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Structure-Activity Relationships of Adrenergic Compounds on the Adenylate Cyclase of Frog Erythrocytes

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SUMMARY

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Frog erythrocyte adenylate cyclase possesses characteristics of a *beta*-2 adrenergic receptor; this enzyme was used to test various adrenergic compounds for their effects on cyclic 3′,5′-AMP formation. Agonists demonstrate different EC_{50} values and intrinsic activities. A large amino substituent and a hydroxyl group in the *levo* configuration at the β-carbon increase the potency of agonists and antagonists, but neither modification is essential for activity. Compounds containing a methyl group at the α-position with the *erythro* configuration retain their agonist or antagonist activities. Agonists must have two functional groups on the phenyl ring; a functional group other than hydroxyl can be substituted only at position 3. The effect of ring substitution on the activity of an antagonist depends on the nature of the adjacent functional group. Studies with trimethoquinol suggest correlation of the observed EC_{50} with affinity. There is an excellent correlation between activation of frog erythrocyte adenylate cyclase by the agonists studied and the physiological responses in mammalian preparations.

INTRODUCTION

Structure-activity relationships among adrenergic agents have been used to identify different classes of *beta* receptors (1–3) and to explain the action of catecholamines at their receptor sites (4). The pharmacological responses to catecholamines in whole animal or intact organ preparations are complex, and the effects of any given drug may depend on routes of administration, transport to the site of action, oxidative and conjugative metabolism, and interaction with non-adrenergic receptors mediating similar physiological responses. Adrenergic compounds may also release endogenous stores of neurotransmitter, thereby producing indirect sympathomimetic effects (5). Chronically denervated, cocaine-treated, or reserpine-treated preparations have been developed

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to obviate this problem (5, 6), but variability in responsiveness (5) limits the usefulness of these models for defining a direct *beta* adrenergic response.

Adenosine cyclic 3′,5′-monophosphate mediates the *beta* adrenergic response to catecholamines in many tissues (7). Previous studies in our laboratory (8, 9) demonstrated that stimulation of frog erythrocyte adenylate cyclase activity by catecholamines possesses the following characteristics *of beta* adrenergic stimulation: (a) formation of cyclic AMP is stimulated by catecholamines but not by other hormones, (b) the relative order of potency among catecholamines is isoproterenol > epinephrine > norepinephrine, and (c) stimulation by catecholamines is inhibited by the *beta* adrenergic blockers propranolol and dichloroisoproterenol, but not by the *alpha* adrenergic blockers phentolamine and phenoxybenzamine.

We have utilized frog erythrocyte adenylate cyclase as a model system to study the direct interaction of drugs with a *beta* adrenergic receptor even though the function of cyclic AMP in the cell is not known (8). Earlier structure-activity studies have been published (10, 11); we now report more detailed studies of how substitutions on the ring and ethylamine moieties of various adrenergic agents affect activation and inhibition of activation of adenylate cyclase. Characterization of purified *beta* adrenergic receptors must ultimately include correlation of binding by the receptor complex with the complete structure-activity relationships documented for adenylate cyclase.

MATERIALS AND METHODS

Adenylate cyclase was partially purified from the erythrocytes of *Rana pipiens* and assayed as previously described (8, 9). The reaction mixture (0.2 ml) contained 0.05 M Tris-HCl buffer (pH 8.1), 3 mM $MgSO_4$, 0.02 M dithiothreitol, 1.0 mM cyclic AMP, 1.0 mM [^{14}C]ATP (2500 cpm/nmole), and the appropriate adrenergic compounds at the final concentrations indicated below. The reaction was initiated by the addition of 10 μg of enzyme and incubated for 15 min at 35°. Enzyme preparations catalyzed the formation of 6–7 nmoles of cyclic AMP per milligram of protein in 15 min when maximally stimulated by isoproterenol. Despite some variability in the activity of different enzyme preparations, the relative potency of activators and blockers remained constant. Most experiments were performed using a single enzyme preparation. Adenylate cyclase formed essentially no cyclic AMP in the absence of added catecholamines or sodium fluoride and was not contaminated with cyclic nucleotide phosphodiesterase (8, 9). Proteins were determined by the method of Lowry *et al.* (12), using bovine serum albumin as a standard.

