with the benzylic hydroxyl group eclipsed with the ortho proton, which places the carbon–nitrogen bond out of the plane of the aromatic ring. While electrostatic repulsion in A and C would be most severe in the eclipsed conformation, obviously intermediate conformations would also be destabilized. However, the skewed rotamer having the amino group antiplanar to the aromatic ring would not seem likely to be dramatically affected by electrostatic repulsion. The proposal that adrenergic agonist specificities result from conformational changes produced by repulsive interactions of the benzylic hydroxy group and fluorine in the 2- or 6-position also is consistent with our previous observation that ring-fluorinated dopamines, which lack a benzylic hydroxy group, do not exhibit selective adrenergic agonist specificities.[4,16] We are in the process of preparing additional analogues designed to test this proposal.

A further point concerns the fact that in the NE and ISO series, no fluorinated analogue exhibited significant increases in adrenergic agonist potency, with the exception of increased β-adrenergic activity seen in some systems with 5-FNE. It is possible that PE, as a relatively weak agonist, is more sensitive to favorable conformational effects than ISO or NE. It is not surprising that the very potent and specific β-adrenergic agonist ISO, the specificity of which is conferred by the *N*-isopropyl group, which blocks interaction with the α-adrenergic receptor and enhances interaction with the β-adrenergic receptor, would not exhibit enhanced α-adrenergic activity in spite of these postulated conformational effects.

PE is itself an important pharmacological agent with widespread applications as a selective α-adrenergic agonist. Fluorine substitution at the 6-position results in an increase in potency at both α_1- and α_2-adrenergic receptors and a marked increase in selectivity due to a decrease in interaction at β-adrenergic receptors. From a practical standpoint, 6-FPE thus should have considerable potential as a *specific* α-adrenergic agonist. The results of a series of pharmacological studies designed to explore this potential with responses involving α_1-, α_2-, and β-adrenergic receptors will be published shortly.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Initial identification of all intermediates and products was ascertained by chemical ionization mass spectrometry using a Finnigan 1015 mass spectrometer. NMR spectra were obtained with a Varian Model XL 300-MHz spectrometer. Thin-layer chromatography was performed on silica gel GF_{254} (Analtech), and spots were made visible by a UV lamp. Ultraviolet spectra were recorded on a Beckman DU-7 spectrophotometer.

2-Fluoro-5-hydroxybenzaldehyde (5c). Hydrolysis of Diazonium Salt. A solution of 8.45 g (0.05 mol) of 2-fluoro-5-nitrobenzaldehyde (**4c**)[9] in 250 mL of absolute methanol was stirred overnight with 5 g of Dowex 50 H^+ ion exchange resin. The solution was filtered, 0.5 mL of triethylamine added, and the nitro group hydrogenated over 200 mg of PtO_2 (Parr apparatus, overnight). Removal of catalyst and solvent gave 2-fluoro-5-aminobenzaldehyde dimethyl acetal, used in the next step without purification.

Following the procedure of Cohen et al.,[8] the amino acetal from above was dissolved in 50 mL of concentrated sulfuric acid and 50 g of ice was added. The solution was cooled to 0 °C and, with stirring, 4.4 g of sodium nitrite in 50 mL of water was added dropwise under the surface of the liquid, while the temperature was maintained between 0 and 5 °C. After stirring an additional 5 min, excess nitrite was decomposed with a few crystals of urea. To this solution was added 189 g of cupric nitrate dissolved in 1.77 L of water. With vigorous stirring, 6.69 g of cuprous oxide was added. After evolution of nitrogen had ceased, the solution was extracted 4 times with ether. The ether was extracted with 1 N sodium hydroxide until thin-layer chromatography showed complete extraction of phenol. Acidification of the basic extract and extraction with ether, after drying (sodium sulfate), removal of solvent, and silica gel chromatography (ethyl acetate/carbon tetrachloride, 1:9), gave pure **5c**: 3.01 g (43%); mp 74–75 °C

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(ether/petroleum ether); NMR ($CDCl_3$) δ 10.04 (s, 1 H, CHO), 7.31 (br dd, 1 H, H-2, J_{HF}^m = 4.7 Hz, J_{HH}^m = 2.7 Hz), 7.05–7.15 (m, 2 H, H-4,5). Anal. ($C_7H_5FO_2$) C,H.

