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EFFECTS OF FULL AND PARTIAL β-ADRENERGIC AGONISTS AND ANTAGONISTS ON HUMAN LUNG ADENYLATE CYCLASE

M. DELHAYE, G. TATON, J. C. CAMUS, P. CHATELAIN*, P. ROBBERECHT, M. WAELBROECK and J. CHRISTOPHE†

Department of Biochemistry and Nutrition, Medical School, Université Libre de Bruxelles, Bld. de Waterloo 115, B-1000 Brussels, Belgium

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Abstract—The β-adrenergic stimulation of adenylate cyclase in membranes from human lung was compared to that of adenylate cyclase in membranes with a majority of β_2-adrenergic receptors (from rat lung) and in membranes with a homogeneous population of β_2-adrenergic receptors (from rat erythrocytes and reticulocytes). In terms of adenylate cyclase stimulation, three full agonists (isoproterenol, epinephrine and norepinephrine), four partial agonists (procaterol, salbutamol, fenoterol and zinterol), and four antagonists (propranolol, metoprolol, atenolol and practolol) were tested. The potency (K_{act} or K_i) of the eleven β-adrenergic agents, and the Hill coefficient (of 1) for the four antagonists tested indicated that the activation of human lung adenylate cyclase occurred through receptors of the β_2-subtype only. Partial β-adrenergic agonists were efficiently discriminated by the human lung preparation, as shown by distinct intrinsic activities. The mediocre efficacy and the relatively low potency of all β-adrenergic agonists on adenylate cyclase suggested a relatively low density of β_2-adrenergic receptors, as compared to the enzyme density.

Strong pharmacological [1, 2] and biochemical [3, 4] evidence indicates that the response of a tissue to β-adrenergic agonists is mediated by the occupancy of β- and/or β_2-adrenergic receptors. These two classes of receptors may co-exist, in variable proportions, in the same tissue (for example, lung tissue contains a majority of β_2-adrenergic receptors whereas the heart possesses a majority of β_1-adrenergic receptors [4]) and sometimes even as a mixed population in the same cells [5].

The kinetic parameters of ligand recognition and binding to β_1- and β_2-adrenergic receptors are identical in all mammalian tissues studied so far [6]. Differences in potency K_{act} and maximal efficacy (intrinsic activity) are, however, obvious when considering the biological response of a given tissue to β-adrenergic agonists: the number of β-adrenergic receptors and the nature of post-receptor membranous and intracellular events may conceivably be responsible for these differences. The coupling between the occupied receptor and the membrane adenylate cyclase effector system is the main early step modulating the biological response to β-adrenergic agonists [7]. The efficiency of this coupling process can determine the potency and efficacy of full and partial β-adrenergic agonists. This efficiency depends on the tissue considered [8]. It was of interest to investigate this parameter in *in vitro* preparations from human lung, especially when considering the therapeutic use of β-adrenergic drugs. A particulate fraction from rat lung and membranes from rat erythrocytes and rat reticulocytes served as reference.

MATERIALS AND METHODS

Lung specimens. Normal human lung tissue was obtained from four patients during pulmonary segmentectomy or lobectomy for bronchopulmonary cancer. These patients did not receive any β-adrenergic treatment before operation. As soon as possible after devascularisation of the tissue, the tumour was dissected out and the remaining healthy tissue was frozen in liquid nitrogen. This part of the research programme was approved by the Ethic Committee of the Medical School of the Université Libre de Bruxelles.

Lungs obtained from exsanguinated rats, killed by decapitation, were immediately frozen in liquid nitrogen.

