F. A. Brown Jr. is gratefully acknowledged.

Registry No. 3, 101126-73-2; 4, 102587-79-1; 5, 102587-80-4; 6, 102587-81-5; 7, 102587-82-6; 9, 102587-83-7; 11, 102587-84-8; 14, 102587-86-0; 15, 102587-87-1; 16, 102587-88-2; 17, 102613-60-5; 18, 102587-89-3; 19, 102587-90-6; 20, 102587-91-7; 1,3-dipropyl-5,6-diaminouracil, 81250-34-2; *p*-anisaldehyde, 123-11-5; 4-acetoxybenzaldehyde, 878-00-2; *p*-amino benzoic acid, 150-13-0; *p*-[(trifluoroacetyl)amino]benzoic acid, 404-26-2; 1,3-dipropyl-6-amino-5-(*p*-trifluoroacetylaminobenzamido) uracil, 102587-85-9; 4-acetamidobenzaldehyde, 122-85-0; 1,3-dipropyl-6-amino-5-[(4-acetamidobenzylidene)amino]uracil, 102587-92-8; *p*-(dimethylamino)benzaldehyde, 100-10-7; *p*-sulfamoylbenzoic acid, 138-41-0; 1,3-dipropyl-6-amino-5-(*p*-sulfamoylbenzamido)uracil, 102613-61-6; *p*-carboxybenzaldehyde, 619-66-9; 1,3-dipropyl-6-amino-5-[(*p*-carboxybenzylidene)amino]uracil, 94781-77-8; 2,3-dihydroxybenzaldehyde, 24677-78-9; 1,3-dipropyl-6-amino-5-[(2,3-dihydroxybenzylidene)amino]uracil, 102587-93-9; 2,4-dihydroxybenzaldehyde, 95-01-2; 2,5-dihydroxybenzaldehyde, 1194-98-5; 1,3-dipropyl-6-amino-5-[(2,5-dihydroxybenzylidene)-amino]uracil, 102587-94-0; 2-hydroxy-4-methoxybenzaldehyde, 673-22-3; 1,3-dipropyl-6-amino-5-(2-methoxy-4-chlorobenzamido)uracil, 102587-95-1; 2,6-difluorobenzoic acid, 385-00-2; 1,3-dipropyl-6-amino-5-(2,6-difluorobenzamide)uracil, 102587-96-2; 3-chloro-4-acetoxybenzoic acid, 70679-89-9; 1,3-dipropyl-6-amino-5-(3-chloro-4-hydroxybenzamido)uracil, 102587-97-3; 2-methoxy-4-chlorobenzoic acid, 57479-70-6.

Synthesis and β-Adrenergic Receptor Blocking Potency of 1-(Substituted amino)-3-(4-indolyloxy)propan-2-ols

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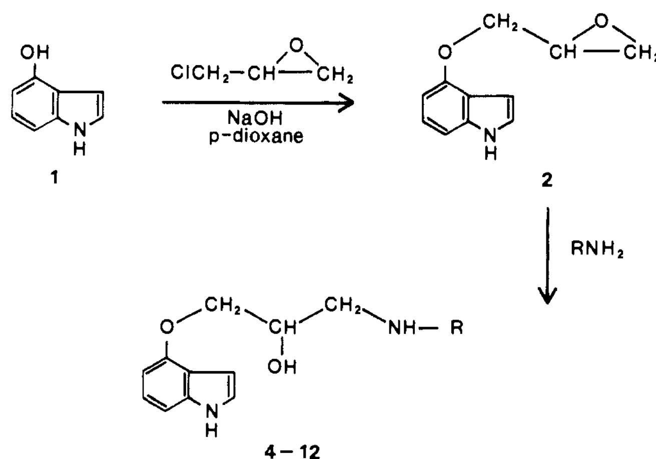
Although (−)-[^{125}I]iodopindolol (IPIN) can be used to label β-adrenergic receptors in the central nervous system in vivo, use of this ligand for receptor imaging studies in humans may be limited due to its relatively poor penetration into the brain. As a first step toward the development of radioligands for imaging studies, we report the synthesis and measurement of in vitro binding affinity to β-receptors of a series of 1-(substituted amino)-3-(4-indolyloxy)-propan-2-ol derivatives. The synthesized compounds vary widely in their lipophilicity as measured by their distribution coefficients between phosphate buffer and octanol at pH 7.4. The affinity of these compounds for β-receptors, as measured by their inhibition of binding of IPIN to rat cortical and cerebellar membranes in vitro, ranges from 2- to 100-fold less potent than pindolol; the most potent compounds have K_i values of 2–5 nM. The radiolabeled analogues of some of these compounds may prove useful for receptor imaging studies.

