

## The Pharmacological Specificity of Beta-1 and Beta-2 Adrenergic Receptors in Rat Heart and Lung *in Vitro*

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### SUMMARY

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The potency and selectivity of a variety of agonists and antagonists were determined for  $\beta$ -1 and  $\beta$ -2 adrenergic receptors on membranes prepared from rat ventricular muscle and lung. Activation or inhibition of  $\beta$ -adrenergic receptor stimulated adenylate cyclase activity and inhibition of specific ( $^{125}$ I)-iodohydroxybenzylpindolol binding were used as *in vitro* measurements of receptor occupancy. With both assays the relative potencies of isoproterenol, epinephrine and norepinephrine with cardiac membranes was approximately 1:10:10, indicating a population of mainly  $\beta$ -1 adrenergic receptors. With membranes from lung the order of potency of these compounds was approximately 1:10:100, indicating a population mainly of  $\beta$ -2 adrenergic receptors. Several drugs previously reported to be  $\beta$ -2 selective agonists (salbutamol, soterenol, salmefamol, zinterol and fenoterol) activated adenylate cyclase in the lung but not in the heart. These compounds turned out to be partial agonists and isoproterenol-stimulated adenylate cyclase activity was inhibited by them in both tissues. Several compounds previously reported to be either  $\beta$ -1 (dobutamine) or  $\beta$ -2 (terbutaline and metaproterenol) selective agonists had similar potencies for stimulation of adenylate cyclase from both tissues. A series of compounds reported to be  $\beta$ -1 selective antagonists were also investigated. Metoprolol and practolol were 10-fold, and atenolol was 3-fold more potent in the heart than the lung. Butoxamine, a  $\beta$ -2 antagonist, was 2-4 fold more potent in the lung than the heart, while H35/25 showed no specificity. The ability of antagonists to inhibit [ $^{125}$ I]-iodohydroxybenzylpindolol binding to membranes prepared from the heart and lung agreed well with their effects on adenylate cyclase. The  $\beta$ -2 selective agonists zinterol and salmefamol also showed a 10-50 fold greater potency in inhibiting [ $^{125}$ I]-iodohydroxybenzylpindolol binding in the lung than the heart. However salbutamol, soterenol and fenoterol, which selectively activated adenylate cyclase in the lung, inhibited [ $^{125}$ I]-iodohydroxybenzylpindolol binding in the two tissues with equal potency. This apparent discrepancy appears to be due to the fact that these drugs which are partial agonists in the lung are competitive antagonists in the heart, and that the  $K_i$  values in the heart are very similar to the  $K_{act}$  values in the lung.

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### INTRODUCTION

Catecholamines have been implicated in the regulation of a wide variety of physiological processes. Ahlquist (1) has proposed the existence of two classes of receptors for catecholamines (termed  $\alpha$  and  $\beta$ ) with differing pharmacological specificities. As defined by Ahlquist (1)  $\alpha$ -adrenergic receptors are stimulated by agonists with the potency order of epinephrine > norepinephrine > isoproterenol while  $\beta$ -adrenergic receptors are stimulated by agonists with the potency order of isoproterenol > epinephrine > norepinephrine.

Further work showed that  $\beta$ -adrenergic receptors in a number of tissues still showed significant differences in their pharmacological specificity. For example, the  $\beta$ -adrenergic receptors regulating lipolysis and myocardial contractility showed similar pharmacological specificities for a series of substituted catecholamines (2). Those mediating bronchodilation and vasodilation, however, showed agonist potencies which were different from the receptors involved in regulating adipose and cardiac tissue. It has been suggested that  $\beta$ -adrenergic receptors should be further subdivided into at least two different subtypes (termed  $\beta$ -1 and  $\beta$ -2) (3).  $\beta$ -1 Adrenergic receptors are found in the heart (controlling contractility and rate) and in adipose tissue (controlling lipolysis) and are stimulated by isoproterenol > epinephrine ≥ norepinephrine.  $\beta$ -2 Adrenergic receptors are found in skeletal muscle (controlling glycogenolysis) and smooth muscle (controlling relaxation) as well as in the liver (controlling glycogenolysis (4)). The order of potency of catecholamines in stimulating  $\beta$ -2 adrenergic receptors is isoproterenol > epinephrine > norepinephrine (5).

Some evidence suggests that even single organs may not have homogeneous populations of  $\beta$ -1 or  $\beta$ -2 adrenergic receptors but that these two types of receptor can coexist in the same organ (6). There is also some evidence consistent with the idea that there are more than two types of  $\beta$ -adrenergic receptor (7). Ahlquist (8) has even proposed that  $\beta$ -adrenergic receptors in each tissue may have their own unique

pharmacological specificity. These hypotheses have proved difficult to investigate due to the lack of a method for biochemically measuring the relative proportions and properties of the different receptor types, as well as due to controversy over the relative specificities of particular drugs.

$\beta$ -Adrenergic receptors cause most of their physiological effects by activating adenylate cyclase and thereby increasing intracellular levels of cyclic AMP (9). Catecholamine-stimulated adenylate cyclase activity can often be demonstrated in tissue homogenates and can be used as a measure of receptor occupancy (10). In addition it has recently become possible to study  $\beta$ -adrenergic receptors *in vitro* by monitoring the specific binding of radiolabelled high affinity ligands (11). In this study these two techniques have been used to determine the *in vitro* pharmacological specificity of  $\beta$ -adrenergic receptors in rat heart (which should be mainly  $\beta$ -1) and in rat lung (which should be mainly  $\beta$ -2). The potencies of a variety of agonists and antagonists as inhibitors of the specific binding of IHYP<sup>3</sup> to  $\beta$ -receptors in the heart and lung was examined, as was the ability of these compounds to stimulate or inhibit adenylate cyclase activity in homogenates of these tissues.