4-Fluoro-3-hydroxybenzaldehyde (5b). By an analogous procedure, from 5.85 g (35 mmol) of 4-fluoro-3-nitrobenzaldehyde **4b**,[7] there was prepared 228 mg (5%) of **5b**: mp 109–110 °C (ether/petroleum ether); NMR ($CDCl_3$) δ 9.90 (s, 1 H, CHO), 7.56 (dd, 1 H, H-2, J_{HF}^m = 8.4 Hz, J_{HH}^m = 2.0 Hz, H-2), 7.45 (octet (ddd), 1 H, J_{HF}^m = 5.0 Hz, J_{HH}^o = 8.3 Hz, J_{HH}^m = 2.0 Hz, H-6), 7.25 (dd, 1 H, J_{HF}^o = 10.0 Hz, J_{HH}^o = 8.3 Hz, H-5). Anal. ($C_7H_5FO_2$) C,H.

2-Fluoro-3-hydroxybenzaldehyde (5a) Using the Photochemical Schiemann Reaction. 3-Methoxy-2-nitrobenzaldehyde (9 g, 0.05 mol) was converted to the dimethyl acetal and hydrogenated as above. The resulting 2-amino-3-methoxybenzaldehyde dimethyl acetal was dissolved in 250 mL of 50% fluoroboric acid; the solution was cooled to −10 °C, and 3.8 g (0.055 mol) of sodium nitrite dissolved in 10 mL of water was added. After storing at −4 °C overnight, the solution was irradiated (Hanovia 450-W medium-pressure mercury lamp, Corex filter) at 5–10 °C for 4 h.[17] Neutralization of the chilled solution and extractions as described above gave crude product containing **6e** and **6f**. Preparative thin-layer chromatography (silica gel; ether/petroleum ether, 1:5; five elutions) gave 300 mg (4.0%) of 2-fluoro-3-methoxybenzaldehyde (**6e**) as a partially crystalline solid.

A 248-mg sample of **6e** in 3 mL of methylene chloride was chilled to −78 °C under argon and, while stirring, was treated with 0.2 mL of boron tribromide. After stirring at room temperature overnight, the solution was cooled and excess boron tribromide was decomposed with water. Extraction as described above gave 179 mg (79%) of **5a**: mp 112–113 °C (ether/petroleum ether); NMR ($CDCl_3$) δ 10.06 (s, 1 H, CHO), 7.40 (heptet (overlapping ddd), 1 H, J_{HF}^m = 6.8 Hz, J_{HH}^o = 8.4 Hz, J_{HH}^m = 1.6 Hz, H-6), 7.29 (hextet (overlapping ddd), 1 H, J_{HF}^m = 7.8 Hz, J_{HH}^o = 8.2 Hz, J_{HH}^m = 1.6 Hz, H-4), 7.17 (br t, 1 H, J_{HH}^o = 8.2, 8.4 Hz, H-5). Anal. ($C_7H_5FO_2$) C, H.

2-Fluoro-3-hydroxybenzaldehyde (5a) from 2-Fluorophenol. A solution of 11.2 g (0.10 mol) of 2-fluorophenol in 50 mL of dimethylformamide was treated with 7.5 g (0.11 mol) of imidazole and 16.58 g (0.11 mol) of *tert*-butyldimethylsilyl chloride. A mildly exothermic reaction ensued. After the solution was stirred overnight, water (250 mL) was added and the mixture was extracted 3 times with petroleum ether. The organic layer was washed once with water, 3 times with 10% sodium carbonate, and 3 times with water and was dried (sodium sulfate). Removal of solvent gave chromatographically pure **10**, used without further purification in the next step.

