Synthesis and Characterization of Iodoazidobenzylpindolol

ABBAS RASHIDBAIGI and ARNOLD E. RUOHO ˣ

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Abstract □ A high affinity β-adrenergic ligand, iodoazidobenzylpindolol, was synthesized and characterized. The absorption spectrum of this compound changed markedly upon photolysis, consistent with decomposition of the azide group. This compound has a K_D of 5–7 × 10^{-10} *M* for the duck erythrocyte ghost β-adrenergic receptor when measured in a competitive binding assay.

Keyphrases □ Iodoazidobenzylpindolol—synthesis and characterization, β-adrenergic receptor competitive binding assay □ β-Adrenergic blocking agents—iodoazidobenzylpindolol, synthesis and characterization □ Photoaffinity labeling—iodoazidobenzylpindolol label for β-adrenergic receptor binding sites

To identify the polypeptides that comprise the catecholamine receptor, suitable derivatives of β-receptor ligands must be available for forming covalent bonds in the binding site. This method for specifically tagging a binding site in an otherwise heterogeneous mixture of binding sites utilizing chemically reactive site-directed ligands has been described as affinity labeling (1–3) or the more recently used method of "photoaffinity labeling" (4–7).

The present study describes the synthesis and characterization of a potent β-adrenergic ligand containing an azide functional group. This compound, which is called iodoazidobenzylpindolol (VI), has been used as a photoaffinity label for β-adrenergic receptor binding site(s) (8).

EXPERIMENTAL[1]

Materials—2-Nitropropane[2], *p*-nitrobenzylchloride[2], thallium trichloride[3], nitromethane[4], *m*-nitrophenol[5], L-alprenolol-D-tartrate[5], and DL-propranolol hydrochloride[5] were all pure grade. DL-[^{125}I]Iodohydroxybenzylpindolol was synthesized according to a slightly modified method of Brown (9) from hydroxybenzylpindolol[6].

Instruments—Photolysis was performed with a high-pressure mercury lamp[7]. Melting points were determined on a capillary melting-point apparatus[8]. The IR spectra were obtained on a grating spectrophotometer[9] as potassium bromide disks. NMR spectra were determined on a 90 MHz Fourier transform spectrometer[10]. The chemical shifts reported are relative to an internal tetramethylsilane standard. Mass spectra were run on a spectrometer[11] at 70 ev.

Synthesis of 4-(2,3-Epoxypropoxy)indole (II)—6-Nitrosalicylaldehyde was prepared from *m*-nitrophenol according to the method of Ando and Emoto (10), with an overall yield of 7.2%. 5-Nitro-1,3-benzodioxane was purified by vacuum distillation (instead of column chromatography) at 1-mm Hg followed by crystallization in ethanol. In the process, 7-nitro-1,3-benzodioxane was removed and discarded.

4-Hydroxyindole (I)—Compound I was prepared from 6-nitrosalicylaldehyde according to the method of Beer *et al.* (11), with an overall yield of 26.0%.

4-(2,3-Epoxypropoxy)indole (II)—4-Hydroxyindole (I) (722 mg, 5.43 mmoles) was added to a solution of sodium hydroxide (216 mg, 5.40 mmoles) in 5 ml of water. Freshly distilled epichlorhydrin, 0.54 ml, was then added under nitrogen, and the reaction was stirred for 22 hr at ambient temperature. The mixture was extracted with ether, decolorized with activated charcoal, and dried over anhydrous sodium sulfate. The ether extract was evaporated to give 570 mg (55.5%) of an oily product. This compound was purified further using a silica gel column[5] (60–200 mesh) equilibrated with petroleum ether. The product was eluted from the column with increasing amounts of chloroform to give a white solid material (II); mp 64–65° [lit. (12), 65–67°]; NMR (deuterochloroform): δ 8.60–8.35 (s, 1H, indole-NH), 7.0–6.3 (m, 5H, aromatic H), and 4.3–2.5 (m, 5H, CH_2, epoxide-H) ppm; mass spectrum: *m/z* 189 (M^+).