### METHODS

*Preparation of heart and lung homogenates.* Male Sprague-Dawley rats were killed by decapitation or cervical dislocation. The ventricles were dissected free of the atria and great vessels and used without further dissection as a source of cardiac tissue. Ventricles (average weight 0.8 g) or whole lung (average weight 1.4 g) were homogenized with a Brinkmann Polytron (setting 5-7) for 10-15 sec in 20 volumes of 0.9% NaCl containing 20 mM Tris HCl, pH 7.5 (isosaline). The homogenates were used without further purification for measurement of adenylate cyclase activity. To study IHYP binding pellets resulting from centrifugation at 20,000  $\times g$  for 10 min were resuspended in 200 (heart) or 600 (lung)

<sup>3</sup> The abbreviations used are: IHYP, [<sup>125</sup>I]-iodohydroxybenzylpindolol, SDS, sodium lauryl sulfate.

volumes of isosaline per gram wet weight. The recovery of specific IHYP binding sites in the  $20,000 \times g$  pellets ranged from 91–96%.

**Adenylate cyclase assay.** Adenylate cyclase activity was determined by measuring the conversion of ( $^{32}$ P)-labelled ATP to ( $^{32}$ P)-cyclic AMP and isolating the product using a modification of the method of Salomon *et al.* (12). The reaction was carried out in a final volume of 0.2 ml including tissue homogenate (5 mg tissue wet weight), and 50 mM Tris-maleate (pH 7.5), 0.25 mM ATP, 1.0 mM MgCl<sub>2</sub>, 0.5 mM EGTA, 5 mM cyclic AMP, 0.75 mM 3-isobutylmethylxanthine, 0.1 mg/ml creatine kinase, 10 mM creatine phosphate, 1–2 million cpm  $\alpha$ -( $^{32}$ P)-ATP, and appropriate drugs. Incubations were initiated by the addition of tissue and were carried out for 10 min at 30°C. The reaction was terminated by the addition of 0.1 ml of a solution containing 50 mM Tris-maleate (pH 7.5), 5 mM ATP and 10% SDS. The reaction tubes were placed in a boiling water bath for 10–20 min to solubilize the tissue so that membrane proteins did not subsequently impede the flow of the columns. The volume of the samples was then increased to 1.0 ml by the addition of 0.7 ml distilled water, and the ( $^{32}$ P)-cyclic AMP was purified using consecutive Dowex and alumina columns essentially as described by Salomon *et al.* (12).

Samples were poured into plastic columns (0.8 × 25 cm, Kontes Glass Co.) containing 0.8 × 2.5 cm of Dowex analytical grade cation exchange resin AG50W-X4, 200–400 mesh, previously washed with 2 ml of 1 N HCl and 5 ml of deionized water. Samples were allowed to drip through and the eluates were discarded. The columns were washed with 3 ml of deionized water and were then placed directly above similar plastic columns containing 0.6 g neutral alumina. The ( $^{32}$ P)-cyclic AMP was then eluted with water (4 ml) from the Dowex onto the alumina columns. The eluate from the alumina columns was discarded. The alumina columns were then placed directly over scintillation vials and the ( $^{32}$ P)-cyclic AMP was eluted in 2 ml of 50 mM Tris HCl (pH 8.0). Fifteen milliliters of a Triton-

based scintillation fluid was added and radioactivity determined. Recovery of cyclic AMP through the column purification was determined by running duplicate samples containing ( $^3$ H)-cyclic AMP instead of ( $^{32}$ P)-ATP and calculating the percentage recovered. The recovery for the entire procedure was routinely 60% and results reported have been corrected for recovery.

The Dowex and the alumina columns could be used upwards of fifty times with appropriate recycling procedures. After each use the Dowex columns were washed with 2 ml of 1 N HCl and then with 5 ml of deionized water. The columns were then stored in deionized water and were reused without further manipulation. After eluting the ( $^{32}$ P)-cyclic AMP from the alumina columns, the columns were stored dry without further manipulation. Immediately before the next experiment the alumina columns were washed with 10 ml of 50 mM Tris HCl (pH 8.0). Reaction blanks (which were normally 30–40 cpm per 1.5 million cpm added) did not increase over a period of two months with daily use of the same columns. Adenylate cyclase activity was linear under these conditions with time up to at least 12 min and linear with tissue concentration up to at least 20 mg tissue (wet weight) per tube.

**$\beta$ -Adrenergic receptor stimulated adenylate cyclase activity in heart and lung.** Isoproterenol-stimulated adenylate cyclase activity was measured in homogenates of heart and lung. This stimulation was stereospecific, was stereoselectively blocked by 1-propranolol, and was apparently mediated through a  $\beta$ -adrenergic receptor. Basal adenylate cyclase activity in the heart was  $6.1 \pm 0.86$  pmole cyclic AMP formed/min/mg protein and activity was stimulated from 200–400% by isoproterenol. Basal adenylate cyclase activity in the lung was  $2.9 \pm 0.66$  pmole cyclic AMP formed/min/mg protein and was routinely stimulated 80–120% by isoproterenol. While 98% of both basal and isoproterenol-stimulated adenylate cyclase activity from the heart were recovered in the pellet after centrifugation for 10 min at  $20,000 \times g$ , only 8–10% of the adenylate cyclase activity in the lung

was recovered after this procedure. Half of this loss of activity in the lung was due to removal of GTP, which activated both basal and isoproterenol-stimulated activity in the pellet by approximately 5-fold while having no effect on activity in the crude homogenate. The remainder of the original activity was restored in a dose-dependent manner by the readdition of the supernatant to the assay tubes. There are apparently one or more factors in lung homogenates in addition to GTP which are required for maximal levels of adenylate cyclase activity. This is not a  $\beta$ -adrenergic receptor mediated activation, since the fold stimulation by isoproterenol is the same in the presence and absence of the supernatant, even though basal activity is doubled (unpublished observations). To retain an easily measurable amount of activity, adenylate cyclase activity was routinely determined on crude homogenates of both heart and lung, while binding studies were performed on the  $20,000 \times g$  pellets to improve the percentage of specific binding and the reproducibility of the assays. Control experiments showed no difference in the potency or efficacy of norepinephrine, isoproterenol, epinephrine, or propranolol on adenylate cyclase activity in the crude homogenates as compared to the  $20,000 \times g$  pellets of heart or lung.