To a solution of 5.65 g (25 mmol) of **10** in 25 mL of dry tetrahydrofuran stirred under argon and cooled in a dry ice–acetone bath was added dropwise over 10 min 19.5 mL (27.3 mmol) of 1.4 M *sec*-butyllithium in hexane. After stirring for 0.5 h, an excess (2 mL) of DMF was added. After 5 min, the reaction was treated dropwise over 10 min with 30 mL of 1 M tetrabutylammonium fluoride in tetrahydrofuran. The reaction was allowed to warm to room temperature, and 50 mL of water was added. After removal of most of the tetrahydrofuran in vacuo, 25 mL of 1 N sodium hydroxide was added and the basic solution was washed 3 times with 50 mL of ether. The ether was washed 2 times with 25 mL of 1 N sodium hydroxide. The combined sodium hydroxide solutions were acidified with 3 N hydrochloric acid, and the product was extracted 4 times with 50 mL of ether. After drying (sodium sulfate), removal of solvent gave 3.03 g (21.6 mmol, 86%) of **5a**. This material was identical (mp, mmp, TLC, and spectral data) to a sample prepared in the previous procedure.

2-Fluoro-5-hydroxybenzaldehyde (5c) from 4-Fluorophenol. By a similar procedure, 5.65 g of 4-fluorophenol was converted to **5c** in 67% yield, identical to the material prepared by the alternate procedure above.

3-Benzyloxy-2-fluorobenzaldehyde (3a). To a solution of 1.10 g (7.85 mmol) of **5a** in 5 mL of acetone was added 1.4 g (10 mmol) of potassium carbonate and 1.47 g (8.6 mmol) of benzyl bromide. The solution was stirred at room temperature overnight, after which time thin-layer chromatography (silica gel; ether/petroleum ether, 1:1) showed the reaction had reached completion. Water (20 mL) was added, and the acetone was removed in vacuo at room temperature. The solid product was recovered by filtration and recrystallized to give 1.25 g of **3a** (69%): mp 88–89 °C (cyclohexane). Anal. ($C_{14}H_{11}FO_2$) C, H.

3-Benzyloxy-4-fluorobenzaldehyde (3b) and 5-benzyloxy-2-fluorobenzaldehyde (3c) were prepared from **5b** and **5c**, respectively, by the method described above for the synthesis of **3a**. From 328 mg of **5b** (2.34 mmol) there was obtained 398 mg (74%) of **3b**: mp 66–67 °C (cyclohexane). Anal. ($C_{14}H_{11}FO_2$) C, H. From 560 mg of **5c** (4.0 mmol) there was obtained 695 mg (76%) of **3c**: mp 72–74 °C (cyclohexane). Anal. ($C_{14}H_{11}FO_2$) C, H.

Preparation of Fluorinated 3-Benzyloxyphenethanolamines (11a–c). The elaboration of the ethanolamine side chain was carried out by using the procedure described in our synthesis of fluorinated norepinephrines[2] and detailed here for the synthesis of **11c**. A mixture of 600 mg of **3c** and 1 mL of trimethylsilyl cyanide, stirred under argon, was treated with ~10 mg of anhydrous zinc iodide. A mildly exothermic reaction ensued, and the mixture became homogenous. After stirring overnight, the excess trimethylsilyl cyanide was removed under vacuum. The residue was dissolved in 10 mL of ether and was added to a suspension of 300 mg of lithium aluminum hydride in 25 mL of ether. The reaction was refluxed for 3 h, then cooled, and the excess hydride decomposed using the procedure of Fieser.[18] After filtration, the insoluble aluminum salts were washed 3 times with 25 mL of hot ethyl acetate. The combined organic filtrates were dried (sodium sulfate) and evaporated. Recrystallization of the crude product gave 438 mg (64%) of **11c**: mp 108–109 °C (cyclohexane/ethyl acetate). Anal. ($C_{15}H_{16}FNO_2$) C, H, N.

Similarly, 1.25 g of **3a** was converted to 975 mg (69%) of **11a**: mp 93–94 °C (cyclohexane/ethyl acetate). Anal. ($C_{15}H_{16}FNO_2$) C, H, N. From 580 mg of **3b** there was obtained 491 mg (75%) of **11b**: mp 66–68 °C (cyclohexane/ethyl acetate). Anal. ($C_{15}H_{16}FNO_2$) C, H, N.

