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HETEROGENEITY OF ADENYLATE CYCLASE-COUPLED β-ADRENERGIC RECEPTORS

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Abstract—The pharmacological characteristics of adenylate cyclase-coupled β-adrenergic receptors were determined by examining potency series of β-adrenergic agonists and antagonists on adenylate cyclase activity in membranes from six tissues: canine myocardium, diaphragmatic skeletal muscle, liver, lung, rat paraovarian fat and frog erythrocytes. Agonists were: isoproterenol, epinephrine, norepinephrine, soterenol and dobutamine. Antagonists were: *d,l*-propranolol, dichlorisoproterenol, practolol (a selective β_1 antagonist) and butoxamine (a selective β_2 antagonist). Catecholamine stimulation of adenylate cyclase ranged from 1·7-fold in lung membranes to 9·1-fold in frog erythrocyte membranes. Isoproterenol was the most potent agonist and propranolol the most potent antagonist in all tissues. Norepinephrine was a potent agonist in cardiac and adipose membranes (1/3–1/5 isoproterenol) but was much weaker in the other tissues (1/50–1/1000 isoproterenol). Soterenol was very weak in cardiac membranes but equipotent with norepinephrine and epinephrine in adipose membranes. Soterenol was more potent than norepinephrine in muscle, liver, lung and erythrocyte membranes. Practolol was a more potent antagonist than butoxamine in heart and adipose membranes, but the reverse was true in the other tissues. The potency ratio of practolol to butoxamine was 10 in fat, 5·8 in heart, 0·16 in muscle, 0·15 in liver and 0·07 in erythrocyte membranes. These results correlate reasonably well with previously determined results in more intact preparations. They suggest that adenylate cyclase-coupled β-adrenergic receptors reflect the specificity of physiological β-adrenergic receptors. The receptors in cardiac and adipose membranes appear to be β_1- and those in muscle, liver, lung and erythrocyte β_2-adrenergic receptors. The data also indicate heterogeneity of the receptors within each subclass (e.g. potency of soterenol in adipose vs cardiac membranes).

Murad *et al.* [1] first described the stimulation of adenylate cyclase by epinephrine and other β-adrenergic agents. Subsequently, an impressive amount of evidence has indicated that the characteristics of the receptors which mediate adrenergic stimulation of the enzyme are generally those of a typical β-adrenergic receptor [2]. Thus, in a variety of tissues isoproterenol is a more potent agonist than norepinephrine, and catecholamine stimulation is competitively antagonized by β-adrenergic blockers such as propranolol but not by α-adrenergic antagonists such as phentolamine [2].

Recently, pharmacologic data obtained with either intact animal or intact organ preparations have suggested that β-adrenergic receptors are not homogeneous [3]. These studies have utilized selective β-adrenergic agonist and antagonist drugs which preferentially interact with certain of the β-adrenergic receptors. Although results with agonists and antagonists have not always agreed, two broad subclasses of β-adrenergic receptors have been delineated, β_1 and β_2. The former are typified by the receptors mediating adrenergic effects on cardiac contractility and rate and on lipolysis in adipose tissue [3]. The latter are exemplified by those mediating effects of catecholamines on glycogenolysis in liver and skeletal muscle [4], and relaxation of tracheal and uterine smooth muscle [3].

Since adenylate cyclase stimulation may be the mechanism by which many [2], if not all [5], β-adrenergic effects are mediated, it seemed reasonable to ask to what extent β-adrenergic receptors linked to adenylate cyclase reflect the specificity of adrenergic receptors in intact tissues.

The information available in the literature to date already indicates that adenylate cyclase-coupled β-adrenergic receptors are not a homogeneous group [6, 7]. Nonetheless there has been no comprehensive study of adenylate cyclase-linked β-adrenergic receptors utilizing both conventional catecholamines (isoproterenol, epinephrine, norepinephrine) and antagonists (propranolol, DCI)* as well as newer selective agents. Accordingly, the studies reported here were undertaken to determine the characteristics of adenylate cyclase-coupled adrenergic receptors in membrane fractions from a variety of tissues.

Though complete agreement on criteria for classification of adrenergic receptors into β_1 and β_2 subgroups has not been reached, certain criteria are generally accepted [3, 6, 8].

