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Research Article

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Direct Relationship between Mononuclear Leukocyte and Lung β -Adrenergic Receptors and Apparent Reciprocal Regulation of Extravascular, but Not Intravascular, α - and β -Adrenergic Receptors by the Sympathochromaffin System in Humans

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Abstract

To examine putative relationships between adrenergic receptors on accessible circulating cells and relatively inaccessible extravascular catecholamine target tissues, we measured mononuclear leukocyte (MNL) and lung β -adrenergic receptors and platelet and lung α -adrenergic receptors in tissues obtained from 15 patients undergoing pulmonary resection. Plasma catecholamine concentrations were measured concurrently to explore potential regulatory relationships between the activity of the sympathochromaffin system and both intravascular and extravascular adrenergic receptors. MNL and lung membrane β -adrenergic receptor densities were correlated highly ($r = 0.845$, $P < 0.001$). Platelet α_2 -adrenergic receptor and lung α_1 -adrenergic receptor densities were not. Lung α_1 -adrenergic receptor densities were positively related to plasma norepinephrine ($r = 0.840$, $P < 0.01$) and epinephrine ($r = 0.860$, $P < 0.01$) concentrations; in contrast, lung β -adrenergic receptor densities were not positively related to plasma catecholamine concentrations (they tended to be inversely related to plasma norepinephrine and epinephrine [$r = -0.698$, $P < 0.05$] levels). This apparent reciprocal regulation of α - and β -adrenergic receptors by the sympathochromaffin system was only demonstrable with adrenergic receptor measurements in the extravascular catecholamine target tissue. Neither MNL β -adrenergic receptor nor platelet α -adrenergic receptor densities were correlated with plasma catecholamine levels. Thus, although measurements of β -adrenergic receptors on circulating mononuclear leukocytes can be used as indices of extravascular target tissue β -adrenergic receptor densities (at least in lung and heart), it would appear that extravascular tissues should be used to study adrenergic receptor regulation by endogenous catecholamines in humans. These data provide further support for the concept of up regulation, as well as down regulation, of some adrenergic receptor populations during short-term activation of the sympathochromaffin system in humans.

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Introduction

The pharmacology, physiology, biochemistry, and cellular and molecular biology of the adrenergic receptors (adrenoceptors) that mediate the diverse actions of the catecholamines have been studied extensively in vitro and to a lesser extent in vivo in animals (1-3). Application of this data base to the study of adrenergic receptor regulation in humans is limited by the relative inaccessibility of relevant catecholamine target tissues such as heart, lung, etc. Therefore, most investigators studying human adrenergic receptors have measured these on accessible intravascular tissues, circulating mononuclear leukocytes (β_2 -adrenergic receptors), platelets (α_2 -adrenergic receptors), or both, and used these measurements as indices of adrenergic receptor status on extravascular target tissues (3), i.e., those tissues that are not exposed directly to the intravascular compartment. This approach rests on the critical assumption that adrenergic receptor status on circulating cells reflects faithfully that on catecholamine target cells throughout the body. Although one can marshal a body of evidence to support this assumption, certain apparent exceptions led one of us to urge caution (4). For example, estrogen administration has been reported to decrease platelet but increase myometrial α -adrenergic receptor density in rabbits (5, 6), and thyroid hormone excess has been reported to increase human mononuclear leukocyte (7) and rat myocardial (8) β -adrenergic receptor densities but decrease rat hepatic β -adrenergic receptor density (9). Furthermore, regulation of extravascular adrenergic receptors by catecholamines has been found to be tissue and receptor subtype selective (10, 11). To our knowledge, the only published direct comparison of adrenergic receptors on circulating and extravascular cells in humans is the report of Brodde et al. (12) that human myocardial and intact mononuclear leukocyte β -adrenergic receptor densities are correlated.