Preparation of human and rat lung membranes. The same methodology was followed for preparing membranes from human and rat lung. Defrosted tissue (1 g) was dissected out (in the case of human lung, visible bronchioles were discarded) and homogenized at 2° by seven strokes using a glass–Teflon homogenizer in 10 ml of a 10 mM Tris–HCl (pH 7.5) buffer containing 2 mM dithioerythritol and 0.25 M sucrose. After filtration through two layers of medical gauze, the homogenate was centrifuged at 6000 *g* for 15 min. The resulting pellet was homogenized in the same buffer, filtered again through two layers of medical gauze and diluted in order to obtain a protein concentration of 3–5 mg/ml as determined according to Lowry *et al.* [9], using bovine serum albumin as a standard. The crude membrane prep-

* Permanent address: Continental Pharma Research Laboratories, B-1348 Mont-Saint-Guibert, Belgium.
† To whom correspondence should be addressed.

aration was used immediately for adenylate cyclase assay.

Preparation of rat erythrocyte and reticulocyte membranes. Erythrocyte membranes were prepared from normal Wistar albino rats (200–250 g) and reticulocyte membranes from similar rats treated with phenylhydrazine according to Dickinson *et al.* [10]. The methodology of Dickinson *et al.* [10] was also followed for red cell membrane preparation with only two modifications: (a) blood was collected by aortic puncture under ether anaesthesia with a heparinized syringe; (b) the solution used for red blood cell lysis was made of 5 mM Tris–HCl (pH 7.8) buffer containing 0.5 mM EGTA. The membranes were rapidly frozen in liquid nitrogen and stored at −80° until use.

Adenylate cyclase assay. Adenylate cyclase activity was determined with minor modifications of the Salomon *et al.* procedure [11]. Membrane protein (80–100 μg for human and rat lung membranes, 50–60 μg for rat reticulocyte membranes, and 200–300 μg for rat erythrocyte membranes) was incubated in a total volume of 60 μl containing 0.5 mM [α-^{32}P]ATP (10^6 cpm/assay), 5 mM $MgCl_2$, 0.5 mM EGTA, 1 mM cyclic AMP, 0.5 mM theophylline, 10 mM phospho(enol)pyruvate, 30 μg/ml pyruvate kinase, 10 μM GTP and 30 mM Tris–HCl at a final pH of 7.5. The reaction was initiated by addition of the membranes and was terminated after an 8 min incubation at 37° by adding 0.5 ml of a 0.5% sodium dodecyl sulfate solution containing 0.5 mM ATP, 0.5 mM cyclic AMP and 20,000 counts/min of cyclic [8-^{3}H]AMP (for determination of cyclic nucleotide recovery). When red blood cell membranes were used, the samples were then boiled for 3 min to facilitate the separation procedure. Cyclic AMP was separated from ATP by two successive chromatographies on Dowex 50 W × 8 and neutral alumina. Under all conditions tested, cyclic AMP production was linear for at least 8 min.

Determination of K_{act} and K_i values for adenylate cyclase activity. K_{act} values were determined as the drug concentration achieving 50% of maximal enzyme stimulation. K_i values for the inhibition of isoproterenol-stimulated adenylate cyclase by various antagonists were determined by correcting IC_{50} values using the equation of Cheng and Prusoff [12], after incubation in the presence of a submaximal dose of isoproterenol and increasing concentrations of antagonist.

Drugs and chemicals. Cyclic [8-^{3}H]AMP (24 Ci/mmole) was obtained from the Radiochemical Centre (Amersham, U.K.) and [α-^{32}P]ATP (25 Ci/mmole) was from New England Nuclear Corporation (Boston, MA). D.,L-Isoproterenol, L-epinephrine, L-norepinephrine, phospho(enol)pyruvate, pyruvate kinase, cyclic AMP, GTP and ATP (sodium salt, grade I) were purchased from Sigma Chemical Co. (St. Louis, MD). L-Propranolol, practolol and atenolol were from ICI Ltd. (Alderly Park, U.K.), salbutamol was from Glaxo Group Research (Ware, U.K.), fenoterol was from Boehringer (Ingelheim, F.R.G.), procaterol was from Otsuka (Tokushima, Japan), zinterol was from Mead Johnson (Bristol

