Biochemical Pharmacology, Vol 27, pp. 1455–1461.

0006-2952/78/0515-1455/$02.00/0

STRUCTURE-BINDING–ACTIVITY ANALYSIS OF BETA-ADRENERGIC AMINES—II.

BINDING TO THE BETA RECEPTOR AND INHIBITION OF ADENYLATE CYCLASE*

JOHN P. BILEZIKIAN,† ALAN M. DORNFELD and DONALD E. GAMMON

Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY 10032, U.S.A.

(*Received* 23 *June* 1977; *accepted* 7 *September* 1977)

Abstract—Over forty adrenergic analogues were tested for their ability to bind to the beta receptor and to inhibit adenylate cyclase in membranes of the turkey erythrocyte. Over a range of five log orders, the affinity of a compound for the beta receptor, as measured by the specific binding of [^{125}I]hydroxybenzylpindolol, correlated significantly with its ability to inhibit adenylate cyclase. Potency was related to the size of the substituent on the secondary amine, the levo-rotatory configuration, and to the nature of the aromatic group. In contrast to agonists, antagonists permitted a great latitude of aromatic groups. The methoxy bridge linking aromatic and ethanolamine functions was also important but not essential in determining inhibitory activity. The results establish that the structural requirements for activation and inhibition of beta-adrenergic-directed activities are reflected in the interaction at the beta receptor and suggest models to accommodate their effects.

In the accompanying paper [1], the first detailed structure-binding–activity analysis for a large series of beta-adrenergic agonists in a beta$_1$-adrenergic tissue was presented. The affinity with which agonists interacted at beta-receptor sites (K_D) in the turkey erythrocyte correlated significantly with their ability to stimulate adenylate cyclase (K_A). Specific structural features which contributed to receptor occupation and adenylate cyclase responsiveness were denoted. It was also possible to consider the ability of agonists to stimulate adenylate cyclase maximally (relative to isoproterenol) with regard to structural determinants. The K_A for activation and the K_D for binding correlated significantly with the intrinsic activity but less well than the K_A and K_D values correlated with each other.

This companion paper presents the first detailed structure-binding–inhibition analysis for a large series of beta-adrenergic antagonists in a beta$_1$ target tissue. Over forty adrenergic compounds have been studied with respect to their ability to bind to the beta receptor—as monitored by [^{125}I]hydroxybenzylpindolol, and to inhibit catecholamine-responsive adenylate cyclase. The combined analyses represent the most comprehensive structure-binding–activity study yet reported. The results provide further insight into the molecular features of receptor occupation and suggest mechanisms by which these compounds influence the biological coupling of the beta receptor and adenylate cyclase.

* Supported by Research Grants HL 17813, HL 20859 and a Grant-in-Aid from the New York Heart Association.

† Molly Berns Senior Investigator, New York Heart Association; recipient of Research Career Development Award KO4 HL 00383.