**$\beta$ -Adrenergic receptor binding assay.** Hydroxybenzylpindolol (13) was iodinated and IHYP was purified to theoretical specific activity ( $2.2 \text{ Ci}/\mu\text{mole}$ ) as previously described (14, 15). An aliquot (0.15 ml) of the crude membrane preparation of heart or lung was incubated with IHYP (40,000–80,000 cpm), 0.154 M NaCl and 20 mM Tris-HCl, pH 7.5 in a total volume of 0.25 ml (16). Binding assays were routinely carried out in new disposable polypropylene tubes (Sarstedt). To determine the efficacy of the various drugs in inhibiting IHYP binding, samples were incubated with various concentrations of each drug in the media described above. Since the concentration of IHYP (54–108 pM) was close to the  $K_d$  of IHYP (0.1 nM), the concentration of drug that inhibited 50% of the specific binding of IHYP is approximately twice the  $K_d$  value

of the drug (17).  $K_d$  values were calculated as described below.

To determine the density of binding sites, the amount of specifically bound IHYP was determined at 8 concentrations of IHYP. The data were analyzed by the method of Scatchard (19) to provide a value for the density of receptors and the dissociation constant ( $K_d$ ) of IHYP. The concentration of IHYP was varied over a concentration range of 30–400 pM (22,000–300,000 cpm).

Samples were incubated for 30 min at 37°. Reactions were then stopped by adding 10 ml of 0.154 M NaCl in 20 mM Tris buffer (37°, pH 7.5) to each assay tube, and the samples were rapidly filtered through Gelman type AE glass fiber filters. Each filter was washed with an additional 10 ml of buffer, and radioactivity determined in a gamma counter.

Specific binding of IHYP was defined as the amount of IHYP bound in the absence of competing ligand minus the amount bound in the presence of 10  $\mu\text{M}$  *l*-isoproterenol. This concentration of *l*-isoproterenol is one hundred times its  $K_d$  value and with observed Hill coefficients of 0.7–0.9 corresponds to occupancy of 94–98% of the receptors. Specific binding routinely represented 60–70% of total IHYP binding in both heart and lung.

**Determination of  $K_d$ ,  $K_i$ , and  $K_{act}$  values.**  $K_d$  values for the inhibition of IHYP binding for the various drugs were calculated by the method of Cheng and Prusoff (18) using the equation

$$K_d = IC_{50}/(1 + S/K_m)$$

where  $IC_{50}$  = concentration of drug inhibiting IHYP binding by 50%;  $S$  = concentration of IHYP; and  $K_m$  =  $K_d$  value for IHYP determined by Scatchard analysis (19).  $IC_{50}$  values for the drugs were determined from dose response curves for the inhibition of IHYP binding by the drug.

$K_i$  values for the inhibition of isoproterenol-stimulated adenylate cyclase activity by various drugs were determined from the same equation where  $IC_{50}$  = concentration of drug necessary to inhibit isoproterenol stimulation by 50%;  $S$  = concentration of isoproterenol; and  $K_m$  =  $K_{act}$  value for the

stimulation of adenylate cyclase by isoproterenol.  $K_i$  values for partial agonists were calculated by determining the  $IC_{50}$  as the concentration of drug which inhibited the stimulation of isoproterenol over the maximal stimulation by the partial agonist.

$K_{act}$  values were determined as the concentration necessary for half maximal activation of adenylate cyclase. For partial agonists which did not activate adenylate cyclase to as great an extent as did full agonists like isoproterenol, the  $K_{act}$  values were the concentrations necessary to achieve 50% of the maximal stimulation observed for that particular drug.

#### DRUGS

The following drugs were generously provided as gifts: Metoprolol and terbutaline sulfate (CIBA GEIGY); soterenol, sotalol and zinterol (MJ-9184-1; N-(5(2-((1,1-dimethyl-2-phenyl-ethyl)amino)-1-hydroxyethyl)-2-hydroxyphenyl)-monohydrochloride) (Mead Johnson); salbutamol and salmefamol 1-(4-hydroxy-3-hydroxymethyl-phenyl)-2-(4-methoxy- $\alpha$ -methylphenethyl-amino)-ethanol (Allen and Hanbury's); fenoterol (3,5 dihydroxy- $\alpha$ -((p-hydroxy- $\alpha$ -methyl phenethyl)amino)methyl)benzyl alcohol and metaproterenol sulfate (Boehringer Ingelheim, Ltd); pindolol and hydroxybenzylpindolol (Sandoz Pharmaceuticals); KL 255 (Bupranolol, Sanolarzneimittel); H35/25 ((1-(4-methylphenyl)-2-isopropyl aminopropranolol) (Hassle); atenolol (ICI Americas); dobutamine (Eli Lilly and Co.); MK-950 (timolol; Merck, Sharpe and Dohme); Cc-25 (+ and -, hydroxyphenylisoproterenol) (N.V. Phillips Duphar Weesp); l- and d-propranolol (Ayerst). Butoxamine was purchased from Burroughs Wellcome; and Cc-34 (hydroxybenzylisoproterenol) and practolol were kindly provided by Dr. R. J. Lefkowitz. Other drugs and reagents were commercially available.

#### RESULTS

*Characteristics of IHYP binding to heart and lung membranes.* Scatchard analysis (19) showed that IHYP bound to a single class of high affinity receptors in both heart and lung membranes. The cal-

culated  $K_d$  values for IHYP binding to  $\beta$ -adrenergic receptors (mean  $\pm$  S.E.M. from 6 experiments) in heart ( $6.5 \pm 0.68 \times 10^{-11}$  M) and lung ( $4.6 \pm 0.47 \times 10^{-11}$  M) were similar. The density of binding sites (Bmax) in the heart was  $29.0 \pm 2.58$  fmole IHYP bound/mg protein; while the density of binding sites in the lung was substantially higher:  $186 \pm 7.9$  fmole IHYP bound/mg protein).

