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

BINDING TO THE BETA RECEPTOR AND ACTIVATION OF ADENYLATE CYCLASE*

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Abstract—Over fifty catecholamines and related analogues were tested for their ability to bind to the beta-adrenergic receptor and to activate adenylate cyclase in membranes of the turkey erythrocyte. Using [^{125}I]hydroxybenzylpindolol, a radioligand that has successfully been used to detect beta-adrenergic receptors, binding to erythrocyte membranes was highly specific and showed strict selectivity for (−) antipodes. Over a range of four log orders, the affinity of a compound for the beta receptor correlated significantly with its ability to activate adenylate cyclase. Potency both for specific binding and adenylate cyclase activation was related to the size of the substituent on the secondary ethanolamine and to the configuration of the aromatic group. Full intrinsic activity required the presence of the catechol and beta-hydroxyl groups. These results confirm that [^{125}I]hydroxybenzylpindolol is a direct probe for the detection of beta-adrenergic receptors and further delineate the structural requirements for occupation and activation of the beta-adrenergic receptor.

Abundant physiological data led to and have long since supported the hypothesis that the actions of catecholamines are mediated by alpha and beta receptors [1, 2]. Murad *et al.* [3] first showed that for their effects in target tissues, beta-adrenergic catecholamines stimulated adenylate cyclase activity [ATP-pyrophosphate-lyase (cyclizing); EC 4.6.1.1.] and the accumulation of intracellular cyclic 3,5-adenosine monophosphate (cyclic AMP). Using adenylate cyclase as a receptor marker, structure–activity analyses of agonists and antagonists have validated more indirect approaches [4–10] and emphasized features of adrenergic compounds which are critical for potency, stimulation and inhibition [11–13]. It has been assumed that the structural specificity of these compounds is related to acceptor geometry of the beta receptor.

More direct evaluation of functional groups as determinants of agonist properties has become possible with the introduction of radioligands which bind to the beta receptor [14, 15]. The potent beta-adrenergic inhibitors, [^{125}I]hydroxybenzylpindolol [16–20], [^{3}H]dihydroalprenolol [21, 22], and [^{3}H]propranolol [23, 24] have all been shown to interact with membranes of target tissues with extremely high affinity, low capacity, and stereospecificity. These adrenergic ligands have permitted the direct correlation of binding and biological parameters. The close corroboration between binding and activation or inhibition constants in a number of target organs has supported the contention that these radioligands are detecting a beta receptor [16, 18, 20, 21]. Only one detailed study, however, has addressed itself to the structural features of the catecholamine molecule that contribute to binding and activation or inhibition of adenylate cyclase [25].

The membrane preparation used in this study, the turkey erythrocyte, contains a beta-adrenergic receptor and a catecholamine-sensitive adenylate cyclase [26–30]. It is subclassified as a beta$_1$-adrenergic system. The data reported herein and in the accompanying paper [31] represent the most comprehensive structure-binding–activity analysis of adrenergic agonists and antagonists in a beta$_1$-adrenergic system using [^{125}I]hydroxybenzylpindolol and provides further insight into the molecular features of the beta-adrenergic receptor.

MATERIALS AND METHODS

Materials

The materials and their sources are as follows: [^{32}P]ATP (100 Ci/m-mole) (ICN, Irvine, CA); [^{3}H]cyclic AMP (New England Nuclear, Boston, MA); [^{125}I]carrier-free (Amersham-Searle, Chicago, IL); cyclic AMP (CalBiochem, La Jolla, CA); alumina, neutral (Brinkman Instruments, Westbury, NY); imidazole (Pierce Chemicals, Rockford, IL); Gelman A/E glass filters (A. H. Thomas, Philadelphia, PA); (−)norepinephrine, dopamine, (+/−)octopamine, (+/−)synephrine, 3-methoxytyramine, (+/−)metanephrine, (+/−)normetanephrine, (+/−)phenylephrine, (+/−)ephedrine, (+/−)dopa, (+/−)dihydroxymandelic acid, (+/−)dihydroxyphenylglycol, (+/−)dihydroxyphenylacetic acid, (+/−)4-hydroxy, 3-methoxy mandelic acid, (+/−)4-

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hydroxy, 3-methoxy phenylacetic acid, (+/−)dichloroisoproterenol, ATP and dithiothreitol (Sigma Chemicals, St. Louis, MO); and S35179-2, (+/−)-S38537-9, (+/−)*p*-hydroxyephedrine, tyramine, 5-hydroxydopamine, (+/−)S37260-9 and (+/−)-S35985-8 (Aldrich Chemicals, Milwaukee, WI).

