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Beta$_1$- and *Beta*$_2$-Adrenergic Receptors Responsible for Cyclic AMP Accumulation in Isolated Heart and Lung Cells

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SUMMARY

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Cells isolated from adult rat heart and lung by the collagenase method were incubated with the addition of general or selective *beta*-adrenergic agonists and antagonists and were analyzed for changes in the cyclic AMP content as an index of the cellular adenylate cyclase activity. On the basis of concentrations required for half-maximal increase in the cyclic AMP content, norepinephrine was as potent in heart cells as, but much less potent in lung cells than, epinephrine. Practolol, a selective *beta*$_1$ antagonist, was more potent in heart cells than in lung cells, whereas butoxamine, a selective *beta*$_2$ antagonist, was more potent in lung cells than in heart cells. Thus, adenylate cyclase-linked *beta*-adrenergic receptors were characterized as *beta*$_1$ in heart cells and as *beta*$_2$ in lung cells. All of the *beta*$_2$ agonists tested, salbutamol, trimetoquinol and procaterol, showed much lower intrinsic activities than epinephrine, norepinephrine or isoproterenol in increasing cyclic AMP contents in heart cells, indicating that the intrinsic activity observed in heart cells may serve as an additional criterion for classification of *beta*$_1$ and *beta*$_2$ agonists. Concentrations of adrenergic agonists required for the half-maximal increase in cyclic AMP, as well as concentrations of adrenergic antagonists required for the half-maximal decrease of the epinephrine-induced accumulation of the nucleotide, in heart and lung cells were well correlated with the dissociation constants for their specific binding to membrane preparations of the same tissues. Thus, measurement of cyclic AMP in isolated heart and lung cells may provide a simple and convenient assay system for screening new, potentially specific, *beta*-adrenergic agonists and antagonists.

INTRODUCTION

Beta-adrenergic receptors have been differentiated into *beta*$_1$ and *beta*$_2$ subtypes based on measurement of pharmacological responses of selected tissues to various catecholamines (1). Biochemical properties, such as adenylate cyclase activity (2–4) and ligand binding affinity (5), of broken-cell preparations and slices of these tissues have also been used as criteria for the subdivision of receptors. These studies *in vitro* showed that *beta* receptors in cardiac muscles were of *beta*$_1$ type and those in bronchus and lung were of *beta*$_2$ type.

New techniques have been proposed for isolation of cardiac cells (6–10) and lung cells (11–13) from adult rats. These isolated cell preparations, in which the spatial organization of the cell membrane and the physiological milieu of adenylate cyclase units are expected to be maintained, appear to be suitable for the study of the adenylate cyclase-linked *beta*-adrenergic receptors. The purpose of the present paper is to characterize *beta*-adrenergic receptors in the isolated heart and lung cells by virtue of the cyclic AMP responses to various *beta*-adrenergic agents. Affinities of these agents to *beta*-adrenergic receptors were also determined based on their potencies in competing for the binding of [^{3}H]dihydroalprenolol to the membrane preparations.

MATERIALS AND METHODS

Materials. Sources of reagents used are as follows. [^{3}H]Dihydroalprenolol, New England Nuclear Corporation; collagenase (type 2), Worthington Biochemical Corporation; bovine serum albumin (Fraction No. V), isoproterenol and norepinephrine, Sigma Chemical Company; epinephrine, E. Merck Japan Ltd.; IBMX[1], Aldrich Chemical Company, procaterol, propranolol, pindolol, carteolol, practolol, and butoxamine, gifts from Otsuka Pharmaceutical Company; trimetoquinol, a gift from Tanabe Seiyaku Company; glucagon, a gift from Eli Lilly and Company. Other reagents were of analytical grade from commercial sources.

[1] The abbreviation used is: IBMX, 3-isobutyl-1-methylxanthine.

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Isolation of cells. Male rats of the Wistar strain weighing 200–250 g were used as tissue donors. Heart cells were isolated by the method of Powell and Twist (10) with slight modifications. Hearts were removed quickly from rats killed by decapitation and placed in ice-cold saline. Blood cells were first washed out of the heart by means of "flow-through" perfusion with 10 ml of perfusate. The perfusate was prepared by adding 0.05 mM $CaCl_2$ and 1% albumin to the "basal medium" which consisted of 133 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 15 mM Tris buffer (pH 7.4), and 10 mM glucose. The organ was then perfused by recirculation with 25 ml of the same medium for 5 min; it was followed by a subsequent 17-min perfusion with the addition of collagenase (0.05% in a final volume of 35 ml). Perfusions were carried out at a rate of 8 ml/min at 37° under vigorous aeration with an atmosphere of 100% oxygen.