There is considerable evidence that catecholamines modulate adrenergic receptors (1-4). The most extensively studied pattern is an inverse relationship between agonist levels and adrenergic receptor function. For example, high catecholamine levels generally lead to desensitization of the tissue response to β -adrenergic agonists; among other possibilities, this involves uncoupling of β -adrenergic receptors from adenylate cyclase, sequestration of the receptors from the cell surface into another plasma membrane or intracellular compartment, and an absolute decrease in the number of receptors (2). Attempts to document these phenomena in humans have relied upon studies of adrenergic receptors on circulating cells; the results have not been clear cut. There is evidence that physiologic activation of the sympathochromaffin system and nor-

epinephrine infusion are associated with uncoupling of leukocyte β -adrenergic receptors (13, 14). Sequestration of β -adrenergic receptors from the surface of leukocytes in response to increased endogenous catecholamine release *in vivo* has been difficult to demonstrate in humans (15). An inverse relationship between endogenous plasma catecholamine concentrations and leukocyte β -adrenergic receptor density in normal humans has been reported (14, 16), but others have not found these to be correlated (7, 17, 18). In patients with markedly elevated plasma catecholamine concentrations due to catecholamine-secreting pheochromocytomas, reduced leukocyte β -adrenergic receptor density has been reported (19, 20). Platelet α -adrenergic receptor density has not been found to be related to plasma catecholamine concentrations in normal humans (21, 22) and has been reported to be normal (20, 23) or decreased (19, 24) in patients with pheochromocytomas. Do these seemingly contradictory data indicate that adrenergic receptor down regulation by endogenous catecholamines is of little physiologic relevance in humans, or do they reflect limited utility of the use of adrenergic receptors on intravascular circulating cells as indices of the regulation of adrenergic receptors on extravascular catecholamine target tissues by the sympathochromaffin system?

In contrast to desensitization, there is some evidence that catecholamines may up regulate adrenergic receptors in humans. For example, mononuclear leukocyte β -adrenergic receptor densities have been reported to increase initially during infusions of the agonists isoproterenol and epinephrine (25, 26) and during exercise (27, 28). The interpretation of the latter data (25–28) is difficult, however, since one cannot be certain that the populations of cells examined were not altered by the interventions. There is, to our knowledge, no precedent for agonist-induced up regulation of α -adrenergic receptors.

To examine the putative relationships between adrenergic receptors on circulating cells and those on an additional human extravascular catecholamine target tissue, we quantitated mononuclear leukocyte and lung β -adrenergic receptors and platelet and lung α -adrenergic receptors in tissues obtained from patients undergoing pulmonary resection. Plasma catecholamine concentrations were measured concurrently to explore potential regulatory relationships between the activity of the sympathochromaffin (sympathoadrenal) system and both intravascular and extravascular adrenergic receptors.

Methods

Subjects. 15 patients (5 women and 10 men, ages 20 to 75 yr) undergoing thoracotomy were studied. Three had benign lesions, the remainder were scheduled for lobectomy for suspected carcinoma of the lung. All patients were ambulatory before surgery without respiratory impairment. The mean forced expiratory volume in one second was 83% of predicted. Three patients were taking propranolol and one patient was taking metaproterenol by inhalation. A common approach to general endotracheal anesthesia was followed in all patients. Induction was with sodium pentathol and anesthesia was provided by nitrous oxide and volatile inhalation agents (halothane, isoflurane, or enflurane), morphine and non-depolarizing muscle relaxants. This study was approved by the Washington University School of Medicine Human Studies Committee. All patients provided written informed consent prior to surgery.

Sample collection and preparation. Arterial blood (2 ml) was obtained for catecholamine determinations before induction of anesthesia, 10 min after induction and at the time of pulmonary resection. At

the latter time an additional 150 ml of blood was obtained for isolation of mononuclear leukocytes (MNL)¹ and platelets (PLT). This blood was placed in 50-ml plastic tubes containing 5 ml of 3.8% sodium citrate and 0.5 ml of 0.5 M EDTA and transported immediately to the laboratory. The blood was centrifuged at 400 *g* for 10 min, the platelet-rich plasma removed and ice-cold PBS added to the remaining leukocytes and erythrocytes. From this point on, all steps involving MNL were performed at 4°C. The cells were subfused with Ficoll-Hypaque and centrifuged at 400 *g* for 30 min. The MNL were harvested and washed twice with PBS by centrifugation at 400 *g* and resuspension. The final pellet was resuspended in Dulbecco's modified Eagle's medium with 25 mM Hepes (DMEH). An aliquot of this was removed for studies of β -adrenergic receptors on intact MNL and supplemented with 1 mg/ml bovine serum albumin, with the remainder used for preparing membranes. The typical yield was 2–3 × 10⁸ MNL/150 ml whole blood, with ~ 80% lymphocytes and 20% monocytes. Greater than 95% of these MNL excluded trypan blue at the time of preparation, and cell survival was greater than 90% after 40 h of incubation in DMEH at 4°C.