*Effects of catecholamines on IHYP binding and adenylate cyclase activity in rat heart and lung.* Isoproterenol, epinephrine and norepinephrine caused an increase in adenylate cyclase activity and inhibited specific IHYP binding in heart and lung (Fig. 1). The effects of these compounds were stereoselective, with the (d)-isomers being much less potent than the corresponding (l)-isomers (Table 1). For each drug, the  $K_d$  value for inhibiting IHYP binding was in relatively good agreement with the  $K_{act}$  value for stimulating adenylate cyclase activity in the same tissue. The potencies of isoproterenol and epinephrine were similar in heart and lung, with isoproterenol being about 10-fold more potent in both tissues. However, while norepinephrine was approximately equipotent with epinephrine in the heart, it was about 10-fold less potent than epinephrine in the lung. The order of potency of these catecholamines in inhibiting IHYP binding and activating adenylate cyclase in the heart (isoproterenol > epinephrine = norepinephrine) is that expected of a  $\beta$ -1 adrenergic receptor. In the lung, on the other hand, the order of potency of these compounds (isoproterenol > epinephrine > norepinephrine) suggests a population of mainly  $\beta$ -2 adrenergic receptors.

*Effect of "specific"  $\beta$ -1 and  $\beta$ -2 adrenergic agonists on adenylate cyclase activity in rat heart and lung.* A number of drugs have been reported to be specific agonists for  $\beta$ -2 adrenergic receptors on isolated organ preparations or *in vivo*. Five of these drugs (soterenol, zinterol, fenoterol, salmefamol and salbutamol) selectively activated adenylate cyclase activity in rat lung (Table 2). The stimulation of adenylate cyclase activity caused by these compounds was

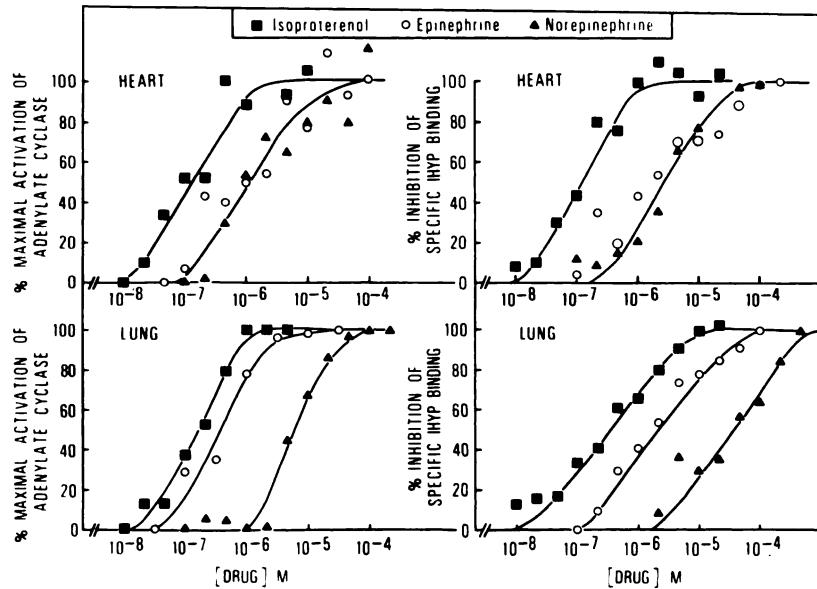


FIG. 1. Effect of isoproterenol, epinephrine and norepinephrine on adenylate cyclase activity and specific IHYP binding in rat heart and lung

The effects of *L*-isoproterenol (■—■), *L*-epinephrine (○—○), and *L*-norepinephrine (▲—▲) were determined on adenylate cyclase activity (left) and specific IHYP binding (right) in membranes from rat heart (top) and lung (bottom). Each graph is representative of at least three similar experiments. The three agonists showed equal efficacy in that adenylate cyclase was activated to the same extent in each case.

TABLE I  
Potency of catecholamines on  $\beta$ -adrenergic receptors in rat heart and lung *in vitro*

The  $K_d$  value for the inhibition of specific IHYP binding and the  $K_{act}$  for activation of adenylate cyclase were determined for the *L*- and *d*-isomers of isoproterenol, epinephrine and norepinephrine in membranes prepared from heart and lung as described in METHODS. For the *L*-isomers the values are the means  $\pm$  S.E.M. of 3 or more determinations. For the *d*-isomers the values are the average of 2 determinations.

Drug	$K_d$ for inhibition of specific IHYP binding			$K_{act}$ for activation of adenylate cyclase		
	Heart	Lung	Ratio	Heart	Lung	Ratio
<i>L</i> -Isoproterenol	0.050 $\pm$ .008	0.101 $\pm$ .021	2.02	0.117 $\pm$ .042	0.178 $\pm$ .015	1.52
<i>d</i> -Isoproterenol	13.4	24.6	1.84	40	60	1.50
<i>L</i> -Epinephrine	0.90 $\pm$ .211	1.46 $\pm$ .167	1.62	1.50 $\pm$ .289	0.60 $\pm$ .150	0.40
<i>d</i> -Epinephrine	210	35.7	0.17	30	50	1.67
<i>L</i> -Norepinephrine	0.82 $\pm$ .238	9.18 $\pm$ .769	11.19	1.10 $\pm$ .231	7.75 $\pm$ 1.03	7.04
<i>d</i> -Norepinephrine	168	179	1.06	300	700	2.33

not additive with the stimulation caused by isoproterenol and appeared to be mediated through a  $\beta$ -adrenergic receptor (Figs. 2, 3 and 4). Four of these compounds, soterenol, zinterol (Figs. 2 and 3) and salbutamol and salmefamol (Fig. 4) had no effect on adenylate cyclase activity in the heart. Fenoterol did stimulate adenylate cyclase activity in the heart (Fig. 3), but the  $K_{act}$  value was higher in the heart than in the lung

(Table 2). Zinterol and soterenol (Figs. 2 and 3, Table 2) were partial agonists in the lung, and at higher concentrations inhibited isoproterenol-stimulated adenylate cyclase activity. These compounds were competitive antagonists in the heart and dose-dependently inhibited adenylate cyclase activity (Figs. 2 and 3). Fenoterol, on the other hand was a full agonist in the lung but a partial agonist in the heart (Fig. 3).