(1) "Standard catecholamines"—norepinephrine is significantly more potent at β_1 receptors (approximately equipotent with epinephrine, 1/3—1/10 as

* DCI = dichlorisoproterenol.

potent as isoproterenol) than at β_2 receptors where it is very weak.

(2) Selective agonists e.g. soterenol or salbutamol [9], have much higher affinity for β_2 than β_1 receptors. No clear-cut example of a selective β_1 agonist has been reported, though a new drug, dobutamine, had been reported to be selective for cardiac receptors, hence its inclusion in the present study [10].

(3) Selective antagonists—butoxamine [11] appears to be relatively more potent at certain β_2 receptors (uterus, liver) and practolol [12] at certain β_1 receptors (heart).

These considerations dictated the choice of drugs in this study. Propranolol and DCI were also included as relatively general β-adrenergic antagonists for comparative purposes.

Tissues were selected so as to include examples of both β_1-adrenergic receptors (heart and adipose) and β_2-adrenergic receptors (skeletal muscle and liver). Attempts to obtain a relatively pure smooth muscle preparation (β_2) for study were of limited success. Repeated attempts to utilize uterine muscle from several species failed. Although basal adenylate cyclase activity could be detected, no stimulation by catecholamines was observed. Although lung homogenate membranes represent a mixture of cell types, these membranes did contain catecholamine-sensitive adenylate cyclase and were used for these studies. Frog erythrocyte adenylate cyclase was also studied because of the presence of a classical β-adrenergic receptor in these and other erythrocyte membranes [13].

MATERIALS

l-Isoproterenol bitartrate, l-epinephrine bitartrate, l-norepinephrine bitartrate, *d,l*-propranolol, myokinase, phosphoenolpyruvate, pyruvate kinase, cyclic adenosine 3',5'-monophosphate (cAMP) and ATP were purchased from Sigma.

Soterenol (2'-hydroxy-5'-[l-hydroxy-2-(4-methoxyphenethylamino)-propyl] methanesulfonanilide hydrochloride) was a gift of Mead Johnson, dichlorisoproterenol and dobutamine (±)-4-[2-3-(*p*-hydroxyphenyl)-1-methylpropyl amino ethyl pyrocatechol hydrochloride] of Eli Lilly Co., practolol of Ayerst and butoxamine of Burroughs-Wellcome.

[α-^{32}P]ATP (1–10 Ci/m-mole) was from New England Nuclear Co. [^{3}H]cAMP was from Schwarz-Mann.

Dowex AG 50W-X2 (200–400 mesh) was from BioRad, and (Woelm, neutral grade) was obtained from Nutritional Biochemicals.

METHODS

Tissue samples. Samples of left ventricular myocardium, liver, lung and diaphragmatic skeletal muscle were obtained from adult mongrel dogs (20–40 kg) sacrificed by injection of pentobarbital. Adipose tissue was paraovarian fat from 200 to 225-g rats. Erythrocytes were obtained from Southern Grass frogs (Carolina Biological) by cardiac puncture.

Membrane preparations. All procedures were performed at 0–4°.

Myocardium, liver, skeletal muscle and lung were handled in an identical fashion. Tissue (0·5 g) minced with scissors was suspended in 10 ml of cold 0·25 M sucrose, 0·005 M Tris-HCl, pH 7·4. The tissue mince was disrupted by a 10-sec exposure to a "Tissuemizer" (Tekmar Co.) operating at one-half maximal speed. The thick slurry obtained was further homogenized with 10 strokes of a motor-driven Teflon glass Potter Elvehjem homogenizer. The tissue homogenate was passed through a single layer of cheesecloth and then centrifuged at 12,000 *g* for 10-min in a Sorvall RC2B centrifuge at 2°. The pellet was resuspended in twice the original volume of sucrose buffer and recentrifuged at 12,000 *g* for 10 min. The washing procedure was repeated three or four times. Ultimately, the pellet was resuspended and homogenized in a volume of 75 mM Tris-HCl, pH 7·4, and 25 mM $MgCl_2$ equal to the original homogenate volume. Membrane fractions were assayed immediately.