The following compounds were the generous gifts of the companies indicated: (+)norepinephrine, (+)epinephrine, (−)- and (+)isoproterenol, (+)ethylnorepinephrine, (+/−)cobefrin, (+/−)isoetharine and beta-deoxyisoproterenol (Sterling-Winthrop Research Institute, Rensselaer, NY; (+/−)salbutamol and (+/−)AH2923 (Allen & Hanbury Ltd., Ware, England); (+/−)protokylol (Lakeside Laboratories, Milwaukee, WI); (+/−)MJ9910, (+/−)isoxyprine, (−)- and (+)soterenol, (+/−)MJ9184-1, (+/−)MJ8798-1, (+/−)MJ6987-1 and (+/−)MJ7434-1 (Mead Johnson Co., Evansville, IN); (+/−)nylidrin (USV Pharmaceuticals, Tuckahoe, NY); (+/−)W9803A, (+/−)W10773A, (+/−)W1055A and (+/−)W10470A (Warner-Lambert Research Institute, Morris Plains, NJ); (+/−)MI39, (+/−)metaproterenol and (+/−)PI39 (Boehringer Ingelheim, Ltd., Elmsford, NY); dobutamine (Eli Lilly, Indianapolis, IN); (+/−)sulfonterol (Smith, Kline & French, Philadelphia, PA); (−)- and (+)alprenolol and (+/−)H6452 (Hassle, Molndal, Sweden); quinterenol (Pfizer Central Research, Groton, CT); trimethoquinol (Tanabe Seiyaku Co., Ltd., Osaka, Japan); tazolol (Syntex Corporation, Palo Alto, CA); (+/−)CC-25 and (+/−)CC34 (Philips Duphar, Weesp, Holland); *N*-methyldopamine and *N*-isopropyldopamine (Hoffman-LaRoche Pharmaceuticals, Nutley, NJ); (+/−)hydroxybenzylpindolol (Drs. D. Hauser and R. Berthold, Sandoz Pharmaceuticals, Basel, Switzerland); and (+/−)metaraminol (Merck, Sharp & Dohme, West Point, PA).

Methods

Preparation of erythrocyte membranes. Catecholamine-sensitive turkey erythrocyte membranes were prepared according to methods previously published [26, 28]. The membrane-enriched fraction (3.0 mg/ml) was stored in sucrose (0.25 M)–Tris (0.05 M) buffer at −80°. The membranes retained stable hormone responsiveness and binding properties for at least 6 months. Binding and adenylate cyclase assays were performed on identical membrane preparations.

Assay for adenylate cyclase activity. The formation of [^{32}P]cyclic AMP from the substrate [^{32}P]ATP was measured in an assay previously described [28, 32]. The incubation mixture contained Tris (0.05 M), pH 7.5, ATP, 0.143 mM, an ATP-regenerating system (creatine phosphate 10 mM, creatine phosphokinase, 14 μg) [^{32}P]ATP (approximately 10^6 cpm/assay tube), guanylimidodiphosphate [Gpp(NH)p] 70 μM or as otherwise indicated, stimulators or inhibitors as indicated, and membrane protein (25–50 μg). The assay was started by adding membrane protein and carried out for 10 min at 37°. The isolation of cyclic AMP was performed by sequential Dowex and Alumina chromatography according to the method of Salomon *et al.* [33]. All determinations were made in triplicate; the coefficient of variation was 10 per cent. The recovery of [^{32}P]cyclic AMP as monitored by [^{3}H]cyclic AMP averaged 40 per cent.

Preparation of [^{125}I] hydroxybenzylpindolol ([^{125}I]HYP). The iodination and isolation of [^{125}I]HYP were performed according to previously published methods [16, 18, 32]. The iodinated ligand (2200 Ci/m-mole) was stored in ethyl acetate containing phenol (10 mg/ml) at −80°. For use in the binding assay, an aliquot was reduced to dryness under nitrogen and reconstituted in the assay buffer.

Binding assay. Membranes (0.3 to 0.5 mg/ml) were incubated in Tris buffer (0.05 M) with bovine serum albumin (1 mg/ml), [^{125}I]HYP (50 pM; 15,000–20,000 cpm/0.1 ml) and designated concentrations of unlabeled compounds for 30 min at 37°. (Previous studies have shown that binding reaches equilibrium under these conditions [16, 17, 20].) Aliquots (0.10 ml each) were removed in triplicate, immediately filtered by suction, and washed with 12 ml of Tris buffer (0.01 M) at room temperature. Filtration and washing required 12–15 sec. The assay blank, 1–2 per cent of radioactivity applied, was less than 10 per cent of the maximal binding, which averaged 25 per cent. Specific binding, defined as that component of total binding which was inhibited by (−)propranolol (1 μM), was 75–80 per cent of total binding and averaged 33 fmoles/mg of protein. The data are expressed in terms of specific binding. For every binding experiment, a separate, complete competitive binding curve for (−)propranolol was performed.

Determination of the activation constant. The activation constant (K_A) is defined as the concentration of agonist at which half-maximal activation of adenylate cyclase in the present of Gpp(NH)p is achieved. If maximal activation was less than 10 per cent of that achieved in the presence of (−)isoproterenol, the compound was not considered to be a significant agonist. The dose–response curve for most agonists was established on four separate occasions. For every adenylate cyclase assay performed, a complete dose–response relationship for (−)isoproterenol was determined.

Intrinsic activity. The intrinsic activity (I_A) was determined by establishing the ratio of maximal activity with agonist to that achieved with (−)isoproterenol (both at 0.5 mM) in the presence of Gpp(NH)p (70 μM). The intrinsic activity of (−)isoproterenol was defined as 1.0.

Determination of the dissociation constant. The concentration of analogue leading to half-maximal inhibition of [^{125}I]HYP specifically bound under equilibrium conditions approximates the dissociation constant (K_D) for that compound. Because the concentration of radioligand and membrane protein may influence the K_D [34, 35], the K_D for the parent compound, hydroxybenzylpindolol (HYP), was determined by a careful series of experiments at ligand concentrations below the K_D employing a wide range of membrane protein concentrations. Previous studies have shown that this approach agrees well with the K_D determined by computer analysis [32], and also is in close agreement with the K_D determined by the dissociation and association rate constants ($K_D = K_{-1}/K_{+1}$).