MATERIALS AND METHODS

Materials

Materials for the adenylate cyclase and binding assays were noted previously [1]. (+/−)Dichloroisoproterenol was purchased from Sigma Chemicals, St. Louis, MO. The following compounds were the generous gifts of the companies noted: (−)isoproterenol (Sterling-Winthrop Research Institute, Rensselaer, NY); phentolamine (Ciba-Geigy, Summitt, NJ); (+/−)MJ9910, (+/−)isoxyprine, (+/−)MJ7434-1, and (−) and (+)sotalol (Mead, Johnson Co., Evansville, IN); (+/−)nylidrin (USV Pharmaceuticals, Tuckahoe, NY); (+/−)oxprenolol (Merrell National Laboratories, Cincinnati, OH); (+/−)desisopropyl propranolol, (+/−)propranolol glycol, (+/−)propranolol lactic acid, (+/−)propranolol acetic acid, (+/−)hydroxypropranolol, (+/−)iodophenyloxypropranolisopropylamine, (+/−)4-hydroxyphenyloxypropranolisopropylamine, (+/−)4-aminophenyloxypropranolisopropylamine and (+/−)indoleoxypropranolisopropylamine (Imperial Chemical Industries, Ltd., Alderly Park, Cheshire, England); (−) and (+)propranolol, (+/−)pronetholol and (+/−)practolol (Ayerst Laboratories, New York, NY); (+/−)W9803A, (+/−)W10055A, (+/−)W10470A, (+/−)W9291A and (+/−)bunolol (Warner-Lambert Research Institute, Morris Plains, NJ); (+/−)butoxamine (Burroughs Wellcome Research Laboratories, Research Triangle, NC); (−) and (+)alprenolol, and (+/−)H64-52 (Hassle, Molndal, Sweden); tazolol (Syntex Corporation, Palo Alto, CA); (+/−)ritodrine (Philips Duphar, Weesp, Holland); (+/−)hydroxybenzylpindolol, (+/−)hydroxybenzylpropranolol, (+/−)pindolol, (+)pindolol and (+/−)LL21945 (Drs. D. Hauser and R. Berthold,

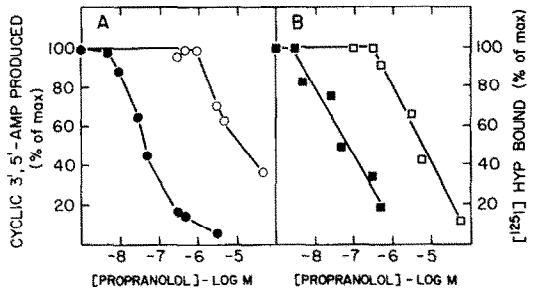


Fig. 1. (A) adenylate cyclase in turkey erythrocyte membranes; inhibition by propranolol. Membranes were incubated under conditions of the adenylate cyclase assay with isoproterenol (50 μM) and increasing concentrations of (−)propranolol (●) or (+)propranolol (○). (B) binding of [^{125}I]HYP to turkey erythrocyte membranes; inhibition by propranolol. Membranes were incubated under binding assay conditions with [^{125}I]HYP (30 pM) and increasing concentrations of (−)propranolol (■) or (+)propranolol (□). The results are the mean of triplicate determinations.

Sandoz Pharmaceutical, Basel, Switzerland); and (+/−)timolol (Merck, Sharp & Dohme, West Point, PA), Protyronol was synthesized by N. Wasserman (Department of Microbiology, Columbia College of Physicians and Surgeons, NY), and J. Bilezikian.

Methods

The following methods employed in this study were described in the accompanying paper [1]: preparation of turkey erythrocyte membranes; iodination of hydroxybenzylpindolol (HYP); adenylate cyclase assay; binding assay; and determination of the equilibrium dissociation constant for binding, K_D.

Determination of the inhibition constant. The inhibition constant (K_I) is defined as the concentration of antagonist at which half-maximal inhibition of (−)isoproterenol-responsive adenylate cyclase at the K_A of (−)isoproterenol is achieved. This relationship is expressed by the following equation:

$$K_I = [I]\frac{K_{dapp}}{K_{dapp} + [A]}\Big/(R_o/R - 1),$$

where $K_I = [I]$ at half-maximal inhibition, K_{dapp} = concentration of agonist leading to half-maximal activation in the presence of guanylimidodiphosphate; R_o = activity of adenylate cyclase in the presence of agonist $[A]$; and R = activity in the presence of antagonist $[I]$ [2]. A dose–response curve for adenylate cyclase inhibition by all inhibitors tested was performed on at least two separate occasions and contained seven different concentrations of antagonists spanning three orders of magnitude. Each antagonist was studied an average of four separate times. In every adenylate cyclase assay a complete dose–response curve for (−)propranolol was established.