Anal.—Calc. for $C_{11}H_{11}NO_2$: C, 69.84; H, 5.82; N, 7.41. Found: C, 70.00; H, 6.02; N, 7.45.

Synthesis of 1-(*p*-Azido-*m*-iodophenyl)-2-methyl-2-propylamine (V)—2-Methyl-2-nitro-1-(*p*-nitrophenyl)propane was prepared according to the method of Hass *et al.* (13), with an overall yield of 47%.

1-(p-Aminophenyl)-2-methyl-2-propylamine (III)—2-Methyl-2-nitro-1-(*p*-nitrophenyl)propane (10 g, 44.6 mmoles) was dissolved in 150 ml of 50% ethanol–water. Zinc powder (42.5 g, 654 mmoles) was mixed with the above solution. While heating the mixture very slowly with fast mechanical stirring, hydrochloric acid (25 ml of 37% mixed with 40 ml of 50% ethanol–water) was added dropwise. The mixture was refluxed for 1 hr after addition of hydrochloric acid and stirring continued during refluxing. After cooling the mixture, a yellow solution was separated from the zinc and was adjusted to pH 9.0 using 2 *N* potassium hydroxide. The amino compound, 1-(*p*-aminophenyl)-2-methyl-2-propylamine (III), was extracted with ether. The ether solution was dried under anhydrous sodium sulfate followed by evaporation of ether and distillation of the compound under vacuum. A yellow solid, 3.45 g (48%), was collected; mp 81–82° [lit. (13), 84–85°]; R_f 0.17 on silica gel plates[12] in chloroform–methanol (70:30); IR (potassium bromide): 3500, 3350, and 3200 cm^{-1} (NH_2); NMR (deuterochloroform): δ 6.7 (d, 2H, aromatic H, *J* = 3 Hz), 6.4 (d, 2H, aromatic H, *J* = 3 Hz), 2.45 (s, 2H, CH_2), and 1.0 (s, 6H, CH_3); mass spectrum: *m/z* 164 (M^+). The dihydrochloride salt of this compound was prepared by passing hydrogen chloride gas through an ether solution.

1-(p-Amino-m-iodophenyl)-2-methyl-2-propylamine (IV)—1-(*p*-Amino-phenyl)-2-methyl-2-propylamine dihydrochloride (III) (1.66 g, 7.0 mmoles) was dissolved in 200 ml of sodium acetate buffer (pH 4.0, 0.1 *M*). To this solution was added sodium iodide (1.05 g, 7.0 mmoles). Thallium trichloride (2.6 g, 7.0 mmoles) in 50 ml of distilled water was slowly added to the above solution under nitrogen. The mixture was heated on a steam bath for 1 hr under nitrogen, then sodium sulfite (0.88 g, 7.0 mmoles) in 20 ml of water was added. The reaction mixture was made alkaline using sodium bicarbonate and extracted with ether. The compound (IV), 0.76 g (37%), was purified using a silicic acid (325 mesh) column, eluting with increasing concentrations of methanol in chloroform. The compound was oily with a brownish color and an R_f of 0.30 on silica gel plate in chloroform–methanol (70:30). NMR (deuterochloroform): δ 7.4 (d, 1H, aromatic H *ortho* to I), 7.0–6.6 (m, 2H, aromatic H), 2.5 (s, 2H, CH_2), 1.1 (s, 6H, CH_3) ppm, and NH exchangeable with deuterium oxide; mass spectrum: *m/z* 290 (M^+).

Anal.—Calc. for $C_{10}H_{15}IN_2$: C, 41.38; H, 5.17, N, 9.66. Found: C, 41.16; H, 5.31, N, 9.42.