**TABLE 2**  
*Effect of  $\beta$ -adrenergic agonists on  $\beta$ -1 and  $\beta$ -2 adrenergic receptors in vitro in rat heart and lung*

The effect of each drug was determined on adenylyl cyclase and IHYP binding in rat heart and lung.  $K_{\text{et}}$ ,  $K_t$ , and  $K_d$  values were determined as described in METHODS. Efficacy refers to the  $V_{\max}$  relative to isoproterenol for the stimulation of adenylyl cyclase by the drug in the particular tissue examined. Fenoterol did not have equal efficacies in heart and lung. The efficacy for each tissue is indicated on the table. For  $K_t$  determinations isoproterenol was present at a concentration of 30  $\mu\text{M}$ . For adenylyl cyclase measurements each value is range or mean (if values did not differ significantly) of 2 determinations. For IHYP binding each value is the mean  $\pm$  S.E.M. of 3 separate experiments.

Drug	Efficacy (Isoproterenol = 100%)	$K_{\text{et}}$ for activation of adenylyl cyclase			$K_t$ for inhibition of isoproterenol stimulated adenylyl cyclase			$K_d$ for inhibition of IHYP binding			Observed specificity
		Heart	Lung	Ratio	Heart	Lung	Ratio	Heart	Lung	Ratio	
Zinterol	67-85%	N.E. <sup>a</sup>	0.03-0.1	1.5	0.033	0.022	1.03 $\pm$ 0.30	0.02 $\pm$ 0.0017	0.019	$\beta_2$ , partial agonist	
Salmefamol	70-71%	N.E. <sup>a</sup>	0.1-0.5	— <sup>b</sup>	— <sup>b</sup>	—	1.37 $\pm$ 0.18	0.11 $\pm$ 0.031	0.080	$\beta_2$ , partial agonist	
Salbutamol	52-64%	N.E. <sup>a</sup>	1.5-2.0	— <sup>b</sup>	— <sup>b</sup>	—	1.51 $\pm$ 0.04	1.55 $\pm$ 0.339	1.03	$\beta_2$ , partial agonist	
Soterenol	50-66%	N.E. <sup>a</sup>	0.7-0.9	0.66	0.33	0.50	1.61 $\pm$ 0.27	0.57 $\pm$ 0.24	0.35	$\beta_2$ , partial agonist	
Fenoterol	45% Heart	10	0.3-0.7	.05	1.66	—	2.11 $\pm$ 0.27	0.84 $\pm$ 0.18	0.40	$\beta_2$ , full agonist	
100% Lung											$\beta_1$ , partial agonist
Terbutaline	20-33%	30-100	30-100	1.0	30	5	0.17	16.3 $\pm$ 5.79	11.8 $\pm$ 2.64	0.72	none
Dobutamine	31-50%	4-10	8-10	1.28	0.50	0.50	1.00	1.13 $\pm$ 0.27	0.35 $\pm$ 0.087	0.31	none
Metaproterenol	57-62%	20	30	1.50	20	5	0.25	5.05 $\pm$ 0.35	11.7 $\pm$ 1.05	2.32	none
Cs-34	100%	0.8-1.0	0.2-0.3	0.27	—	—	0.035 $\pm$ 0.016	0.026 $\pm$ 0.005	0.74	none	
(-)Cc-25	100%	0.03	0.02	0.67	—	—	0.0079 $\pm$ .00019	0.0132 $\pm$ .0070	1.67	none	
(+)Cc-25	40-66%	3	25	0.83	0.10	0.08	0.90	0.28 $\pm$ 0.102	0.45 $\pm$ 0.11	1.61	none

<sup>a</sup> N.E. = no effect observed.

<sup>b</sup> = unable to determine  $K_t$  value due to non- $\beta$ -adrenergic activation of adenylyl cyclase at high drug concentration. All values are expressed as  $\mu\text{M}$ .

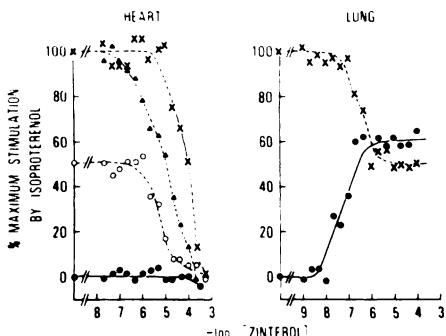


FIG. 2. Effect of zinterol on basal and isoproterenol-stimulated adenylate cyclase activity in rat heart and lung

The effect of zinterol on adenylate cyclase activity in rat heart (left) and lung (right) was examined in the absence (●—●) or the presence of  $10^{-7}$  M (○—○),  $10^{-6}$  M (▲—▲), or  $10^{-5}$  M (×—×) *l*-isoproterenol.

Two drugs (salbutamol and salmefamol) further increased adenylate cyclase at higher concentrations. This effect was apparently unrelated to  $\beta$ -adrenergic receptors (i.e., additive with isoproterenol stimulation) (Fig. 4).

Other drugs which have been previously reported to be specific  $\beta$ -1 (dobutamine) or  $\beta$ -2 (metaproterenol, terbutaline) agonists activated adenylate cyclase with equal potency in heart and lung (Table 2). These compounds were only partial agonists. Cc-34 and Cc-25 were full agonists in both heart and lung. They were also equipotent in heart and lung (Table 2).

**Effect of "specific"  $\beta$ -1 and  $\beta$ -2 adrenergic agonists on IHYP binding in rat heart and lung.** Two  $\beta$ -2 agonists (zinterol and salmefamol) which selectively stimulated adenylate cyclase activity in rat lung also showed a significantly greater potency in inhibiting IHYP binding in rat lung than rat heart (Fig. 5). Zinterol was 50-fold more potent in inhibiting IHYP binding in rat lung than in rat heart, and salmefamol was 10-fold more potent against IHYP binding in the lung than in the heart (Table 2). On the other hand, several other drugs (soterenol, salbutamol and fenoterol), which selectively activated adenylate cyclase in the lung but not in the heart, were approximately equipotent in inhibiting specific IHYP binding in heart and lung (Table 2).