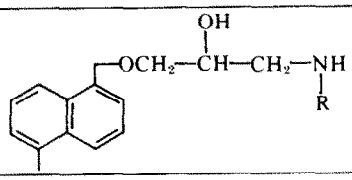
RESULTS

Catecholamine-responsive adenylate cyclase activity (Fig. 1A) and binding of [^{125}I]HYP to turkey erythrocyte membranes (Fig. 1B) are inhibited by (−)propranolol. Half-maximal inhibition for adenylate cyclase and binding is similar. Both binding and adenylate cyclase displayed stereospecific preference for the (−) isomer.

Naphthylmethoxyethanolamines

The compounds listed in Table 1 all contained a primary or substituted naphthylmethoxy group attached to an ethanolamine side chain. These compounds differ from typical beta-adrenergic agonists by the non-catechol, naphthyl group and a methoxy bridge, but share similar ethanolamine determinants. They are potent beta-adrenergic inhibitors with binding and inhibition constants as low as 5.7 and 3.9 nM respectively. These values are approxi-

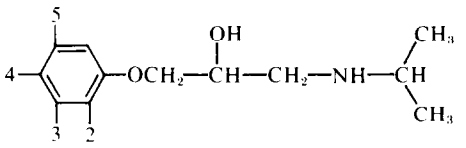
Table 1. Naphthylmethoxyethanolamines*



Compound		R	Binding K_D (nM)	Adenylate cyclase K_I (nM)
(+/−)Desisopropylpropranolol		H	42 ± 4	175 ± 26
(−)Propranolol		$CH(CH_3)_2$	5.7 ± 1	3.9 ± 0.7
(+/−)Protyronol		$(CH_2)_2$-C$_6$H$_4$-OH	40 ± 1	30 ± 5
(+/−)Hydroxybenzylpropranolol		$C(CH_3)_2CH_2$-C$_6$H$_4$-OH	26 ± 0.8	9.6 ± 0.7
(+/−)W9291A	=O	$(CH_2)_2$-C$_6$H$_3$-(OCH$_3$)$_2$	20 ± 1	7.7 ± 0.4
(+/−)4-OH propranolol	−OH	$CH(CH_3)_2$	25 ± 2	11 ± 4
(+/−)Bunolol	=O	$C(CH_3)_3$	8.1 ± 0.4	24 ± 2
(+/−)Pronetholol†		$CH(CH_3)_2$	830 ± 390	506 ± 142
(+)Propranolol		$CH(CH_3)_2$	1210 ± 265	2870 ± 737

* K_D and K_I were determined according to Methods. The data are expressed as mean ± S. E. M.
† 2-α-Naphthol lacking methoxy bridge.

Table 2. Phenylmethoxyisopropylethanolamines*



Compound	2	3	4	5	Binding K_D (nM)	Adenylate cyclase K_I (nM)
(+/−)Iodophenyliso-propylamine	I	H	H	H	2.0 ± 0.1	6.4 ± 2
(−)Alprenolol	$CH_2CH{=}CH_2$	H	H	H	3.0 ± 0.2	22 ± 1
(+/−)Oxprenolol	$OCH_2CH{=}CH_2$	H	H	H	13.9 ± 2	3 ± 2
(+)Alprenolol	$CH_2CH{=}CH_2$	H	H	H	101 ± 6	757 ± 31
(+/−)W10055A†	H	OH	H	OH	227 ± 12	375 ± 79
(+/−)H64/52	H	$CH_2{=}CHCH_2$	H	H	1,220 ± 139	656 ± 201
(+/−)MJ9910	H	OH	OH	H	740 ± 41	1,200 ± 69
(+/−)4-Aminophenyliso-propylamine	H	H	NH_2	H	971 ± 53	2,390 ± 1,149
(+/−)4-OH phenylisopro-pylamine	H	H	OH	H	1,060 ± 748	1,230 ± 481
(+/−)Practolol	H	H	$NHCOCH_3$	H	9,710 ± 533	11,000 ± 1,380

* K_D and K_I were determined according to Methods. The data are expressed as mean ± S. E. M.
† Butyl group replaces isopropyl group.