EC_{50} values of agonists were calculated from the drug concentration required for 50% maximal stimulation of enzyme activity for that drug; intrinsic activity for a given drug was determined by calculating the ratio of the maximum stimulatory effect for a given drug to that of isoproterenol (13). Inhibition of activation was measured by determining the effect of blocking agents on the stimulation of enzymatic activity induced by 50 μM *l*-isoproterenol; IC_{50} is defined as the concentration of drug required to reduce that activity by 50%. Variations in the absolute level of blockade were standardized by comparing each inhibitor with *dl*-sotalol and *dl*-dichloroisoproterenol, which were equipotent. Relative inhibition is expressed as the ratio of IC_{50} for *dl*-sotalol to that determined simultaneously for a given inhibitor. Complete dose-response curves were determined for each agonist and antagonist, and the values presented represent the averages of two separate experiments. The EC_{50} for isoproterenol averaged 3.7 $\pm$ 0.2 μM (mean $\pm$ SE) for 32 determinations.

Dithiothreitol was purchased from Calbiochem; cyclic AMP, from Sigma; [^{14}C]ATP and Omnifluor, from New England Nuclear; and *l*-isoproterenol, dichloroisoproterenol, dopamine, phenisonone, and S-40045-9, from Aldrich. The following drugs were gifts: soterenol, sotalol, metalol, amidephrine, β-deoxysoterenol, α-methylsotalol, MJ-9910, MJ-9643, MJ-6987, trimethoquinol, isoxsuprine, and their respective isomers, courtesy of Dr. W. T. Comer of Mead Johnson; salbutamol, from Allen and Hansbury, Ltd.; propranolol, from Ayerst Laboratories; quinterenol, from Charles Pfizer & Com-

pany; metaproterenol, from Geigy Pharmaceuticals; *d*- and *l*-alprenolol and H64/52, from A. B. Hassle; β-deoxyisoproterenol, from Sterling-Winthrop Research Institute; butoxamine, from Burroughs Wellcome; and practolol, from Ayerst Laboratories. All drugs were racemic mixtures unless otherwise specified. Compounds were shown to be homogeneous by thin-layer chromatography (14) on silica gel and cellulose (Eastman), using water-saturated 1-butanol and 1-butanol acetic acid water (66:17:17) as developing solvents.

RESULTS

Activation of adenylate cyclase. Activation of adenylate cyclase by a catecholamine (isoproterenol), a sulfonanilide (soterenol), and the β-deoxy congener of soterenol is shown in Fig. 1. Intrinsic activities and EC_{50} values for the other agonists were determined by similar experiments. Data for compounds possessing various substituents on the β-carbon are presented in Table 1. Two compounds lacking hydroxyl groups on the β-carbon, but containing *N*-isopropyl substituents, have a limited ability to stimulate adenylate cyclase activity. β-Deoxyisoproterenol has 25% of the intrinsic activity but only 1% of the apparent affinity of *l*-isoproterenol. β-Deoxysoterenol has 60% of the intrinsic activity and 4% of the apparent affinity of *l*-soterenol. The *d* isomer of soterenol has a lower activity and a higher EC_{50} than the *l* isomer. Dopamine, lacking both a β-hydroxyl and an *N*-isopropyl group, and phenisonone, a β-ketone, are not agonists at concentrations up to 1 mM.

Effect of various substituents on phenyl ring. Compounds possessing a single ring hydroxyl in position 4 (nylidrin and isoxsuprine) or in position 3 (S-40045-9) do not stimulate adenylate cyclase activity (Table 2). Compounds containing only a methanesulfonamide at position 3 or 4 (amidephrine and sotalol) are also inactive.

Phenylethanolamine derivatives possessing a methanesulfonamide or a hydroxymethyl group in position 3 and a hydroxyl in position 4 (soterenol and salbutamol) are potent agonists. When the positions of the methanesulfonamide and hydroxyl groups are reversed (MJ-6987, an isomer of soterenol), the resulting congener is inactive. Both soterenol and salbutamol, with larger substituents in position 3, have lower intrinsic activities than isoproterenol, but the EC_{50} of soterenol is slightly higher than that of isoproterenol. Methylation of the sulfonamide nitrogen of soterenol (MJ-9643) abolishes agonist activity. Metaproterenol, an isomer of isoproterenol containing hydroxyls in positions 3 and 5, is also a weak agonist with a very high EC_{50}.