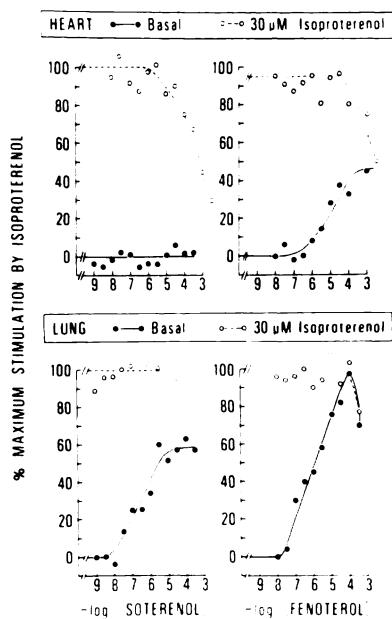


FIG. 3. Effect of  $\beta$ -2 selective agonists on adenylate cyclase activity in rat heart and lung

The effect of soterenol (left) and fenoterol (right) on adenylate cyclase activity in crude homogenates of rat heart (top) and rat lung (bottom) were determined in the absence (●—●) and presence (○—○) of 30  $\mu$ M *l*-isoproterenol. Each point is the mean of duplicate determinations on a single animal and each graph is representative of at least two similar experiments. Basal activity in the heart was  $6.1 \pm 0.86$  pmole cAMP formed/minute/mg protein and activity was routinely stimulated 200–400% by 30  $\mu$ M *l*-isoproterenol. Typical basal activity in the lung was  $2.9 \pm 0.66$  pmole cAMP formed/minute/mg protein and was routinely stimulated 80–120% by 30  $\mu$ M *l*-isoproterenol.

These drugs were more potent in inhibiting IHYP binding in the heart than had been expected from their ability to activate adenylate cyclase activity (i.e.,  $K_d < K_{act}$ ). However, as noted above, soterenol is a competitive antagonist in the heart and fenoterol is a partial agonist, and when the potencies of these compounds as inhibitors of adenylate cyclase were compared to their potencies as inhibitors of IHYP binding, a good correlation was observed (i.e.,  $K_d = K_i$ ). Salbutamol showed no inhibition of adenylate cyclase activity up to 1 mM even though its  $K_i$  for displacing IHYP was 1.51  $\mu$ M. However, this drug also had nonspecific effects on adenylate cyclase (Fig. 4) which

may explain this apparent discrepancy.

The drugs dobutamine, metaproterenol, terbutaline, Cc-34 and Cc-25 which showed

no specificity in activating adenylate cyclase in heart or lung also showed no selectivity in inhibiting IHYP binding in heart and lung (Table 2). Good agreement was observed between  $K_d$  and  $K_{act}$  for these agents.

**Effect of "specific"  $\beta$ -1 and  $\beta$ -2 adrenergic antagonists on adenylate cyclase activity in rat heart and lung.** Several drugs which have been reported to selectively antagonize either  $\beta$ -1 or  $\beta$ -2 adrenergic receptors *in vivo* or in isolated organs were tested for their ability to inhibit isoproterenol-stimulated adenylate cyclase activity in rat heart and lung. The  $\beta$ -1 adrenergic receptor antagonists metoprolol and practolol showed a marked selectivity, and were 10-fold more potent in inhibiting isoproterenol-stimulated adenylate cyclase activity in the heart than in the lung (Table 3). Atenolol, which has also been reported to be a  $\beta$ -1 specific antagonist showed only a slightly greater potency (2-fold) in the heart than in the lung.

Although butoxamine has been reported to specifically antagonize  $\beta$ -2 adrenergic receptors, it was only slightly more potent in the lung than in the heart (Table 3). H35/25, which has also been reported to specifically inhibit  $\beta$ -2 adrenergic receptors, showed approximately equal potency in inhibiting isoproterenol-stimulated adenylate cyclase activity in membranes prepared from lung and heart (Table 3). Other  $\beta$ -

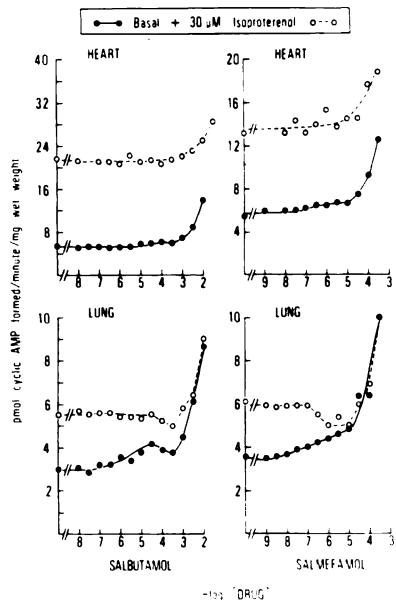


FIG. 4. Effects of salbutamol and salmefamol on adenylate cyclase activity in rat heart and lung

The effect of increasing concentrations of salbutamol (left) and salmefamol (right) was determined on adenylate cyclase activity in crude homogenates of rat heart (top) and rat lung (bottom) in the absence (●—●) or presence (○—○) of 30  $\mu$ M *l*-isoproterenol. Each point is the mean of duplicate determinations on a single animal and each graph is representative of at least two similar experiments.