Considerable effort is being made in the development of techniques for the imaging of human brain receptors in vivo using single photon emission computed tomography (SPECT) and positron emission tomography (PET). A key step in the development of such techniques is the synthesis of suitable radioligands; measurements in humans are now being reported using for example (*R*)-3-quinuclidinyl-4-[^{123}I]iodobenzilate for muscarinic cholinergic receptors using SPECT[1] and 3-*N*-[^{11}C]methylspiperone for brain dopamine receptors using PET.[2]

We are engaged in a program to develop radioligands suitable for the measurement of β-adrenergic receptors in human brain using PET and SPECT. Central β-receptors have been implicated in the etiology and/or treatment of a number of psychiatric and neurologic disorders as well as in the mechanism of action of a number of drugs used in psychiatry.[3,4]

Recently, binding in vivo in the central nervous system (CNS) of rats of the β-adrenergic receptor radioligand (−)-[^{125}I]iodopindolol (IPIN), whose use was first described in vitro by Barovsky and Brooker,[5] was reported.[6,7]

Scheme I



However, IPIN itself does not appear to be a good ligand for use with imaging techniques in humans since its penetrability into the brain is limited.[7] Consequently, it seemed that derivatives of pindolol, particularly those that might have greater penetrability into the CNS, might be useful ligands for β-receptors in brain for use with PET or SPECT.

In this report, the synthesis of a series of derivatives of pindolol in which the group attached to the N-terminal end of the molecule is varied is described as well as their binding affinity to β-receptors in the CNS in vitro. It is hoped that by varying the nature of the N-terminal end of these molecules a compound would be found that has both high affinity for β-receptors and good penetration into the brain. Since lipophilicity is likely to be important in this regard, the distribution coefficient for each compound was also measured between phosphate buffer and octanol at pH 7.4.

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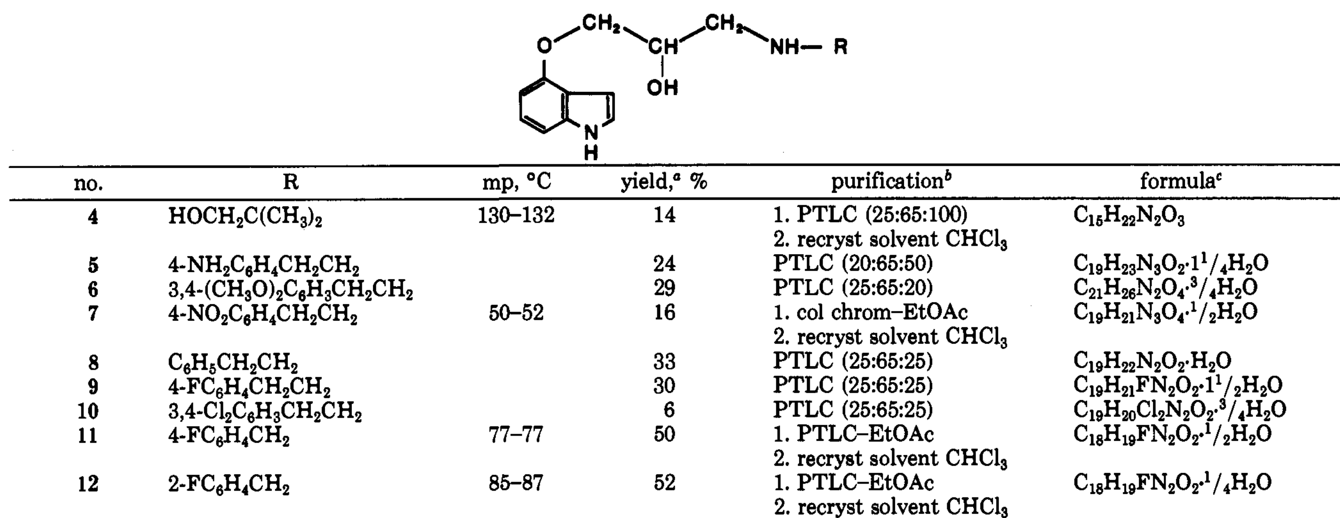
Table I. Chemical Data