1-(p-Azido-m-iodophenyl)-2-methyl-2-propylamine (V)—1-(*p*-Amino-*m*-iodophenyl)-2-methyl-2-propylamine (IV) (220 mg, 0.758 mmoles) was dissolved in 6.2 ml of water containing 0.207 ml of 96% sulfuric acid. The mixture was cooled in crushed ice, and sodium nitrite (58 mg, 0.840 mmoles) in 8 ml of water was added dropwise while stirring the mixture. When the addition was completed, the reaction was allowed to proceed for 15 min, then sodium azide (54 mg, 0.83 mmoles) in 1 ml of water was added. After another 15 min, the mixture was made alkaline

[1] Elemental analyses were performed by the Galbraith Laboratories, Knoxville, Tenn.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] K & K Laboratories, Plainview, N.Y.
[4] Fisher Scientific Co., Itasca, Ill.
[5] Sigma Chemical Co., St. Louis, Mo.
[6] A gift from Sandoz Pharmaceutical Co., East Hanover, N.J.
[7] AH-6 lamp from Advanced Radiation Corp., Santa Clara, Calif.
[8] Buchi, Switzerland.
[9] Model 700, Perkin-Elmer Corp., Norwalk, Conn.
[10] HX-90 Fourier transform NMR spectrometer, Bruker Instruments, Inc., Billerica, Mass.
[11] Model 1015 Finnigam spectrometer with Finnigam 6000 data system.
[12] Silica gel GF254, Type 60, E. Merck, was used to prepare plates with a 0.5-mm thickness.

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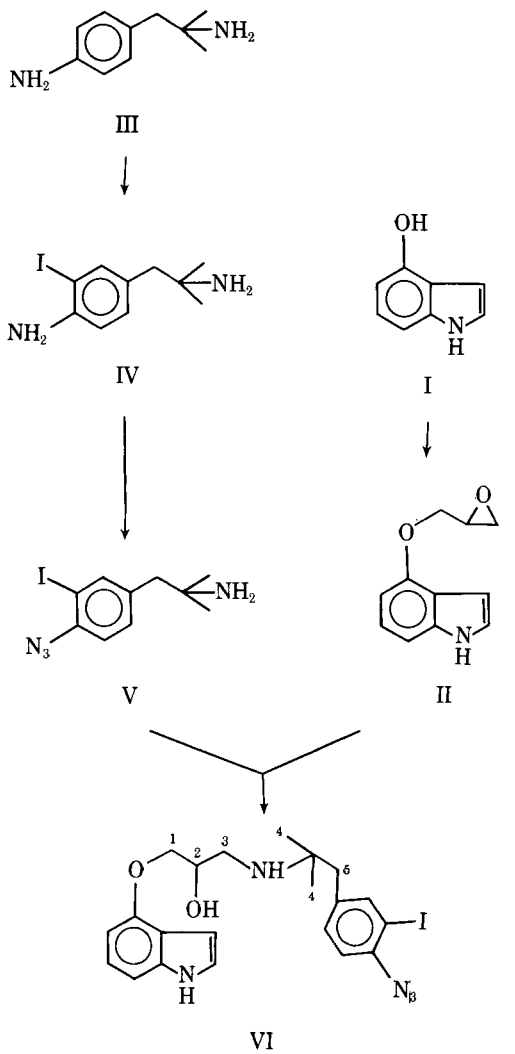

Scheme I—Synthetic scheme for iodoazidobenzylpindolol (VI).

