Biochemical Pharmacology, Vol. 36, No. 15, pp. 2557–2564, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

(−)[^{125}I]PINDOLOL BINDING TO HUMAN PERIPHERAL LUNG BETA-RECEPTORS*

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(*Received* 9 *August* 1986; *accepted* 12 *January* 1987)

Abstract—Beta-adrenergic receptors in human peripheral lung were characterized by biochemical and radioligand assays employing binding of the beta-antagonist (−)[^{125}I]pindolol to plasma membrane preparations. The specific binding of (−)[^{125}I]pindolol reached equilibrium by 45 min with an initial rate constant of 0.0282 min^{-1}. Binding was reversible with a kinetic dissociation rate constant of 0.0146 min^{-1}. The calculated kinetic K_d (dissociation constant) was 430 pM which agreed very well with the K_d of 394 pM obtained by Scatchard analyses of equilibrium binding data. Computer analyses of equilibrium binding experiments revealed a similar K_d of 336 ± 24 pM. The binding capacities calculated by computer analyses (155 ± 7 fmol/mg protein) and Scatchard analyses (113 fmol/mg protein) were also in close agreement. By all three methods (kinetic, Scatchard, and computer analyses), the data were most compatible with a single (−)[^{125}I]pindolol binding site. Analyses of equilibrium binding data from ten different human lungs revealed values for the K_d ranging from 79 to 360 pM (mean, 136 pM), and for the receptor concentration ranging from 58 to 196 fmol/mg protein (mean, 118 fmol/mg protein). The displacement of (−)[^{125}I]pindolol binding by various agents exhibited stereoselectivity and the expected rank order of potency predicted for interactions with beta-receptors. Isoproterenol induced a rapid and dose-related increase in cyclic AMP that was prevented by specific beta-antagonists. Approximately 70% of the beta-receptors were found to be of the beta$_2$-subtype by both radioligand binding and biochemical assays. Thus, (−)[^{125}I]pindolol appears to be an excellent ligand for characterizing human lung beta-receptors since accurate and reproducible results can be obtained with this radioligand using limited tissue sample quantities.

Beta-adrenergic receptors have been reported to modulate a variety of lung functions, including the control of airway [1, 2] and vascular tone [3], fluid and protein exchange [4], ion transport [5], mucus secretion [6], mast cell mediator release [7, 8], and surfactant secretion [9]. Since target cell response begins with the number and affinity of the surface receptors for their neurotransmitter, it is important to characterize human lung beta-receptors in an attempt to elucidate the function of these receptors in peripheral lung responses. Investigators have previously used [^{3}H]dihydroalprenolol, a beta-antagonist, to characterize the lung beta-receptor system [10–13]. However, due to the low specific activity of [^{3}H]dihydroalprenolol, it has been difficult to measure beta-receptors present in low amounts. This problem is most evident when tissue samples are not available in large quantities (e.g. fresh human lung tissue). Moreover, [^{3}H]dihydroalprenolol has been found to exhibit nonstereoselective binding to nonphysiologic sites which may result in inaccurate estimates of binding parameters for beta-receptors [14, 15]. In an attempt to more accurately determine the concentration and affinity of beta-receptors on various tissues, iodinated pindolol ligands have been developed [16, 17]. These molecules have much higher specific activity than [^{3}H]dihydroalprenolol and could potentially facilitate the identification of beta-receptors present in low concentrations. We, therefore, extensively studied the binding of (−)[^{125}I]pindolol to human peripheral lung membranes. We found (−)[^{125}I]pindolol to be an excellent ligand for characterizing the human lung beta-receptor system. The binding parameters estimated by kinetic studies, traditional Scatchard analyses [18], and computer modeling techniques [19] agreed well with each other. Moreover, accurate and reproducible binding parameters were obtained using as little as 2 g (wet weight) of fresh lung tissue. The radioligand binding data were most compatible with a single affinity binding site of high concentration. By both radioligand and biochemical assays (measurement of stimulated cyclic AMP), approximately 70% of human peripheral lung beta-receptors were found to be of the beta$_2$-subtype.

MATERIALS AND METHODS

Reagents

Bovine serum albumin (BSA), isoproterenol, atenolol, pindolol, isobutylmethylxanthine (IBMX), metropolol, epinephrine, histamine and atropine were purchased from the Sigma Chemical Co., St. Louis, MO; and sucrose and $MgCl_2$ from the Fisher Scientific Co., Fair Lawn, NJ. Tris was purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN; and Dulbecco's medium from Hazel-

* Supported in part by a VA Merit Review Award.
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ton, Denver, PA. (−)- and (+)Propranolol were gifts from Ayerst Laboratories, New York, NY. (−)[^{125}I]Pindolol (2200 Ci/mmol) and [^{125}I]-radioimmunoassay kits for cyclic AMP determination were purchased from New England Nuclear, Boston, MA.