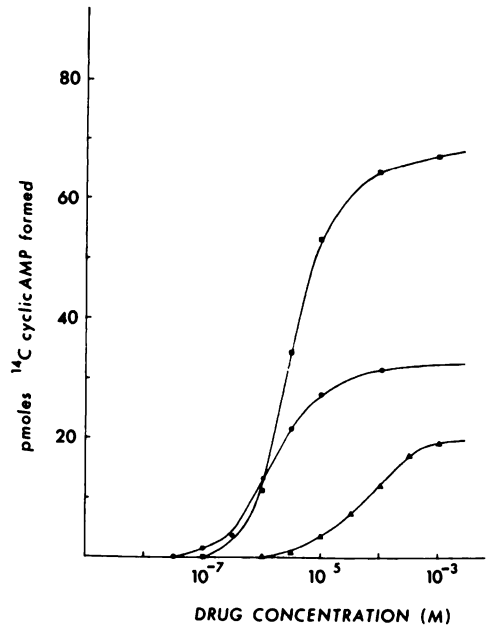


FIG. 1. *Activation of adenylate cyclase by beta adrenergic agonists*

Assays were performed as described in MATERIALS AND METHODS. ■, *l*-Isoproterenol; ●, *l*-soterenol; ▲, β-deoxysoterenol.

Effect of variations in ring structure. In addition to the simple phenylethylamines, three other compounds listed in Table 2 possess agonist activity. Quinterenol, a 5-substituted 8-hydroxyquinoline, exhibits a weaker EC_{50} and intrinsic activity than isoproterenol. The phenoxypropylamine MJ-9910, a catechol congener of propranolol, is an even weaker agonist. Trimethoquinol, a tetrahydroisoquinoline, is also a weak agonist and has a low EC_{50}.

Inhibition of isoproterenol-induced stimula-

TABLE 1

Activation of adenylate cyclase by compounds with different substitutions at the β-carbon

Assays were performed as described in MATERIALS AND METHODS.

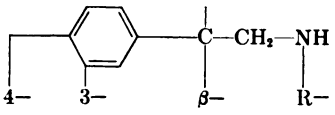
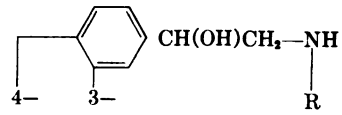
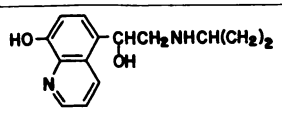
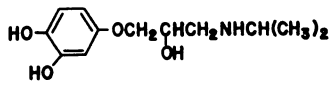
Compound					EC_{50}	Intrinsic activity
	4	3	β	R	μM	
l-Isoproterenol	—OH	—OH	—OH, —H	$—CH(CH_3)_2$	3.7	1
β-Deoxyisoproterenol	—OH	—OH	—H, —H	$—CH(CH_3)_2$	260	0.25
Soterenol	—OH	$—NHSO_2CH_3$	—OH, —H	$—CH(CH_3)_2$	1.6	0.49
β-Deoxysoterenol	—OH	$—NHSO_2CH_3$	—H, —H	$—CH(CH_3)_2$	71	0.31
d-Soterenol	—OH	$—NHSO_2CH_3$	—OH(*d*), —H	$—CH(CH_3)_2$	20	0.16
Dopamine	—OH	—OH	—H, —H	—H		0
Phenisonone	—OH	—OH	=O	$—CH(CH_3)_2$		0

TABLE 2

Effect of ring substituents on activation of adenylate cyclase

Compound				EC_{50}	Intrinsic activity
	4	3	R	μM	
l-Isoproterenol	—OH	—OH	$—CH(CH_3)_2$	3.7	1.0
Nylidrin	—OH	—H	$—CH(CH_3)CH_2CH_2\phi$		0
Isoxsuprine	—OH	—H	$—CH(CH_3)CH_2O\phi$		0
S-40045-9	—H	—OH	$—CH(CH_3)_2$		0
Sotalol	$—NHSO_2CH_3$	—H	$—CH(CH_3)_2$		0
Amidephrine	—H	$—NHSO_2CH_3$	$—CH_3$		0
l-Soterenol	—OH	$—NHSO_2CH_3$	$—CH(CH_3)_2$	1.6	0.49
Salbutamol	—OH	$—CH_2OH$	$—C(CH_3)_3$	10	0.81
MJ-6987	$—NHSO_2CH_3$	—OH	$—CH(CH_3)_2$		0
MJ-9643	—OH	$—N(CH_3)SO_2CH_3$	$—CH(CH_3)_2$		0
Dichloroisoproterenol	—Cl	—Cl	$—CH(CH_3)_2$		0
Metaproterenol	—H	3-OH,5-OH	$—CH(CH_3)_2$	150	0.25
Quinterenol[a]				16	0.37
MJ-9910[b]				0.54	0.13
Trimethoquinol[c]				0.33	0.17