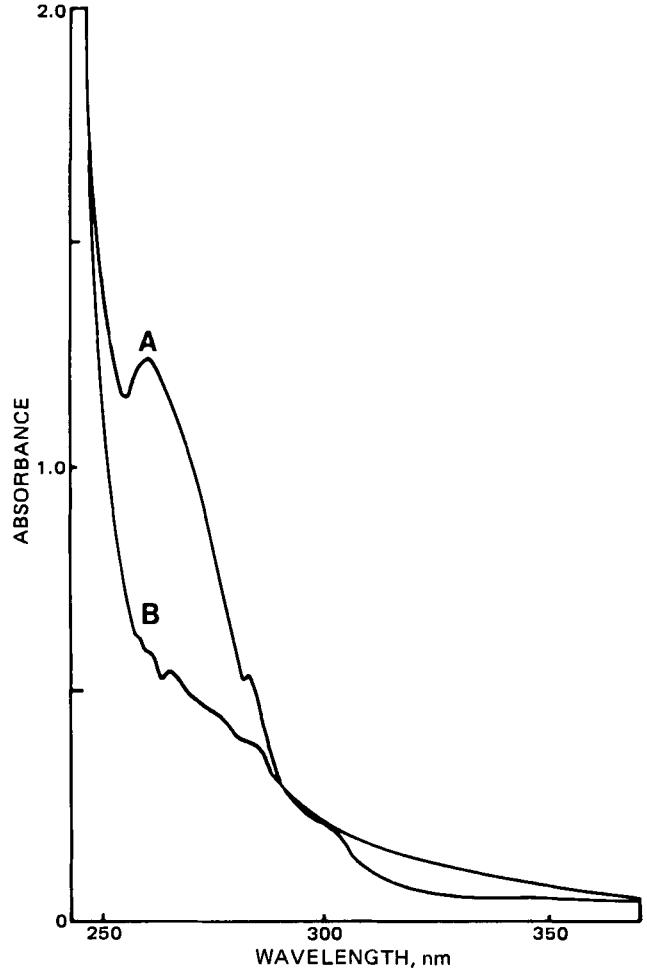

Figure 1—*Ultraviolet spectra for iodoazidobenzylpindolol (VI) before and after photolysis. A, VI (5.7×10^{-5} M) in ethanol; B, VI (5.7×10^{-5} M) photolyzed for 5 sec in ethanol using a high-pressure mercury lamp.*

using sodium bicarbonate and extracted with ether. All reactions were performed in the dark, and 72 mg (30%) of the oily product was purified by preparative TLC using silica gel in chloroform–methanol (70:30) with an R_f of 0.59; IR (potassium bromide): 2150 cm^{-1} (N_3); NMR (deuterochloroform): δ 7.60 (d, 1H, aromatic H *ortho* to I), 7.26–6.97 (m, 2H, aromatic H), 2.5 (s, 2H, CH_2), and 1.1 (s, 6H, CH_3) ppm.

Synthesis of DL-1-(Indol-4-yloxy)-3-[1-*p*-(azido-*m*-iodophenyl)-2-methyl-2-propylamine]-2-propanol (Iodoazidobenzylpindolol, VI)—4-(2,3-Epoxypropoxy)indole (II) (56 mg, 0.296 mmoles) and 1-(*p*-azido-*m*-iodophenyl)-2-methyl-2-propylamine (V) (35 mg, 0.110 mmoles) were dissolved in 1.5 ml of absolute ethanol. The reaction was heated in a sealed tube at 60° in the dark for 7 days. The products of the reaction mixture were separated on preparative silica gel plates with benzene–acetonitrile–triethylamine (100:75:1) to yield 44 mg (78.8%) of a solid compound (VI) with an R_f of 0.39 and a mp of 70–73° (dec. at 140–150°); IR (potassium bromide): 2150 cm^{-1} (N_3); NMR (deuterochloroform): δ 8.9 (s, 1H, indole-NH), 7.78 (d, 1H, aromatic H *ortho* to I), 7.57–6.45 (m, 7H, aromatic H), 4.20 [m, 5H, NH, OH, CH_2(1), CH(2)]; deuterium oxide exchange: 4.10 [m, 3H, CH_2(1), CH(2)], 3.01 [m, 2H, CH_2(3)], 2.70 [s, 2H, CH_2(5)], and 1.12 [s, 6H, CH_3(4)] ppm; UV (ethanol): 300, 288, and 262 nm (Fig. 1A) with ϵ262 = 19,000 and ϵ288 = 6920 M^{-1} cm^{-1}.

Anal.—Calc. for $C_{21}H_{24}IN_5O_2$: C, 49.90; H, 4.75; N, 13.86. Found: C, 50.03; H, 5.24; N, 13.10.

The %H and %N values differ by 0.5 and 0.7%, respectively, from the calculated values. This is probably due to decomposition that occurred before analysis, resulting from exposure of the compound to light.