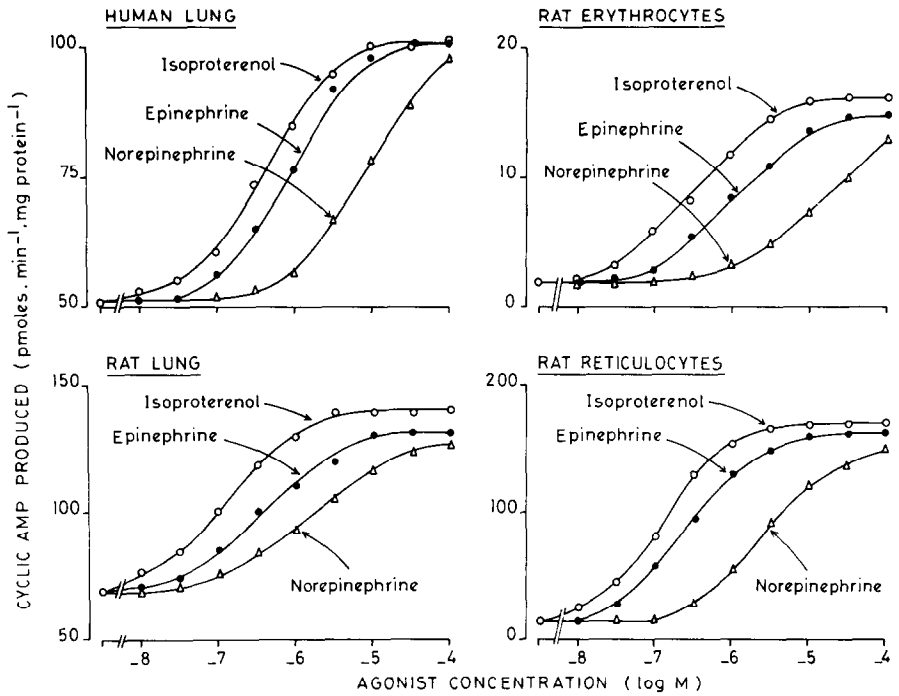

Fig. 1. Dose–effect curves of adenylate cyclase activation of human lung membranes (upper left panel), rat lung membranes (lower left panel), rat erythrocyte membranes (upper right panel) and rat reticulocyte membranes (lower right panel) in the presence of isoproterenol (○), epinephrine (●) and norepinephrine (△). The results, expressed in pmole cyclic AMP produced/min per mg protein, were obtained in the presence of 10 μM GTP (as described in Materials and Methods) and were the mean of four experiments (human and rat lung membranes) or three experiments (erythrocyte and reticulocyte membranes) performed in duplicate.

Myers, Evansville, IN) and metoprolol was from Ciba–Geigy Corp. (Basel, Switzerland). Other drugs and reagents were commercially available.

RESULTS

Evidence for adenylate cyclase activation through β_2-adrenergic receptors in human lung

The effects of isoproterenol and of two natural catecholamines on adenylate cyclase activity in membranes from human lung were compared with those observed in membranes from rat lung (taken as a reference system with a predominance of β_2-receptors), rat reticulocytes (taken as a reference system with an homogeneous population of highly coupled β_2-receptors) and rat erythrocytes (taken as a reference system with a homogeneous population of poorly coupled β_2-receptors). The results are illustrated in Fig. 1 and K_{act} values for adenylate cyclase are reported in Table 1. In the four systems investigated, adenylate cyclase activation displayed a pattern compatible with stimulation through β_2-adrenergic receptors, based on the relative agonist potencies: isoproterenol > epinephrine > norepinephrine. Besides, K_{act} values for the three full agonists were almost identical in human lung and rat erythrocyte membranes and 3- to 7-fold higher than in membranes from rat lung and rat reticulocytes.

