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β-Adrenergic Receptor: Stereospecific Interaction of Iodinated β-Blocking Agent with High Affinity Site

Abstract. *An iodine-labeled β-adrenergic inhibitor (*^{125}*I-hydroxybenzylpindolol) binds specifically to a site on turkey erythrocyte membranes. A series of β-adrenergic agonists and inhibitors compete for this binding site, with apparent affinities paralleling biological effectiveness as activators or inhibitors of catecholamine-stimulated adenylate cyclase. The activity of* d-(+) *agonists or inhibitors was 1 percent (or less) than that of the corresponding* l-(−) *isomers in competing for binding of the iodinated blocker as well as in affecting catecholamine-stimulated adenylate cyclase.* l-(−)-*Norepinephrine was about one-tenth as active as* l-(−)-*isoproterenol in competing for the β-blocking agent site. The stereospecificity of the interaction with the iodinated β-blocking agent and the correspondence between affinity for site and biological potency of analogs suggested that this interaction is involved in function of the β-adrenergic receptor.*

Interaction of β-adrenergic agonists with specific receptors accounts for a variety of biological phenomena in diverse tissues; and in each, adenylate cyclase is activated with consequent generation of adenosine 3′,5′-monophosphate (cyclic AMP), which in turn produces the ultimate physiological response (*1*). The generality of this sequence has prompted many investigators to analyze directly for the receptor. These several studies (*2*, *3*) to date have depended on determining binding of ligands of low specific activity (tritiated catecholamines, 2 to 15 c/mmole) and have detected a site that recognizes primarily the catechol function of the molecule. This site does not distinguish between active or inactive stereoisomers of the agonist (*2*, *3*). Conversely, the typical β-adrenergic blocking agents (β-blockers) show low affinity for the catechol-specific site but high affinity as inhibitors of adenylate cyclase and biological activity (*2*, *3*). The β-blockers are ethanolamine analogs, not catechol analogs, and show the same required stereoconfiguration as do the catecholamines for biological activity. Thus it appeared (*3*) that receptor recognition of the stereospecific ethanolamine function was common to both the β-agonists and β-blockers. We now report identification of such a stereospecific site by determining binding of an iodine-labeled β-blocker to a high affinity site on turkey erythrocyte membranes. The apparent affinities of either β-adrenergic agonists or blockers for this site parallel biological effectiveness of the compounds as activators or inhibitors of catecholamine-responsive adenylate cyclase in the turkey erythrocyte membrane.

Hydroxybenzylpindolol (HYP) (*4*) was iodinated by a modification (*5*) of the Hunter-Greenwood technique (*6*) and purified by extraction (equal volumes in upper and lower phases) into ethyl acetate from a mixture of 0.02*M* potassium iodide and 1*M* acetic acid. Specific activity of the labeled compound in the ethyl acetate phase was in the range of 200 to 300 c/mmole. The preparation and storage of turkey erythrocyte membranes were as described earlier (*3*). Binding of ^{125}I-labeled HYP was determined by sedimenting the membranes in microcentrifuge tubes (*7*). The amount of ^{125}I-labeled HYP bound to membranes was more than ten times that in the blank (that is, the radioactivity trapped in the centrifuge tube in the absence of added membranes). Adenylate cyclase was assayed (Fig. 1, legend) according to Salomon *et al.* (*8*).

The iodine-labeled inhibitor bound rapidly to erythrocyte membranes. Binding was maximal within 8 minutes at 25°C, and within 4 minutes at 37°C. The biological significance and specificity of binding were determined with catecholamines, analogs, and inhibitors by comparing apparent affinities for the receptor with potency as inhibitors of isoproterenol-stimulated adenylate cyclase. These studies were carried out in parallel under virtually identical conditions of incubation (see Fig. 1). The compounds recognized as β-blockers were effective as inhibitors of isoproterenol-stimulated adenylate cyclase and showed an order of apparent affinity for the binding site on the membranes virtually identical to the order of potencies as inhibitors of isoproterenol-stimulated adenylate cyclase (Fig. 1). The *dl*-HYP is more potent than *l*-(−)-propranolol in adenylate cyclase inhibition as well as in inhibition of binding of ^{125}I-labeled HYP (*9*). Inhibition of binding and inhibition of adenylate cyclase were both dependent on stereochemical configuration; *l*-(−) analogs were greater than 100 times more effective than *d*-(+) analogs. The following compounds tested at $10^{-4}M$ did not inhibit iodine-labeled HYP binding: dihydroxyphenylglycol, dihydroxymandelic acid, and octopamine. Dopamine, metanephrine, and phentolamine (all at $10^{-4}M$) inhibited binding by 7, 13, and 17 percent, respectively. All β-blockers that were effective as inhibitors of isoproterenol-stimulated adenylate cyclase activity as well as inhibitors of specific binding of the iodinated β-blocker were derivatives of ethanolamine. Conversely, nonagonist catechols—for example, dihydroxyphenylglycol or dihydroxymandelic acid—which interact strongly with a catechol-specific site on the membrane (*3*) were ineffective as inhibitors of iodine-labeled HYP binding. On the other hand, biologically effective catecholamines *l*-(−)-isoproterenol or *l*-(−)-