Preparation of Fluorinated *N*-Formyl-3-benzyloxyphenethanolamines (12a–c). Refluxing solutions of **11a–c** in ethyl formate produced the corresponding *N*-formyl derivatives, **12a–c**. Thus, a suspension of 600 mg of **11c** in 30 mL of ethyl formate was refluxed for 6 h, after which time the solution was homogenous and thin-layer chromatography (silica gel, ethyl acetate) showed the reaction was complete. Ethyl formate was removed in vacuo, and the solid residue was recrystallized from ether/petroleum ether to give 480 mg (72%) of **12c**: mp 97–98 °C (ether/petroleum ether). Anal. ($C_{16}H_{16}FNO_3$) C, H, N. In similar fashion, 975 mg of **11a** was converted to 874 mg (81%) of **12a**: mp 89–91 °C (cyclohexane/ethyl acetate). Anal. ($C_{16}H_{16}FNO_3$) C, H. From 490 mg of **11b** there was produced 307 mg (57%) of **12b**: mp 76–78 °C (ether/petroleum ether). Anal. ($C_{16}H_{16}FNO_3$) C, H, N.

Preparation of Fluorinated *O*-Benzylphenylephrines (13a–c). Lithium aluminum hydride reduction of **12a–c** produced the corresponding *N*-methyl analogues. The preparation of **13c** illustrates the procedure. A solution of 600 mg of **12c** in 30 mL of anhydrous tetrahydrofuran was added dropwise to a cold stirred suspension of 300 mg of lithium aluminum hydride in 50 mL of tetrahydrofuran. The mixture was refluxed for 4 h, then cooled in ice, and the excess hydride was decomposed by the method of Fieser.[18] The inorganic salts were filtered and washed 3 times with hot ethyl acetate. The combined organic solution was dried (sodium sulfate) and evaporated. The solid residue was recrystallized from cyclohexane to give 465 mg (81%) of **13c**: mp 97–98 °C (cyclohexane). Anal. ($C_{16}H_{11}FNO_2$) C, H, N.

By the same procedure, 1.16 g of **12a** was converted to 976 mg (88%) of **13a** as a noncrystalline solid. This was characterized as the oxalate (**12a**·$H_2C_2O_4$), prepared in 54% yield from **12a** by adding equimolar amounts of oxalic acid dissolved in ethanol to an ethanol solution of **12a**, chilling, and isolating the white crystals by filtration: mp 171–172 °C (ethanol). Anal. ($C_{16}H_{18}FNO_2$·$H_2C_2O_4$) C, H, N. C: calcd, 59.17; found, 59.81.

As above, 307 mg of **12b** was converted to 205 mg (70%) of **13b**:

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mp 96–97 °C (cyclohexane/ethyl acetate). Anal. ($C_{16}H_{18}FNO_2$) C, H, N.

2-Fluorophenylephrine·0.5$H_2C_2O_4$ (2a·0.5$H_2C_2O_4$). A solution of 260 mg of 13a·$H_2C_2O_4$ in 100 mL of ethanol was hydrogenated over 50 mg of 5% Pd on charcoal at 20 psi overnight. Removal of the catalyst and evaporation of the solvent gave a noncrystalline glass. This was dissolved in hot 2-propanol. Chilling gave a white semisolid. The solvent was removed by decanting, and the procedure was repeated 2 times. After drying, the product, 64 mg (39%), was homogenous by thin-layer chromatography (silica gel; butanol/ethyl acetate/acetic acid/water, 1:1:1:1). A 12-mg sample of the noncrystalline material obtained by evaporation of a methanol solution gave a satisfactory elemental analysis for the neutral oxalate containing one molecule of methanol: NMR (D_2O) δ 6.85–7.18 (m, 3 H, aromatic), 5.30–5.34 (m, 1 H, X portion of ABX multiplet (not resolved), CHOH), 3.28–3.37 (m, 2 H, AB portion of ABX multiplet (not resolved), CH_2NH_2), 2.79 (s, 3 H, NCH_3). Anal. ($C_9H_{12}FNO_2·0.5C_2H_2O_4·CH_3OH$) C, H, N.

4-Fluorophenylephrine (2b). A 100-mg sample of 13b in 20 mL of ethanol was hydrogenated over 20 mg of 5% Pd on carbon for 4 h. The product, 2b, had precipitated during the reaction. The solution was diluted with ethanol, warmed, and filtered to remove the catalyst. Evaporation of solvent and recrystallization from ethanol gave 52 mg (77%) of pure **2b**: mp 211–214 °C dec (ethanol); NMR (CD_3OD) δ 6.88–7.02 (m, 2 H, aromatic), 6.69–6.79 (m, 1 H, aromatic), 4.65–4.69 (m, 1 H, X portion of ABX multiplet (not resolved), CHOH), 2.67–2.80 (m, 2 H, AB portion of ABX multiplet (not resolved) CH_2NH), 2.44 (s, 3 H, NCH_3). Anal. ($C_9H_{12}FNO_2$) C, H, N.