no.	R	mp, °C	yield,[a] %	purification[b]	formula[c]
4	$HOCH_2C(CH_3)_2$	130–132	14	1. PTLC (25:65:100) 2. recryst solvent $CHCl_3$	$C_{15}H_{22}N_2O_3$
5	$4\text{-}NH_2C_6H_4CH_2CH_2$		24	PTLC (20:65:50)	$C_{19}H_{23}N_3O_2 \cdot 1^1/_4H_2O$
6	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$		29	PTLC (25:65:20)	$C_{21}H_{26}N_2O_4 \cdot {}^3/_4H_2O$
7	$4\text{-}NO_2C_6H_4CH_2CH_2$	50–52	16	1. col chrom–EtOAc 2. recryst solvent $CHCl_3$	$C_{19}H_{21}N_3O_4 \cdot {}^1/_2H_2O$
8	$C_6H_5CH_2CH_2$		33	PTLC (25:65:25)	$C_{19}H_{22}N_2O_2 \cdot H_2O$
9	$4\text{-}FC_6H_4CH_2CH_2$		30	PTLC (25:65:25)	$C_{19}H_{21}FN_2O_2 \cdot 1^1/_2H_2O$
10	$3,4\text{-}Cl_2C_6H_3CH_2CH_2$		6	PTLC (25:65:25)	$C_{19}H_{20}Cl_2N_2O_2 \cdot {}^3/_4H_2O$
11	$4\text{-}FC_6H_4CH_2$	77–77	50	1. PTLC–EtOAc 2. recryst solvent $CHCl_3$	$C_{18}H_{19}FN_2O_2 \cdot {}^1/_2H_2O$
12	$2\text{-}FC_6H_4CH_2$	85–87	52	1. PTLC–EtOAc 2. recryst solvent $CHCl_3$	$C_{18}H_{19}FN_2O_2 \cdot {}^1/_4H_2O$

[a] Yield is based on the epoxide. [b] Preparative thin-layer chromatography (PTLC) was carried out with a mixture of solvents, heptane–chloroform–methanol, in the ratios stated. [c] All compounds were analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values.

Chemistry. Scheme I illustrates the synthetic route for the compounds reported. 4-Hydroxyindole (1) was treated with epichlorohydrin to afford the intermediate epoxide **2**.[8] The epoxide was then reacted with the appropriate amines (commercially available) to give the desired products **4–12**. The compounds synthesized are listed in Table I. With the exception of compounds **4**, **11**, and **12**, most of the compounds were hygroscopic semisolids. A representative synthesis for the preparation of the derivative **6** is described under the Experimental Section.