Preparation of human lung tissue

Human lung tissue was obtained at the time of resection of lung cancer. Macroscopically normal areas of peripheral lung tissue were dissected free of pleura, large bronchi (greater than 3–5 mm) and large blood vessels and were washed in Dulbecco's medium. The tissue was dissected further into 200 mg replicates (wet weight) for experiments involving the determination of cyclic AMP content. Tissue for the radioligand binding assays was frozen at −70° until used.

Membrane preparation for radioligand binding assays

Preliminary studies were performed to determine how to optimally prepare the lung plasma membranes for the radioligand binding studies (see Results). Based on these studies, the following membrane preparation was employed. Frozen lung tissue was minced and placed in ice-cold 10 mM Tris (pH 7.4) containing 0.25 M sucrose and 0.5% bovine serum albumin (10 ml/g tissue). The tissue was homogenized using a Polytron-PCU homogenizer at 22,000 rpm for 1 min. The homogenate was filtered through double-layered gauze cloth and then centrifuged twice at 900 *g* for 10 min at 4° to remove cellular debris, unbroken cells and nuclei. The supernatant fraction was centrifuged further at 40,000 *g* for 45 min at 4°. The resulting pellet was resuspended in the buffer used for the radioligand binding assays (125 mM Tris with 25 mM $MgCl_2$, pH 7.4). The protein content of this plasma membrane preparation was adjusted to 0.1–1.0 mg/ml with the Tris $MgCl_2$ buffer. Protein concentration was determined by the method of Lowry *et al.* [20].

Radioligand binding assays

Radioligand binding assays were done as described previously [21]. Aliquots (100 μl) of the plasma membrane preparations suspended in the Tris $MgCl_2$ buffer were used in the binding assays in a final volume of 160 μl. The assay mixtures contained either increasing concentrations of radioligand, ((−)[^{125}I]pindolol), or a fixed concentration of radioligand and different concentrations of various agonists and antagonists. In the kinetic and drug competition studies, the lung membranes were incubated for various intervals of time with 20 μl of 400 pM (−)[^{125}I]pindolol at 22°. In the equilibrium studies, the lung membranes were incubated for 60 min with (−)[^{125}I]pindolol at 22° with and without 40 μl of unlabeled pindolol. Incubations were terminated by adding 4 ml of ice-cold incubation buffer followed by rapid vacuum filtration of the samples through pindolol-presoaked Whatman GF/C glass filters. The filters were washed immediately thereafter with 20 ml of ice-cold buffer, dried, and then assayed in a Beckman 5500 gamma counter (mean efficiency, 80%). All samples were run in duplicate to quadruplicate; replicates differed from each other by less than 10%. Specific binding of (−)[^{125}I]pindolol was defined as the difference in the amount of radioligand bound in the absence and presence of an unlabeled ligand (>80%). Initial binding experiments done at 4°, 22°, and 37° demonstrated significantly less binding at 4° versus 22° and 37°, but no significant difference in binding at 22° versus 37°. All assays were done at 22° as a matter of convenience.

Analysis of radioligand binding data

Kinetic studies. The observed initial rate constant(k_{ob})* for the binding of (−)[^{125}I]pindolol to lung membranes was calculated from the equation, $k_{ob}t = \ln[Be/Be - Bt]$. The dissociation rate constant (k_{-1}) was calculated from the equation, $k_{-1}t = \ln[Bt/Be]$. *Be* is the amount of radioligand specifically bound at equilibrium, and *Bt* represents the amount of radioligand specifically bound at each time interval, *t*. The slopes of these regression lines, calculated by the method of least squares, equal k_{ob} and k_{-1} respectively. The second-order rate constant, k_{+1}, was calculated from the equation, $k_{+1} = k_{ob} - k_{-1}/$ [(−)[^{125}I]pindolol] where [(−)[^{125}I]pindolol] is 400 pM. The $K_d = k_{-1}/k_{+1}$.

Equilibrium studies. The data generated from equilibrium binding studies were analyzed with either Scatchard plots [18] or the computer program LIGAND [19]. The LIGAND program analyzes the data using a weighted, nonlinear, least-squares curve fitting. The binding curves were first re-expressed in terms of bound (−)[^{125}I]pindolol concentration versus total concentration added, considering both the labeled and unlabeled ligand. Since this analysis permitted a wide range of ligand concentrations to be evaluated, the presence or absence of low-affinity receptor sites could be determined. Contrary to common custom for binding studies (e.g. Scatchard analyses), nonspecific binding (*N*) was not measured for each individual concentration by use of a 100-fold excess unlabeled ligand concentration. Rather, *N* was modeled directly as an extra, very low-affinity, nonsaturable class of receptors, and expressed as the ratio of nonspecifically bound to free ligand [19].