The effects of the non-selective β-adrenergic antagonist propranolol and of the three selective β_1-adrenergic antagonists metoprolol, atenolol and practolol were compared in human lung and rat reticulocyte membranes (Fig. 2). Rat reticulocyte membranes were used instead of rat erythrocyte membranes as their higher adenylate cyclase activity allows higher precision when using antagonists and as the efficacy of coupling between receptors and adenylate cyclase is irrelevant when antagonists molecules are considered. The Hill coefficients for the four antagonists (mentioned in Fig. 2) were not different from one suggesting that the inhibition of isoproterenol-activated adenylate cyclase in membranes from human lung and rat reticulocytes involved a single homogeneous class of receptors. The K_i values for the non-selective antagonist propranolol and for the three β_1-selective antagonists

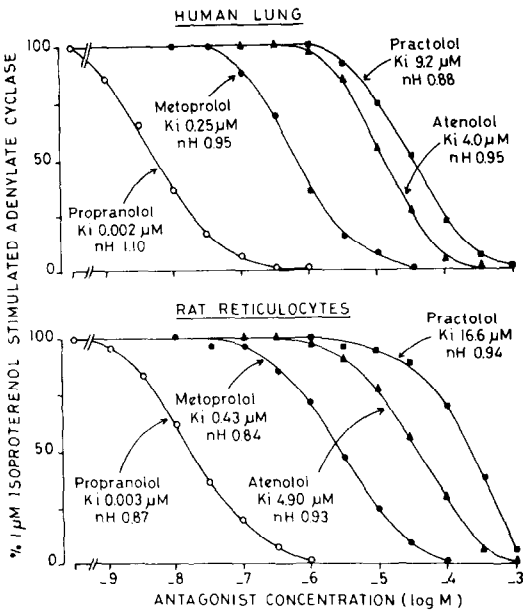

Fig. 2. Dose–effect curves of inhibition of 1 μM isoproterenol-stimulated adenylate cyclase from human lung membranes (upper panel) and rat reticulocyte membranes (lower panel) in the presence of increasing concentrations of L-propranolol (○), metoprolol (●), atenolol (▲) and practolol (■). The results expressed in % of adenylate cyclase activity observed in the presence of 1 μM isoproterenol alone were the mean of three experiments (human lung membranes) or two experiments (rat reticulocyte membranes) performed in duplicate. On the same preparations, a complete dose–effect curve of isoproterenol-stimulated adenylate cyclase was performed in order to establish the K_{act} of the agonist and to calculate the K_i for antagonists according to [12]. The Hill coefficients (nH) and K_i values for antagonists are also mentioned in the figures.

metoprolol, atenolol and practolol were similar for membranes from human lung and rat reticulocytes (Fig. 2) and comparable also to those reported for rat lung membranes [13]. They differed from those established for rat cardiac membranes and other tissues possessing a majority of β_1-adrenergic receptors [13].

Table 1. Effect of full and partial β-adrenergic agonists on adenylate cyclase activation in particulate fractions from human lung and rat lung, and in membranes from rat erythrocytes and rat reticulocytes

	K_{act} of adenylate cyclase (in μM)			
Drug	Human lung	Rat lung	Rat erythrocyte	Rat reticulocyte
D,L-Isoproterenol	0.35 ± 0.02	0.12 ± 0.03	0.40 ± 0.03	0.12 ± 0.03
L-Epinephrine	1.00 ± 0.20	0.28 ± 0.08	1.00 ± 0.30	0.25 ± 0.04
L-Norepinephrine	8.00 ± 3.00	1.20 ± 0.30	10.00 ± 4.00	2.00 ± 0.6
Procaterol	0.20 ± 0.04	0.10 ± 0.03	0.30 ± 0.09	0.10 ± 0.02
Salbutamol	0.40 ± 0.03	0.30 ± 0.08	0.80 ± 0.20	0.10 ± 0.03
Fenoterol	0.80 ± 0.05	0.20 ± 0.07	0.75 ± 0.30	0.10 ± 0.03
Zinterol	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.02 ± 0.01

The results were derived from those presented in Figs. 1 and 3 and were the mean ± S.E.M. of three or four experiments performed in duplicate.