Pharmacology. To determine IC_{50} and K_i values of the derivatives for β-receptors in cortex and cerebellum, displacement curves of the binding of IPIN measured in vitro were done. Measurement of the binding of IPIN in vitro was done as described previously.[9] The ability of at least nine concentrations of these compounds ranging from 5×10^{-10} to 10^{-6} M to inhibit IPIN binding (30 pM) in the presence of GTP (250 μM) was measured. IC_{50} values were determined from a plot of the percentage inhibition of specific binding against the logarithm of the concentration of competing compound. GTP was included in the binding assay to eliminate the contribution of multiple affinity states of β-receptors to the calculated parameters.[9,10]

Pharmacological Results and Discussion

The affinities of the compounds for β-receptors in vitro were determined by measuring the inhibition of IPIN binding to β-receptors in rat cortex and cerebellum. These tissues contain β-adrenergic receptors primarily of the β_1- and β_2-subtypes, respectively. The results of this experiment are shown in Table II. The affinity of the pindolol derivatives ranged from approximately 2- to 100-fold less than that of pindolol itself.

The lipophilicity of the compounds studied was estimated by determining distribution coefficients between 1-octanol and phosphate buffer (pH 7.4). The distribution coefficients (Table II) showed a wide range of values ranging from −0.60 (log value) for compound **4** to 2.5 for compound **10**; this represents a range of over 1000-fold. The distribution coefficients were not significantly related

Table II. In Vitro Binding Data and Distribution Coefficients for Derivatives of Pindolol

no.	R	K_i,[a] nM cortex	K_i,[a] nM cerebellum	distribn coeff (log)
3	$(CH_3)_2CH$[b]	1	1	−0.3
4	$HOCH_2C(CH_3)_2$	2	2	−0.6
5	$4\text{-}NH_2C_6H_4CH_2CH_2$	25	22	0.3
6	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$	3	7	1.1
7	$4\text{-}NO_2C_6H_4CH_2CH_2$	5	8	1.5
8	$C_6H_5CH_2CH_2$	5	4	1.7
9	$4\text{-}FC_6H_4CH_2CH_2$	5	4	2.0
10	$3,4\text{-}Cl_2C_6H_3CH_2CH_2$	6	11	2.5
11	$4\text{-}FC_6H_4CH_2$	125	80	1.9
12	$2\text{-}FC_6H_4CH_2$	60	45	2.0

[a] K_i values determined from IC_{50} values (see text). Hill coefficients for all the compounds ≥0.9. [b] Pindolol.

to the affinities of the compounds for β-receptors (r_s = +0.61 and +0.51 for cortex and cerebellum, respectively). The absence of a relationship between affinity for β-receptors and lipophilicity for a series of compounds has also been noted before by Rzeszotarski et al.[11] The wide range of lipophilicities of the compounds with high affinity for β-receptors will be important when studies are performed measuring the penetration and interaction of these compounds with β-receptors in the brain in vivo.

The present study is one of the first that examines the relationship of structural changes on the potency of β-adrenergic antagonists for binding to β-adrenergic receptors. As discussed by Rzeszotarski et al.,[11] use of in vitro receptor binding has several advantages over other physiological testing methods when structure–activity relationships are being examined since direct receptor binding measures eliminate confounding factors such as varying metabolism and distribution.

Overall the compounds are tolerant to the structural changes at the amino end of the molecule. A hydroxyalkyl derivative **4** was synthesized due to previous reports that hydroxyalkyl derivatives of a number of β-antagonists were nearly as potent as the parent isopropyl or *tert*-butyl compounds.[12–14] In the present study, compound **4** is the

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most potent of the compounds synthesized.

Six phenethyl derivatives were also synthesized. Previously, a large number of phenethyl-substituted β-adrenergic antagonists have been studied and found to have a potency similar to the corresponding isopropyl or *tert*-butyl derivatives.[11-15] With the exception of compound **5** the phenethyl derivatives have an affinity for β-receptors only slightly lower than that of pindolol itself.

The high potency of the *p*-nitrophenethyl **7**, *p*-fluorophenethyl **9**, and 3,4-dichlorophenethyl **10** compounds is of interest, since to our knowledge only one such derivative (a 3,4-dichlorophenethyl derivative with weak activity) has been reported for any β-antagonist.[15] The *p*-fluorophenethyl compound **9** is a potential candidate for PET studies while the fact that a *p*-nitro group can be tolerated may be important for synthetic purposes as a promising method for incorporating ^{18}F with high specific activity into phenyl rings involves the formation of compounds that contain both NO_2 and F groups in the benzene ring.[16] The 3,4-dichlorophenethyl compound is of interest due to both its high potency and lipophilicity.