Equilibrium binding models with one, two, or three classes of specific binding sites were fitted to the data, and the best-fitting model was chosen on the basis of the "extra sum-of-squares" F-test criterion [19]. That is, the addition of a new class of receptors into the model had to result in a significantly better fit ($P < 0.05$) of the data before the new class was considered part of the model. Finally, values for the dissociation constants (K_d), binding capacities (*R*), and *N* for the chosen model were estimated, along with their standard errors. The predicted curve was then plotted superimposed on the data, and the goodness-of-fit was evaluated using a "number-of-runs" test on the residuals and by

* Abbreviations include: *Be*, amount of radioligand specifically bound at equilibrium; *Bt*, amount of radioligand specifically bound at a time point; *C*, proportionality (scaling) factors; k_{ob}, observed initial rate constant; k_{-1}, kinetic dissociation rate constant; k_{+1}, second-order rate constant; K_d, dissociation constant; *N*, ratio of nonspecifically bound to free ligand; *R*, binding capacity; and *RMS*, root-mean-square.

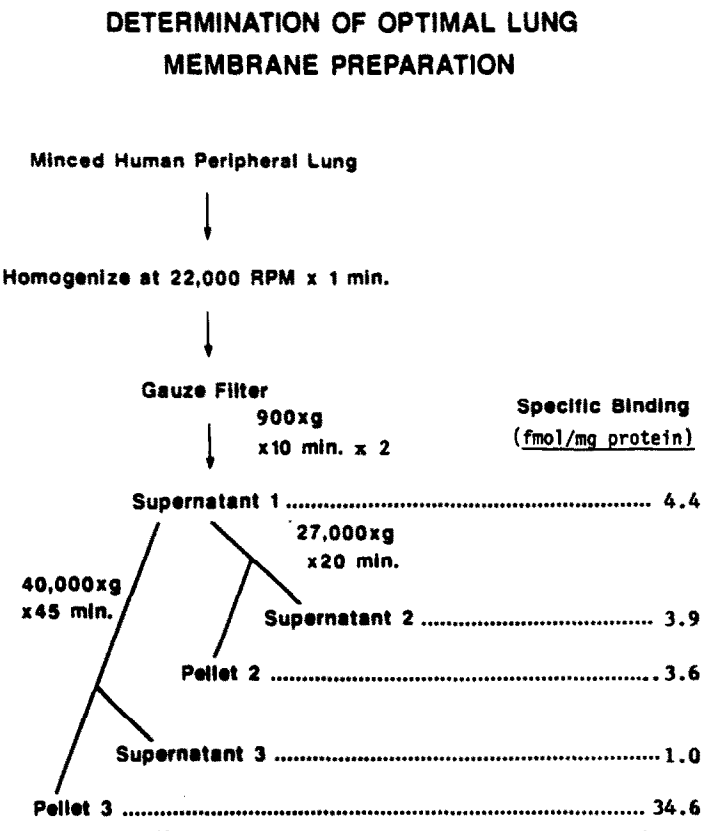

Fig. 1. Specific binding of (−)[125I]pindolol to five different human lung plasma membrane preparations. (−)[125I]Pindolol (400 pM) and the human peripheral lung membrane preparations were incubated in the absence and presence of 40 nM unlabeled pindolol for 60 min, and specific binding was compared.

evaluation of the root-mean-square (*RMS*) residual error.

When experiments involved different lung membrane preparations, some variability in the values for R was observed, even after the binding data were normalized for protein concentration. To reduce the effect of this variability, a set of proportionality (scaling) factors (C) was estimated for each series of experiments. These parameters essentially allowed for proportional fluctuations in the R and N values from experiment to experiment, but required that the K_d values remain constant. By convention, the scaling factor for the first experiment in a set (C_1) was set to 1. For example, if $C_2 = 2.05$, we would then understand that the second experiment had approximately twice the receptor concentration of the first.

During the computer analysis, R was calculated in molar concentration based in the incubation medium. For presentation, however, the R values were divided by the average protein concentration (mg protein/liter) so that comparable units for R (mol/mg protein) were obtained. The reported value of R is thus the average value obtained within a given set of experiments. Significant between-experiment variability can be handled effectively via this analytic method, as previously demonstrated [19].

Measurement of cyclic AMP content

After a 5-min preincubation with a 100 μM concentration of the phosphodiesterase inhibitor, IBMX, the lung fragments were incubated with the beta-agonist isoproterenol, a beta antagonist, or both for 10 min in a 37° shaking water bath. At the end of the incubation period, the lung fragments were transferred to ice-cold 10% perchloric acid. The fragments were homogenized subsequently for 30 sec at 22,000 rpm using a Polytron-PCU homogenizer. To the homogenates in the 10% perchloric acid, 4000 cpm of [3H]cyclic AMP (0.1 pmol) was added to permit quantification of recovery. The samples where then centrifuged at 2000 *g* for 15 min at 4°. The precipitates were digested in 0.1 N NaOH at 22° for 12 hr for protein determination [20], and the supernatant fractions were neutralized with 5 N KOH. After centrifugation at 2000 *g* for 15 min at 4°, the resulting supernatant fractions were assayed for cyclic AMP content using the [125I]-radioimmunoassay kits from New England Nuclear. The values of cyclic AMP concentrations reported herein have been corrected on the basis of the recovery of 0.1 pmol of [3H]cyclic AMP in each sample, with the actual recoveries usually exceeding 70%. The values are reported as pmol cyclic AMP per mg of protein.