At the end of the perfusion period, two hearts, perfused side by side, were cut into small pieces in a plastic beaker to be further incubated for 10 min at 37° in 5 ml of the gassed fresh perfusate-containing collagenase. After incubation, the tissue suspension was filtered through two layers of nylon stocking and centrifuged at 200*g* for 2 min. The pellets, suspended in 5 min of the fresh perfusate containing no collagenase, were again centrifuged under the same conditions. The final cell pellets were resuspended in 10 ml of the "basal medium" supplemented with 2.5 mM $CaCl_2$ and 1.5% albumin (approximately 5×10^5 cells/ml).

Lung cells were isolated by modifications of the previous method of Pérez-Diaz *et al.* (12, 13) and Ayuso *et al.* (11). Rats were anesthetized with pentobarbital (40 mg/kg body wt), and the portal vein was exposed by a midline abdominal incision. The basal medium was infused into the portal vein at a rate of 20 ml/min for 5 min. The aorta and cava vessels were severed close to their bifurcation to allow the blood to evacuate. The lung tissues made free of blood cells were then removed, finely minced with scissors, and suspended in 3 ml/one pair of the tissues of the basal medium fortified with 0.5% collagenase and 1% albumin. The suspension was incubated under vigorous stirring in an atmosphere of 100% oxygen at 37°. After 30 min, the free cells were filtered through double-layer nylon stocking, and a second 30-min incubation was performed with the remaining tissue. The filtrates from both incubations were combined and spun at 200*g* for 2 min. The pellets were again suspended in the "basal medium" supplemented with 1% albumin and recentrifuged under the same conditions. The final cell pellets were dispersed in the same medium used for cardiac cells with the aid of a Teflon pestle. Generally, two pairs of rat lung were pooled for each preparation yielding 7–8 ml of approximately 5×10^5 cells/ml.

Incubation of isolated cells and determination of the cyclic AMP content. Each incubation tube contained 100 μl of cell suspensions (5×10^4 cells of heart or lung) and 100 μl of the basal medium supplemented with 2.5 mM $CaCl_2$ and drugs. Unless otherwise stated, IBMX (0.5 mM) was added to inhibit phosphodiesterase activity in the cells. Incubation was carried out at 37° usually in duplicate. At the end of incubation (usually for 5 min), 200 μl of 0.2 N HCl was quickly added and the tube was immersed in boiling water for 3 min to extract cyclic AMP as described elsewhere (14). After centrifugation at 600*g* for 10 min, cyclic AMP in the supernatant was succinylated quantitatively and was then measured by the sensitive radioimmunoassay procedure described by Honma *et al.* (15).

The supernatant thus prepared contained no detectable amount of cyclic AMP after it was incubated with bovine heart phosphodiesterase (Sigma). The recovery of the authentic cyclic AMP added to the supernatant was essentially 100%. Based on these results, this simple technique was adopted for cyclic AMP determination in the present study.

Heart and lung membrane preparations. Heart and lung were taken from decapitated rats. The tissue was homogenized for 30 sec in 20 vol of the ice-cold Tris buffer (50 mM, pH 7.7) containing 10 mM $MgCl_2$ in a Polytron tissue disrupter. The homogenates were filtered through two layers of nylon stocking and the filtrates were centrifuged at 50,000*g* for 10 min in the cold. The sediment was suspended in 10 vol of the same medium and recentrifuged under the same conditions. The pellets thus obtained were dispersed in the same medium (10 ml per 0.1 g of the original tissue weight) with the aid of a Polytron disrupter (10 sec).