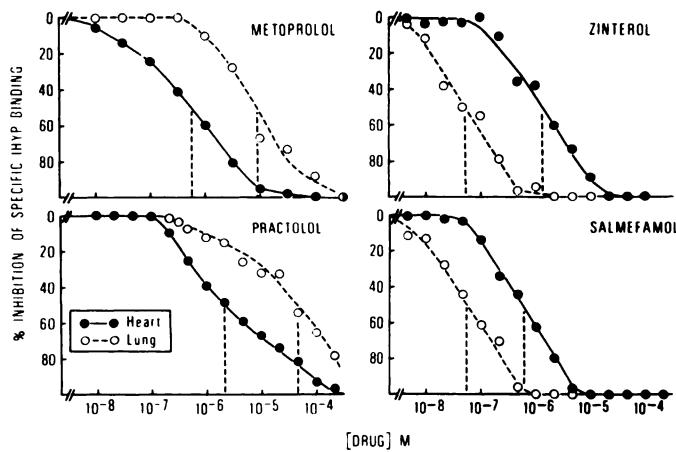


FIG. 5. Effect of  $\beta$ -1 and  $\beta$ -2 selective drugs on specific IHYP binding in rat heart and lung

The effect of metoprolol, practolol, zinterol and salmefamol on specific IHYP binding was measured in membranes from rat heart (●—●) and rat lung (○—○). Each point is the mean of duplicate determinations from three animals.

TABLE 3

*Effect of  $\beta$ -adrenergic antagonists on  $\beta$ -1 and  $\beta$ -2-adrenergic receptors in vitro in rat heart and lung*

The effect of each drug was determined on adenylate cyclase activity and IHYP binding in rat heart and lung.  $K_i$  and  $K_d$  values were determined as described in METHODS. For adenylate cyclase measurements each value is the range or mean (if values did not differ significantly) of 2 determinations. For IHYP binding each value is the mean  $\pm$  S.E.M. of 3 separate experiments. All values are expressed as  $\mu\text{M}$ .

Drug	$K_i$ for inhibition of adenylate cyclase stimulated by 10 $\mu\text{M}$ isoproterenol			$K_d$ for inhibition of specific IHYP binding			Observed specificity
	Heart	Lung	Ratio	Heart	Lung	Ratio	
Practolol	0.5–1.0	8.0–11.0	12.66	1.10 $\pm$ 0.32	26.0 $\pm$ 11.7	23.6	10–20 fold more potent on $\beta_1$
Metoprolol	0.07–0.17	1.0–1.2	9.17	0.22 $\pm$ 0.048	3.63 $\pm$ 0.98	16.5	10 fold more potent on $\beta_1$
Atenolol	0.5–1.0	1.5–2.0	2.33	1.59 $\pm$ 0.57	6.76 $\pm$ 0.89	4.25	3–4 fold more potent on $\beta_1$
Butoxamine	3.3–10	1.0–1.7	0.21	7.03 $\pm$ 2.09	3.55 $\pm$ 0.58	0.50	none
H35/25	0.7–2.0	0.7–1.0	0.63	1.97 $\pm$ 0.79	0.84 $\pm$ 0.18	0.43	none
<i>l</i> -propranolol	0.0056	0.0014	0.25	0.0017 $\pm$ 0.0009	0.00064 $\pm$ .000055	0.38	none
<i>d</i> -propranolol	0.61	0.52	0.85	0.20 $\pm$ 0.037	0.14 $\pm$ 0.017	0.70	none
Pindolol	0.0083	0.0075	0.90	0.0012 $\pm$ 0.00016	0.0010 $\pm$ 0.00021	0.83	none
Sotalol	2.31	1.21	0.52	1.18 $\pm$ 0.33	0.75 $\pm$ 0.10	0.64	none
MK950	0.0049	0.0040	0.82	0.0018 $\pm$ 0.00069	0.00093 $\pm$ 0.00027	0.51	none
KL255	0.0033	0.0033	1.00	0.0040 $\pm$ 0.00060	0.0014 $\pm$ 0.00026	0.35	none

adrenergic receptor antagonists which have not been reported to be specific for  $\beta$ -1 or  $\beta$ -2 receptors (*d*- and *l*-propranolol, pindolol, sotalol, MK 950, and KL 255) also showed equal potency on adenylate cyclase from both heart and lung (Table 3).

*Effect of "specific"  $\beta$ -1 and  $\beta$ -2 adrenergic receptor antagonists on IHYP binding in rat heart and lung.* The effects of  $\beta$ -adrenergic receptor antagonists on inhibiting specific IHYP binding in heart and lung agreed well with the effects observed on isoproterenol-stimulated adenylate cyclase activity (Table 3). The  $K_d$  values for inhibiting IHYP binding were well correlated with the  $K_i$  values for inhibiting isoproterenol-stimulated adenylate cyclase activity. The  $\beta$ -1 antagonists metoprolol and practolol, which were about 10-fold more potent inhibitors of adenylate cyclase activity in the heart than in the lung, were also about 10-fold more potent in inhibiting IHYP binding in the heart than in the lung (Fig. 5, Table 3). Atenolol again showed a small specificity for heart  $\beta$ -receptors; this drug was 4-fold more potent in inhibiting IHYP

binding in the heart than in the lung (Table 3). The  $\beta$ -2 receptor antagonist butoxamine was slightly (2-fold) more potent in the lung than the heart, and again H35/25 showed no apparent selectivity.

## DISCUSSION

The existence of two subtypes of  $\beta$ -adrenergic receptors was first postulated by Lands *et al.* (3, 4). According to this classification, the  $\beta$ -adrenergic receptors in cardiac and adipose tissue, which have a high affinity for norepinephrine, are called  $\beta$ -1 receptors, while the  $\beta$ -adrenergic receptors mediating smooth muscle relaxation in the trachea, vascular beds, bronchial smooth muscle and uterus, which have a relatively low affinity for norepinephrine, are called  $\beta$ -2 receptors.

Frequently, however, the pharmacological specificity of receptors in different organ preparations are not identical (7). This has led to the suggestion that there may be more than two subtypes of  $\beta$ -adrenergic receptors. In particular, it has been suggested that the  $\beta$ -2 receptors mediating

bronchial relaxation may be distinct from the  $\beta$ -2 receptors mediating vasodilation (7). These conclusions were based on the relative potencies of various drugs in *in vivo*, *in situ* or isolated organ preparations. It has been shown, however, that the apparent selectivity of a large number of drugs for tracheal  $\beta$ -receptors as compared to vascular  $\beta$ -receptors is actually due to differences in the extra-neuronal uptake of these compounds (20). In the presence of inhibitors of extraneuronal uptake (such as phenoxybenzamine), these compounds show little or no selectivity. In any case, pharmacological data obtained with intact preparations is frequently open to alternative interpretations, since the potency of a particular drug on a particular preparation can be influenced by a number of factors in addition to the affinity of the drug for the receptor. These factors may include access of the drug to the receptor, the relative lipophilic or hydrophilic nature of the drug, the chemical stability or the susceptibility of the drug to degradative enzymes, and membrane-stabilizing effects of the drug.