a 

b 

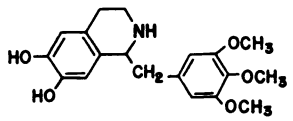
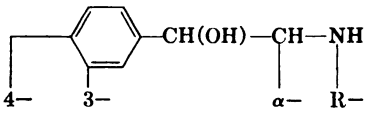
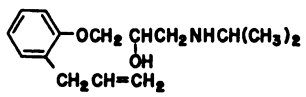
c 

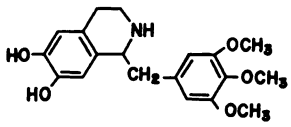
TABLE 3

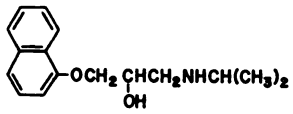
Inhibition of activation of adenylate cyclase by isoproterenol

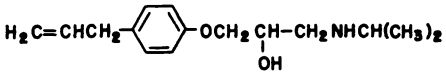
The reaction was carried out in the presence of 50 μM isoproterenol. Relative inhibition was calculated as described in MATERIALS AND METHODS.

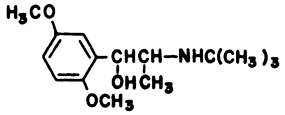
Compound					Relative inhibition
	4	3	α	R	
Dichloroisoproterenol	—Cl	—Cl	—H	—$CH(CH_3)_2$	1
Sotalol	—$NHSO_2CH_3$	—H	—H	—$CH(CH_3)_2$	1
α-Methylsotalol	—$NHSO_2CH_3$	—H	—CH_3	—$CH(CH_3)_2$	1
Metalol	—$NHSO_2CH_3$	—H	—CH_3	—CH_3	0.35
threo-Metalol	—$NHSO_2CH_3$	—H	—CH_3	—CH_3	0.011
Amidephrine	—H	—$NHSO_2CH_3$	—H	—CH_3	0.058
MJ-9643	—OH	—$N(CH_3)SO_2CH_3$	—H	—$CH(CH_3)_2$	0.92
MJ-6987	—$NHSO_2CH_3$	—OH	—H	—$CH(CH_3)_2$	0.02
l-Sotalol	—$NHSO_2CH_3$	—H	—H	—$CH(CH_3)_2$	2.6
d-Sotalol	—$NHSO_2CH_3$	—H	—H	—$CH(CH_3)_2$	0.056
S-40045[g]	—H	—OH	—H	—$CH(CH_3)_2$	0.27
Nylidrin	—OH	—H	—H	—$CH(CH_3)CH_2CH_2\phi$	2.7
l-Alprenolol[a]					745
d-Alprenolol[a]					24
Trimethoquinol[b,g]					20
Propranolol[c]					36
H64/52[d]					0.75
Butoxamine[e]					1
Practolol[f]					0.1

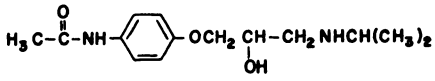
a 

b 

c 

d 

e 

f 

[g] Inhibition of isoproterenol-stimulated cyclic AMP formation by trimethoquinol never exceeded 80%, owing to the intrinsic activity of this drug.

tion of adenylate cyclase. The capacity of certain adrenergic compounds to inhibit the stimulation of adenylate cyclase by *l*-isoproterenol (50 μM) is shown in Table 3. Inhibition is expressed relative to that of racemic sotalol. At a concentration of 0.1 mM, *dl*-sotalol, *dl*-α-methylsotalol, and *dl*-dichloroisoproterenol block the response to *l*-isoproterenol by 50%. α-Methylsotalol, an *erythro* diastereomer, has the same inhibitory effect as sotalol. The *threo* diastereomer of metalol, however, is a weaker inhibitor than metalol itself.