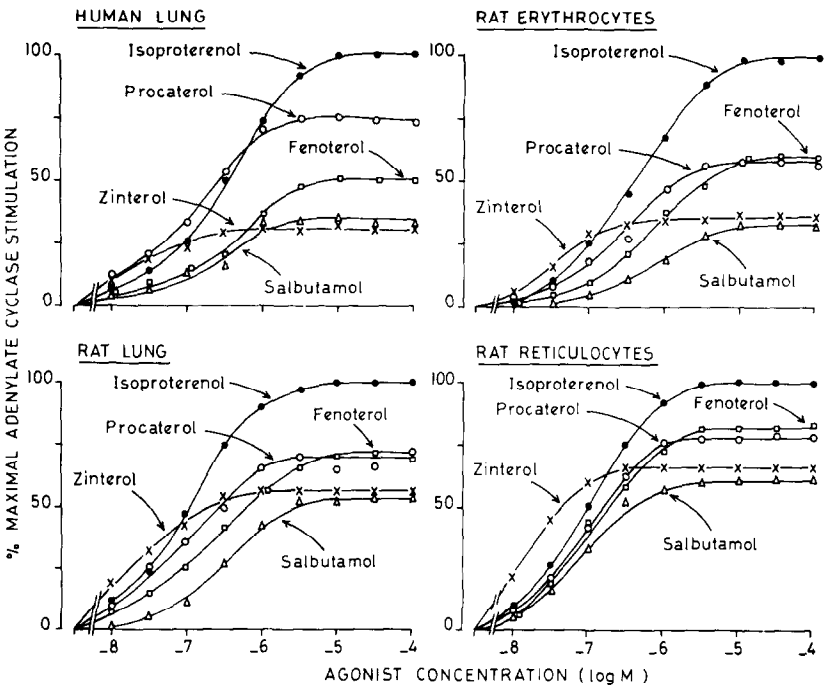

Fig. 3. Dose–effect curves of adenylate cyclase activation of human lung membranes (upper left panel), rat lung membranes (lower left panel), rat erythrocyte membranes (upper right panel) and rat reticulocyte membranes (lower right panel) in the presence of isoproterenol (●), procaterol (○), fenoterol (□), salbutamol (△), and zinterol (×). The results, expressed in % of adenylate cyclase activation achieved with 100 μM isoproterenol, were the mean of four experiments (human and rat lung membranes) or three experiments (erythrocyte and reticulocyte membranes) performed in duplicate.

Comparison of the stimulatory effects exerted by four partial β-adrenergic agonists on adenylate cyclase stimulation in membranes from human lung, rat lung, rat erythrocytes and rat reticulocytes

Dose–effect curves of adenylate cyclase activation are illustrated in Fig. 3, the corresponding K_{act} values for adenylate cyclase activation are mentioned in Table 1, and the efficacy of the partial agonists (as compared to isoproterenol) in Table 2. The relative potencies of the partial agonists, as shown by their K_{act} values, were similar in the four systems tested, decreasing in the order: zinterol > procaterol ≥ salbutamol ≥ fenoterol. The absolute values of K_{act} were generally higher in membranes from human lung and rat erythrocytes than in membranes from rat lung and rat reticulocytes. These K_{act} values were in good agreement with the reported effects of these four partial agonists on several β_2-adrenergic receptors [4, 13].

The relative efficacies (intrinsic activities) of partial agonists were similar in the four systems tested: procaterol ≥ fenoterol > salbutamol = zinterol. The efficacy of a given partial agonist as related to isoproterenol was lower in human lung and rat erythrocyte membranes than in rat lung and rat reticulocyte membranes, with one exception: the potency of procaterol was identical in membranes from human lung, rat lung and rat reticulocytes.