Another hypothesis that has been advanced to explain observed differences in the exact pharmacological specificity of  $\beta$ -adrenergic receptors in different tissues suggests that all the  $\beta$ -adrenergic receptors in a particular organ may not be of a single subtype, but that both  $\beta$ -1 and  $\beta$ -2 receptors may coexist in the same organ and that stimulation of both types of receptors will result in the same physiological response (6). If this were the case, differences in the relative percentage of  $\beta$ -1 and  $\beta$ -2 receptors in different tissues would result in variations in the exact pharmacological specificity for a particular physiological response.

One approach to obtaining information on the pharmacological specificity of  $\beta$ -adrenergic receptors invokes direct *in vitro* determination of receptor properties in tissue homogenates. Since  $\beta$ -adrenergic receptors are usually linked to adenylate cyclase, the stimulation or inhibition of stimulation of this enzyme in tissue homogenates is usually taken as a measure of receptor occupancy. Studies using these methods to characterize the pharmacological specificity of  $\beta$ -adrenergic receptors in various tis-

sues (5, 22-25) have generally concluded that practolol and metoprolol are about 10-fold more potent in inhibiting isoproterenol-stimulated adenylate cyclase activity in the heart than in the lung, tracheal ring or liver. There is also agreement that salbutamol and soterenol activate adenylate cyclase in the lung but not in the heart. The effects of butoxamine, on the other hand, are controversial. Mayer (5) reported that butoxamine was 20-fold more potent in the liver than the heart while Burges and Blackburn (22) found butoxamine to be only 4-fold more potent in the lung than in the heart, and Lefkowitz (25) found that butoxamine was equipotent in heart and lung and only 2-fold more potent in the liver.

The recent development of techniques for monitoring the specific high affinity binding of radiolabelled  $\beta$ -adrenergic antagonists to tissue fragments has made it possible to directly measure  $\beta$ -adrenergic receptors *in vitro* (11). This technique offers significant advantages over studies of  $\beta$ -adrenergic receptors carried out by measuring the stimulation of adenylate cyclase activity since it does not depend on the assumption that all  $\beta$ -adrenergic receptors are linked to adenylate cyclase, or that receptor occupancy is linearly associated with adenylate cyclase activation.

In this paper the effects of a number of  $\beta$ -adrenergic agonists and antagonists on adenylate cyclase and specific IHYP binding in rat heart and lung have been examined. IHYP showed no selectivity for the  $\beta$ -adrenergic receptors in the heart or lung, as it bound to a single class of high-affinity binding sites with the same affinity in both tissues. The  $K_{act}$  or  $K_i$  values of these drugs for the activation or inhibition of adenylate cyclase activity and the  $K_d$  values for the inhibition of specific IHYP binding showed a good correlation in these tissues, suggesting that the two techniques are measuring the same receptor population.

In confirmation of previous reports (22, 24), the  $\beta$ -1 antagonists practolol and metoprolol were 10-fold more potent in inhibiting isoproterenol-stimulated adenylate cyclase activity in the heart than in the lung. These drugs were also more potent inhibi-

tors of specific IHYP binding in the heart than the lung. Atenolol, another  $\beta$ -1 antagonist, showed a smaller but similar selectivity for the  $\beta$ -adrenergic receptors in the heart. Butoxamine, which has been classified as a  $\beta$ -2 antagonist *in vivo*, showed only a small (2-4-fold) selectivity for the  $\beta$ -2 adrenergic receptors in the lung *in vitro*. H35/25, a drug of the same chemical class as butoxamine which has been reported to be a  $\beta$ -2 selective antagonist, showed no selectivity between heart and lung.

Compounds that have been reported to be selective  $\beta$ -2 agonists (salbutamol, soterenol, fenoterol, zinterol and salmefamol) showed a marked selectivity in activating adenylate cyclase in the lung but not in the heart. These drugs were not nearly as selective in inhibiting IHYP binding, however, as only zinterol and salmefamol were significantly more potent in the lung than the heart. The reason for this apparent discrepancy is that these drugs are partial agonists in the lung but competitive antagonists in the heart. The  $K_d$  values for these drugs in the heart agree very well with the  $K_i$  values calculated from the inhibition of adenylate cyclase. Since the inhibition of IHYP binding does not distinguish between antagonists and agonists, these drugs are equipotent in the two tissues.

On the other hand, some compounds reported to be either  $\beta$ -1 (dobutamine) or  $\beta$ -2 (terbutaline and metaproterenol) selective agonists in physiological studies showed no selectivity in these *in vitro* tests. The reported selectivity of these drugs may be due to factors other than differences in receptors.

The results suggest that the  $\beta$ -adrenergic receptors in rat heart and lung display different pharmacological specificities *in vitro*, consistent with the existence of more than one type of  $\beta$ -adrenergic receptor. Although not all drugs reported to be selective *in vivo* or *in situ* displayed selectivity *in vitro*, those drugs which did show selectivity *in vitro* showed the predicted selectivity, that is,  $\beta$ -1-selective compounds were more potent in the heart than in the lung, and  $\beta$ -2-selective compounds were more potent in the lung than in the heart. The goal of these studies was to develop a method for mea-

suring the relative concentrations of  $\beta$ -1 and  $\beta$ -2 adrenergic receptors and to determine the affinities of a variety of drugs for  $\beta$ -1 and  $\beta$ -2-adrenergic receptors in tissues with heterogeneous populations of the two receptor subtypes. Such a method has been developed and the results are presented in the following paper.

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