The rat cortex contains β-receptors that are predominantly of the β_1-subtype. However, it also contains a small percentage of β_2-receptors.[17] The presence of β_2-receptors (approximately 20%) could alter the K_i values determined in the cortex for the compounds tested if they have selectivity for the subtypes of the β-receptor. To investigate this further, competition experiments for derivatives **3**, **4**, **6**, and **8** were performed in the cortex in the presence of ICI 118,551, a highly selective β_2-antagonist.[18] A concentration of 20 nM of ICI 118,551 was chosen on the basis of preliminary experiments in cortical membranes using 18 concentrations of the β_2-antagonist. These experiments determined that ICI 118,551 bound to two sites in the cortex with IC_{50} values of 1.6 (for β_2-receptors) and 140 nM (for β_1-receptors), respectively. These values were determined by using PROPHET, an interactive computer system that utilizes the modeling program MLAB. At an ICI 118,551 concentration of 20 nM, 93% of the β_2-receptors and only 12% of the β_1-receptors in the cortex would be blocked, and experiments performed in the presence of this concentration of ICI 118,551 will therefore represent almost exclusively binding to β_1-receptors. Results indicated that the K_i values for compounds **3**, **4**, and **8** were essentially unchanged when the binding reaction was carried out in the presence or absence of ICI 118,551 in the rat cortical membranes (Table II). This confirms the lack of subtype selectivity for the majority of the compounds listed in Table II.

Two of the compounds, the 3,4-dimethoxyphenethyl **6** and the isosteric 3,4-dichlorophenethyl **10** derivatives, have a slightly higher affinity for β-receptors in the cortex than the cerebellum, which is probably due to their selectivity for binding to β_1-receptors. The K_i value for **6** was decreased from 3 nM in the absence of ICI 118,551 to 1 nM in the presence of ICI 118,551 (20 nM), indicating an approximately 7-fold selectivity for this compound. The β_1-selectivity of 3,4-dimethoxyphenethyl-substituted β-antagonists has been found previously although the extent of this selectivity varies widely.[11,15,19] The apparent β_1-selectivity of the 3,4-dichlorophenethyl compound **10** would appear to warrant further investigation of the effect of this substituent on β_1-selectivity for other β-antagonists.

The relatively weak affinity of the *p*-aminophenethyl compound **5** is somewhat surprising given previous reports of very high β-receptor binding affinity for similar derivatives of carazolol[20] and propranolol.[21] Finally, two fluorobenzyl derivatives **11** and **12** were studied. These compounds were the least potent, and again this is in accord with a previous study that showed that a benzyl-substituted β-antagonist was weaker than the corresponding phenethyl compound.[19]

In summary, results from the above study indicate that the compounds appear to be quite tolerant to a number of structural changes that were made at the terminal amino end of the molecule. This can be seen from the number of derivatives that exhibit high affinity for the β-receptors in the cortex and cerebellum. Furthermore, compounds **6** and **10** have a higher affinity for β-receptors in the cortex than the cerebellum, and this is probably due to their selectivity for binding to β_1-receptors. The wide range of lipophilicities of these compounds with high affinity for β-receptor will be important when studies are performed measuring the penetration and interaction of these compounds with β-receptors in the brain in vivo. In addition it should prove possible to prepare and study radioiodinated derivatives of these pindolol derivatives.