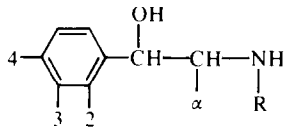
mately 100-fold lower than the binding and activation constants of the most potent beta-adrenergic agonists. The 4-hydroxyl and keto-analogues of propranolol, 4-hydroxy propranolol and bunolol, retained significant potency. The affinity of these inhibitors was influenced by the size of the R substituent on the secondary amine in a manner similar to beta-adrenergic agonists. Propranolol and hydroxybenzylpropranolol, containing large R groups, were more potent than desisopropylpropranolol, a free amine. The weakest compounds in this group were the (+) antipode of propranolol and pronetholol. Pronetholol differs from propranolol by being a 2-α naphthol derivative and by lacking the methoxy bridge.

Naphthylmethoxy compounds which lacked the complete ethanolamine side chain were ineffective inhibitors of binding and adenylate cyclase. The K_I and K_D for propranolol glycol, propranolol acetic acid, and propranolol lactic acid were all greater than 100 μM. These results are similar to those obtained for similarly oxidized catecholamines [1]. The loss of the amine group was, therefore, associated with the loss of the activity of compounds that otherwise would have been effective agonists or antagonists.

Phenylmethoxyisopropylethanolamines

Aromatic groups besides primary or substituted naphthyl determinants could be associated with significant antagonist properties (Table 2). Phenylmethoxy compounds substituted in position 2 with iodine, methyl ethylene (alprenolol) or methoxy ethylene (oxprenolol) groups were as potent as the naphthyl derivatives described in Table 1. However, substitution of the phenyl group in position 3 or 4, or both, led to a marked reduction in the affinity of binding and the inhibitory activity of these

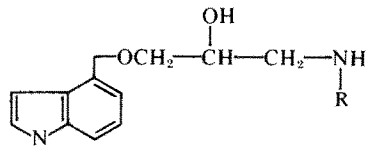
Table 3. Phenylethanolamines*

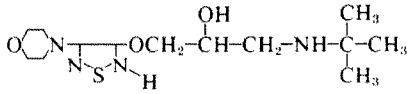
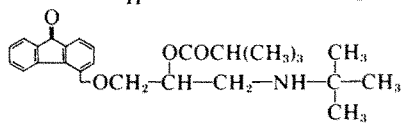
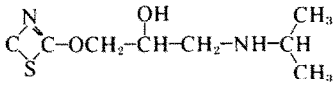


Compound	2	3	4	α	R	Binding K_D (nM)	Adenylate cyclase K_I (nM)
(+/−)Nylidrin	H	H	OH	CH_3	$CH(CH_3)CH_2CH_2$⟨⬡⟩	370 ± 50	471 ± 30
(+/−)Ritodrine	H	H	OH	CH_3	$(CH_2)_2$⟨⬡⟩−OH	900 ± 140	370 ± 145
(+/−)PI39	H	H	OH	H	$CH(CH_3)_2$	970 ± 200	980 ± 57
(+/−)Isoxyprine	H	H	OH	H	$CH(CH_3)(CH)_2$⟨⬡⟩	5,000 ± 900	5,900 ± 341
(+/−)Butoxamine	OCH_3	OCH_3	H	CH_3	$C(CH_3)_3$	1,500 ± 82	2,040 ± 538
(−)Sotalol	H	H	$NHSO_2NH_3$	H	$CH(CH_3)_2$	1,100 ± 31	838 ± 148
(+/−)Dichloroisoproterenol	H	Cl	Cl	H	$CH(CH_3)_2$	1,210 ± 600	1,210 ± 117
(+/−)MJ7434-1	H	H	CH_3SO_2NH	CH_2CH_3	$CH(CH_3)_2$	12,000 ± 659	7,800 ± 451
(+)Sotalol	H	H	$NHSO_2CH_3$	H	$CH(CH_3)_2$	4,000 ± 220	1,080 ± 62

* K_D and K_I were determined according to Methods. The data are expressed as mean ± S. E. M.