The 4-substituted methanesulfonamide, metalol, is a more potent inhibitor than the 3-substituted methanesulfonamide, amide-

phrine. The reverse is true for the compounds with two ring substitutions. MJ-9643, an *N*-methyl-substituted congener of soterenol, is a potent inhibitor, whereas MJ-9687, with a methanesulfonamide in position 4 and a hydroxyl in position 3, is one of the weakest inhibitors. Nylidrin, a compound with a single ring hydroxyl in position 4, is a potent inhibitor. In general, the *d* isomers tested are substantially less inhibitory than the comparable *l* isomers. Trimethoquinol and propranolol are also potent inhibitors. Alprenolol, the most potent inhibitor, is 1000 times stronger than its congener, H64/52. Butoxamine is more potent than practolol.

DISCUSSION

Structure-activity relationships. Substitutions at the ethylamine moiety of phenylethylamines appear to be important but not essential for stimulation of enzymatic activity. Bulky substitutions on the amine enhance the activity of these drugs on adenylate cyclase, as they do in intact organisms or whole organ preparations (15, 16). Primary amines also stimulate enzyme activity (10). Dopamine and other β-deoxy compounds without large *N*-alkyl substituents were shown in our previous paper to be inactive in this preparation (10). From the present study it appears that compounds which either lack a β-hydroxyl or possess the *d* configuration at the β-carbon but have isopropylamine substitutions can act as agonists. However, their EC_{50} values are higher and intrinsic activities lower than those of the *l* analogues. This is in contrast to the mixed *alpha* and *beta* receptor linked to rat erythrocyte adenylate cyclase (17), which is stimulated by dopamine and shows the same intrinsic activity for isoproterenol and β-deoxyisoproterenol (18). Adenylate cyclase of the frog erythrocyte, unlike that of the rat erythrocyte (17), shows no inhibition of catecholamine stimulation by the *alpha* adrenergic antagonists dibenzyline (8) and phentolamine.[3]

Trimethoquinol, a tetrahydroisoquinoline with pharmacological activity as a bronchodilator, has been viewed as a β-deoxy analogue of the catecholamines (19, 20). Unlike β-deoxyisoproterenol and β-deoxysoterenol, trimethoquinol has a high potency in terms of its EC_{50}, despite low intrinsic activity. The potent blockade by trimethoquinol of enzymatic stimulation by isoproterenol shows that the EC_{50} is a good indicator of affinity. Studies performed on organ preparations have shown that a bulky substituent at position 1 enhances the agonist activity of tetrahydroisoquinoline (19, 20). This substitution may be comparable to *N*-substitution of catecholamines, in which case the ethylamine moiety of trimethoquinol is part of a rigid ring system that may contribute to its low intrinsic activity.

The most critical structural requirements for stimulation appear to involve the substitutions on the phenyl ring. Nylidrin, isoxsuprine, and S-40045-9, compounds that possess single ring hydroxyls, are often classified as *beta* sympathomimetic agents (16, 21–23); however, they do not stimulate this adenylate cyclase preparation. These compounds probably bind to the adrenergic receptor in frog erythrocyte membranes, since they are potent inhibitors of catecholamine-stimulated adenylate cyclase (this paper and ref. 10). Our studies suggest that agonists require more than one functional group on the phenyl ring and that the nature of those two groups is also critical.

The bulky methanesulfonamide group cannot be substituted for the hydroxyl group in position 4 without destroying the ability of the compound to stimulate adenylate cyclase. Similar conclusions have been derived from studies of adrenergic agents in intact organ preparations (24, 25, 26). A hydroxyl group at position 4 is not an absolute requirement for activity; metaproterenol possesses two *m*-hydroxyls and is a weak agonist.

Although a functional group in position 3 is required for agonist activity, the data do not allow a precise definition of its chemical role in stimulating adenylate cyclase activity. Substitution of a hydroxyl (catecholamines), a hydroxymethyl (salbutamol), or a methanesulfonamide (soterenol) in position 3 suffices for agonist activity. Each of these three functional groups is a potential proton donor. The inactivity of MJ-9643

[3] C. Grunfeld, unpublished observations.

(in which the amide is substituted by a methyl group) in the enzyme assay and the inactivity of 3-methoxy metabolites of catecholamines *in vivo* (27) are consistent with a role for the 3-substituent as a hydrogen donor. The function of quinterenol, as an agonist, is difficult to define because of the unusual nature of the quinoline ring system and its severely restricted rotation about the ethylamine moiety.

