Synthesis and Binding to β-Adrenergic Receptors of *p*-Aminobenzyl Analogues of Practolol and Atenolol

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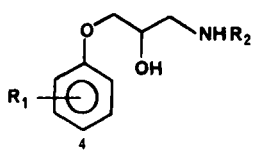
Abstract □ The *p*-aminobenzyl analogues (**8a** and **8b**, respectively) of the cardioselective β-adrenergic receptor antagonists practolol and atenolol were prepared from the corresponding phenoxymethyloxiranes in 30 and 13% yields, respectively. The dissociation constants for the β-adrenergic receptor were measured in membrane preparations of rat heart and lung. In membranes from the heart (which contain mostly β_1-adrenergic receptors), the affinities of the derivatives and parent compounds were similar. By contrast, in membranes from the lung (which contain mostly β_2-adrenergic receptors), the derivatives were more potent than the parent compounds. Thus, the cardioselectivities of the *p*-aminobenzyl analogues **8a** and **8b** were about one-sixth those of the respective parents.

The synthesis of high-affinity, cardioselective β-adrenergic receptor antagonists into which a radionuclide (e.g., iodine-123) can be introduced by electrophilic substitution may lead to new radiodiagnostic agents for imaging the myocardium.[1-3] Several studies have shown that it is possible to enhance cardioselectivity in the aryloxypropanolamine class of β-adrenergic receptor antagonists via appropriate substitution of the basic amine.[4-9] For example, Hoefle et al.[8] reported that the 2-(3,4-dimethoxyphenyl)ethylamine analogue (2) of the cardioselective β-adrenergic receptor antagonist practolol (1) had improved affinity for the β_1-adrenergic receptor and vastly improved cardioselectivity, results that were later confirmed by Gibson et al.[10] However, the same 2-(3,4-dimethoxyphenyl)ethyl substituent reduced in vitro the β_1-adrenergic receptor blocking activity of the cardioselective antagonist betaxolol (3).[11] The cardioselectivity index of 4 was not reported.



4

1 R_1 = 4-NHCOCH$_3$ R_2 = CH(CH$_3$)$_2$

2 R_1 = 4-NHCOCH$_3$ R_2 = CH$_2$CH$_2$-(3,4-dimethoxyphenyl)

3 R_1 = 4-CH$_2$CH$_2$OCH$_2$-cyclopropyl R_2 = CH(CH$_3$)$_2$

4 R_1 = 4-CH$_2$CH$_2$OCH$_2$-cyclopropyl R_2 = CH$_2$CH$_2$-(3,4-dimethoxyphenyl)

5 R_1 = 2,3-CH=CH CH=CH- R_2 = CH(CH$_3$)$_2$

6 R_1 = 2,3-CH=CH CH=CH- R_2 = C(CH$_3$)$_2$CH$_2$-(4-aminophenyl)

It is also well documented that, in this same class of antagonists, substitution of the basic amine with an α-branched arylalkyl group results, in general, in increased potency, especially when the aryl ring possesses a polar substituent.[4] Because the *p*-aminobenzyl derivative (6) of the nonselective β-adrenergic receptor antagonist propranolol (5) is a potent, long-lasting antagonist,[12] we examined the effects of including the potentially versatile *p*-aminobenzyl moiety into an already cardioselective antagonist.[13] We herein describe the synthesis and binding affinities to β-adrenergic receptors of the *p*-aminobenzyl analogues of the cardioselective β-adrenergic receptor antagonists practolol and atenolol.

Experimental Section

Melting points (mp) were determined with a Thomas Hoover capillary melting point apparatus and are uncorrected. The NMR spectra were recorded on a Varian A-60 spectrometer. Mass spectra were obtained on a Finigan 4021B mass spectrometer. Elemental analyses were determined by Schwarzkopf Microanalytical Laboratory, Woodside, NY.