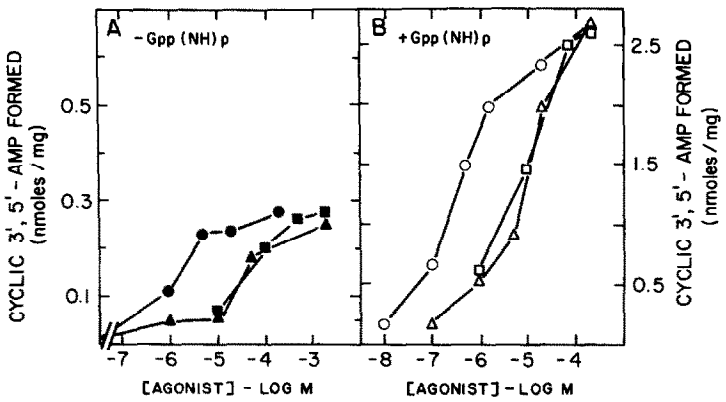


Fig. 1. Adenylate cyclase in turkey erythrocyte membranes; activity of agonists with or without Gpp(NH)p. Panel A: erythrocyte membranes were exposed to increasing concentrations of (−)isoproterenol (●), (−)epinephrine (▲), or (−)norepinephrine (■) in the absence of Gpp(NH)p. Panel B: erythrocyte membranes were exposed to increasing concentrations of (−)isoproterenol (○), (−)epinephrine (△), or (−)norepinephrine (□) in the presence of Gpp(NH)p (70 μM).

RESULTS

Gpp(NH)p as an amplifier and expressor of beta-adrenergic activity

Although turkey erythrocyte membranes respond to (−)isoproterenol with an increase in adenylate cyclase activity, Gpp(NH)p markedly improved maximal isoproterenol responsiveness and decreased the concentration required for half-maximal activation (K_A). Maximal adenylate cyclase activity stimulated by isoproterenol was ten times greater with Gpp(NH)p (2.7 nm/mg; Fig. 1, panel B) than without (0.28 nm/mg; Fig. 1, panel A). The K_A was decreased from 1.5 to 0.3 μM in the presence of isoproterenol and Gpp(NH)p. It has previously been shown [36, 37] and confirmed by us, under these conditions, that the increment in adenylate cyclase activity afforded by Gpp(NH)p and isoproterenol remained identifiable as beta adrenergic because it was competitively inhibited by propranolol (Fig. 2). In addition, the adrenergic subclassification of the turkey erythrocyte adenylate cyclase as beta$_1$ was not altered by Gpp(NH)p because the order of potency for agonists (IS > EPI = NE) remained unchanged in the presence of Gpp(NH)p (Fig. 1).

The importance of using Gpp(NH)p in a structure–activity analysis of beta-adrenergic compounds could be further supported by the observation that beta-adrenergic responsiveness was improved (Table 1, group A), unmasked (Table 1, group B) or unchanged (Table 1, group C) by Gpp(NH)p. Thus, the activity of potential agonists could be undetected or underestimated if Gpp(NH)p was not routinely used in this membrane preparation.

Although Gpp(NH)p had major effects upon the beta-adrenergic responsiveness of the turkey erythrocyte adenylate cyclase, maximal specific binding of [^{125}I]HYP and the dissociation constant for HYP were unaffected. Also, Gpp(NH)p had no effect upon the ability of agonists to compete with [^{125}I]HYP for receptor sites (Fig. 3).

Catecholamines with a hydroxyl group on the beta carbon

Compounds in this group (Table 2) were potent stimulators of adenylate cyclase. The determinant most obviously influencing the K_A was the size of the substituent (R) on the secondary amine. Compounds CC-25 and CC-34, which contain very large and bulky R groups, were more potent than iso-

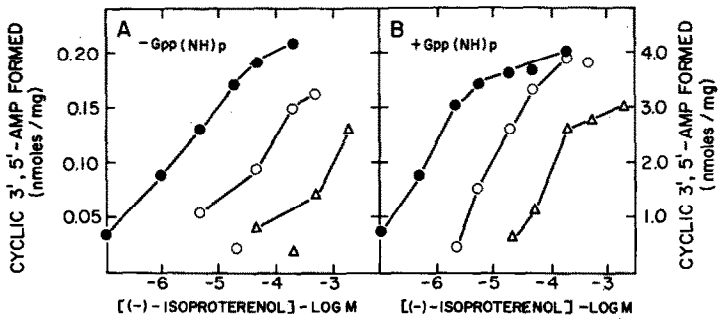


Fig. 2. Competitive inhibition of catecholamine-responsive adenylate cyclase by propranolol. Panel A: without Gpp(NH)p, erythrocyte membranes were assayed for adenylate cyclase activity with increasing concentrations of (−)isoproterenol and no propranolol (●), or with (−)propranolol at 1 μM (○) or 10 μM (△). Panel B: erythrocyte membranes were assayed under the same conditions except for the presence of Gpp(NH)p (70 μM).

Table 1. Effect of Gpp(NH)p upon the intrinsic activity of catecholamines and analogues*

Group	I_A without Gpp(NH)p	I_A with Gpp(NH)p (70 μM)
A		
CC-25	0.48 ± 0.03	0.96 ± 0.09
MJ9184	0.44 ± 0.02	0.82 ± 0.06
Trimethoquinol	0.23 ± 0.01	0.75 ± 0.10
Deoxyisoproterenol	0.15 ± 0.01	0.41 ± 0.14
B		
Isoetharine	< 0.10	0.51 ± 0.06
Dopamine	< 0.10	0.16 ± 0.04
Dobutamine	< 0.10	0.34 ± 0.10
S 35179-2	< 0.10	0.34 ± 0.06
PI 39	< 0.10	0.35 ± 0.06
Octopamine	< 0.10	0.26 ± 0.09
Ritodrine	< 0.10	0.30 ± 0.06
C		
S38537-9	< 0.10	< 0.10
5-OH Dopamine	< 0.10	< 0.10
MJ6987	< 0.10	< 0.10
Hydroxyephedrine	< 0.10	< 0.10