Table 4. Indolemethoxyethanolamines; other analogues*



Compound	R	Binding K_D (nM)	Adenylate cyclase K_I (nM)
(+/−)Pindolol	$C(CH_3)_3$	0.6 ± 0.03	6 ± 0.04
(+/−)Isopropylpindolol	$CH(CH_3)_2$	1.4 ± 0.08	10 ± 0.6
(+/−)Hydroxybenzylpindolol	$C(CH_3)_2CH_2$–C$_6$H$_4$–OH	0.085 ± 0.02	0.28 ± 0.06
(+)Pindolol	$C(CH_3)_3$	> 1000	> 1000
Timolol		6.5 ± 0.4	2.5 ± 0.1
LL21945		150 ± 8	250 ± 53
Tazolol		1636 ± 90	230 ± 93

* K_D and K_I were determined according to Methods. The data are expressed as mean ± S. E. M.

compounds. Compound W10055A, a 3′,5′-dihydroxy analogue, and MJ9910, a catecholamine, differed from their agonist counterparts, metaproterenol and isoproterenol, only by the methoxy bridge. The methoxy bridge, therefore, conferred significant antagonist properties on compounds which otherwise would have been agonists. Similar to 4-hydroxyl adrenergic compounds which were weaker agonists than their 3-hydroxyl counterparts, 4-substituted phenyl compounds were weaker inhibitors than their 3-substituted derivatives.

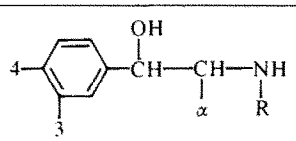
Phenylethanolamines

3- and 4-Substituted phenyl derivatives lacking the methoxy bridge (Table 3) were much weaker antagonists than the methoxy analogues described in Table 2. The importance of the methoxy group is evident again. Within this group, compounds with 4-hydroxyl groups (nylidrin, ritodrine, isoxyprine and PI39) were more potent than compounds with other substitutions in position 4 (sotalol, MJ7434-1 and dichloroisoproterenol). Because these compounds were analogous to the 4-hydroxyl-containing agonists, it seemed likely that these analogues with bulky R groups but without the methoxy bridge might also be partial agonists (see below).

Indolemethoxyethanolamines and other aryl analogues

The great latitude of aromatic groups which was associated with significant inhibitory properties is further illustrated in Table 4. The indole-containing compounds, pindolol, isopropylpindolol and hydroxybenzylpindolol, were among the most potent beta-adrenergic inhibitors as reflected by their ex-

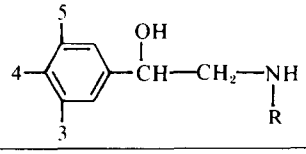
Table 5. Compounds with agonist and antagonist properties*



Compound	3	4	α	R	Binding K_D (μM)	Adenylate cyclase K_I (μM)	K_A (μM)	I_A
MJ9910†	OH	OH	H	$CH(CH_3)_2$	0.74 ± 0.04	1.2 ± 0.07	1.4 ± 0.6	0.20 ± 0.03
Nylidrin	H	OH	CH_3	$CH(CH_3)CH_2CH_2$–C$_6$H$_5$	0.37 ± 0.05	0.47 ± 0.03	1.3 ± 0.06	0.32 ± 0.06
Isoxyprine	H	OH	H	$CH(CH_3)CH_2$–C$_6$H$_5$	5.0 ± 0.9	5.9 ± 0.3	8.4 ± 0.07	0.21 ± 0.03
Ritodrine	H	OH	CH_2CH_3	CH_2CH_2–C$_6$H$_4$–OH	0.90 ± 0.14	0.37 ± 0.1	8.7 ± 1	0.30 ± 0.06
PI39	H	OH	H	$CH(CH_3)_2$	0.97 ± 0.02	0.98 ± 0.06	30 ± 9	0.35 ± 0.06

* K_D, K_I, K_A and I_A were determined according to Methods. The data are expressed as mean ± S. E. M.
† Methoxy group bridges phenyl and ethanolamine groups.