Adipose tissue was paraovarian fat from 200 to 225-g Charles River (CD strain) rats fed *ad lib.* Animals were killed by cervical dislocation, paraovarian fat was dissected and membranes were prepared essentially as described by Czech and Lynn [14]. In brief, approximately 25 g adipose tissue from 8 to 10 rats was pooled and cut into small pieces. The tissue was divided into 4 to 5-g portions and placed in plastic bottles containing 8–10 ml of 3% albumin in "buffer" (128 mM NaCl, 1·8 mM $CaCl_2$, 4·5 mM $MgSO_4$, 5·2 mM KCl, 10 mM Na_2HPO_4, pH 7·4) with 1 mg/ml of crude collagenase Type 1 (clostridium histolyticum, Worthington) and was incubated for 60 min at 37°. After this digestion, cells were filtered through cheesecloth and washed twice with albumin buffer, then once with warm 0·25 M sucrose and finally added to a prechilled glass homogenizer with 25 ml of ice-cold "buffer" and homogenized (Teflon pestle) with seven up-and-down strokes. The homogenate was centrifuged for 15 min at 18,000 *g*. The resulting pellet was washed by resuspension and centrifugation in ice-cold 0·25 M sucrose, 0·005 Tris-HCl, pH 7·4, three times and finally suspended in 75 mM Tris-HCl, pH 7·4, and 25 $MgCl_2$ by homogenization.

Blood from cold or warm adapted Southern Jumbo Grass frogs (Carolina Biological, Burlington, N.C.) was collected and the red cells were washed three times with saline. Cells were lysed in 5 mM Tris-HCl, pH 8·1, buffer and the membranes centrifuged at 18,000 *g* for 15 min. The lysis process was repeated three times. Membranes were finally suspended in 75 mM Tris-HCl, pH 7·4, and 25 mM $MgCl_2$ by homogenization.

Adenylate cyclase assay. The assay was performed using a modification of the method of Krishna *et al.* [15, 16]. Assays were carried out in a volume of 50 μl which contained Tris-HCl buffer, 30 mM (pH 7·4); $MgCl_2$, 10 mM; cyclic AMP, 0·1 mM; ATP, 1·5 mM;

[α-^{32}P]ATP, 1–2 × 10^6 cpm; phosphoenolpyruvate, 5 mM; pyruvate kinase, 40 μg/ml; myokinase, 20 μg/ml and agonists and/or antagonists at the indicated concentrations. Incubations were for 10 min at 37°, and were stopped by addition of 1 ml of a solution containing [^{3}H]cAMP (15,000 cpm/ml), ATP 100 μg and cAMP 50 μg. ^{32}P-cAMP formed was isolated by chromatography either on Dowex AG 50W-X2 (followed by two precipitations with $BaSO_4$) as previously described [15, 16] or by chromatography on neutral alumina as described by Ramachandran [17]. Recovery was 50 per cent on Dowex and 70–75 per cent on the alumina columns. Labeled material isolated by either procedure was >95 per cent cAMP as documented by thin-layer chromatography on Silica gel plates as described by Ramachandran [17]. Samples were added to 15 ml scintillation fluid [18], and counted in a Packard liquid scintillation spectrometer. All results were corrected for product recovery on the basis of recovery of [^{3}H]cAMP.

For all membrane preparations, it was determined that reactions were linear over the course of the 10-min incubation. Enzyme activity was directly proportional to protein concentration over the range of proteins used in the assays. Membranes were prepared fresh each day for each experiment.

As noted in the legends to the figures and tables, all experiments were repeated in duplicate on four to six separate membrane preparations. In each experiment, stimulation by agonists was expressed as per cent above basal activity. In experiments with antagonists, "maximum response" refers to activity in the presence of isoproterenol 1 × 10^{-5} M alone. The "0% maximal response" refers to basal enzyme activity or complete inhibition of isoproterenol stimulation by the antagonist.

Protein was determined by the method of Lowry *et al.* [19].

Drugs. All compounds were crystalline solids freshly dissolved in water for each experiment. Practolol was dissolved in dilute acetic acid.

Calculations. Relative potencies of agonists were calculated by comparing concentrations which produced comparable enzyme stimulation, and those of antagonists by comparing concentrations which produced 50 per cent inhibition of isoproterenol-stimulated adenylate cyclase.

RESULTS

Basal adenylate cyclase activity and sensitivity to catecholamine stimulation of the membrane preparations are tabulated in Table 1. Stimulation by isoproterenol ranged from only 65 per cent above basal in the lung preparations to 900 per cent above basal in the erythocyte membranes.