RESULTS

Plasma membrane preparation

To determine the optimal lung plasma membrane preparation for the radioligand binding assays, the

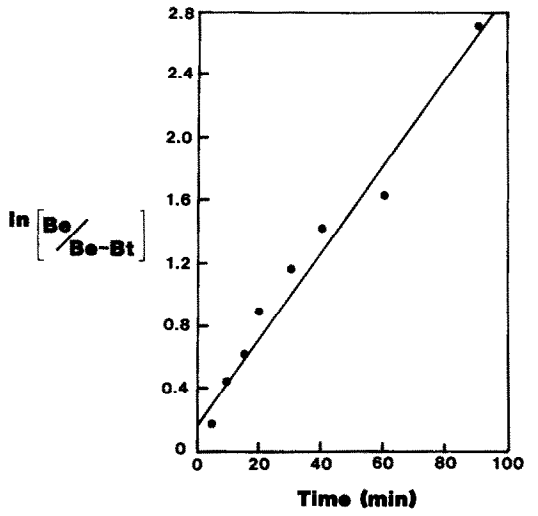

Fig. 2. Pseudo-first-order kinetic plot of association time course for [^{125}I](−) pindolol binding to human lung. Lung plasma membranes and 400 pM (−)[^{125}I]pindolol were incubated in the absence and presence of 40 nM unlabeled pindolol. *Be* is the amount (63 fmol/mg protein) of (−)[^{125}I]pindolol bound at equilibrium, and *Bt* represents the amount bound at each period of time, *t*. The line, determined by least squares linear regression analyses (r = 0.99), has a slope of 0.0282 min^{-1} which is the k_{ob}.

specific binding of (−)[^{125}I]pindolol to five different plasma membrane preparations was compared (Fig. 1). Specific binding was calculated as the difference in binding when 400 pM (−)[^{125}I]pindolol was incubated in the absence and presence of 40 nM unlabeled pindolol. The lung was minced, placed in a Tris-sucrose buffer, homogenized, filtered, and centrifuged slowly. The resultant supernatant fraction was tested directly for specific binding or was centrifuged further either at 27,000 *g* for 20 min or at 40,000 *g* for 45 min. The pellets and supernatants were then resuspended in Tris $MgCl_2$ buffer, equilibrated for protein content, and analyzed for specific binding. As shown in Fig. 1, the highest specific (−)[^{125}I]pindolol binding occurred in pellet 3 (34.6 fmol/mg protein). Therefore, all subsequent radioligand binding studies used the plasma membrane isolation procedure outlined for pellet 3 (Fig. 1).

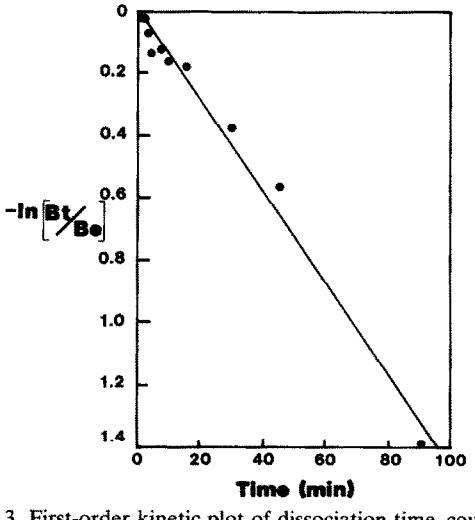

Fig. 3. First-order kinetic plot of dissociation time-course for (−)[^{125}I]pindolol binding to human lung. Lung plasma membranes were incubated for 60 min with 400 pM (−)[^{125}I]pindolol in the absence and presence of 40 nM unlabeled pindolol. At the 0 time point, unlabeled pindolol was added to all tubes to a final concentration of 4 μM. The natural log of the ratio of (−)[^{125}I]pindolol bound at each time point (*Bt*), to that bound at equilibrium (*Be*, 55 fmol/mg protein), is plotted as a function of time. The line, determined by least squares linear regression analysis (r = −0.99), has a slope of 0.0146 min^{-1} which is the k_{-1}.

Kinetics of (−)[^{125}I]*pindolol binding*

The specific binding of (−)[^{125}I]pindolol to human lung plasma membranes occurred rapidly (Fig. 2) and was reversible (Fig. 3). At 22° the association reaction reached equilibrium by 45 min with an initial rate constant (k_{ob}) of 0.0282 min^{-1} (Fig. 2). When equilibrium was reached, the addition of unlabeled pindolol resulted in displacement of the specifically bound (−)[^{125}I]pindolol with a k_{-1} of 0.0146 min^{-1} (Fig. 3). From the k_{ob} and k_{-1}, a second-order rate constant (k_{+1}) of 0.034 min^{-1} nM^{-1} was calculated. The dissociation constant ($K_d = k_{-1}/k_{+1}$) determined from the kinetic studies was 430 pM.