Structure-activity data for inhibition of isoproterenol-induced activation of adenylate cyclase suggest the following relationships. Whereas an α-methyl substitution in the *erythro* configuration does not reduce potency of an inhibitor, an α-methyl substitution resulting in a *threo* configuration drastically reduces potency. Sulfonanilides with a single ring substituent are more potent when the methanesulfonamide is in position 4 (metalol compared to amidephrine). Finally, the *d* isomers of inhibitors are weaker than the *l* isomers.

Comparison of adenylate cyclase data with adrenergic response. Correlation between potency of drugs in this system and the adrenergic response in whole animals and tissue preparations is not simple, since the amount of cyclic AMP formed in a tissue following stimulation of adenylate cyclase may have a variable relationship to the level necessary to initiate and maintain a *beta* adrenergic response (28). Drugs that stimulate adenylate cyclase display different apparent affinities and intrinsic activities. While both parameters must be used to characterize the response of adenylate cyclase, the apparent affinity of drugs for the enzyme may be more relevant to the physiological adrenergic response. Thus the ability of drugs like soterenol, quinterenol, and trimethoquinol to stimulate maximally a *beta* adrenergic response in whole tissue studies (19, 20, 26, 29) is not inconsistent with their having lower intrinsic activities than isoproterenol. The level of cyclic AMP required for a maximal physiological response and the concentration of a drug needed to achieve that level must be determined for each tissue.

Evidence has previously been presented that frog erythrocyte adenylate cyclase has characteristics of a *beta* adrenergic receptor (8, 9). Recently several authors have reported that the adenylate cyclase preparations from heart and lung show hormone responsiveness compatible with *beta*-1 and *beta*-2 adrenergic receptors, respectively (30–32). Frog erythrocyte adenylate cyclase appears to possess the structure-activity relationships similar to the *beta*-2 receptor responsible for bronchodilation and vasodepression (1–3, 30, 32), because α-methyl substitutions do not decrease activity in agonists, such as cobefrine (α-methylnorepinephrine) (10), or in antagonists, such as α-methylsotalol; epinephrine is a more potent activator than is norepinephrine (8, 10); three predominantly bronchodilating drugs, salbutamol (24), quinterenol (29), and trimethoquinol (19, 20), stimulate enzymatic activity; and the *beta*-2 selective antagonists butoxamine (32, 33) and alprenolol (34) are more potent than the *beta*-1 antagonists practolol (30, 33) and H64/52 (34).

In Table 4 the apparent affinities of many of the adrenergic drugs we have tested are compared with their pharmacological effects in tissue preparations. There is excellent correlation, with two exceptions. (a) Dopamine, which is ineffective in this adenylate cyclase preparation, is active *in vivo*, although more often in tissues containing *beta*-1 (35) rather than *beta*-2 (36) adrenergic receptors. Sheppard and Burghardt (17, 18) have shown dopamine to stimulate the mixed *alpha* and *beta* receptor of rat erythrocyte adenylate cyclase. The degree of direct adrenergic response to dopamine, however, has been questioned (5, 6, 35). (b) Nylidrin and isoxsuprine, which also do not stimulate this adenylate cyclase, are traditionally classified as *beta* adrenergic agents (21, 22). *Beta* blockers, however, cannot inhibit the vasodilation induced by these drugs (37) or the uterine relaxation observed *in vitro* (38). Uterine relaxation *in vivo* (39) and the weaker cardiac stimulant and bronchodilation effects of isoxsuprine (40, 41) are partially susceptible to *beta* blockade. Since isoxsuprine can induce relaxation of smooth muscle which is not relaxed by other *beta* stimulants and has the ability to inhibit *alpha* adrenergically induced smooth muscle contractions, its

TABLE 4

Comparison of activity of adrenergic agents as shown with adenylate cyclase and beta adrenergic tissue preparations

The symbols +++, ++, +, and ± are used to denote decreasing affinity. Agonists are expressed relative to isoproterenol, and antagonists, relative to propranolol. 0 refers to drugs that were tested but found to be inactive. Wherever possible, tissue preparations cited were *beta*-2 receptors. Results from adenylate cyclase are taken from this paper and the preceding one (10).