Binding studies. In all binding experiments, 1-ml aliquots of the crude membrane preparation (approximately 0.5 mg of protein) were mixed with 0.1 ml of water containing [^{3}H]dihydroalprenolol at a final concentration of 1 nM and nonradioactive ligands at various concentrations. Incubation was allowed to proceed with constant shaking (120 strokes/min) for 20 min at 25°. The time and temperature were chosen to assure that equilibrium of specific binding was reached. At the end of incubation, the reaction mixture was filtered under reduced pressure through a glass fiber filter (Whatman GF/C, 24 mm diameter). The filters were washed twice with 10 ml of ice-cold Tris buffer per wash and then dried by being kept in an oven at 80° for 2 hr. The time required to filter and wash a sample was less than 20 sec. Finally, the filters were put in vials, toluene-type scintillator was added, and the samples were counted in a liquid scintillation counter. Each determination was always done in duplicate.

The binding of [^{3}H]dihydroalprenolol in the absence of propranolol minus the binding in its presence at 45 μM was considered as specific or receptor binding. This specific binding was usually greater than 75% of total radioactivity bound to membrane protein. Protein was measured by the method of Lowry *et al.* (16) with bovine serum albumin as standard. The apparent dissociation constant of *beta*-adrenergic receptors for [^{3}H]dihydroalprenolol (K_d(DHA)) was determined by Scatchard analysis. Then, K_d for the receptor binding of a nonradioactive agonist or antagonist was calculated by the following equation (17),

$$K_d = \frac{ID_{50}}{1 + [C]/K_d(DHA)},$$

where ID_{50} is the concentration of the agonist or antagonist to cause the 50% inhibition of the [^{3}H]dihydroalprenolol binding at its concentration of [C].

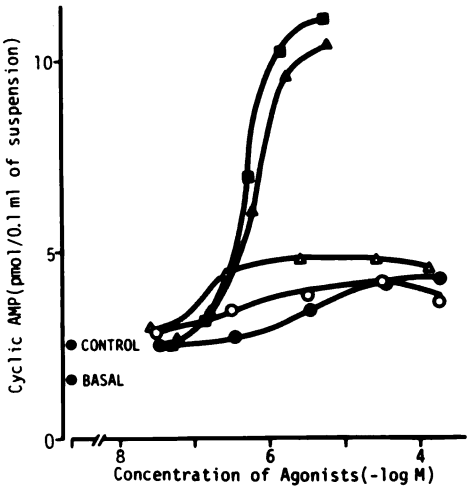

FIG. 1. *Cyclic AMP production during incubation of isolated heart cells as a function of concentration of beta-adrenergic agonists added*

Heart cell suspensions were incubated at 37° for 5 min in the presence of 0.5 mM IBMX with various concentrations of epinephrine (▲), norepinephrine (■), salbutamol (●), trimetoquinol (△), or procaterol (○). CONTROL: the cyclic AMP content of cells incubated with IBMX alone; BASAL: the cyclic AMP content of cells before incubation. Each point is the mean of duplicate observations.

RESULTS

The content of cyclic AMP in heart cells isolated from adult rats was 4.2 ± 0.3 pmol/mg protein or 3.2 ± 0.2 pmol/10^5 cells on an average of five experiments. In all figures in the present paper, the cyclic AMP values will be presented as pmol/0.1 ml of the cell suspension which contains 5 × 10^4 cells. The cyclic AMP content of heart and lung cells increased slightly after a 5 min incubation with 0.5 mM IBMX. When 4 μM isoproterenol was added, much more cyclic AMP accumulated very rapidly during incubation. The accumulation was so rapid that it almost leveled off at 5 min. The variation of cyclic AMP data was found to be small within a set of experiments; the standard error of the mean obtained from 5 observations under the similar conditions was usually less than 5% of the mean value either for the control (incubated with IBMX alone) values or for the values increased by the addition of catecholamines. Hence, the mean value from duplicate observations, agreeing to each other within 10%, will be presented in all figures in the present paper. The reproducibility of the data, in both the cyclic AMP and the binding studies, was also ascertained by repeating experiments at least two or three times under the similar conditions.