* Intrinsic activity was determined by comparing the adenylate cyclase activity of turkey erythrocyte membranes stimulated by the compounds indicated to the activity stimulated by (−)isoproterenol. All compounds were tested at 0.5 mM. The data are expressed as mean ± S. E. M.

proterenol bearing a smaller isopropyl moiety and the free amine norepinephrine. The K_D of agonists for the beta receptor closely reflected the concentration at which these compounds half-maximally activated adenylate cyclase. Intrinsic activity, however, did not appear to be as greatly influenced by the R group. For example, norepinephrine and CC-25 had markedly different R groups and affinities but similar intrinsic activities. In this series, catechol-containing compounds with (−) orientation had generally equivalent intrinsic activities. The ethyl substituent either on the amine (ethylnorepinephrine) or on the alpha carbon (isoetharine) decreased both the intrinsic activity and the K_A when compared to their corresponding analogues, norepinephrine and isoproterenol, respectively.

Stereospecificity for (−) isomers was a feature of both adenylate cyclase activation and binding to the beta receptor (Table 2). Note also that the catechol-containing compound MJ9910, which differs from isoproterenol only by a methoxy bridge between

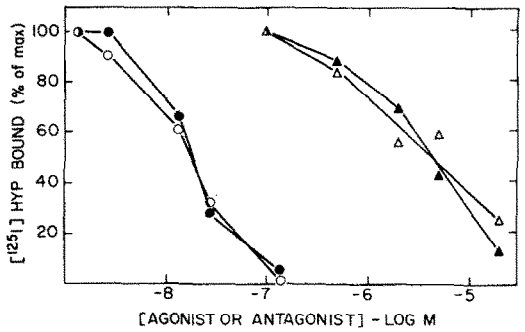
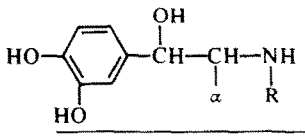
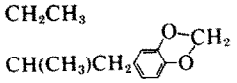
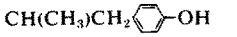
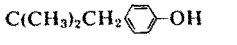


Fig. 3. Effect of Gpp(NH)p on the ability of propranolol and isoproterenol to compete with [^{125}I]HYP for binding sites on turkey erythrocyte membranes. Membranes (0.15 mg/ml) were exposed to [^{125}I]HYP (50 pM) and increasing concentrations of (−)propranolol (circles) or (−)isoproterenol (triangles) with (closed symbols) or without (open symbols) Gpp(NH)p (70 μM). The binding assay was performed as in Methods.

Table 2. Catecholamines with an intact beta-hydroxyl group*

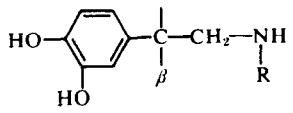


Compound	α	R	Binding K_D (μM)	Adenylate cyclase K_A (μM)	I_A
(−)Norepinephrine	H	H	7.6 ± 0.2	3.2 ± 0.2	0.97 ± 0.1
(+/−)Cobefrin	CH_3	H	0.32 ± 0.02	1.6 ± 0.07	1.04 ± 0.06
(+/−)Epinephrine	H	CH_3	5.8 ± 0.1	2.3 ± 0.1	0.99 ± 0.09
(−)Isoproterenol	H	$CH(CH_3)_2$	0.34 ± 0.06	0.30 ± 0.1	1.00
(+/−)MJ9910†	H	$CH(CH_3)_2$	0.74 ± 0.04	1.4 ± 0.6	0.20 ± 0.03
(+/−)Isoetharine	CH_2CH_3	$CH(CH_3)_2$	4.5 ± 0.2	15 ± 1	0.51 ± 0.06
(+/−)Ethylnorepinephrine	H	CH_2CH_3	8.9 ± 0.2	18 ± 1	0.62 ± 0.04
(+/−)Protokylol	H		0.22 ± 0.01	0.20 ± 0.01	1.04 ± 0.06
(+/−)CC-25	H		0.09 ± 0.01	0.14 ± 0.01	0.96 ± 0.09
(+/−)CC-34	H		0.18 ± 0.01	0.14 ± 0.01	0.97 ± 0.07
(+)Isoproterenol	H	$CH(CH_3)_2$	13 ± 4	92 ± 26	0.25 ± 0.07
(+)Epinephrine	H	CH_3	137 ± 8	100 ± 35	0.35 ± 0.11
(+)Norepinephrine	H	H	137 ± 10	101 ± 20	0.27 ± 0.05

* K_D, K_A and I_A for the compounds listed were determined according to Methods. Data are expressed as mean ± S. E. M.

† Methoxy bridge links catechol and ethanolamine groups.

Table 3. Catecholamines without a beta-hydroxyl group*



Compound	β	R	Binding K_D (μM)	Adenylate cyclase K_A (μM)	I_A
Dopamine	H.H	H	134 ± 19	114 ± 11	0.16 ± 0.04
5-OH dopamine	H.H	H	103 ± 5	> 200	< 0.10
S35-179	=O	$CH(CH_3)_2$	71 ± 3	61 ± 7	0.34 ± 0.03
n-Methyldopamine	H.H	CH_3	55 ± 2	39 ± 2	0.26 ± 0.08
β-Deoxyisoproterenol	H.H	$CH(CH_3)_2$	12 ± 2	13 ± 0.5	0.41 ± 0.14
Dobutamine	H.H	$C(CH_3)_2CH_2$-⬡-OH	4.5 ± 0.3	1.4 ± 0.1	0.34 ± 0.10

* K_D, K_A and I_A for the compounds listed were determined according to Methods. Data are expressed as mean ± S. E. M.

catechol and ethanolamine portions of the molecule, had markedly reduced activity as an agonist. Its affinity for the beta receptor, however, was not significantly different from isoproterenol.