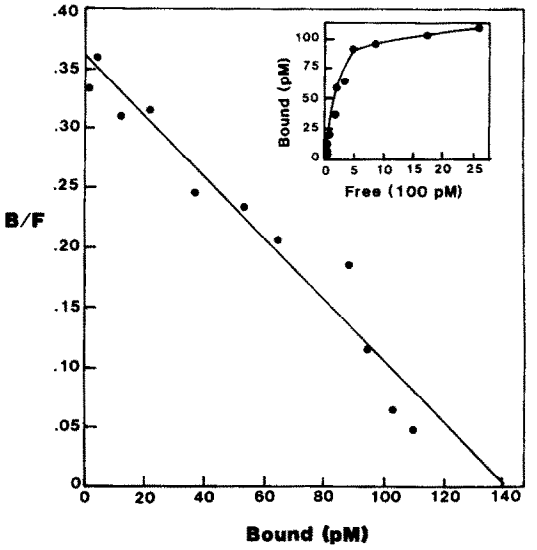

Fig. 4. Specific binding of (−)[^{125}I]pindolol to human lung plasma membranes as a function of increasing (−)[^{125}I]pindolol concentrations. Lung membranes were incubated in the absence and presence of 200 nM unlabeled pindolol for 60 min. In the inset, the specific binding of (−)[^{125}I]pindolol (ordinate) saturates with increasing radioligand concentration (abscissa). Scatchard analyses of the binding data plots the ratio *B/F* of specifically bound (−)[^{125}I]pindolol to free (−)[^{125}I]pindolol on the ordinate, versus specifically bound (−)[^{125}I]pindolol on the abscissa. The straight line is the least squares regression fit of these data (r = −0.97). From these data, a K_d (−1/slope) of 394 pM and a receptor concentration (X-intercept) of 113 fmol/mg protein were calculated.

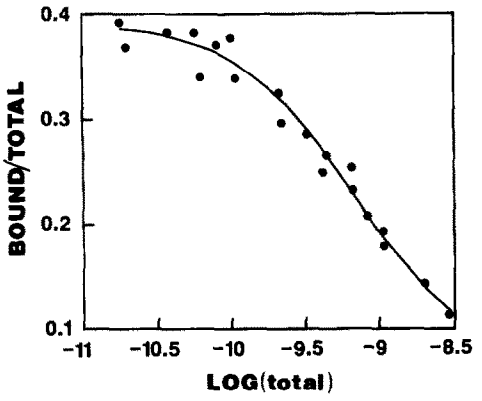

Fig. 5. Computer analyses of equilibrium binding of (−)[125I]pindolol to human lung plasma membranes. Various concentrations of (−)[125I]pindolol were incubated with lung membranes for 60 min at 22°. The computer generated curve plots the amount of (−)[125I]pindolol bound over the total added versus the log of the total (−)[125I]pindolol concentration. The data from two experiments were combined and analyzed using the LIGAND program. The best fit curve was superimposed over the data points. The calculated values for the binding parameters are: K_d = 336 ± 24 pM; R = 155 ± 7 fmol/mg protein; N = 6.7 ± 0.6%; and *RMS* residual error = 5.3%.

Equilibrium binding studies

The specific binding of (−)[125I]pindolol to human lung membranes was a saturable process reaching half-maximal saturation (K_d) at 394 pM (−)[125I]pindolol (Fig. 4, inset). A Scatchard plot (Fig. 4) of the specific binding to human lung membranes was linear and suggestive of a single class of binding sites with a concentration of 113 fmol/mg protein.

To confirm that there was only a single (−)[125I]pindolol binding site, we used a computer program, LIGAND [19], to combine and analyze the data generated from two equilibrium binding experiments spanning a wide range of radioligand concentrations. The data were most compatible with a single affinity binding site with a K_d of 336 ± 24 pM and a receptor concentration of 155 ± 7 fmol/mg protein (Fig. 5). Using the F-ratio test, there was no significant reduction in the *RMS* residual error for more complex models involving multiple binding sites as compared to a single site model.

Table 1 shows a comparison of the (−)[125I]pindolol binding parameters generated by the three previously detailed techniques. The kinetic K_d agrees very well with the K_d values derived by the Scatchard and computer analyses of the equilibrium binding data. The receptor concentrations calculated from the Scatchard and computer analyses were also in good agreement. The data were most compatible with a single (−)[125I]binding site by all three methods.

Table 1. Comparision of (−)[125I]pindolol binding parameters

Method	K_d (pM)	R (fmol/mg protein)
Kinetics of association and dissociation	430	—
Equilibrium binding, Scatchard analyses	394	113
Equilibrium binding, computer analyses	336	155

Using computer analyses of equilibrium binding experiments, the binding parameters for (−)[125I]pindolol to ten different human lung samples (2–5 g wet weight) were obtained. The K_d values ranged from 79 to 360 pM with the geometric mean = 136 pM. The receptor concentrations ranged from 58 to 196 fmol/mg protein with the geometric mean = 118 fmol/mg protein. The *RMS* residual errors for the experiments ranged from 17 to 50%, indicating good fits for each data set. Moreover, when binding experiments were repeated, estimated binding parameter values between experiments usually agreed within 25% of each other.