The pattern of sensitivity of the adenylate cyclase to a variety of β-adrenergic agonists was determined in each tissue and is presented in Fig. 1, a–f. Five agonists were used: three "classical" agonists—isoproterenol, epinephrine and norepinephrine—and two newer agents—soterenol, a "selective β_2 agent" [9] and dobutamine, a putative cardioselective agent [10].

In all membrane preparations, isoproterenol was the most potent agonist. Marked variation in the patterns of response to the other agonists was found, however. In cardiac and adipose membranes, norepinephrine was quite potent (1/3–1/5 isoproterenol, almost equipotent with epinephrine), whereas in other membranes it was relatively much weaker (1/50–1/100 isoproterenol in skeletal muscle, liver and lung and 1/1000 isoproterenol in frog erythrocytes). Moreover, in these membranes norepinephrine was less than 1/10 as potent as epinephrine.

The selective β-adrenergic agonist soterenol was quite potent in skeletal muscle, liver, lung and erythrocyte membranes, where it was more effective than norepinephrine. Interestingly, this drug was also an effective agonist in adipose membranes where its potency was virtually identical to that of norepinephrine and epinephrine. In heart membranes, soterenol was very weak. Dobutamine was a relatively weak partial agonist in all membrane preparations, and did not demonstrate any specificity for the cardiac enzyme. The potencies of each agonist in each tissue, relative to isoproterenol are summarized in Table 2.

Table 1. Catecholamine-sensitive adenylate cyclase in membranes prepared from various tissues*

Tissue	Species	Basal adenylate cyclase (pmoles/min/mg protein)	Mean maximal stimulation by isoproterenol (x basal)	n
Heart	Canine	66 ± 6	2·2	8
Liver	Canine	46 ± 3	2·6	8
Lung	Canine	69 ± 7	1·7	12
Skeletal muscle	Canine	21 ± 2	2·6	9
Adipose	Rat	42 ± 3	4·3	7
Erythrocyte	Frog	30 ± 3	9·1	5

* Values shown for basal adenylate cyclase represent mean ± S. E. of duplicate determinations performed on 5–12 separate membrane preparations; "n" refers to the number of different membrane preparations tested.