6-Fluorophenylephrine·0.5$H_2C_2O_4$ (2c·0.5$H_2C_2O_4$). A 368-mg sample (1.33 mmol) of 13c in 100 mL of ethanol was hydrogenated at 20 psi over 20 mg of 5% Pd on carbon overnight. The solution was filtered and 98 mg (0.77 mmol) of oxalic acid dihydrate was added. Evaporation of the solvent and recrystallization of the residue from acetone/water gave 109 mg (36%) of **2c** as the neutral oxalate: mp 188–190 °C (acetone/water); NMR (D_2O) δ 6.82–7.10 (m, 3 H, aromatic), 5.26–5.30 (m, 1 H, X portion of ABX multiplet (not resolved), CHOH), 3.29–3.39 (m, 2 H, AB portion of ABX multiplet (not resolved), CH_2NH), 2.79 (s, 3 H, $NHCH_3$). Anal. ($C_9H_{12}FNO_2·0.5H_2C_2O_4$) C, H, N. C: calcd, 52.16; found, 51.63.

Determination of Phenolic Ionization Constants of 2a–c. Stock aqueous solutions of 1 and of **2a–c** were prepared. Phenolic ionization was determined by measuring the ultraviolet absorption spectrum of each as a function of pH by diluting 50-μL aliquots of the stock solution to 3.00 mL in 1 M carbonate buffer. The absorption maxima of **2a–c** and of 1 in neutral and basic solutions are given in Table I. The change in absorption as a function of pH at the wavelength corresponding to the absorption of the phenolate anion was used to calculate the phenolic pK_a.

Determination of [³H]WB 4101 Binding to Guinea Pig Cerebral Cortical Membranes. Measurement of [³H]WB 4101 (2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxan; sp act. = 19.8 Ci/mmol, New England Nuclear, Boston, MA) binding to α_1-adrenergic receptors and displacement by FPEs 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 disrupted in a Polytron (20 s at maximal speed). The homogenate was centrifuged at 10000g for 20 min and washed 2 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 WB 4101 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 [³H]WB 4101.

Determination of [³H]Clonidine Binding to Guinea Pig Cerebral Cortical Membranes. Measurement of [³H]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 [³H]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 [³H]clonidine.

Determination of [³H]Prazosin Binding to Guinea Pig Cerebral Cortical Membranes. Measurement of [³H]prazosin (sp act. = 18.8 Ci/mmol, New England Nuclear, Boston, MA) binding to α_1-adrenergic receptors in guinea pig cerebral cortical membranes (see above) was carried out according to the procedure of Glossman and Hornung.[20] Under these conditions (30 min, 30 °C) the K_d for [³H]prazosin was 0.5 nM, and specific binding, defined as that blocked by 10 μM phentolamine, was 90–95% of total binding. Inhibition curves were performed with 0.5 nM [³H]prazosin.

Determination of Specific Binding of [³H]Dihydroalprenolol to Rat Cerebral Cortical Membranes. Measurement of [³H]dihydroalprenolol (sp act. = 35.6 Ci/mmol, New England Nuclear, Boston, MA) to β-adrenergic receptors in rat cerebral cortical membranes (see above procedure for guinea pig) was carried out in a protocol based on procedures of Bylund and Snyder[21] as modified by Kirk et al.[2] The buffer was 50 mM Tris·HCl (pH 7.4) containing 10 mM $MgCl_2$ and 1 mM EDTA. Under these conditions (30 min, 30 °C) the K_d for [³H]dihydroalprenolol was 1 nM, and specific binding, defined as that blocked by 1 μM propranolol, was 75–82% of total binding. Inhibition curves were performed with 1 nM [³H]dihydroalprenolol.