Dose-dependent stimulation by beta-adrenergic agonists of cyclic AMP production in heart and lung cells. The cyclic AMP contents of heart cells after 5 min incubation are plotted in Fig. 1 as a function of concentrations of *beta*-adrenergic agonists. Norepinephrine was as potent as epinephrine in increasing cyclic AMP levels in heart cells. Moreover, the maximum responses to these catecholamines were essentially the same as the response to the maximum dose (4 μM) of isoproterenol. In contrast, the intrinsic activities or the maximum activities of salbutamol (18), trimetoquinol (19), and procaterol (20), which were reported as selective $beta_2$ stimulants, were far smaller than the intrinsic activity of these general agonists; the ratios of the maximal increase in the cyclic nucleotide achieved with salbutamol, trimetoquinol, and procaterol to that achieved with isoproterenol were 0.20, 0.28, and 0.17, respectively.

Figure 2 shows increases in the cyclic AMP content of lung cells elicited by various *beta* agonists. In lung cells, norepinephrine was much less potent than isoproterenol and epinephrine (i.e., concentrations causing the half-maximal increase, ED_{50}, were much higher with norepinephrine than with these two catecholamines), whereas selective $beta_2$ agonists were nearly "full" agonists; the intrinsic activities were 0.74 for trimetoquinol, 0.70 for salbutamol and 0.85 for procaterol relative to that for isoproterenol. Procaterol was most potent among these three selective agonists; ED_{50} was much lower with procaterol than with isoproterenol as well as with the other two. Thus, the results in Figs. 1 and 2 show that *beta*-adrenergic receptors linked to cyclic AMP generation can be classified as $beta_1$ in heart cells and as $beta_2$ in lung cells.

Inhibition of epinephrine-induced cyclic AMP generation in heart and lung cells by general and selective beta-adrenergic antagonists. Propranolol, a nonselective *beta*-adrenergic antagonist, was very effective in inhibiting epinephrine-induced increases in cyclic AMP contents of both heart and lung cells (Fig. 3); the concentration required for the 50% inhibition (ID_{50}) was around 0.01 μM in both cases. In lung cells, the action of epinephrine was inhibited by butoxamine, a selective $beta_2$ antagonist (21), but not at all by practolol, a $beta_1$ antagonist (22), at concentrations up to 100 μM. In heart cells, ID_{50} of about 2 μM observed with practolol was much less than ID_{50} of 100 μM with butoxamine. Thus, *beta*-adrenergic receptors in heart and lung cells are characterized as $beta_1$ and $beta_2$, respectively, on the basis of their sensitivity to selective antagonists.

By combining ID_{50} values obtained in Fig. 3 with the ED_{50} value for epinephrine used as an agonist in this experiment, the apparent dissociation constants for the

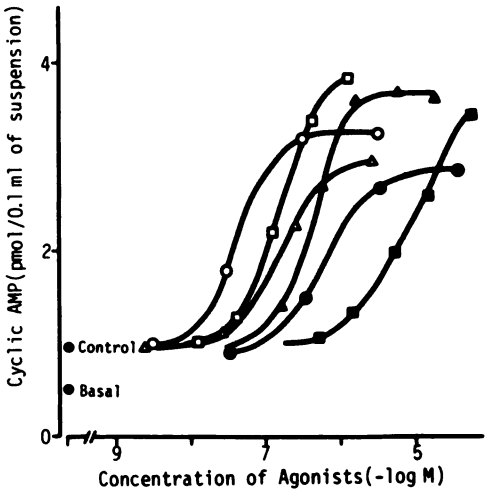

FIG. 2. *Cyclic AMP production during incubation of isolated lung cells as a function of concentrations of beta-adrenergic agonists added*

Lung cell suspensions were incubated at 37° for 5 min in the presence of 0.5 mM IBMX with various concentrations of epinephrine (▲), norepinephrine (■), isoproterenol (□), salbutamol (●), trimetoquinol (△), or procaterol (○). See legend to Fig. 1 for more details.