MNL membranes were prepared by incubation of MNL in hypotonic buffer (5 mM Tris and 1 mM MgCl₂) followed by homogenization with a Polytron (Brinkmann Instruments, Westbury, NY). The homogenate was washed by centrifugation at 35,000 *g* for 15 min and resuspension, followed by centrifugation at 400 *g* for 15 min. The membrane-rich supernatant contained < 2% of the original number of intact cells and was stored in 50 mM Tris and 10 mM MgCl₂ buffer at –70°C.

Platelet membranes were prepared as described previously (17) except that the Polytron was used for homogenization instead of a motor-driven mortar and pestle. Storage was at –70°C in 50 mM Tris, 10 mM MgCl₂, and 5 mM EDTA buffer.

A piece of peripheral lung (typically 2–4 g wet weight) was obtained from a region free of gross tumor and placed in 5 mM Tris, 1 mM MgCl₂, and 0.25 M sucrose buffer at 4°C. The lung tissue was minced with scissors and homogenized with the Polytron as described above. The resulting suspension was centrifuged at 400 *g* for 15 min and the pellet discarded. The supernatant was washed twice by centrifugation at 35,000 *g* for 15 min and resuspension in 50 mM Tris and 10 mM MgCl₂ buffer and stored at –70°C.

Receptor binding studies. Assay conditions as described below provided the appropriate properties for ligand-receptor interactions (saturability, reversibility, stereospecificity, rank order potency and typical kinetics). In saturation binding studies, the concentration of unlabeled competitor used to determine nonspecific binding was ~ 100 times the *K_i* as determined from competition experiments. Radioligand binding to membranes or intact cells was < 10% of that added to the reaction mixture. Guanosine triphosphate (GTP) (100 μ M) was included in the β - and α_2 -adrenergic receptor membrane studies to minimize retained catecholamine binding and to inhibit the accumulation of the ternary complex. When catecholamines were used as competitors, oxidation protection was afforded by 1.1 mM ascorbic acid for membrane studies, or 20 μ g/ml each of superoxide dismutase and catalase for intact MNL studies (29). Protein concentration was determined by the copper-bicinchoninic acid method (30) using bovine serum albumin standards, and cell counts were obtained from an automated cell counter (model S plus IV; Coulter Electronics, Hialeah, FL). After incubation for the specified times, the reactions were terminated by the addition of excess buffer, and the contents filtered over Whatman GF/C glass fiber filters (Whatman International, Maidstone, UK). The tubes and filters were then washed with additional buffer. In studies of intact MNL, a 5-min incubation with excess cold hypotonic PBS was used before filtering (see below). In experiments

1. *Abbreviations used in this paper:* *B*_{max}, receptor density; CYP, cyanopindolol; DMEH, DME with 25 mM Hepes; ISO, isoproterenol; MNL, mononuclear leukocytes; PIN, pindolol; PLT, platelet; PRP, propranolol; PRZ, prazosin; YOH, yohimbine.

with ^{125}I -pindolol (^{125}I -PIN) the wet filters were counted in a gamma counter for 1 min. In studies with tritiated ligands filters were dried and then counted for 5 min in vials containing scintillation cocktail (3a70; Research Products International, Mt. Prospect, IL). The decay of the ^{125}I moiety of ^{125}I -PIN was assumed to be catastrophic as described by Doyle, Buhler, and Burgisser (31). Thus, with decay it is assumed that the binding properties of the pindolol are altered such that it does not bind to β -adrenergic receptors with high affinity. Therefore, the "decay factor" is 1.0 regardless of the age of the ligand and increasing amounts of stock preparation are required to obtain the same concentration of labeled pindolol as the ligand ages. We confirmed this phenomenon by performing saturation binding studies on stock canine lung membranes using ^{125}I -PIN 1 d and 4 wk after iodination. During the study of human tissues, a fresh batch of ^{125}I -PIN was obtained every 4 wk and β -adrenergic receptor assays on samples from any given patient were performed within 3 d of each other.

The B_{max} (receptor density) and K_d (apparent dissociation constant) from saturation binding studies were derived by the method of Scatchard (32). Except for two cases of [^3H]prazosin ([^3H]PRZ) binding to lung α_1 receptors, the Scatchard plots were linear with Hill coefficients approximating 1.0. Thus, computer assisted multiple iterative nonlinear analysis of the untransformed data, as detailed by De Lean et al. (33), gave the same results as linear regression of Scatchard plots.