Cyclic AMP Accumulation in Guinea Pig Cerebral Cortical Synaptoneurosomes. The stimulation of [³H]cyclic AMP accumulation was determined essentially according to the procedure of Daly et al.[22] Nonfiltered guinea pig cerebral cortical synaptoneurosomes were prepared according to Hollingsworth et al.[23] and the nucleotide pool prelabeled by incubation with 0.6 μM [³H]adenine (sp act. = 13 Ci/mmol, New England Nuclear, Boston, MA) in a modified Krebs–Henseleit buffer. The buffer had the following composition (mM): NaCl, 118.5; KCl, 4.7; $MgSO_4$, 1.18; $CaCl_2$, 2.5; KH_2PO_4, 1.18; $NaHCO_3$, 24.9; and glucose, 10; aerated with O_2/CO_2 (95:5%) to adjust the pH to 7.4. [³H]Cyclic AMP accumulation was elicited by a 10-min incubation at 37 °C with various concentrations of PE or FPE in the presence of 10 μM 2-chloroadenosine. [³H]Cyclic AMP was isolated as described by Salomon et al.,[24] and [³H]cyclic AMP formation was expressed as percent conversion of total [³H]adenine nucleotides to [³H]cyclic AMP as described.[23]

Determination of Phosphatidylinositol Turnover in Guinea Pig Cerebral Cortical Synaptoneurosomes. Nonfiltered guinea pig cerebral cortical synaptoneurosomes were prepared according to Hollingsworth et al.[23] The preparation from one guinea pig was suspended in 20 mL of Krebs–Henseleit buffer and incubated for 60 min at 37 °C with 250 μCi of 1 μM [³H]inositol (sp act. = 14 Ci/mmol, Amersham, Arlington Heights, IL) with gentle bubbling with O_2/CO_2 (95:5%). The prelabeled synaptoneurosomes were washed 1 time (1000g × 10 min) and resuspended in fresh buffer (10–15 mL) containing 10 mM LiCl. The preparation was distributed in polypropylene tubes (approximately 1 mg of protein/tube) and the PE or FPE added. The tubes were gassed briefly, capped, and incubated for 90 min at 37 °C. The reaction was stopped by centrifugation (microfuge) and washed with fresh buffer to remove the free [³H]inositol. Trichloroacetic acid was added (0.75 mL, 6%), followed by mixing and centrifugation. [³H]Inositol phosphates were analyzed in the supernatants according to Berridge.[25]

Measurement of Agonist Response in the Isolated Guinea Pig Aorta. The agonist response of the FPEs in isolated guinea

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pig aorta strips was performed by the following modification of the method of Shepperson et al.[26] Guinea pig thoracic aortae (male, Hartley, 200–250 g) were removed and cut into rings of approximately 3 mm width and immediately bathed in Krebs–Ringer buffer bubbled with O_2/CO_2 (95:5%) at 37 °C. The Krebs–Ringer buffer had the following composition (mM): NaCl, 118.0; KCl, 4.7; $MgCl_2$, 1.2; $CaCl_2$, 2.6; NaH_2PO_4, 1.0; $NaHCO_3$, 25; glucose, 11.1; with the inclusion of 10 μM cocaine. The rings were suspended in 20-mL organ baths under a basal tension of 3 g and washed approximately 10 times over a period of 60 min. After this period, cumulative concentration–response curves were obtained to the agonists at concentrations from 3 to 200 μM in approximately 2–3-fold steps. Each agonist concentration was increased to the next step after the response to the preceding step had reached plateau. Maximum response was obtained before and after each agonist concentration–response curve by adding a supramaximal concentration of (–)-NE (30–50 μM). The tension developed by the guinea pig aortic rings was measured with Grass FT03C force displacement transducers and recorded on Grass 79D polygraphs. Potency (EC_{50}) was based on the concentration of PE or FPE that afforded a contraction one-half of the maximal contraction afforded by (–)-NE.

Acknowledgment. We would like to acknowledge the technical assistance of Brian Collins (Washington Center for Learning Alternatives) and the excellent clerical assistance of Colleen LePore and Judy Thomas-Younis.