Experimental Section

Chemistry. Proton magnetic resonance (^1H NMR) and low- and high-resolution mass spectra were obtained on all compounds. Proton NMR were recorded on a Bruker WH 360.04-MHz spectrometer at the University of Pennsylvania. Proton magnetic resonance ^1H NMR spectra were consistent with structures and assignments. Both low- and high-resolution MS were obtained on a Kratos MS 9/50 instrument. Low- and high-resolution mass spectra were performed at Pennsylvania State University, and the molecular ion and fragmentation patterns were consistent with the proposed structures. Results of high-resolution mass spectra were within the tolerance limit of ±10 ppm. Ultraviolet spectra were run on a Perkin-Elmer Lambda 3A UV/vis spectrophotometer. Melting points for compounds that were solids were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on Baker-Flex silica gel IB2-F sheets (Baker), and detection of pure compounds as a single spot on TLC was done by short-wavelength UV light. Preparative thin-layer chromatography was performed on 20 × 20 silica gel GF plates (250 or 1000 μm) (Analtech). Column chromatography was carried out with silica gel (60–200 mesh) (Baker). Evaporation of solvents was achieved under reduced pressure with a rotary evaporator. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA, and the results obtained were within ±0.4% of the theoretical values.

4-(2,3-Epoxypropoxy)indole (2). 4-Hydroxyindole (1; 500 mg, 3.8 mmol) was added to a solution of NaOH (150 mg, 3.8 mmol) dissolved in 5 mL of H_2O. Epichlorohydrin (2.5 mL, 32 mmol) and *p*-dioxane (2 mL) were added to the mixture, and the reaction was stirred for 22 h at room temperature under a slow stream of N_2. The reaction mixture was extracted with methyl *tert*-butyl ether (20 mL) to afford a green solution that was dried over Na_2SO_4 (anhydrous). The solvent was evaporated under

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reduced pressure to near dryness to yield a crude brown oil that was purified by column chromatography (silica gel, 60–200 mesh) using petroleum ether initially as solvent and later increasing amounts of $CHCl_3$ to afford 500 mg (65%) of a gray solid **2** after solvent evaporation: mp 63–65 °C (lit.[8] mp 65–67 °C); ^{1}H NMR ($CDCl_3$) δ 8.2 (s, 1, indole NH), 7.0–6.2 (m, 5, arom H), 4.1–2.7 (m, 5, aliph H).

4-[2-Hydroxy-3-[[(3,4-dimethoxyphenyl)ethyl]amino]-propoxy]indole (6). 4-(2,3-Epoxypropoxy)indole (**2**; 550 mg, 2.7 mmol) and 3,4-dimethoxyphenethylamine (690 mg, 3.8 mmol) were dissolved in MeOH (5 mL) and the reaction mixture was stirred under a steady stream of N_2 at ambient temperature for 1.5 days. The products of the reaction mixture were separated by preparative layer chromatography (silica gel plates, 1000 μm) with heptane–chloroform–methanol (25:65:20) to yield 300 mg (29%) of **6** homogeneous by TLC analysis in Hept–$CHCl_3$–MeOH (25:65:20): R_f 0.39; ^{1}H NMR ($CDCl_3$) δ 8.2 (s, 1, indole H), 7.31–6.4 (m, 8, arom H), 4.1 (br d, 2, CH_2), 3.84 (s, 3, OCH_3), 3.82 (s, 3, OCH_3), 2.8–2.7 (m, 8, aliph H), 1.9 (br s, 1, OH); mass spectrum, m/z 370.1874 (M^+ calcd for $C_{21}H_{26}N_2O_4$, 370.1892). Anal. ($C_{21}H_{26}N_2O_4 \cdot {}^3/_4H_2O$) C, H, N.

Distribution Coefficients. Distribution coefficients were determined for partitioning between 1-octanol and phosphate buffer using ultraviolet (UV) spectroscopy. Each compound was dissolved in potassium phosphate buffer (0.1 M, pH 7.4) presaturated with 1-octanol, at a concentration of 0.1–1.0 mg/mL. An aliquot of this solution was shaken with an aliquot of 1-octanol (presaturated with the buffer) for 30 min, allowed to stand for 10 min, and centrifuged at 600*g* for 10 min at room temperature, and the organic phase was discarded. The absorbance of the aqueous layer both before and after partition was determined by spectrophotometric analysis. Absorbance was measured within a wavelength range of 225–290 μm. The wavelength was chosen for each compound to provide a reading for the unextracted solutions in the range of 0.5–1.5 absorbance units. The distribution coefficient was calculated according to

$$D = \frac{A_{orig} - A_{extr}}{A_{extr} f}$$

where A_{orig} is the absorbance of the drug in buffer before extraction, A_{extr} is the absorbance of the drug in buffer after extraction, and f is the ratio of volumes of 1-octanol and buffer.