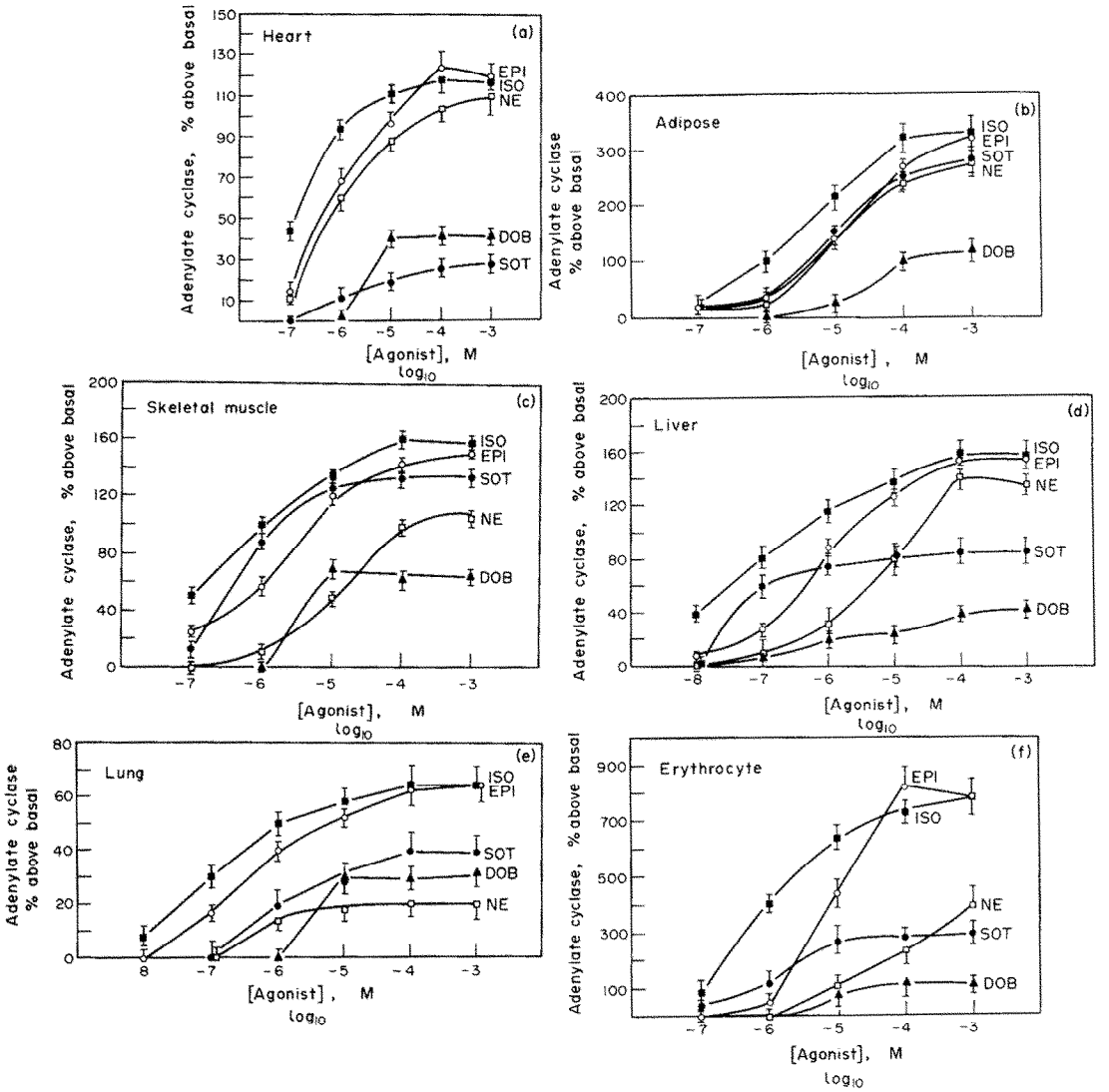

Fig. 1. Activation of adenylate cyclase by β-adrenergic agonists in membrane fractions from: (a) heart, (b) fat, (c) skeletal muscle, (d) liver, (e) lung and (f) erythrocytes. In panels a–e each point is the mean ± S.E.M. of determinations from three to five separate membrane preparations, and of two separate membrane preparations in panel f. All determinations were performed in duplicate. ISO = isoproterenol, EPI = epinephrine, NE = norepinephrine, SOT = soterenol and DOB = dobutamine.

The intrinsic activity [21] or maximum activity of each agonist in each tissue is tabulated in Table 3. Isoproterenol and epinephrine generally had equal activity with norepinephrine slightly less active, except for lung and erythrocyte where norepinephrine had significantly less activity. Dobutamine had weak activity in all tissues. The activity of soterenol varied widely, generally being highest in those tissues where its relative potency was greatest.

In order to further study the characteristics of the adenylate cyclase-coupled β-adrenergic receptors, the ability of several β-adrenergic antagonists to inhibit enzyme stimulation by 1×10^{-5} M isoproterenol was investigated. Four antagonists were used, *d,l*-propranolol and DCI which are relatively general [6, 7], and butoxamine (β_2) [11] and practolol (β_1) [12] which are more selective in their pharmacologic effects.

None of the antagonists produced significant stimulation or inhibition of basal enzyme activity when added to incubations in the concentrations used in this study (10^{-7} to 10^{-3} M). Others have previously reported a slight stimulatory effect of DCI on adenylate cyclase at high concentrations, but it was not noted whether the differences were significant [1].

Table 2. Relative potencies of adrenergic agonists in stimulating membrane adenylate cyclase in various tissues*

Compound	Potency relative to isoproterenol					
	Canine heart	Rat fat	Canine muscle	Canine liver	Canine lung	Frog erythrocyte
Isoproterenol	1	1	1	1	1	1
Epinephrine	0·26	0·33	0·16	0·11	0·25	0·13
Norepinephrine	0·18	0·33	0·01	0·01	0·02	0·001
Soterenol	†	0·33	0·5	0·3	0·04	0·07
Dobutamine	0·01	0·01	0·02	†	0·01	†