Compound	Agonism		Blockade		References for tissue preparations
	Adenylate cyclase	Tissue preparations	Adenylate cyclase	Tissue preparations	
Isoproterenol	+++	+++			15, 16
Epinephrine	++	++			15, 16
Norepinephrine	++	+			15, 16
Dopamine	0	+, 0			5, 35, 36
Phenisonone	0				
β-Deoxyisoproterenol	+				23
β-Deoxysoterenol	+				
Nylidrin	0	+, 0	++		16, 21, 22, 37
Isoxsuprine	0	+, 0	+		21, 22, 37–41
S-40045-9	0	+	+		23
Soterenol	+++	+++			26, 42–44
MJ-6987	0		±	±	26
MJ-9643	0		++		
Salbutamol	++	++			24, 25, 43–45
Trimethoquinol	+++	+++	+++		19, 20
Metaproterenol	+	+			43–45
Quinterenol	++	++			29
MJ-9910	+++	++			46
Sotalol	0		++	++	47, 48
α-Methylsotalol	0		++	++	47, 48
Metalol	0		+	+	47, 48
threo-Metalol	0		±		
Amidephrine	0	±	±	±	48
Dichloroisoproterenol	0		++	++	49, 50
Alprenolol	0		+++	+++	34
Propranolol	0		+++	+++	51
H64/52	0		+	+	34
Butoxamine	0		++	++	33
Practolol	0		+	+	33

effects are probably mediated by several mechanisms (39). Thus specific functional groups are required in positions 3 and 4 on the ring; a β-hydroxyl and one ring hydroxyl are not sufficient, as has been previously proposed (4, 52).

Re-examination of hypotheses concerning chemical nature of adrenergic receptor. Preparations of adenylate cyclase linked to *beta* adrenergic receptors would appear to be a more direct way of determining the functional requirements for adrenergic stimulation than studies using intact tissue or whole animals. Since the latter studies were used to formulate various hypotheses concerning the chemical nature of the *beta* adrenergic receptor (4, 53–55), it may be valuable to re-examine the proposals in light of our present data.

Many authors have assumed that the function of the catechol group is to chelate a metal ion, such as Mg^{2+} (53–55) or Fe^{2+} (56, 57). However, we have shown that metaproterenol, which cannot chelate a metal, is a weak activator, while MJ-6987, which has the same chelating potential as soterenol, is inactive.

Larsen (4) has proposed a chemical

mechanism based on the "common reactivity" of adrenergic agonists. Our results indicate that two compounds that cannot undergo the proposed quinone-methide transition (metaproterenol and MJ-9910) are active. Aryloxypropanolamines (including MJ-9910) have been previously cited as exceptions to the quinone-methide hypothesis (55). We have also shown different requirements for ring substitutions than those assumed by Larsen, in that phenylethanolamines with a single hydroxyl on the ring are not agonists.

A similar lack of specificity for the ring substituents was assumed by those proposing a direct catalytic role for catecholamines in the formation of cyclic AMP. Experiments from this laboratory (8, 9) and others (58–62) indicate that the catecholamine receptor is probably distinct from the active site of adenylate cyclase.

CONCLUSIONS

We draw the following conclusions from our studies. Agonism requires two functional groups on the ring. Most adrenergic compounds have substituents *meta* and *para* to the ethylamine; a compound with two *meta* groups is a weak agonist. Certain functional groups (hydroxymethyl and methanesulfonamide) may be substituted in position 3, but not in position 4, without loss of activity. A hydroxyl group with the *l* configuration at the β-carbon and a bulky amine substituent are important but not essential for agonism and antagonism. α-Methyl substituents, in the *erythro* configuration at the α- and β-carbons, do not decrease the potency of either agonists or antagonists. The effect of a bulky ring substituent on the potency of antagonists is dependent upon its position and the nature of adjacent functional groups. Each of the modifications of the basic catecholamine structure tested produced differences in both components of agonism: intrinsic activity and EC_{50}. Results with trimethoquinol suggest that the value for EC_{50} correlates with affinity.

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ADDENDUM

Since this manuscript was completed, Gardner *et al.* (63) have presented evidence that catecholamine-stimulated cyclic AMP formation increased sodium fluxes in turkey erythrocytes, which, like those from frogs, are nucleated. The relationship of this finding to the physiological response in the frog erythrocyte remains to be explored.

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