1-[2-(4-Aminophenyl)-1,1-dimethylethylamino]-3-(4-acetamidophenoxy)propan-2-ol (8a)—A mixture of 4-(2,3-epoxypropoxy)acetanilide[14] (207 mg, 1.0 mmol, **7a**) and α,α-dimethyl-*p*-aminophenethylamine[13,15] (164 mg, 1.0 mmol) in 10 mL of CH_3CN (freshly distilled from CaH) was stirred at reflux under nitrogen. Heating was discontinued after 7 days, and the reaction mixture was concentrated to a syrup under reduced pressure. Upon trituration of the syrup with diethyl ether, a white solid was obtained, which was chromatographed on silica gel. Elution with 10% methanol in $CHCl_3$ afforded a white powder, which was rechromatographed with the same eluant to give 110 mg (30%) of **8a**: mp 85–90 °C; NMR ($CDCl_3$): δ 1.0 (s, 6H, $(CH_3)_2C$-), 2.0 (s, 3H, -C(O)CH_3), 2.45 (s, 2H, -CH_2 phenyl), 2.70 (br, 2H, -CH_2NH), 2.93 (br, 4H, -NH_2, -NH, -OH exchange with D_2O), 3.72 (s, 3H, -CH_2O, -CHOH), 6.25 (d, 2H, ArH), 6.55 (q, 4H, ArH), 7.03 (d, 2H, ArH), 7.52 (s, 1H, -NHC(O) exchange with D_2O); low-resolution, electron impact MS: *m/e* 265 (100%); low-resolution, field desorption MS: *m/e* 371 (M^+).

Anal.—Calc. for $C_{21}H_{29}N_3O_3 \cdot \frac{1}{2}CH_3OH$ (387.5): C, 66.64; H, 8.06; N, 10.85. Found: C, 67.00; H, 8.18; N, 10.75.

1-[2-(4-Aminophenyl)-1,1-dimethylethylamino]-3-(4-carbamoylmethylphenoxy)propan-2-ol (8b)—A mixture of 4-(2,3-epoxypropoxyl)phenylacetamide[16] (207 mg, 1.0 mmol, **7b**) and α,α-dimethyl-*p*-aminophenethylamine (164 mg, 1.0 mmol) in 15 mL of CH_3CN was stirred at reflux under nitrogen for 7 days. Concentration of the reaction mixture gave a syrup, which was chromatographed on silica gel. Elution with 10% methanol in $CHCl_3$ provided a white solid which was crystallized from a mixture of diethyl ether:CH_3CN to give 50 mg (13%) of **8b**: mp 99–102 °C; NMR ($CDCl_3$ + DMSO-d_6): δ 1.03 (s, 6H, $(CH_3)_2C$-), 2.58 (s, 2H, -CH_2phenyl), 2.83 (br, 2H, -CH_2NH), 3.48 (br, 2H, -$CH_2C(O)$), 3.97 (s, 3H -CH_2O, -CHOH), 6.0 (br, 2H, NH_2), 6.57 (d, 2H, ArH, 6.92 (q, 4H, ArH), 7.20 (d, 2H, ArH).

Anal.—Calc. for $C_{21}H_{29}N_3O_3$ (371.5): C, 67.90; H, 7.86; N, 11.32. Found: C, 67.99; H, 7.60; N, 11.22.

β-Adrenergic Receptor-Binding Assay—An in vitro, competitive, radioligand-binding assay with membrane preparations of rat

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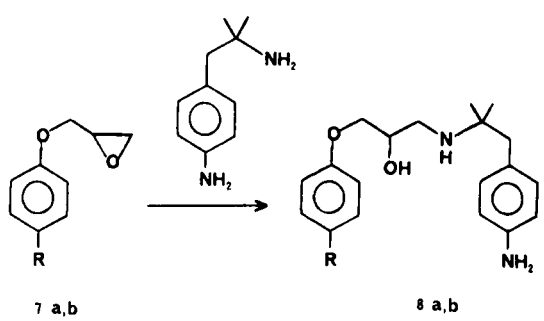
heart and lung was conducted essentially according to the procedure of Bylund and Snyder.[17] Thus, adult rat heart and lungs were homogenized in a Polytron homogenizer (model PT 10-35) for two 20-s bursts (with 10-s interval) at high speed in 20–25 volumes of ice-cold 50 mM tris(hydroxymethyl)aminomethane–HCl buffer (pH 8.0 at 25 °C). The homogenate was then centrifuged at 49 000 × *g* for 10 min. The pellet was suspended in 20–25 volumes of the same buffer, and the homogenate was filtered through a 53-μm nylon mesh. The homogenization and centrifugation were repeated under the same conditions, and the tissue concentration was adjusted to yield ~0.3 mg of protein per milliliter. For the inhibition experiments, 970 μL of the tissue preparation was incubated with 0.5 nM [^{3}H]dihydroalprenolol (20 μL; 95 Ci/mmol) and 5.0 nM drug (10 μL) at 23 °C for 30 min. Specific binding was defined as the difference of binding in the absence or presence of 100 μM norepinephrine. Incubations were terminated with rapid filtration through Whatman GF/B glass fiber filters. The filters were rinsed with 9 mL of the ice-cold buffer, the filtrate was transferred to vials containing 10 mL of scintillation cocktail, and the radioactivity was determined by scintillation spectroscopy.