* "Potencies" were determined as the ratio of concentrations of compounds required to produce an equivalent response [20], generally that caused by a one-half maximally effective concentration of isoproterenol. When intrinsic acitivity [21] of an agonist was less than one-half that of isoproterenol, a lower fractional response was selected on a portion of the isoproterenol dose–response curve which was approximately parallel to the dose–response curve of that particular agonist. Values were determined independently in 3–5 separate membrane preparations and the means are shown. Statistical analysis was performed by comparing the means in a *t*-test. The following differences were significant ($P < 0·05$): heart, isoproterenol > epinephrine = norepinephrine > dobutamine > soterenol; fat, isoproterenol > epinephrine = norepinephrine = soternol > dobutamine; muscle, isoproterenol = soterenol > epinephrine > norepinephrine = dobutamine; liver, isoproterenol > soterenol > epinephrine > norepinephrine = dobutamine; lung, isoproterenol > epinephrine > soterenol > norepinephrine = dobutamine; and erythrocyte, isoproterenol > epinephrine = soterenol > norepinephrine = dobutamine.

† indicates that a compound was so weak that no meaningful potency ratio could be calculated.

Also, inhibition by antagonists could always be overcome by increasing isoproterenol to 10^{-3} M (data not shown).

Propranolol and DCI were potent β-adrenergic antagonists in all the membrane preparations (Fig. 2, a–f). Sensitivity to the other two compounds varied appreciably from tissue to tissue. Practolol was most effective in cardiac membranes where it was about 1/7 as potent as propranolol. Its potency relative to propranolol was 1/50 in fat, 1/125 in liver and skeletal muscle and less than 1/1000 in lung and erythrocyte. Conversely, butoxamine was least effective in heart and adipose membranes and considerably more potent in skeletal muscle, liver, lung and erythrocyte membranes. Concentrations of each antagonist producing 50 per cent inhibition of isoproterenol stimulation in each of the tissues are summarized in Table 4. Of particular note was the ratio of the concentration of butoxamine to that of practolol necessary for 50 per cent inhibition of isoproterenol response. This was 10 in fat, 5·8 in heart, 0·16 in muscle, 0·15 in liver and 0·07 in erythrocyte membranes. Practolol was so weak in the lung membranes that a valid ratio could not be calculated.

DISCUSSION

Since Ahlquist [22] first suggested classification of adrenergic receptors into alpha and beta types, this scheme has been successfully applied to adrenergic receptors in a wide variety of tissues. Recently, subclassification of adrenergic receptors in β_1 and β_2 subtypes has gained increasing acceptance. Since adenylate cyclase may be the physiologically relevant target of β-adrenergic catecholamines in a number of tissues [2], careful classification of β-adrenergic receptors linked

Table 3. Intrinsic activity of adrenergic agonists for stimulation of adenylate cyclase*

Compound	Canine heart	Rat fat	Canine muscle	Canine liver	Canine lung	Frog erythrocyte
Isoproterenol	1	1	1	1	1	1
Epinephrine	1	1	0·95	1	1	1
Norepinephrine	0·86	0·83	0·66†	0·88	0·31†	0·5†‡
Soterenol	0·24†	0·85	0·83†	0·53†	0·63†	0·37†
Dobutamine	0·34†	0·36†	0·40†	0·26†	0·47†	0·14†

* All activities represent the ratio of the maximal enzyme stimulation achieved with a compound to that achieved with a maximally effective concentration of isoproterenol [21]. Each value shown is the mean of determinations on 3–5 separate membrane preparations. Mean values were compared in a *t*-test.

† Indicates those values which were significantly ($P < 0·05$) less than that of isoproterenol.

‡ Indicates that, in erythrocytes, activity with norepinephrine had not plateaued even at 10^{-3} M.