Preparation of Duck Erythrocyte Ghosts—All steps were performed at 4°. Heparinized whole duck blood was diluted in an equal volume of washing buffer (145 m*M* NaCl, 10 m*M* tromethamine[5], 20 m*M* glucose, pH 7.4). The cells were centrifuged at 500×*g* and the supernate and buffy coat were removed. The packed cells were washed twice more in washing buffer and then lysed by stirring in lysis buffer (10 m*M* tromethamine, 4 m*M* magnesium chloride, pH 7.4). The resulting lysate was centrifuged at 20,000×*g* and the supernate was removed. The ghosts were stored at −80° at a concentration of ~15 mg/ml. The protein content of the ghosts was determined by the Lowry method (14) using bovine serum albumin as a standard.

[^{125}I]Iodohydroxybenzylpindolol Competitive Binding Assay—Nucleated duck ghosts were diluted to a protein concentration of 3–4 mg/ml with 50 m*M* tromethamine buffer, pH 7.4, containing 10 m*M* magnesium chloride. Each condition in the incubation contained ~0.2 mg of nucleated ghosts in 0.1 ml of total incubation volume, various concentrations of the competitive ligands, and 0.6 n*M* [^{125}I]iodohydroxybenzylpindolol. Incubation proceeded for 30 min at 32°. The binding was terminated by diluting 35-μl aliquots in duplicate into 10 ml of lysis buffer at 35°, filtering quickly (10 sec) through a glass fiber filter paper[13] under vacuum, and washing with an additional 10 ml of lysis buffer (5 sec) at 27°. The filter papers were counted using a gamma counter[14] with 70% efficiency, and the amount of bound [^{125}I]iodohydroxybenzylpindolol was determined.

The amount of nonspecific binding in all experiments was determined by incubating membranes and [^{125}I]iodohydroxybenzylpindolol in the presence of 1 μ*M* (L)-alprenolol. Nonspecific binding was generally 5–10% of the total binding and was subtracted from all experimental values. The K_D for each ligand was calculated from the equation (15):

$$K_D = IC_{50}/(1 + L/K_L) \qquad \text{(Eq. 1)}$$

where IC_{50} is the concentration of nonradioactive ligand which half maximally inhibits [^{125}I]iodohydroxybenzylpindolol binding, L is the concentration of [^{125}I]iodohydroxybenzylpindolol in the incubation, and

[13] GF/A Whatman glass microfilter paper, England.
[14] Model 5230, Packard Instrument, Downers Grove, Ill.

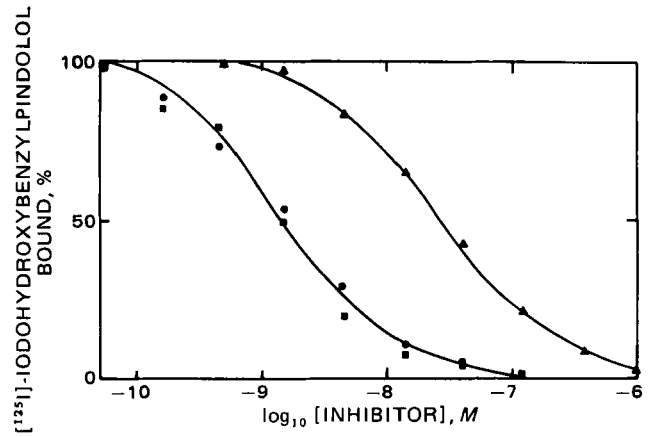

Figure 2—*Competitive inhibition of specific β-receptor binding of [125I]iodohydroxybenzylpindolol by DL-iodoazidobenzylpindolol (VI), DL-hydroxybenzylpindolol, and DL-propranolol. Data for each compound were obtained in duplicate, and the mean was plotted as percent of initial specific binding of [125I]-iodohydroxybenzylpindolol. Key: DL-iodoazidobenzylpindolol, ●; DL-hydroxybenzylpindolol, ■; and DL-propranolol, ▲.*

K_L is the equilibrium dissociation constant of [125I]iodohydroxybenzylpindolol determined from Scatchard analysis (16) (5×10^{-10} *M*).