Registry No. **2a**, 103439-04-9; **2a**·0.5oxalate, 103439-05-0; **2b**, 103439-06-1; **2c**, 103439-07-2; **2c**·0.5oxalate, 103439-08-3; **3a**, 103438-90-0; **3b**, 103438-91-1; **3c**, 103438-92-2; **4b**, 42564-51-2; **4c**, 27996-87-8; **5a**, 103438-86-4; **5b**, 103438-85-3; **5c**, 103438-84-2; **6e**, 103438-88-6; **6f**, 591-31-1; **10**, 103438-89-7; **11a**, 103438-94-4; **11b**, 103438-95-5; **11c**, 103438-93-3; **12a**, 103438-97-7; **12a**·oxalate, 103439-02-7; **12b**, 103438-98-8; **12c**, 103438-96-6; **13a**, 103439-01-6; **13a**·oxalate, 103439-03-8; **13b**, 103438-99-9; **13c**, 103439-00-5; 2-fluoro-5-aminobenzaldehyde dimethyl acetal, 103438-83-1; 3-methoxy-2-nitrobenzaldehyde, 53055-05-3; 2-amino-3-methoxybenzaldehyde dimethyl acetal, 103438-87-5; 2-fluorophenol, 367-12-4; 4-fluorophenol, 371-41-5.

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Cyclic Analogues of 2-Amino-4-phosphonobutanoic Acid (APB) and Their Inhibition of Hippocampal Excitatory Transmission and Displacement of [^{3}H]APB Binding

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Conformationally restricted analogues of 2-amino-4-phosphonobutanoic acid (APB, **2**) were prepared where the structure of APB was incorporated into cyclopentane (**3**) or cyclohexane (**4**) rings. Hydrophosphinylation of the appropriate cycloalkenones followed by Strecker amino acid syntheses provided the desired analogues. Assignments of the relative configurations for **3a** (trans), **3b** (cis), **4a** (cis), and **4b** (trans) were determined through ^{13}C NMR studies. Compounds **3b**, **4a**, and **4b** possessed low activity as inhibitors of excitatory synaptic field potentials in the rat hippocampal perforant path. Analogues **4a** and **4b** also showed little activity in displacing [^{3}H]APB from synaptic plasma membranes. The cyclopentyl APB analogue **36**, on the other hand, was extremely potent in inhibiting the binding of [^{3}H]APB, possessing an IC_{50} = 4.7 μM, thus giving further credence to the idea that the APB binding site in the rat brain synaptosomal membrane preparation is not the same as the receptor mediating APB-induced inhibition of the lateral perforant path. Of the four cyclic APB analogues, **3a** most resembled APB in its spectrum of biological activity. It showed significant potency (IC_{50} = 130 μM) in inhibiting lateral entorhinal projections to hippocampal granule cells. Analogous to APB, **3a** also showed selectivity for the lateral perforant path over the medial perforant path. Its activity in the radioligand binding assay paralleled its activity in inhibiting the lateral perforant path. It thus appears that **3a** comes closest to mimicking the active conformation of APB and suggests that a folded conformation wherein the amino and phosphonate moieties are in a cis relationship to one another may approximate the active conformation of APB.

It has been proposed that L-glutamic acid (**1**) is one of the major excitatory neurotransmitters in the central nervous system.[1–3] While L-glutamate meets many of the proposed criteria for neurotransmitter status, there still exists a great need for the development of potent and specific antagonists for neuronal pathways thought to use glutamate as a neurotransmitter. The glutamic acid analogue L-2-amino-4-phosphonobutanoic acid (**2**, APB) has been shown to antagonize excitatory synapses in the lateral perforant path of the rat hippocampal slice with an apparent K_d of 2.5 μM.[4] The inhibitory effect of **2** shows stereoselectivity, since the D isomer of **2** is some 40 times less potent as an antagonist. Furthermore, **2** possesses high pathway selectivity, since it is 18 times less potent in antagonizing the synaptic transmission in the medial perforant path (the lateral and medial perforant paths originate from adjacent lateral and medial areas of the entorhinal cortex and terminate distally and medially, respectively, on the dendritic field of dentate granule cells).

It also has been shown that a class of L-[^{3}H]glutamate binding sites exist that are localized in synaptic plasma membranes and are distinguished by Ca^{2+}/Cl^- dependence. [^{3}H]Glutamate is displaced from these binding sites by concentrations of L-APB that are in the same range (1–10 μM) as those required for the inhibition of synaptic transmission.[5–9] This observation has led to the suggestion

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