The following catecholamine metabolites lacking the amine function were unable to bind to the beta receptor or to activate adenylate cyclase: dopa, dihydroxymandelic acid, dihydroxyphenylglycol, dihydroxyphenylacetic acid, 3-methoxymandelic acid and 4-hydroxy-3-methoxyphenylacetic acid.

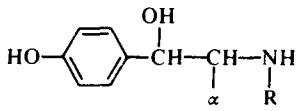
Catecholamines without a hydroxyl group on the beta carbon

The catecholamines listed in Table 3 lack the hydroxyl group on the beta carbon. In general, these compounds were much less active and markedly weaker agonists than their (−)hydroxylated counterparts listed in Table 2. For example, beta-deoxyisoproterenol was less active and weaker than isoproterenol. The affinity of these compounds for the beta receptor was also much less than their more potent beta-hydroxyl-containing analogues. Within the context of generally lower activity, however, the ability of individual compounds in this group to interact with the beta receptor and to activate adenylate cyclase was also related to the size of the substituent (R) on the secondary amine. Dobutamine with a bulky butyl derivative was much more potent and bound to the beta receptor with greater affinity than the free amine, dopamine. These compounds, therefore, were weaker and less active by virtue of lacking the stereospecific beta-hydroxyl group. The presence of other groups on the phenyl ring (5-OH dopamine) or on the beta carbon (S35179) seemed to decrease affinity without significantly altering binding.

Catecholamine analogues lacking the 3-hydroxyl group: without a substituent in position 3

The 4-OH-containing compounds in this group (Table 4) were only weakly active despite the presence of a hydroxyl group on the beta carbon. Their average intrinsic activity (0.21) approximated that for the catechol-containing amines which lacked a beta-hydroxyl group (0.25; Table 3). In this series, the K_A and the K_D were also related to the size of

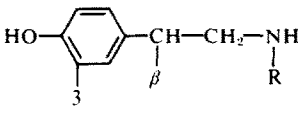
Table 4. Catecholamine analogues lacking the 3-hydroxyl group*



Compound	α	R	Binding K_D (μM)	Adenylate cyclase K_a (μM)	I_A
(+/−)Octopamine	H	H	131 ± 24	> 200	0.26 ± 0.09
(+/−)Synephrine	CH_3	H	76 ± 11	50 ± 5	0.11 ± 0.004
(+/−)S38537-9	H	CH_3	116 ± 37	> 200	< 0.10
(+/−)Hydroxyephedrine	CH_3	CH_3	55 ± 8	57 ± 4	0.10 ± 0.005
(+/−)PI 39	H	$CH(CH_3)_2$	9.7 ± 2	30 ± 9	0.35 ± 0.06
(+/−)Isoxyprine	H	$CH(CH_3)CH_2O$-⬡	5.0 ± 0.9	8.4 ± 0.07	0.21 ± 0.03
(+/−)Nylidrin	CH_3	$CH(CH_3)(CH_2)_2$-⬡	0.37 ± 0.05	1.3 ± 0.06	0.32 ± 0.06
(+/−)Ritodrine	CH_3	CH_2CH_2-⬡	0.90 ± 0.14	8.7 ± 1	0.30 ± 0.06

* K_D, K_A and I_A for the compounds listed were determined according to Methods. Data are expressed as mean ± S. E. M.

Table 5. Catecholamine analogues with a non-hydroxyl substituent at position 3*



Compound	3	β	R	Binding K_D (μM)	Adenylate cyclase K_A (μM)	I_A
Tyramine	H	H	H	> 200	> 200	< 0.10
Methoxytyramine	OCH_3	H	H	> 200	> 200	< 0.10
(+/−)Normetanephrine	OCH_3	OH	H	> 200	> 200	< 0.10
(+/−)Metanephrine	OCH_3	OH	CH_3	37 ± 5	> 200	< 0.10
(+/−)Soterenol	$NHSO_2CH_3$	OH	$CH(CH_3)_2$	13 ± 0.3	3.6 ± 0.1	0.64 ± 0.05
(+/−)Sulfonterol	$CH_2SO_2CH_3$	OH	$CH(CH_3)_2$	5.9 ± 2	12.7 ± 1	0.27 ± 0.02
(+/−)AH2923	CH_2OH	OH	$CH(CH_2)CH_2$⬡OCH_3	8.2 ± 2	4.5 ± 0.5	0.43 ± 0.02
(+/−)Salbutamol	CH_2OH	OH	$CH(CH_3)_2$	3.2 ± 0.4	3.4 ± 0.3	0.46 ± 0.06
(+/−)8798-1	$CH_2SO_2CH_3$	OH	◁	3.4 ± 0.5	3.0 ± 0.1	0.83 ± 0.08
(+/−)MJ9184	$NHSO_2CH_3$	OH	$C(CH_3)_2CH$⬡	0.32 ± 0.05	0.32 ± 0.06	0.82 ± 0.06
(+)Soterenol	$NHSO_2CH_3$	OH	$CH(CH_3)_2$	50 ± 8	> 200	< 0.10

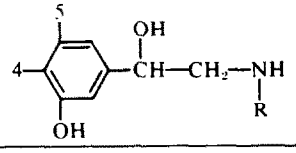
* K_D, K_A and I_A for the compounds listed were determined according to Methods. The data are expressed as mean ± S. E. M.

the amino substituent. Their ability to interact at beta-receptor sites mirrored their K_A for adenylate cyclase. The isoproterenol analogue for this series, PI39, had the greatest intrinsic activity.