RESULTS

Synthesis of Iodoazidobenzylpindolol (VI)—The synthetic scheme for the compound is summarized in Scheme I. The indole-containing epoxide (II) was prepared in a straightforward manner from 4-hydroxyindole (I). The epoxide was reacted with a primary amine to generate the final product (VI). The primary amine that was used in this synthesis contained an aralkyl structure, since it is known that affinity for the β-receptor is increased by increasing the size of substituents on the amino nitrogen (17). This final product, VI, has several structural features that are noteworthy: (*a*) the compound contains an indole ring structure which is characteristic of pindolol (12, 18); (*b*) the propanolamine structure which is characteristic of adrenergic compounds; and (*c*) an alkyl chain terminating with an aromatic group. Iodoazidobenzylpindolol (VI) is an analogue of iodohydroxybenzylpindolol (19), in which the phenolic hydroxyl on the aralkyl chain is replaced by an azido group and an iodine atom is *ortho* to the azido group rather than on the indole ring. An important synthetic reaction to generate 1-(*p*-amino-*m*-iodophenyl)-2-methyl-2-propylamine (IV) was the use of thallium trichloride and sodium iodide to incorporate iodine covalently. This type of reaction was reported previously for the preparation of iodinated polynucleotides (20) and nucleotides (21). In these cases, covalent iodine incorporation was followed by using carrier-free [125I]sodium iodide. This reaction demonstrated that the iodine-containing nucleotides could substitute for the parent nucleotides in polymers synthesized by either DNA or RNA polymerase (21). Chemical characterization of the reaction products was not performed.

Iodoazidobenzylpindolol (VI) has three absorption maxima (262, 288, and 300 nm). The main absorption occurs at 262 nm, whereas the 288 and 300 nm absorbances are secondary peaks (Fig. 1A). The photosensitivity of VI is shown in Fig. 1. After a 5-sec photolysis of VI in ethanol using a 1 kw mercury lamp, the ultraviolet absorption at 262, 288, and 300 nm were all reduced in a manner consistent with the decomposition of the azide group (Fig. 1B).

Biological Characterization of Iodoazidobenzylpindolol (VI)—To determine the binding characteristics of VI to the β-adrenergic receptor, intact duck erythrocyte ghosts were utilized. The β-receptor in this system has been thoroughly characterized for [3H]-L-dihydroalprenolol binding and has been partially purified 5000–10,000-fold over detergent extracts[15]. The apparent equilibrium dissociation constants (K_D) were determined by a previous method (15) utilizing competitive inhibition of [125I]iodohydroxybenzylpindolol binding. Iodoazidobenzylpindolol (VI) and hydroxybenzylpindolol possess the same affinity for the duck erythrocyte β-receptor with an apparent K_D calculated to be 0.5–0.7 n*M* (Fig. 2). From Scatchard analysis (16), it was found that the K_D for [125I]iodohydroxybenzylpindolol is 0.5 n*M* under the same conditions (data not shown). DL-Propranolol inhibited [125I]iodohydroxybenzylpindolol binding with a calculated K_D of 12.5 n*M* (Fig. 2), the same as previously reported for other β-adrenergic receptor systems (22).

[15] Y. Shing, A. Abramson, and A. E. Ruoho, submitted for publication.

DISCUSSION

The successful chemical synthesis of an azide derivative of pindolol, a potent β-adrenergic ligand, was performed. The compound was synthesized from 4-(2,3-epoxypropoxy)indole (II), which was prepared in good yield from *m*-nitrophenol. Thallium trichloride and sodium iodide were used to produce 1-(*p*-amino-*m*-iodophenyl)-2-methyl-2-propylamine (IV). This reaction allowed covalent incorporation of iodine into the phenyl ring *ortho* to the amine functional group which could then be readily converted to the azide derivative of V.

Iodoazidobenzylpindolol (VI) has as high an affinity as hydroxybenzylpindolol for the duck erythrocyte β-adrenergic receptor, and contains an azide functional group which can be incorporated into the β-adrenergic receptor binding site(s) upon photolysis (8). To minimize photodestruction of the biological samples and release of iodine, the majority of the light of wavelength <300 nm was filtered out. Successful photoincorporation of 4-[125I]iodobenzene-1-azide (23) and 5-[125I]iodonapthyl-1-azide (24) have also been reported for probing the lipid bilayer of biological membrane and labeling of the α-subunit of the sodium–potassium adenosinetriphosphatase (25).

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