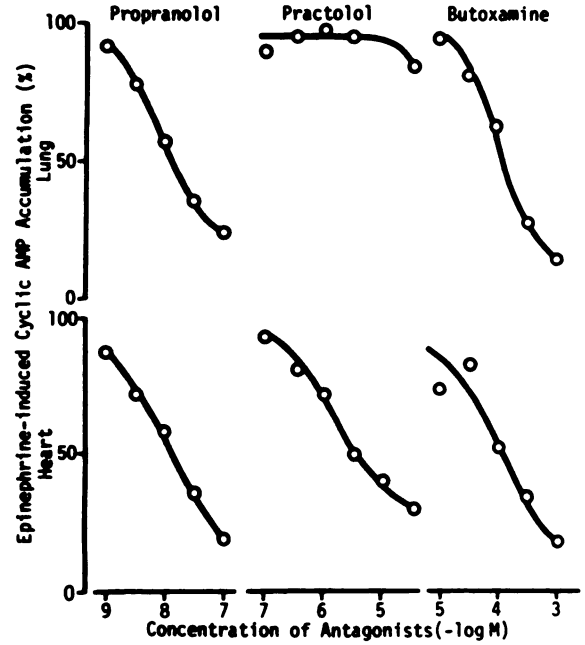

FIG. 3. *Dose-dependent inhibition of epinephrine-induced cyclic AMP production by beta adrenergic antagonists in heart and lung cells*

Lung (top panels) or heart (bottom panels) cells were incubated at 37° for 5 min with 0.5 mM IBMX and 2.8 μM epinephrine in the presence or absence of *beta*-adrenergic antagonists. The epinephrine-induced increased in cyclic AMP in the presence of an antagonist was expressed as a percentage of the increase in its absence, and plotted as a function of concentrations of the antagonist. The data are means of duplicate observations.

antagonist–receptor complexes can be calculated according to the equation described elsewhere (17). These values, together with ED_{50} values for stimulation of cyclic AMP production by agonists which directly reflect apparent dissociation constants for the agonist–receptor complexes, are listed in Table 1. The values are also included in this table for pindolol and carteolol, which have been reported to be more potent than propranolol on a pharmacological basis (17, 23).

Affinities for the receptor of beta-adrenergic agonists and antagonists directly determined by [^{3}H]dihydroalprenolol binding. Specific binding of [^{3}H]dihydroalprenolol to the broken cell preparations of heart and lung was determined as described under MATERIALS AND METHODS. K_d values for [^{3}H]dihydroalprenolol binding were 1.4 and 1.3 nM with heart and lung cell membrane preparations, respectively. Displacement of [^{3}H]dihydroalprenolol from the protein caused by the addition of various *beta*-adrenergic agonists and antagonists afforded the apparent dissociation constants (K_d) for receptor bindings of these ligands. These values are also listed in Table 1. The K_d value for norepinephrine was of the same order in heart as, but was much greater in lung than, the value for epinephrine. Two selective $beta_2$ agonists, salbutamol and procaterol, showed smaller K_d in lung than in heart, but K_d values for trimetoquinol were essentially the same between heart and lung. In lung, K_d value for butoxamine was smaller, but the value for practolol was much greater, than the respective value in heart. The, *beta*-adrenergic receptors in the heart cell membrane were of $beta_1$ subtype, whereas those in the lung cell membrane were of $beta_2$ subtype.

TABLE 1

Apparent dissociation constants (K_d) of agonist(or antagonist)-receptor complex

The data in the two columns under the heading "cyclic AMP" are ED_{50} values for cyclic AMP production which were estimated from plots in Figs. 1 and 2 in the case of agonists (drugs 1–6). In the case of antagonists(drugs 7–11), the values were calculated from ID_{50} values estimated from plots in Fig. 3 (plots not shown for pindolol and carteolol) according to the equation

$$K_d = ID_{50}/(1 + [C]_{ep}/ED_{50_{ep}})$$

where $[C]_{ep}$ is the concentration (2.8 μM) of epinephrine employed in Fig. 3 $ED_{50_{ep}}$ is the ED_{50} value for epinephrine-induced cyclic AMP production. The data in the two columns under the heading "^{3}H-binding" are K_d values calculated from [^{3}H]-dihydroalprenolol binding to membrane preparations of agonists or antagonists. See MATERIALS AND METHODS for experimental details and for calculation.