Catecholamine analogues lacking the 3-hydroxyl group: with a substituent in position 3

The compounds in this series (Table 5) showed a great range of activity from the inactive catecholamine metabolites, normetanephrine and metanephrine, to the very potent MJ9184. Compounds with the methoxy group in position 3 were as inactive as those with a simple H determinant in that position (e.g. octopamine, synephrine and S38537-9; Table 4). The presence of a —CH_2OH group, on the other hand (salbutamol and AH2923), improved both activity and affinity when compared to their analogues without a substitution in position 3 (PI39 and isoxyprine; Table 4). These compounds, however, were only one-half as active as their counterparts with the complete 3,4-dihydroxy catechol function (isoproterenol and protokylol; Table 2). The sulfonilamide group in position 3 (MJ9184 and soterenol) permitted activity and potency that was greater than the compounds with a —CH_2OH group but still less than the true catecholamine equivalents. The —$CH_2SO_2CH_3$ substitution in sulfonterol altered potency only slightly while markedly decreasing intrinsic activity. Binding of these compounds to the beta receptor directly reflected the potency of these compounds to activate adenylate cyclase.

Table 6. Catecholamine anologues with a non-hydroxyl substituent at position 4*

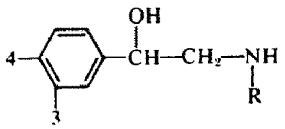


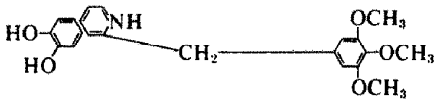
Compound	4	5	R	Binding K_D (μM)	Adenylate cyclase K_A (μM)	I_A
(+/−)MJ6987	$NHSO_2CH_3$	H	$CH(CH_3)_2$	74 ± 12	> 200	< 0.10
(+/−)W9803A	H	CH_2OH	$C(CH_3)_3$	46 ± 13	> 200	< 0.10
(+/−)S40032-7	H	H	CH_2CH_3	35 ± 5	> 200	< 0.10
(+/−)W10773	H	CH_2OH	$C(CH_3)_2CH_2$⬡-OH	8.7 ± 0.6	> 200	< 0.10
(+/−)W10470†	H	CH_2OH	$(CH_2)_2$⬡(OCH_3, OCH_3)	3.7 ± 0.3	> 200	< 0.10
(+/−)Metaraminol	H	H	H	16 ± 1	109 ± 5	0.14 ± 0.01
(+/−)Phenylephrine	H	H	CH_3	37 ± 6	36 ± 2	0.24 ± 0.03
(+/−)Metaproterenol	H	OH	$CH(CH_3)_2$	19 ± 1	43 ± 17	0.28 ± 0.06
(+/−)MI 39	H	H	$CH(CH_3)_2$	4.7 ± 0.3	55 ± 5	0.10 ± 0.01
(+/−)Fenoterol	H	OH	$CH(CH_3)_2$⬡-OH	1.7 ± 0.1	13 ± 5	0.63 ± 0.05

* K_D, K_A and I_A for the compounds listed were determined according to Methods. Data are expressed as mean ± S. E. M.

† Methoxy bridge links phenyl and ethanolamine groups.

Table 7. Catecholamine analogues with non-hydroxyl substituents at positions 3 and 4; other analogues*



Compound	4	3	R	Binding K_D (μM)	Adenylate cyclase K_A (μM)	I_A
(+/−)Ephedrine	H	H	CH_3	33 ± 2	> 200	< 0.10
(+/−)S35985-4	H	H		174 ± 13	> 200	< 0.10
S37260-9	H	H	$(CH_2)_2OH$	> 200	> 200	< 0.10
(+/−)MJ 7434†	$NHSO_2CH_3$	H	$CH(CH_3)_2$	12 ± 0.6	> 200	< 0.10
(+/−)Dichloroisoproterenol	Cl	Cl	$CH(CH_3)_2$	1.2 ± 0.6	> 200	< 0.10
Quinterenol			$CH(CH_3)_2$	0.10 ± 0.02	0.11 ± 0.01	0.12 ± 0.03
Trimethoquinol				0.10 ± 0.02	0.02 ± 0.01	0.75 ± 0.10

* K_D, K_A and I_A for the compounds listed were determined according to Methods. The data are expressed as mean ± S. E. M.

† Ethyl group on α-carbon.

Catecholamine analogues lacking the 4-hydroxyl group

These analogues (Table 6) were generally much less active than their 4-OH counterparts in Table 4 (e.g. MI39 vs PI39; metaraminol vs octopamine). The decreased activity, however, was not associated with a significant difference in potency, the K_A values being essentially equivalent among the analogues in Tables 4 and 6. The ether link between the phenyl and ethanolamine portions of W9803A, W10773 and W10470 essentially destroyed all agonist properties. The retention of significant affinity for the beta receptor, however, suggested that these compounds would be effective beta antagonists [31]. It is also noteworthy that the sulfonilamide group in position 4 destroyed activity (MJ6987) but in position 5 did not alter activity (soterenol; Table 5). The sulfonilamide group in position 4, other groups being equivalent (soterenol vs PI39), markedly improved activity. Compounds with 3,5-dihydroxy groups (metaproterenol and fenoterol) were more active than the analogous 3-hydroxy compounds (MI39, metaraminol and phenylephrine) but were still less active than their analogues with a complete catechol group (isoproterenol and CC-34; Table 2).