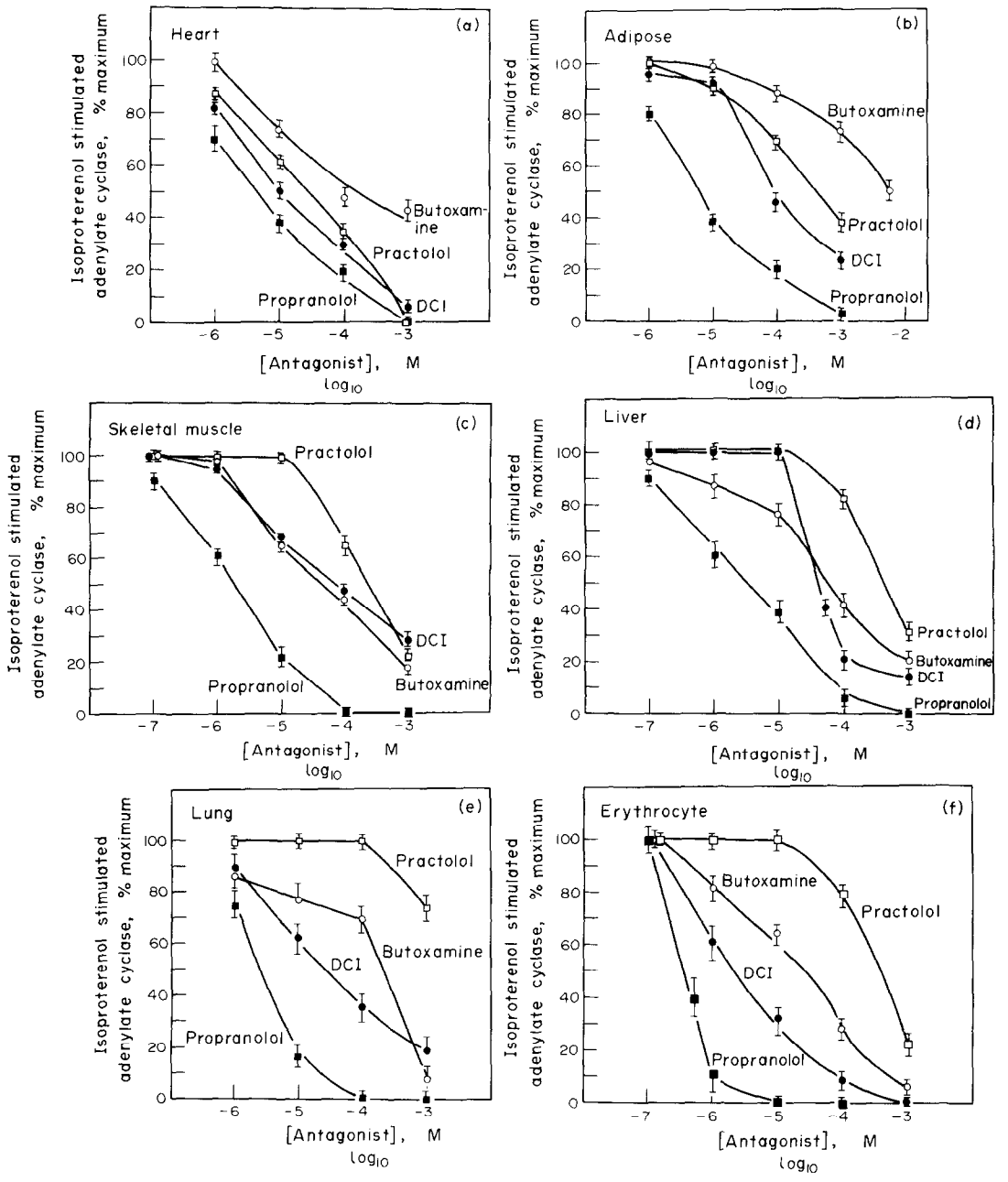

Fig. 2. Inhibition of isoproterenol-stimulated adenylate cyclase by β-adrenergic antagonists in membrane fractions from: (a) heart, (b) fat, (c) skeletal muscle, (d) liver, (e) lung and (f) erythrocytes. Each point is the mean ± S.E.M. of determinations (performed in duplicate) on the following number of membrane preparations: cardiac, four; fat, three; skeletal muscle, four; liver, five; lung, four; and erythrocytes, two. "Maximum" adenylate cyclase refers to enzyme activity in the presence of isoproterenol, 10^{-5} M. "O" refers to basal enzyme activity or complete inhibition of the isoproterenol response by an antagonist. DCI = dichlorisoproterenol.

Table 4. Concentrations of adrenergic antagonists causing 50 per cent inhibition of isoproterenol-stimulated adenylate cyclase*

Antagonist	One-half maximal inhibition (M)					
	Canine heart	Rat fat	Canine muscle	Canine liver	Canine lung	Frog erythrocyte
Propranolol	4×10^{-6}	6×10^{-6}	2×10^{-6}	$3{\cdot}5 \times 10^{-6}$	$1{\cdot}5 \times 10^{-6}$	$3{\cdot}5 \times 10^{-7}$
DCI	1×10^{-5}	9×10^{-5}	7×10^{-5}	4×10^{-5}	$1{\cdot}5 \times 10^{-5}$	2×10^{-6}
Butoxamine	$1{\cdot}5 \times 10^{-4}$	4×10^{-3}	4×10^{-3}	6×10^{-5}	2×10^{-4}	$2{\cdot}5 \times 10^{-5}$
Practolol	$2{\cdot}6 \times 10^{-5}$	4×10^{-4}	$2{\cdot}5 \times 10^{-4}$	4×10^{-4}		$3{\cdot}5 \times 10^{-4}$