Intact MNL. β -Adrenergic receptors of intact MNL were studied with ^{125}I -PIN at 4°C with methods similar to those recently described by Motulsky and colleagues (34). At this temperature, receptors that have become internalized remain trapped in this state and can be differentiated from cell surface receptors by the use of hydrophilic and lipophilic unlabeled competitors. ^{125}I -PIN, which is lipophilic, penetrates the cell membrane and therefore binds to both internalized and cell surface receptors. The difference between binding of this ligand alone and that which occurs in the presence of propranolol (PRP) which is also lipophilic, provides a measure of specific binding to all receptors (internal and external). The difference between ^{125}I -PIN binding alone and that which occurs with 4-(3-*t*-butylamino-2-hydroxypropoxy)-benzimidazole-2-one HCl or CGP-12177 (CGP), which is hydrophilic and unable to penetrate the cell membrane, provides specific binding to external receptors only. The number of internal receptors is the difference between specific binding to all receptors and external receptors as defined.

For the current study, saturation binding experiments on cells harvested at 4°C , as described above, were performed by incubating $2\text{--}5 \times 10^5$ cells with eight concentrations of ^{125}I -PIN ($5\text{--}400$ pM) alone and in the presence of PRP (10^{-6} M) and in the presence of CGP (10^{-6} M) in DMEH for 40 h at 4°C . Preliminary studies showed that this incubation period is required for binding to reach equilibrium with heavily desensitized cells. Prior to filtering, each tube was filled with 10 ml of ice-cold 1:10 PBS and incubated for 5 min at 4°C , a procedure known to decrease nonspecific binding (35). After filtering, the tubes and filters were washed with 20 ml of room temperature PBS.

Lung, MNL, and platelet membranes. β -Adrenergic receptors of lung and MNL were studied with ^{125}I -PIN using methods similar to those described for membrane preparations from other tissues (36). Membranes ($10\text{--}40$ μg) were incubated with nine concentrations of ^{125}I -PIN ($10\text{--}400$ pM for MNL and $10\text{--}500$ pM for lung) at 37°C for 30 min in the absence or presence of 10^{-5} M isoproterenol (ISO) used to define nonspecific binding.

Lung α_1 -adrenergic receptors were assayed by incubating $50\text{--}100$ μg of membranes with eight to nine concentrations ($0.5\text{--}4.0$ nM) of [^3H]PRZ with or without 10^{-5} M phentolamine at 25°C for 30 min (37).

PLT α_2 -adrenergic receptors (17) were studied by incubating $40\text{--}100$ μg of membranes with nine concentrations ($0.5\text{--}18$ nM) of [^3H]yohimbine ([^3H]YOH) at 25°C for 30 min with nonspecific binding determined with 10^{-5} M phentolamine.

Catecholamine determinations. Plasma norepinephrine and epinephrine levels were determined as described previously (38) using a single isotope derivative (radioenzymatic) assay based on the conver-

sion of these catecholamines to their *O*-methyl derivatives in the presence of methyl- ^3H -*S*-adenosyl-*l*-methionine. The detection limit of this assay is 10 pg/ml with a between assay coefficient of variation < 10%.

Statistical analysis. Unless otherwise specified, data are presented as the mean \pm SE. Data management and analysis, including linear regression analysis, were performed using the Washington University GCRC CLINFO System and programs from Biomedical Data Processing Statistical Software Co. (Los Angeles, CA) and Elsevier-Biosoft (Cambridge, UK). *P* values < 0.05 were considered significant (two-tailed test). Statistical power in relation to sample size for the regression analyses was determined as described by Lachin (39).

Chemicals. ^{125}I -pindolol (2200 Ci/mmol), methyl- ^3H -yohimbine (87 Ci/mmol), 7-methoxy- ^3H -prazosin (82 Ci/mmol) and methyl- ^3H -*S*-adenosyl-*l*-methionine ($5\text{--}15$ Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). Phentolamine and CGP-12177 were gifts from Ciba-Geigy (Summit, NJ) as was (+)norepinephrine from Sterling Winthrop Research (Rensselaer, NY) and (+)propranolol and (–)propranolol from Ayerst Laboratories (New York, NY). Dulbecco's modified Eagle's medium with 25 mM HEPES was purchased from Gibco (Grand Island, NY). Superoxide dismutase (3200 U/mg), catalase (2500 U/mg) and (–)isoproterenol were purchased from Sigma Chemical Co. (St. Louis, MO) as were other reagents not specified here.