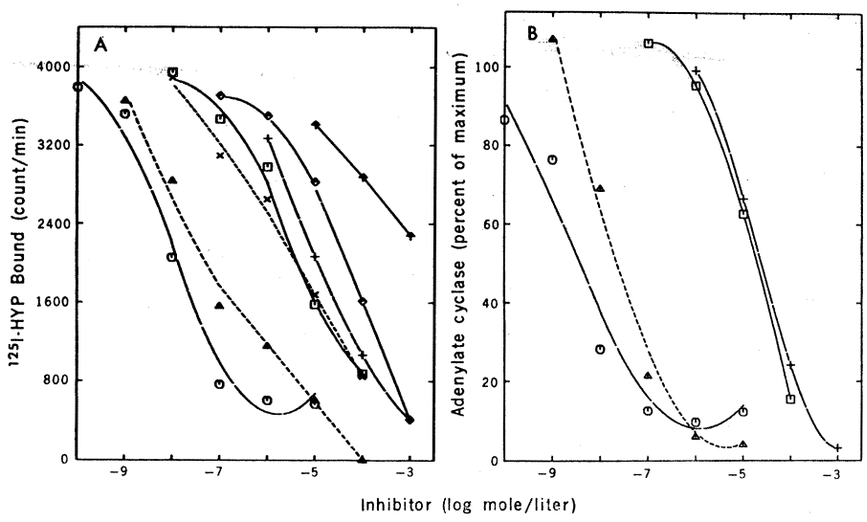

Fig. 1. (A) Comparative effects of catecholamine analogs and inhibitors on binding of ^{125}I-labeled HYP. (B) Inhibition of isoproterenol-stimulated adenylate cyclase. The basic incubation medium for both parts of the experiments was the same: 0.125 m*M* adenosine triphosphate (ATP), 5 m*M* magnesium, 8 m*M* theophylline, 1 μM guanylimidodiphosphate (*10*), 10 m*M* KCl, creatine phosphokinase 100 μg/ml, 5 m*M* creatine phosphate, 1 m*M* ethylene glycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid in 50 m*M* tris, *p*H 7.5., with membrane protein equivalent to 0.4 mg/ml. In part A, ^{125}I-HYP (130,000 count/min per milliliter; approximately $5 \times 10^{-10} M$) was added to the system with other compounds [HYP (⊙——⊙), *l*-(−)-propranolol (▲ - - - ▲), dichloroisoproterenol (⊡——⊡), *d*-(+)-propranolol (+ - - +), *l*-(−)-isoproterenol (× - - - - ×), *l*-(−)-norepinephrine (◇— —◇), *d*-(+)-isoproterenol (⮝ — —⮝)] as indicated and incubated at 37°C for 10 minutes. The amount of iodinated β-blocking agent bound was determined in triplicate with 100-μl samples. The ordinate represents the amount of ligand specifically bound—that is, the total amount bound minus that nonspecifically bound (taken as the amount not displaced by $10^{-4} M$ *l*-(−)-propranolol). The total amount of ^{125}I-HYP specifically bound in the absence of other ligands was 4260 count/min. In (B), AT^{32}P, 7×10^6 count/min per milliliter, 3 μM *l*-(−)-isoproterenol, and other compounds as indicated were added, and the amount of radioactive adenosine 3',5'-monophosphate (cyclic AMP) was determined in duplicate with 70-μl samples taken after incubation at 37°C for 10 minutes. On the ordinate 100 percent represents the amount of cyclic AMP formed (2.55 nmole per milligram of protein) with no added inhibitors. Symbols as in (A).

norepinephrine, which have both catechol and stereospecific ethanolamine moieties, were effective inhibitors at the site of interaction with the labeled antagonist. Characteristically *l*-(−)-isoproterenol is approximately ten times as potent as *l*-(−)-norepinephrine as an activator of adenylate cyclase in turkey erythrocyte membranes. The same high potency ratio of isoproterenol to norepinephrine was apparent in competitive inhibition of ^{125}I-labeled HYP binding (Fig. 1). The dextrorotatory isomer *d*-(+)-isoproterenol showed less than 1 percent the affinity of the corresponding active *l*-(−)-stereoisomer; *dl*-norepinephrine was approximately half as effective (data not shown) as *l*-(−)-norepinephrine. Analyses to date suggest that the high affinity (in the range of 10^9 liter/mole) stereospecific sites are few in number in comparison with the number of catechol sites (*3*). Thus the requirement is readily apparent for a high specific activity tracer such as the iodine-labeled HYP analog used here. The high affinity, the stereospecificity, and the order of affinities paralleling biological potency make it reasonable to propose that the β-blocker binding site identified here represents a function common to the β-adrenergic receptor. The use of high specific activity analogs such as ^{125}I-labeled HYP should be valuable in the search to identify β-adrenergic receptors in other tissues or species as well as for development of receptor assays possibly applicable to research or clinical medicine.

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