Specificity (drug competition) studies

To measure the binding affinity of different drugs relative to the affinity of (−)[125I]pindolol for the beta-receptor, K_d values were calculated from competition binding studies. These experiments involved the displacement of 400 pM (−)[125I]pindolol from the lung beta-receptor site by unlabeled drugs added in concentrations from 10^{-9} to 10^{-3} M. The data were analyzed using the computer program LIGAND [19] and are summarized in Table 2. The K_d values for the beta-antagonists were less than those for the beta-agonists. As expected, histamine and atropine did not interact with the (−)[125I]pindolol-identified binding site. The stereospecificity of the binding to human lung beta-receptors is also shown in Table 2. The K_d of (−)propranolol is 2 logs lower than that of (+)propranolol. The racemic mixture of propranolol had a K_d of 0.147 μM which is intermediate between the K_d values of 0.003 μM and 0.235 μM measured for (−)- and (+)propranolol respectively.

Ratio of beta$_1$- to beta$_2$-receptors

To determine the ratio of beta$_1$- to beta$_2$-receptors on human lung, we performed and combined the

Table 2. Dissociation constants of various drugs for human lung beta-receptors

Drug	Action	K_d (μM)
(−)Propranolol	β-Antagonist	0.003 ± 0.001
(±)Pindolol	β-Antagonist	0.016 ± 0.009
(±)Propranolol	β-Antagonist	0.147 ± 0.081
(+)Propranolol	β-Antagonist	0.235 ± 0.035
(±)Isoproterenol	β-Agonist	2.590 ± 0.480
(±)Epinephrine	β-, α-Agonist	12.30 ± 1.60
Histamine	H_1-, H_2-Agonist	>10,000
Atropine	M_1-, M_2-Antagonist	>10,000

Various concentrations of the listed drugs were incubated with 400 pM (−)[125I]pindolol, and the binding data were analyzed with the LIGAND program. Numbers represent the mean ±SE.

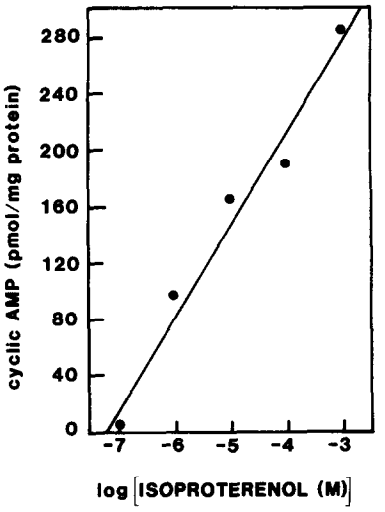

Fig. 6. Effect of adding increasing concentrations of isoproterenol on lung cyclic AMP content. Isoproterenol in the concentrations listed was added to human lung fragments, and the cyclic AMP was measured after 10 min. The control cyclic AMP content was 2.5 ± 0.3 pmol/mg protein (N = 3).

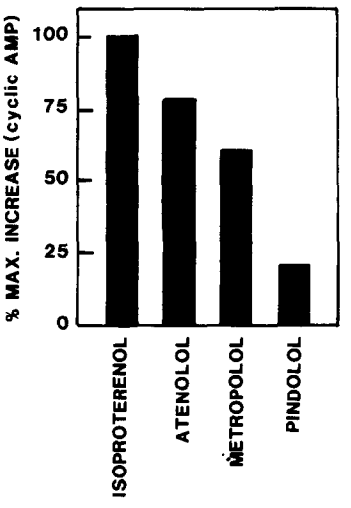

Fig. 7. Effects of beta-antagonists on isoproterenol-induced increases in cyclic AMP. Lung fragments were incubated with the beta-antagonists (100 μM) 5 min before 100 μM isoproterenol was added, and cyclic AMP was measured 10 min after the introduction of isoproterenol. The increase in cyclic AMP content induced by isoproterenol in the absence of antagonists (72 fmol/mg protein, 100%) is compared to the amount of cyclic AMP induced in the presence of the beta-antagonists.

data from two competition experiments with atenolol. The basic experiment involved the displacement of (−)[^{125}I]pindolol, a beta$_1$- and beta$_2$-antagonist, from the lung beta-receptor by atenolol, a beta$_1$-antagonist. The analysis of the computer-modeled data was most compatible with a two-site fit having an *RMS* residual error of only 6.2. The number of runs for each of the two experiments was statistically appropriate, and the calculated parameter estimates for the K_d and receptor concentration values had errors of less than a factor of 2. It is assumed that the higher affinity site is the beta$_1$-receptor, and the lower affinity site is the beta$_2$-receptor. The K_d for atenolol binding to the beta$_1$-receptor was 7 nM, and the K_d for atenolol binding to the beta$_2$-receptor was 12 μM. The receptor concentrations for the high- and low-affinity sites were 16 and 37 fmol/mg protein respectively. Thus, the ratio of beta$_1$- to beta$_2$-receptors is calculated to be 30:70.