Drug no.	Drug	K_d based on			
		Cyclic AMP		^{3}H-binding	
		Heart	Lung	Heart	Lung
		(nM)			
1	Isoproterenol	130	160	74	76
2	Epinephrine	630	450	440	240
3	Norepinephrine	500	11,000	590	2,200
4	Salbutanol	2,800	750	2,600	1,000
5	Trimetoquinol	79	180	15	26
6	Procaterol	140	42	680	65
7	Propranolol	2.6	2.0	4.3	2.0
8	Pindolol	1.9	2.5	2.0	1.9
9	Carteolol	3.1	2.8	0.9	3.0
10	Butoxamine	2,500	1,400	10,000	2,200
11	Practolol	830	>10,000	2,800	56,000

K_d values estimated based on stimulation of cyclic AMP production by agonists or its inhibition by antagonists (values under the heading "cyclic AMP" in Table 1) were plotted as a function of K_d values for the membrane receptor bindings of these compounds (values under the heading "^{3}H-binding" in Table 1) in logarithmic scales (Fig. 4). The slope of the regression line drawn by

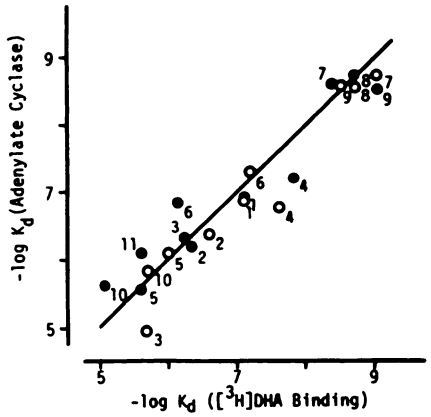

FIG. 4. *Correlation between K_d values estimated based on cyclic AMP changes in isolated cells and those estimated by [^{3}H]dihydroalprenolol binding to membrane preparations of the same tissue.*

The data in Table 1 are plotted in logarithmic scales. Numbers in the panel represent drug numbers in Table 1. ○: lung, ●: heart.

the least-squares fitting method, i.e., the regression coefficient, was 0.89 ± 0.070; it was highly significant ($p < 0.001$) and did not differ from 1.0, showing that affinities of agonists and antagonists for the enzyme responsible for cyclic AMP production were essentially identical to their affinities for membrane receptors. It is concluded, therefore, that adenylate cyclase was activated and inactivated by agonists and antagonists as a result of their binding to *beta*-adrenergic receptors.

DISCUSSION

The addition of *beta*-adrenergic agonists to heart or lung cell preparations gave rise to a marked increase in cyclic AMP content when the enzymic breakdown of the nucleotide was inhibited by IBMX. Thus, adenylate cyclase involved in generation of cyclic AMP must have been activated via *beta*-adrenergic receptors in these cells.

Stimulation *in vitro* of adenylate cyclase by *beta*-adrenergic agonists has been investigated so far in most cases with membrane preparations, broken cell preparations or tissue slices. The advantage of isolated cells over these cell-free preparations and tissue slices appears to reside on much larger magnitude of activation elicited by *beta*-adrenergic agonists. For example, the maximal stimulation of adenylate cyclase caused by isoproterenol in membrane preparations was 120 and 65% for heart and lung, respectively, above the basal activity (3). Norepinephrine caused a 160% increase in adenylate cyclase activity in slices of rat ventricles (24). In our experiments, the maximal increases in cyclic AMP caused by *beta* adrenergic stimulants such as isoproterenol, epinephrine and norepinephrine (in the presence of IBMX) were 900% in heart cells and 700% in lung cells of the increases elicited by IBMX alone in the respective cells (the % increases over the preincubation level were much greater than these values). Powell and Twist (25) recorded a 300% increase by isoproterenol in heart cells under similar conditions. Probably, the structural and functional mechanisms whereby adenylate cyclase is linked to *beta*-adrenergic or hormonal receptors in the cell membrane is liable to undergo such adverse influences during tissue homogenization as to become less sensitive to the agonists later added. In accordance with this view, exposure of isolated cells to a hypotonic medium decreased cyclic AMP production in response to isoproterenol (data not shown).

Lefkowitz summarized criteria generally accepted for classification of adrenergic receptors into $beta_1$ and $beta_2$ subgroups (3), i.e., (i) norepinephrine is more potent at $beta_1$ receptors than at $beta_2$ receptors, (ii) selective agonists such as salbutamol have much higher affinity for $beta_2$ than $beta_1$ receptors, (iii) butoxamine, one of the selective antagonists, is relatively more potent at $beta_2$ receptors and practolol at $beta_1$ receptors. On the basis of these criteria, the concentrations of agonists and antagonists required for the half-maximal increase in cyclic AMP (or the half-maximal suppression of the epinephrine-induced increase) in cells as well as those required for the half-maximal inhibition of the [^{3}H]dihydroalprenolol binding to membrane preparations obtained in the present study can be employed for characterization of *beta* receptors in heart and lung cells. The data in Table 1 show that receptors are $beta_1$ type in heart cells and $beta_2$ type in lung cells, since more potency was observed in heart than in lung with norepinephrine and practolol, and vice versa with salbutamol and butoxamine, regardless of whether ED_{50} (ID_{50}) for cyclic AMP changes or K_d for receptor binding was employed as an index of their relative potencies.