Table 6. Compounds which could bind to beta-receptor sites but were without agonist or antagonist properties*



Compound	3	4	5	R	Binding K_D (μM)
5-OH dopamine	OH	OH	OH	H	103 ± 5
S38537-9	H	OH	H	CH_3	116 ± 37
Metanephrine	OCH_3	OH	H	CH_3	37 ± 5
(+)Soterenol	$NHSO_2CH_3$	OH	H	$CH(CH_3)_2$	50 ± 8
S40032-7	OH	H	H	CH_2CH_3	35 ± 5
W9803A	OH	H	CH_2OH	$C(CH_3)_3$	46 ± 13
W10773	OH	H	CH_2OH	$C(CH_3)_2CH_2$-⬡-OH	8.7 ± 0.6
MJ6987	OH	$NHSO_2CH_3$	H	$CH(CH_3)_2$	74 ± 12
Ephedrine	H	H	H	CH_3	33 ± 2
S35985-4	H	H	H	⬡	174 ± 13

* K_D was determined according to Methods. The data are expressed as mean ± S. E. M.

tremely high affinity and equivalently low K_I values for adenylate cyclase. Other aromatic groups were associated also with potent inhibitory activity as noted by compounds tazolol, timolol and LL21945.

Adrenergic amines with direct agonist and antagonist properties

The compounds listed in Table 5 were both partial agonists and antagonists. Common features included the 4-hydroxyl phenyl configuration, an absent methoxy bridge, and a very bulky R group. In addition, the intrinsic activity of these compounds was between 0.20 and 0.35. The K_D for these compounds was in the range expected for a rather potent agonist and a rather weak antagonist. All other inhibitors depicted in these tables were tested for agonist properties and no others were observed to stimulate adenylate cyclase.

A few compounds which demonstrated a weak but definite affinity for the beta receptor were completely inactive agonists or antagonists within the concentration limits of this assay (Table 6). These compounds shared a common phenylethanolamine configuration. It is not known whether some or all of these compounds would have had demonstrable effects on adenylate cyclase if concentrations greater than 500 μM had been used. For all other compounds tested, if specific competition with [^{125}I]HYP for stereospecific binding sites was demonstrable, the compound was predictably either an agonist or an antagonist.

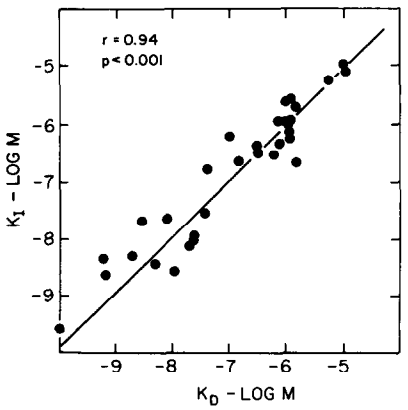


Fig. 2. Correlation between K_D for binding and K_I for adenylate cyclase inhibition among beta-adrenergic antagonists. The values for K_D and K_I are listed in Tables 1–5. Each symbol represents one compound.

Relationship between the K_I and the K_D

The inhibitors reported in this series ranged in binding affinity and in inhibitory potency over six orders of magnitude (100 pM to 50 μM). The correlation between the ability of antagonists to inhibit adenylate cyclase and to occupy the beta receptor was highly significant, the correlation coefficient being $r = 0.94$ ($P < 0.001$) (Fig. 2).