Results and Discussion

Chemistry—The target compounds were prepared as outlined in Scheme I. The requisite substituted phenoxymethyloxiranes (**7a** and **7b**) were prepared according to the method of Crowther et al.[14] Condensation of these epoxides with α,α-dimethyl-*p*-aminophenethylamine[13,15] in acetonitrile gave products **8a** and **8b** in 30 and 13% yields, respectively. The low yields were undoubtedly a consequence of steric crowding at the reaction center, because unreacted epoxide was generally recovered in large amounts (≤60%). Compound **8a** gave a satisfactory elemental analysis for the expected product with inclusion of 0.5 mol of methanol. Low-resolution, field desorption MS of **8a** provided a molecular ion at *m/e* 371, further supporting the structure. Low-resolution, electron impact MS of **8a** did not give a molecular ion. However, the base peak in the spectrum occurred at *m/e* 265, which is compatible with fragmentation of **8a** at the *gem*-dimethyl branch.[12]

Binding Studies—An examination of the dissociation constants for **8a** and **8b** (Table I) demonstrates that these compounds, as might be expected, bind to β-adrenergic receptors with increased affinities relative to their respective parents in the rat lung, which contains predominately β_2-adrenergic receptors. Unfortunately, in rat heart, which contains mostly β_1-adrenergic receptors, the affinities of **8a** and **8b** are comparable to those of the parent compounds. The reason for the unimproved affinities of **8a** and **8b** for β_1-adrenergic receptors is not readily apparent. It is conceivable



7 a,b 8 a,b

a= NHCOCH$_3$

b= CH$_2$CONH$_2$

Scheme I

Table I—Pharmacological Results[a]

Compound	Rat Heart (mostly β_1) K_i, nM	Rat Lung (mostly β_2) K_i, nM	Cardioselectivity Index (Lung:Heart K_i)
8a	650 ± 40 (3)	2200 ± 300 (3)	3.4
8b	360 ± 40 (3)	1900 ± 300 (3)	5.3
d,l-Practolol	620 ± 130 (3)	13 000 ± 2000 (3)	21
d,l-Atenolol	150 ± 40 (7)	4500 ± 200 (3)	30

[a] The dissociation constants (K_i) were calculated from the IC_{50} (concentration of drug causing 50% inhibition of [^{3}H] dihydroalprenolol binding) values by $K_i = IC_{50}/(1 + L/K_D)$, where L is the concentration of radioligand (0.5 nM) and K_D is the dissociation constant for the radioligand, determined in separate experiments to be 0.26 ± 0.04 nM for heart and 0.25 ± 0.02 nM for lung; numbers in parentheses indicate the number of determinations.

that preferential interaction of one of the functionalities (i.e., the hydrogen-bonding substituent or the amine substituent) with the receptor results in a conformational perturbation that precludes optimal interaction of the other functionality with the β_1-adrenergic receptor. In any case, the net result of the difference in affinity is a loss of cardioselectivity, as reflected in the decreased cardioselectivity index (ratio of dissociation constant with lung membranes to that with heart) (Table I). Both practolol and atenolol are sixfold more cardioselective than their respective *p*-aminobenzyl analogues.

In light of the disappointing and significant decrease in cardioselectivity associated with the incorporation of the *p*-aminobenzyl group into the cardioselective β-adrenergic receptor antagonists practolol and atenolol, the title compounds were not pursued as candidates for radioiodination.

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