Table 2. Effect of partial agonists on adenlyate cyclase activity in particulate fractions from human lung, rat lung and in membranes from rat erythrocytes and rat reticulocytes

	Efficacy (isoproterenol = 1.00)			
Drug	Human lung	Rat lung	Rat erythrocyte	Rat reticulocyte
D,L-Isoproterenol	1.00	1.00	1.00	1.00
Procaterol	0.73 ± 0.05	0.72 ± 0.05	0.57 ± 0.04	0.78 ± 0.02
Salbutamol	0.31 ± 0.03	0.54 ± 0.04	0.32 ± 0.02	0.60 ± 0.04
Fenoterol	0.49 ± 0.03	0.72 ± 0.05	0.60 ± 0.07	0.80 ± 0.09
Zinterol	0.29 ± 0.04	0.60 ± 0.03	0.37 ± 0.06	0.60 ± 0.03

The results were derived from those presented in Fig. 3 and were the mean ± S.E.M. of three or four experiments performed in duplicate. The efficacy refers to the V_{max} relative to isoproterenol for the stimulation of adenylate cyclase by the drug in the tissue examined.

DISCUSSION

The aim of the present study was two-fold: (1) to characterize β-adrenergic receptors in human lung and compare their coupling to adenylate cyclase with that observed in three mammalian systems serving as reference; (2) to test *in vitro*, on human lung membranes, the efficacy and potency of four partial β-adrenergic agonists that are already used in human therapy or of potential pharmacological interest.

Our reference systems were mostly or exclusively of the β_2-adrenergic type, based on two grounds: (1) binding studies have demonstrated the presence of a large excess (80%) of β_2-adrenergic receptors over β_1-adrenergic receptors (20%) in rat lung membranes [4], and the existence of a homogeneous (100%) population of β_2-adrenergic receptors in rat erythrocyte and reticulocyte membranes [10]; and (2) these binding data are corroborated by the specificity of adenylate cyclase activation or inhibition.

The present data demonstrated that β-adrenergic receptors coupled to adenylate cyclase in human lung membranes were also of the β_2-subtype. The presence of β_1-adrenergic receptors could not be detected with selected agonists and antagonists, suggesting that such receptors were absent or, as in rat lung, present in such a small number that they remained undetected due to poor adenylate cyclase coupling. Our data on adenylate cyclase stimulation could not be confronted, unfortunately, with adequate binding studies due to the high non-specific binding of radiolabelled ligands on human lung membranes (data not shown).

If we accept that human lung membranes and the three other preparations tested are endowed with a homogeneous population of functional β_2-adrenergic receptors, the potency of the three full agonists and the potency and efficacy (intrinsic activity) of the four partial agonists allow us to distinguish two varieties of β_2-response: in membranes from human lung and rat erythrocytes, the potency and efficacy of partial agonists were lower than in membranes from rat lung and rat reticulocytes.

Three explanations have been forwarded to account for the decreased response of mature erythrocytes to catecholamines: a reduced number of β-adrenergic receptors [10, 14–16], impaired coupling between occupied β-adrenergic receptors and the guanine nucleotide binding protein, and/or a general decrease in enzyme activity [15, 16]. The same mechanisms may be involved in human lung membranes when considering the high K_{act} values for full agonists (Table 1) and reduced intrinsic activity of partial agonists (Table 2, and [8]). Among these mechanisms, a general impairment of adenylate cyclase activity is unlikely, however, as human lung membranes respond adequately (i.e. as well as rat lung membranes) to guanine nucleotides, fluoride, prostaglandins and secretin-like peptides [17]. A more likely explanation for the reduced potency and efficacy of β-adrenergic drugs in human lung membranes (as compared to rat lung membranes) is that the density of β_2-adrenergic receptors was low when compared to that of adenylate cyclase.

Besides these general considerations on their β_2-adrenergic receptors, human lung membranes appeared more discriminative than the three other β-adrenergic systems considered for testing the intrinsic activity of partial β-adrenergic agonists: for instance, procaterol was more efficient than fenoterol in human lung membranes, whereas the intrinsic activities of these two partial β-adrenergic agonists were identical in membranes from rat lung, rat erythrocytes and rat reticulocytes. This may reflect an intrinsic property of a membrane component of human lung membranes distinct from the hormonal receptor and adenylate cyclase [18]. To conclude, the present data illustrate the usefulness of a simple human pulmonary preparation for testing, *in vitro*, β-adrenergic drugs of potential therapeutic interest in human disease.

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