Cell suspensions isolated from rat lung by the collagenase method were found to consist of bronchiolar epithelial cells, alveolar cells, mast cells, macrophages, etc. (11). Our study showed that this heterogeneous preparation, however, may be promising as a system practically applicable for studies *in vitro* on selective *beta*-adrenergic agonists and antagonists; receptors exhibited characteristic of the $beta_2$ subtype as a whole, although Rugg *et al.* (26) recently reported that $beta_1$- and $beta_2$-adrenergic binding sites coexist in rat lung membranes.

In addition to the above-mentioned criteria, the intrinsic activity of selective *beta*-adrenergic agonists in stimulation of intact cell adenylate cyclase was found to be useful for differentiation of receptors into $beta_1$ and $beta_2$. Dose–response curves for *beta* agonist-induced stimulation of cyclic AMP production in heart cells (Fig. 1) showed that intrinsic activity of salbutamol, a selective $beta_2$ agonist, was much lower than the activities of general or $beta_1$ agonists. Conceivably, not only affinity of receptors to an agonist but also the process coupling the agonist–receptor interaction to activation of adenylate cyclase may be determinants of selectivity of the agonist in cardiac cells. Thus, it is very likely that, in cardiac cells, receptor interaction with $beta_1$ agonists would provide more favorable conditions than the interaction with $beta_2$ agonists for generation of a coupling signal or its transduction into the enzyme activation.

Affinities of various *beta* agonists and antagonists for receptors estimated on the basis of cyclic AMP production in isolated cardiac and lung cells showed a good correlation with affinities estimated from the radioactive ligand binding to broken-cell preparations of the same tissues (Fig. 4). Thus, estimation of affinities and intrinsic activities relevant to cyclic AMP generation in isolated heart and lung cells may provide a simple and convenient assay system for screening new, potentially specific, *beta*-adrenergic agonist and antagonist drugs.

REFERENCES

1. Lands, A. M., A. Arnold, J. P. McAuliff, F. P. Luduena and T. G. Brown. Differentiation of receptor systems activated by sympathomimetic amines. *Nature* **214**: 597–598 (1967).
2. Murad, F. Beta-blockade of epinephrine-induced cyclic AMP formation in heart, liver, fat and trachea. *Biochim. Biophys. Acta* **304**: 181–187 (1973).
3. Lefkowitz, R. J. Heterogeneity of adenylate cyclase-coupled beta-adrenergic receptors. *Biochem. Pharmacol.* **24**, 583–590 (1975).
4. Vulliemoz, Y., M. Verosky and L. Triner. Effect of albuterol and terbutaline, synthetic *beta* adrenergic stimulants, on the cyclic AMP system in smooth muscle. *J. Pharmacol. Exp. Ther.* **195**: 549–556 (1975).
5. U'Prichard, D. C., D. B. Bylund and S. H. Snyder. (±)-[^{3}H]-Epinephrine and (−)-[^{3}H]dihydroalprenolol binding to $beta_1$ and $beta_2$ noradrenergic receptors in brain, heart and lung membrane. *J. Biol. Chem.* **253**: 5090–5102 (1978).
6. Vahouny, G. V., R. Wei, R. Starkweather and C. Davis. Preparation of beating heart cells from adult rats. *Science* **167**: 1616–1618 (1970).
7. Kono, T. Roles of collagenases and other proteolytic enzymes in the dispersal of animal tissues. *Biochim. Biophys. Acta* **178**: 397–400 (1969).
8. Glick, M. R., A. H. Burns and W. J. Reddy. Dispersion and isolation of beating cells from adult rat heart. *Anal. Biochem.* **61**: 32–42 (1974).