* Isoproterenol was present at 1×10^{-5} M. Values are the means of determinations performed on 2–5 separate membrane preparations. In a *t*-test, the values for butoxamine differed significantly ($P < 0{\cdot}05$) from that for practolol in each tissue.

to adenylate cyclase and comparison of their properties with those of the β-adrenergic receptors in intact tissues are of some importance.

The adenylate cyclase-coupled β-adrenergic receptors in cardiac membranes like those in intact hearts were β_1. Norepinephrine was 1/5 as potent as isoproterenol and almost as potent as epinephrine. Soterenol was very weak; practolol was considerably more effective as an antagonist than butoxamine. The adipose membranes also appeared to contain β_1-adrenergic receptors. Norepinephrine and epinephrine were 1/3 as potent as isoproterenol, and practolol was a more potent antagonist than butoxamine. Nonetheless, differences between the cardiac and adipose β_1 receptors were present. Thus, soterenol which had very little effect in heart membranes was equipotent with norepinephrine in adipose membranes. Also, practolol was less effective as an antagonist in adipose than in heart membranes. These data are consistent with a recent report that soterenol is equipotent with norepinephrine in stimulating lipolysis in isolated fat cells [23].

The β-adrenergic receptors mediating effects of catecholamines on glycogenolysis in liver and skeletal muscle are known to be of the β_2 variety [4]. In this study, adenylate cyclase was coupled to a β_2-adrenergic receptor in membranes from these two tissues. Norepinephrine was only 1/100 as potent as isoproterenol and significantly less active than epinephrine. Soterenol was even more potent than epinephrine, although its intrinsic activity was somewhat less. In both cases, butoxamine was a more effective antagonist than practolol.

Similarly, as previously reported by Burges and Blackburn [6], we found that the adenylate cyclase-coupled β-adrenergic receptors in lung homogenate membranes were β_2. Soterenol, however, was somewhat less active in these preparations. It should be borne in mind, however, that of the preparations used in these studies, the lung homogenates were by far the most heterogenous. Although the results obtained do seem to reflect the pharmacological characteristics of β_2-adrenergic receptors in bronchial smooth muscle, further data with more homogenous preparations of smooth muscle from several tissues will be required to confirm the exact nature of adenylate cyclase-coupled β-adrenergic receptors in these cells.

There is no well delineated physiological β-adrenergic response in frog erythrocytes to correlate with adenylate cyclase activation. Nonetheless, it seemed of interest to characterize these β-adrenergic receptors for comparative purposes. The data clearly indicate a β_2-adrenergic receptor in these membranes.

Our data are consistent with other published studies on adenylate cyclase activation by catecholamines. Mayer [7] has found that butoxamine and related compounds block hepatic receptors more effectively than cardiac receptors. Murad [24] found that practolol blocked heart receptors better than adipose receptors and was very weak on receptors in tracheal ring preparations. As noted above, Burges and Blackburn [6] found butoxamine to be more potent in lung than heart and the reverse for practolol.

In summary, there appears to be a reasonable correlation between the characteristics of adenylate cyclase-coupled β-adrenergic receptors in membrane fractions, and those of the β-adrenergic receptors in intact tissues. However, considerably higher concentrations of agonists and antagonists were necessary for effects on the membrane preparations than on intact tissues. As has been emphasized in the literature, this is likely due to some distortion of the normal receptor-cyclase coupling mechanisms in the subcellular membranes [25]. The possible presence of "spare receptors" or of incomplete equilibration of drugs with the receptors may also play a role. The order of potencies of agonists and antagonists, however, was in reasonably good agreement with information derived from more intact tissues. These correlations add further weight to the thesis that adenylate cyclase is the physiologically relevant target of β-adrenergic catecholamines in a number of tissues.

The adenylate cyclase system *in vitro* may provide a simple and convenient assay system for screening new, potentially specific, β-adrenergic agonist and antagonist drugs.

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