Effects of isoproterenol upon lung cyclic AMP

Isoproterenol at 10 μM induced a rapid rise in cyclic AMP content which peaked within 5 min and remained elevated for an additional 10 min. The peak level of cyclic AMP was twelve times that of control (i.e. 100 μM IBMX alone). Therefore, a dose-response of isoproterenol (0.1 to 100 μM) was examined 10 min after stimulation. Isoproterenol at these concentrations induced a dose-related increase in cyclic AMP levels ($r = 0.99$) (Fig. 6).

The effects of the beta$_1$-antagonists, atenolol and metropolol, on cyclic AMP generation by added isoproterenol were studied to determine whether beta$_1$-antagonists might have a specific inhibitory capacity (Fig. 7). The beta-antagonists themselves had no effect on the cyclic AMP content. The beta$_1$-antagonists inhibited the isoproterenol-induced rise in cyclic AMP by approximately 30%. However, the nonselective beta-antagonist, pindolol, inhibited the isoproterenol-induced rise in cyclic AMP by 80%. Since we only used a single concentration of the beta-antagonists, we cannot make definitive conclusions about the proportion of cyclic AMP produced by the beta-receptor subtypes. However, these data suggest that the majority of beta-agonist-induced lung cyclic AMP content may be due to beta$_2$-receptor stimulation.

DISCUSSION

These data demonstrate that (−)[^{125}I]pindolol is an excellent radioligand for studying the human lung beta-receptor system. At 22°, (−)[^{125}I]pindolol binding to human lung reached equilibrium by 45 min and remained stable for at least 75 additional min. (−)[^{125}I]Pindolol binding to human lung membranes was readily displaced by unlabeled pindolol with a $T_{\frac{1}{2}}$ of dissociation equal to 47 min. The K_d calculated from the kinetic studies (430 pM) was in excellent agreement with the K_d obtained from saturation experiments (Fig. 4) and Scatchard analyses (394 pM). Specific binding of [^{125}](−)pindolol was consistently >80% of the total binding.

Radioligand binding parameters estimated by Scatchard analyses can be influenced by the radioligand concentrations used in the individual experiments [19, 21]. When the radioligand concentrations are restricted to a narrow range, analyses of the binding data may result in a linear Scatchard plot suggestive of a single binding site. However, estimates for the K_d and R values may be biased and, therefore, inaccurate. By using the LIGAND computer modeling program to analyze the binding data, the investigator is able to pool and fit data from

multiple experiments covering a wider range of ligand concentrations. Another major advantage of the LIGAND computer program is that it provides a statistical method to determine the best fit model for the data [19]. After pooling the data from two different equilibrium binding experiments (20 points) employing a wide range of (−)[125I]pindolol concentrations, we obtained an excellent fit for a model having a single class of binding sites. The model involving only a single class of binding sites was significantly better than fits involving multiple classes of sites. When the "extra sum-of-squares" principle was employed, the fit involving only a single class of binding sites decreased the *RMS* residual error with a corresponding F value that was highly significant ($P < 0.001$). The average scatter of a point around the fitted curve (Fig. 5) corresponded to only a ±5% error in the value of [Bound/Total] for any given value of [Total]. The calculated K_d and R values had errors of only 7 and 5% respectively. The overall value for N (ratio of nonspecifically bound (−)[125I]pindolol to free (−)[125I]pindolol) was also very well determined (6.66 ± 0.61%). The computer-calculated (−)[125I]pindolol binding parameters for human lung were in close agreement to the values calculated by kinetic studies and Scatchard analyses of saturation binding experiments (Table 1).

The abilities of various drugs to displace (−)[125I]pindolol from the beta-receptor agreed with the potencies of these drugs predicted by pharmacologic experiments (Table 2). The beta-antagonists had a greater affinity than the beta-agonists. The K_d of (−)propranolol was 78-fold less than the K_d of (+)propranolol, confirming the stereospecificity of binding. In contrast to the non-selective beta-antagonists pindolol and propranolol, the binding of the $beta_1$-antagonist atenolol was most compatible with a two-site model. From the atenolol competition curves, the percentage distribution of $beta_1$- versus $beta_2$-receptors was calculated to be approximately 30:70. These results agree with Engel's results using [125I]cyanopindolol as the radioligand [22]. However, Engel calculated the K_d values for atenolol to be 0.1 and 2 μM, whereas our data indicated the K_d values to be 7 nM and 12 μM. The differences obtained for the K_d values could have been due to methodology. Engel did not appear to use concentrations of atenolol less than 10 nM. Thus, Engel may not have detected a binding site with an affinity of 7 nM. It is doubtful that these differences in K_d values are due to the use of iodopindolol versus iodocyancopindolol since these radioligands appear to have similar K_d values for human lung [22, 23].