9. Moustafa, E., T. Skomedal, J.-B. Osnes and I. Øye. Cyclic AMP formation and morphology of myocardial cells isolated from adult heart: effect of Ca^{2+} and Mg^{2+}. *Biochim. Biophys. Acta* **421**: 411–415 (1976).
10. Powell, T. and V. W. Twist. A rapid technique for the isolation and purification of adult cardiac muscle cells having respiratory control and a tolerance to calcium. *Biochem. Biophys. Res. Commun.* **72**: 327–333 (1976).
11. Ayuso, M. S., A. B. Fisher, R. Parilla and J. R. Williamson. Glucose metabolism by isolated rat lung cells. *Am. J. Physiol.* **225**: 1153–1160 (1973).
12. Pérez-Diaz, J., A. Martin-Requero, M. S. Ayuso-Parrilla and R. Parrilla. Metabolic features of isolated rat lung cell. I. Factors controlling glucose utilization. *Am. J. Physiol.* **232**: E394–E400 (1977).
13. Pérez-Diaz, J., A. Martin-Requero, R. Parilla and M. S. Ayuso-Parrilla. Relationship between cellular energy production and rates of glucose utilization by lung cells. *Pflüger's Arch.* **371**: 19–24 (1977).
14. Katada, T. and M. Ui. Islet-activating protein. Enhanced insulin secretion and cyclic AMP accumulation in pancreatic islets due to activation of native calcium ionophores. *J. Biol. Chem.* **254**: 469–479 (1979).
15. Honma, M., T. Satoh, J. Takezawa and M. Ui. An ultrasensitive method for the simultaneous determination of cyclic AMP and cyclic GMP in small-volume samples from blood and tissue. *Biochem. Med.* **18**: 257–273 (1977).
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275 (1951).
17. Morita, S., Y. Irie, Y. Saitoh and H. Kohri. Evaluation of a new beta-adrenergic blocking agent, carteolol, based on metabolic responses in rats. I. Blockade *in vivo* of epinephrine- and isoproterenol-induced alterations of blood glucose concentrations of carbohydrate and lipid intermediary metabolites. *Biochem. Pharmacol.* **25**: 1836–1842 (1976).
18. Choo-Kang, Y. F. T., W. T. Simpson and I. W. B. Grant. Controlled comparison of the bronchodilator effects of three *beta*-adrenergic stimulant drugs administered by inhalation to patients with asthma. *Brit. Med. J.* **2**: 287–289 (1969).
19. Iwasawa, Y. and A. Kiyomoto. Tetrahydroisoquinolines. I. Bronchodilator activity and structure-activity relations. *Jap. J. Pharmacol.* **17**: 143–152 (1967).
20. Saitoh, Y., Y. Irie, T. Hosokawa, T. Igawa, F. Hashimura and H. Kohri. Evaluation of a new beta-adrenergic agonist, procaterol, based on metabolic responses in rats. *Biochem. Pharmacol.* **27**: 2531–2535 (1978).
21. Levy, B. The adrenergic blocking activity of N-tert.-butyl-methoxamine(Butoxamine). *J. Pharmacol. Exp. Ther.* **151**: 413–422 (1966).
22. Levy, B. and B. E. Wilkenfeld. An analysis of selective beta receptor blockade. *Eur. J. Pharmacol.* **5**: 227–234 (1969).
23. Nakagawa, K., N. Murakami, S. Yoshizaki, M. Tominaga, H. Mori, Y. Yabuuchi and S. Shintani. Derivatives of 3,4-dihydrocarbostyril as *beta*-adrenergic blocking agents. *J. Med. Chem.* **17**: 529–533 (1974).
24. Laraia, P. J. and W. J. Reddy. Hormonal regulation of myocardial adenosine 3′,5′-monophosphate. *Biochim. Biophys. Acta* **177**: 189–195 (1969).
25. Powell, T. and V. W. Twist. Isoprenaline stimulation of cyclic AMP production by isolated cells from adult rat myocardium. *Biochem. Biophys. Res. Commun.* **72**: 1218–1225 (1976).
26. Rugg, E. L., D. B. Barnett and S. R. Nahorski. Coexistence of *beta*$_1$ and *beta*$_2$ adrenoceptors in mammalian lung: evidence from direct binding studies. *Mol. Pharmacol.* **14**: 996–1005 (1978).

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