Catecholamine analogues without hydroxyl groups

As depicted in Table 7, the compounds without 3- or 4-hydroxyl groups were essentially without significant agonist properties. They also had little affinity for the beta receptor. As shown further in Table 7, non-catecholamines quinterenol and trimethoquinol with only indirect structural resemblance to the catecholamines were associated with significant agonist and binding properties.

Correlation of the K_D *with the* K_A

For the entire series of agonists, the equilibrium dissociation constant for binding, K_D, was correlated with the K_A over a range of four log orders (Fig. 4). The correlation coefficient ($r = 0.90$) is indicative of a highly significant relationship ($P < 0.001$). Although intrinsic activity was not as closely correlated with the K_A or the K_D as these latter two were to each other, the correlations, nevertheless, were significant (Fig. 5). The K_A and the I_A were described by a correlation coefficient of $r = -0.38$ ($P < 0.05$) and that for the K_D and I_A was $r = -0.57$ ($P < 0.01$).

Although the R group was a critical determinant of the K_A and the K_D, the catechol and beta-hydroxyl groups were also influential (Figs. 6 and 7). For any given amino substituent, compounds with the full catechol and beta-hydroxyl groups had greater affinity for the beta receptor and a higher K_A. The lack of 3-, 4- or beta-hydroxyl groups led to a reduction in both the K_A and the K_D. Figures 6 and 7 also depict that for any phenyl-beta-hydroxyl configuration, the K_A and K_D were improved by a increasingly large amino substituent.

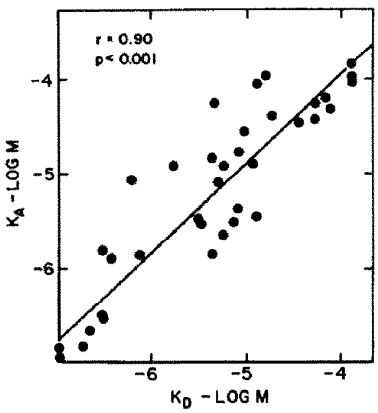


Fig. 4. Correlation between the K_D for binding and the K_A for adenylate cyclase activation among beta-adrenergic agonists. The values for K_D and K_A are listed in Tables 2–7. Each symbol represents one compound.

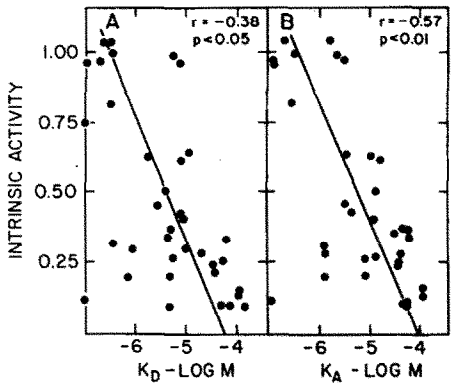


Fig. 5. Correlation between intrinsic activity (I_A) of beta-adrenergic agonists and their binding (panel A) and activation (panel B) constants. Actual values for these compounds are listed in Tables 2–7. Each symbol represents one compound.

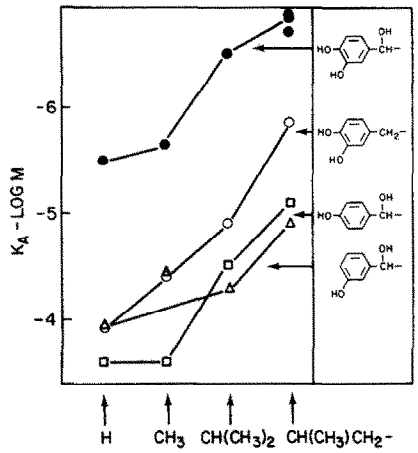


Fig. 7. Relationship between catechol, beta-hydroxyl, and amine substituents in defining the potency of beta-adrenergic agonists as stimulators of adenylate cyclase. For the four basic configurations of catechol and beta-hydroxyl groups, the K_A is noted as a function of an increasingly large amino substituent.

DISCUSSION

Previous structure–activity analyses utilizing catecholamine-responsive membrane preparations have not employed guanyl nucleotides. As shown elsewhere [20] and confirmed here, Gpp(NH)p may unmask or improve adrenergic activity of certain partial agonists. The K_A for adrenergic agonists may also be altered by Gpp(NH)p. It appears, therefore, that a structure–activity analysis of beta-adrenergic catecholamines and their analogues in membrane preparations is incomplete without Gpp(NH)p. This requirement for Gpp(NH)p is not applicable to structure-binding considerations in turkey erythrocytes because Gpp(NH)p does not have significant effects upon the binding capacity or the K_D of beta agonists and antagonists [17].

An overview of this structure-binding–activity analysis of adrenergic agonists has permitted a comparison between the activation constant, K_A, and the binding constant, K_D. Utilizing over fifty compounds with potencies spanning four orders of magnitude, the binding and activation constants displayed a significant relationship to each other. The actual value of the ratio K_A/K_D, which was 1 with- and 10 without- Gpp(NH)p, is less important for the purposes of this analysis than the persistent, predictable relationship described by these constants. The biological role of the binding site detected by [^{125}I]HYP appears to be so pertinent that compounds without affinity for this site will not activate adenylate cyclase. Similarly, the binding constant of a suspected agonist is an accurate predictor of the activation constant.