It is readily apparent from the cyclic nucleotide data (Figs. 6 and 7) that exogenous stimulations of human lung beta-receptors resulted in an increase in cyclic AMP. The rise in cyclic AMP occurred rapidly and was dependent on the concentration of isoproterenol (Fig. 6). The $beta_1$-antagonists atenolol and metropolol prevented only 30% of the isoproterenol-induced increase in cyclic AMP (Fig. 7). These biochemical studies support the results obtained from the radioligand binding studies which indicated that the majority of human lung beta-receptors were of the $beta_2$-subtype.

Previous studies of beta-receptors on human peripheral lung have used radioligands other than (−)[125I]pindolol [10–13, 22, 23]. One major advantage of using (−)[125I]pindolol over tritiated radioligands such as [3H]dihydraoalprenolol is the higher specific activity of the iodinated compound (2200 Ci/mmol versus 35 Ci/mmol). Radioligands with higher specific activity are especially useful for studies involving human tissues where sample quantites are limited. In contrast to radioligand studies with [3H]dihydroalprenolol, we were able to perform binding assays with as little as 0.01 mg protein/sample (i.e. 10% of that used for assays with [3H]dihydroalprenolol [10–13]). In the present study, accurate binding parameter values could be obtained from starting tissue samples of only 2 g. Moreover, the specific binding of (−)[125I]pindolol was consistently >80% which is much higher than that reported for [3H]dihydroalprenolol [10, 13]. In a recent study by Neve and co-workers [24], the binding characteristics of [125I]pindolol, [125I]cyanopindolol, [125I]hydroxybenzylpindolol, and [3H]dihydroalprenolol to cultured C-6 glioma cells and human right atrial tissue were directly compared. [125I]Pindolol and [125I]cyanopindolol were 3.2- and 2.0-fold selective, respectively, and [125I]hydroxybenzylpindolol and [3H]dihydroalprenolol were 5.8- and 2.3-fold selective, respectively, for $beta_2$-receptors. These investigators suggested that, for experiments requiring an accurate estimate of the proportion of each subtype of beta-receptors, [125I]cyanopindolol may be the most appropriate ligand, as it is only 2-fold selective. Similar studies directly comparing the binding characteristics of different beta-receptor radioligands to human or animal lung tissue have not been done. In comparing the present study to previous studies using [125I]-cyanopindolol, there does not appear to be a large difference in the binding characteristics of these two radioligands to human lung [22, 23]. Both iodinated pindolol compounds exhibit high specific binding and can be used with limited tissue quantities.

Since receptor concentrations are dependent on the degree of plasma membrane purification (Fig. 1, Ref. 25), differences in methodology between studies make direct comparisons of calculated binding parameters somewhat difficult. However, the beta-receptor concentration found in the present study (127 ± 36 fmol/mg protein) is in good agreement with the calculated receptor concentration found when using [125I]cyanopindolol [22, 23], but is approximately 35% of the value reported when using [3H]dihydroalprenolol [10, 11, 13]. Moreover, the calculated K_d values for the iodinated ligands are also much lower than those derived with the tritiated ligands [10, 11, 13, 22, 23]. These data imply that, in comparison to [3H]dihydroalprenolol, the iodinated ligands of pindolol recognize a lower concentration of higher affinity lung beta-receptor binding sites. The differences in lung binding parameters derived by the iodinated pindolol compounds and [3H]dihydroalprenolol may be due to their differing liposolubility [14, 15]. The iodinated pindolol compounds have a lower partition coefficient and, therefore, are not as likely as [3H]dihydroalprenolol to interact nonspecifically with the lung membranes.

Our data are most compatible with a single affinity (−)[^{125}I]pindolol binding site, suggesting that the beta-receptors on the various lung cell types all have similar affinities for (−)[^{125}I]pindolol. However, the densities of beta-receptors on the various cell types might be expected to differ. Autoradiographic studies on human lung have demonstrated large differences in the distribution of beta-receptors. The beta-receptors appeared to be localized predominantly to airway epithelium, alveolar walls, and submucosal glands. The densities of beta-receptors were less in the airway and vascular smooth muscle [23]. The beta-receptors of the airway smooth muscle, airway epithelium, and vascular smooth muscle appeared to be of the $beta_2$-subtype. $Beta_1$-receptors were in higher concentrations in the submucosal glands and alveoli (90 and 70% respectively) [23].

We used human peripheral lung tissue, which is composed of multiple cell types, and, therefore, are unable to discern exactly which cell types possess the beta-receptors identified. However,the methodologies employed herein will allow the acurate determination of receptor density and the affinity of the receptors for various drugs and hormones using small amounts of tissue samples. Thus, direct and meaningful comparisons of these parameters between patient groups and in response to experimental manipulations can be done. These future studies should provide much information on the effect of many important clinical parameters such as age, smoking history, allergic state, and drug exposure on the role of the beta-receptor system in peripheral lung responses in health and disease.

Acknowledgements—The writers thank Douglas Wood for technical assistance and Kay Meyer and Darla Bartels for secretarial assistance.

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