The most potent inhibitors of binding (Fig. 3) and

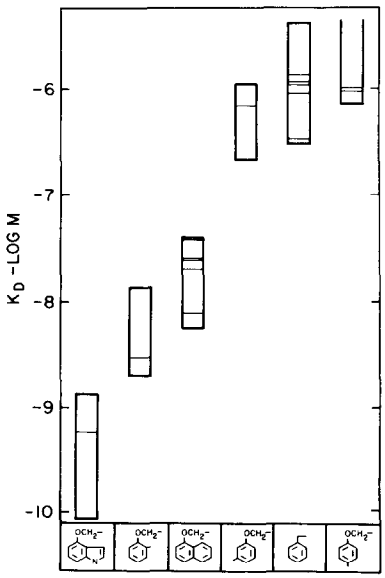


Fig. 3. Role of aromatic groups in defining binding affinity of beta-adrenergic inhibitors. The binding affinity of compounds listed in Tables 1–5 is plotted as a function of their aromatic configuration. Each horizontal line represents one compound.

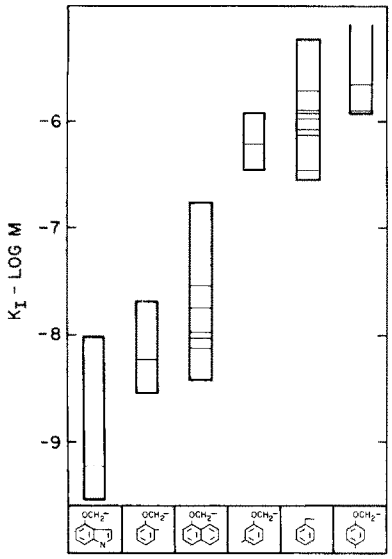


Fig. 4. Role of aromatic groups in defining the inhibitory potency (K_I) of beta-adrenergic inhibitors upon adenylate cyclase. The inhibitory potency of compounds listed in Tables 1–5 is plotted as a function of their aromatic configuration. Each horizontal line represents one compound.

adenylate cyclase (Fig. 4) contained either indole, 2-substituted phenyl, or naphthyl groups with the critical methoxy bridge common to all. Weaker inhibitors contained 3- or 4-substituted phenylated analogues with the methoxy bridge or phenyl-bearing compounds lacking the bridge.

DISCUSSION

The results reported herein and in the accompanying study further delineate the structural features of adrenergic compounds that contribute to their stimulatory and inhibitory properties. For over one hundred adrenergic compounds, the ability to stimulate or inhibit adenylate cyclase correlated significantly with the ability of the compound to interact with binding sites detected by [^{125}I]hydroxybenzylpindolol. The ability to correlate stimulation or inhibition with binding to the beta receptor provides direct validation of more indirect approaches [3–5] and establishes that the beta-adrenergic receptor mirrors the structural requirements of beta-adrenergic functions. Previous studies using adenylate cyclase as an indirect receptor marker [6–8] also appear to be a valid reflection of events occurring at the beta receptor. The relationship between the binding and activation or inhibition constants, the actual value of the constants, and the stereospecificity of binding all provide strong evidence that [^{125}I]HYP interacts with the beta receptor of the turkey erythrocyte.

The common structural denominator of beta-adrenergic agonists and antagonists is the ethanolamine side chain. Isoproterenol and propranolol, prototypes of potent agonists and antagonists, respectively, have identical side chains. Although a great variety of ethanolamine variants was not available in the case of inhibitors, it, nevertheless, appeared that the size of the R substituent was important for potency, as was clearly demonstrated previously for agonists. Also common to both beta-adrenergic agonists and antagonists is a rather strict stereospecificity for the (−) antipode. These data suggest, therefore, that the stereospecific ethanolamine determinant does not define whether the ultimate action of the adrenergic amine is stimulatory or inhibitory. The ethanolamine function, however, is an essential structural component that defines, in part, the extent of binding to the beta receptor and the potency of the activity, whether it is stimulatory or inhibitory.