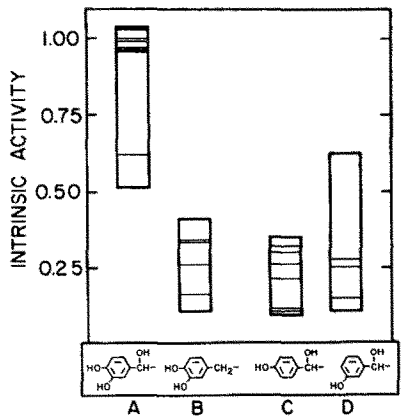


Fig. 6. The role of the catechol and beta-hydroxyl groups in defining intrinsic activity of beta agonists. The intrinsic activity of compounds listed in Tables 2–7 are plotted as a function of their catechol and beta-carbon configurations. In column A are compounds with both the catechol and beta-hydroxyl groups. In column B, compounds possess the catechol group but lack the beta-hydroxyl group. Compounds in the last two columns lack either the 3-hydroxyl (C) or 4-hydroxyl (D) groups but possess the beta-OH group. Each horizontal line represents one compound.

The catechol group is an essential feature of potent adrenergic amines. Compounds with an identical (−)ethanolamine side chain but a non-catechol configuration have decreased affinity for the beta receptor and for adenylate cyclase. Considering (−)isoproterenol as a catechol-containing prototype, the corresponding 4-OH and 3-OH analogues, PI39 and MI39, have decreased binding and activating properties. Similarly, the 3-OH and 4-OH analogues of (−)epinephrine, phenylephrine and S38537-9, are much weaker agonists. This comparison held also for the 3-OH and 4-OH analogues of (−)norepinephrine, metaraminol and octopamine. In general, the 4-OH-containing compounds were less effective than the 3-OH analogues. 4-OH derivatives with a non-methoxy substituent in position 3 (soterenol and salbutamol) were about as potent as analogues without a substituent (PI39). On the other hand, the 3-OH-containing compound with an amino substituent in position 4 (MJ6987) was much less effective than its non-substituted analogue, MI39. Therefore, the catechol group is an important determinant of binding affinity and potency.

Quinterenol and trimethoquinol appear to be exceptional agonists in that they are not catechol-related amines but are significant agonists. Trimethoquinol may be viewed, however, as a dop-

aminergic derivative with a very bulky R group, accounting for its relatively high intrinsic activity. Quinterenol, on the other hand, can be viewed as a 4-OH-containing analogue with a substituent in the 3 position. Its relatively weak activity is consistent with 4-OH analogues. These two compounds illustrate that structurally dissimilar compounds may, nevertheless, be associated with the appropriate spatial geometry or electrical orientation to permit an interaction with the beta receptor.

The beta-hydroxyl group is also an important factor in binding to the beta receptor and activating adenylate cyclase. Stereospecificity denoted at this site for the (−) antipode was evident for all isomer pairs studied. It is uncertain whether the markedly reduced activity of (+) isomers is due to a weaker but, nevertheless, direct interaction with the beta receptor, or rather, to minor chemical admixture of the (−) isomer. A beta-hydroxyl group, however, is not absolutely essential for binding or for stimulation of adenylate cyclase. Dopamine and its analogues can stimulate adenylate cyclase, albeit weakly, in the presence of Gpp(NH)p. Compounds lacking a beta-hydroxyl group are approximately as potent as the (+) isomers of their beta-hydroxyl analogues. Thus, the pairs, dopamine and (+)norepinephrine, *n*-methyl dopamine and (+)epinephrine, and beta deoxyisoproterenol and (+)isoproterenol, are equivalently potent. The dopamine-related compounds are ten to twenty times weaker than their (−)-hydroxylated counterparts.

This study also clearly demonstrates the importance of the R substituent on the secondary amine as a determinant of affinity of catecholamines and their analogues for the beta receptor. For any given composition of aromatic and beta-carbon groups, a bulkier substituent is invariably associated with greater potency. The branched chained isopropyl and tertiary butyl groups are somewhat less potent than the more elongated alkyl groups bearing phenyl or phenol substitutions. These bulky groups, however, are associated with much greater potency than compounds bearing a primary amine or a small methyl determinant. Previous reports have emphasized this observation to the extent of denoting the R group as the affinity-determining moiety [25]. The value of the R group or "cationic head" is viewed as providing a closer spatial orientation to the catechol group and a better dimensional fit for the beta receptor [12].

Although these results demonstrate that the affinity of adrenergic agonists for the beta receptor is a good index of their potency, intrinsic activity is also reflected, albeit not as well, by the K_D for binding. Full intrinsic activity requires the full complement of catechol and (−) beta-hydroxyl groups. Alterations at either site leads to a significant reduction in intrinsic activity. In contrast, the R group had relatively little influence on intrinsic activity.

The data reported herein are in general agreement with those published previously using other target tissues and other radioligands [21, 25]. It appears that labeled beta-adrenergic inhibitors can be used to detect beta receptors and to correlate binding with activation of adenylate cyclase in a $beta_1$ target tissue as shown here as well as in a $beta_2$ target tissue as reported elsewhere [25].

In formulating a model of beta-adrenergic interaction, previous reports have emphasized steric and stereoselective constraints of the phenyl and beta-carbon groups in relation to the alkyl side chain [38], surface changes in electrical charge accompanied by structural alterations [39], the chelating potential of the catechol group [40] and the ability of adrenergic compounds to undergo quinone–methide transitions [41]. None of these hypotheses successfully accommodates the varied agonists described in this report. Further studies on purified beta-receptor preparations will be required to establish the exact molecular geometry of the acceptor region of the beta receptor and the conformational alternations that occur with occupation. Further studies will also be necessary to understand how binding by the agonists in this study is linked to activation of adenylate cyclase and how binding by adrenergic antagonists as described in the subsequent study prevents this biological coupling.

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