All values for A were corrected for the absorbance of 1-octanol-saturated buffer at the appropriate wavelength, and experiments were carried out twice.

Preparation of Membranes. The membranes of cortical or cerebellar tissue for the in vitro studies of IPIN were obtained by the method of O'Donnell et al.[9] Male Sprague–Dawley rats (Ace Animal, Boyertown, PA) weighing 175–225 g were killed by decapitation. Their brains were removed and rapidly dissected. Each region was placed in 10 mL (cortex) or 5 mL (cerebellum) of ice-cold HEPES (2 mM)–EDTA (1 mM), pH 7.5 buffer and homogenized for 15 s with a tissue disrupter (Kinematica). The homogenates were centrifuged for 15 min at 40000*g*, and the supernatant fluid was discarded. This wash procedure was repeated an additional three times, after which the membrane pellet was resuspended in 80 mL (cortex) and 20 mL (cerebellum) of a solution of NaCl (0.15 M)–Tris (10 mM)–$MgCl_2$ (2.5 mM), pH 7.5 buffer (incubation buffer).

β-Adrenergic Receptor Assay. IPIN was prepared by iodination of (–)-pindolol and was purified by a modification of the method of Barovsky and Brooker[5] as described by Wolfe and Harden.[22] Stock solutions of competing drugs (10^{-4} M) were prepared by dissolving the drugs in ethanol–incubation buffer (1:1) and were diluted with the incubation buffer to give nine concentrations for each drug in the range 10^{-6} to 5×10^{-10} M. The binding assays were conducted in the incubation buffer in borosilicate tubes in a total volume of 500 μL containing IPIN (100 μL, 30 pM), GTP (100 μL, 250 μM), and competing compound or buffer (100 μL); aliquots (200 μL) of the membrane suspension were then added to initiate the reaction, and the tubes were incubated for 20 min at 37 °C. Incubation was terminated by the addition of 6 mL of ice-cold NaCl (0.15 M)–Tris (10 mM), pH 7.5 rinse buffer followed by rapid vacuum filtration through a cell harvester (Brandel M24R) using filter strips (2 × 12 in.) (Schleicher and Schuell). The filters were washed twice with 6 mL of rinse buffer, and the radioactivity was counted with a γ counter (Beckman Gamma 4000). Specific binding, which averaged approximately 90% of the total binding, was measured with isoproterenol (25 μM). All experiments were performed at least twice, and reported values are the average of closely related values. IC_{50} values and Hill coefficients were determined from the inhibition data using nonlinear regression analyses. IC_{50} values were determined from the equation $B = B_0/(1 + I/IC_{50})$ where B is the amount of IPIN bound at a competitor concentration I and B_0 is the amount of IPIN bound in the absence of competitor. Hill coefficients were determined from the equation $B = B_0/(1 + I^n/IC_{50}{}^n)$ where n is the Hill coefficient. K_i values were calculated for the active enantiomers (eutomers) from the IC_{50} values, assuming only one stereoisomer of each of the racemates binds with high affinity to β receptors. Recently, evidence to support this assumption has been provided for a number of β-adrenergic agonists and antagonists by Morris et al.[23] The calculation of K_i values also uses the correction factor introduced by Cheng–Prusoff[24] to account for the concentration of IPIN used in the in vitro assay. Values for the affinity of IPIN for β-adrenergic receptors in cortex and cerebellum used in the Cheng–Prusoff correction were obtained in separate saturation experiments (145 and 65 pM, respectively).

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