Whereas fully active beta-adrenergic agonists displayed a requirement for the catechol function, beta-adrenergic inhibitors tolerated a great latitude in aromatic groups. For example, pindolol, alprenolol and hydroxybenzylpropranolol containing indole, phenyl and naphthyl groups, respectively, were equivalently potent inhibitors. Furthermore, timolol, LL945 and tazolol contained even other aromatic configurations.

Potent inhibitors displayed a greater affinity for the beta receptor than potent agonists. The generally greater lipophilicity of the non-catechol aryl groups could facilitate interposition of the inhibitor compound into the membrane milieu and prevent conformational changes necessary for coupling to adenylate cyclase or, alternatively, lead to conformational membrane changes that prevent coupling. In either event, it is clear that the aromatic group is an identity-determining group and is the structural component of adrenergic amines that defines, in part, whether or not the analogue is an agonist or an antagonist.

The central role of the methoxy group which bridges the ethanolamine and aromatic determinants in establishing or improving inhibitory activity is also evident. The insertion of this bridge in isoproterenol leads to a compound with significant inhibitory properties (MJ9910) [9]. All potent inhibitors possess the methoxy link. The methoxy bridge, however, is not essential (Table 3) and does not always increase the potency of analogous beta inhibitors which lack this feature.

This study has also permitted the observation that selected compounds may have dual agonist and antagonist properties. These analogues are 4-OH phenyl derivatives and, therefore, are potential agonists and antagonists, this configuration being found in both types. Furthermore, these compounds are all partial agonists and had very bulky R determinants. In the adenylate cyclase assay they were rather potent agonists and rather weak antagonists.

A few compounds in this analysis interacted with the beta receptor but had neither demonstrable agonist nor antagonist activities. Common to these analogues was a K_D for binding that was very high (10^{-5} M). Although it is possible that these compounds are exceptions to the general observation that interaction with the beta receptor is associated with a significant biological effect, it is more likely that the lack of biological correlation is due simply to the limits of the assay technique and the inability to study these compounds at higher concentrations.

The molecular details by which agonists stimulate

and antagonists inhibit adenylate cyclase activity cannot yet be specified in anything but simple model diagrams. It is possible that interaction with the beta receptors involves sub-receptor sites with distinct specificities for aromatic, beta-hydroxyl and ethanolamine moieties, the various permutations of which lead to one biological effect or another. Alternatively, the three-dimensional or electrical orientation of these small molecules could be critical features of an ultimate single-point receptor interaction. Further investigations are necessary before a clear molecular model emerges. These studies, nevertheless, provide the architectural framework upon which the ultimate molecular orientation of the beta receptor may be elucidated.

REFERENCES

1. J. P. Bilezikian, A. M. Dornfeld and D. E. Gammon, *Biochem. Pharmac.* **27,** 1441 (1978).
2. E. M. Brown, S. A. Fedak, C. J. Woodard and G. D. Aurbach, *J. biol. Chem.* **251,** 1239 (1976).
3. J. Axelrod, *Recent Prog. Horm. Res.* **31,** 1 (1975).
4. R. P. Ahlquist, *Am. J. Physiol.* **153,** 586 (1948).
5. G. A. Robison, R. W. Butcher and E. W. Sutherland, in *Fundamental Concepts in Drug-Receptor Interactions* (Ed. D. J. Triggle), p. 59. Academic Press, NY (1969).
6. O. M. Rosen, J. Erlichman and S. M. Rosen, *Molec. Pharmac.* **6,** 524 (1970).
7. A. J. Kaumann and L. Birnbaumer, *J. biol. Chem.* **249,** 7874 (1974).
8. C. Grunfeld, A. P. Grollman and O. M. Rosen, *Molec. Pharmac.* **10,** 605 (1974).
9. C. Mukherjee, M. G. Caron, D. Mullikin and R. J. Lefkowitz, *Molec. Pharmac.* **12,** 16 (1976).