Results

Representative specific binding curves and Scatchard plots for human MNL and lung membrane β -adrenergic receptors are shown in Fig. 1; those for human platelet membrane α_2 -adrenergic receptors and lung membrane α_1 -adrenergic receptors are shown in Fig. 2. Representative data documenting the ability of the methods used to demonstrate agonist-induced internalization of β -adrenergic receptors in intact MNL are shown in Fig. 3.

Adrenergic receptor densities measured in the MNL, PLT, and lung preparations are listed in Table I. β -Adrenergic receptors in human lung (Fig. 1) have not been measured previously with ^{125}I -PIN but the binding characteristics are similar to those in heart (36) and skeletal muscle (40) and the receptor densities measured with ^{125}I -PIN are similar to those measured with ^{125}I -cyanopindolol (^{125}I -CYP) (41) in human lung. Deter-

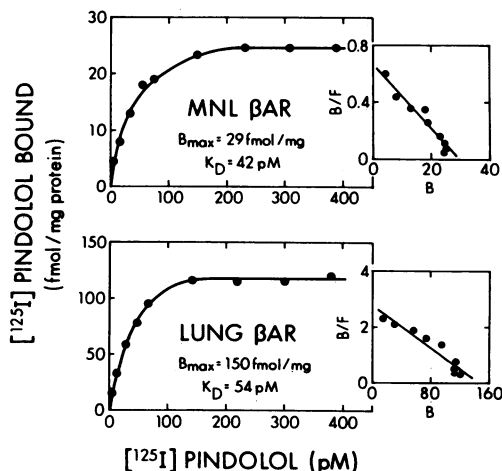


Figure 1. Representative specific binding curves and Scatchard plots for human MNL membrane and lung membrane β -adrenergic receptors (BAR). B/F, bound:free ratio; B, bound.

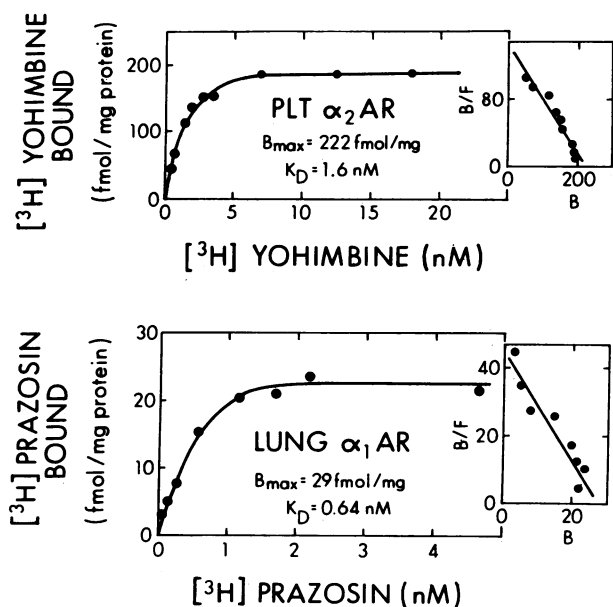


Figure 2. Representative specific binding curves and Scatchard plots for human platelet (PLT) membrane α_2 -adrenergic receptors (α_2 AR) and lung membrane α_1 -adrenergic receptors (α_1 AR). B/F, bound:free ratio; B, bound.

minations of total and cell surface β -adrenergic receptors of intact MNL with ^{125}I -PIN were similar to reported data with ^{125}I -CYP (34), except that nonspecific binding was lower with ^{125}I -PIN (typically 5% and 20% at 10 and 250 pM, respectively). Preliminary in vitro studies verified that the techniques used permitted distinction between cell surface and internalized β -adrenergic receptors in intact MNL. MNL were exposed to 10^{-5} or 10^{-6} M isoproterenol for 10 min at 37°C and then cooled to 4°C and washed extensively. Control cells from the same isolation were treated identically except isoproterenol was omitted. Saturation binding studies on these control cells showed no differences in specific binding of ^{125}I -PIN when PRP or CGP were used as unlabeled competitors. Cells exposed to isoproterenol, however, showed marked differences permitting identification of internalized receptors (Fig. 3). Whole blood incubated with isoproterenol (data not shown) yielded similar results.

The binding characteristics of lung α_1 -adrenergic receptors, measured with ^3H]PRZ, (Fig. 2) were similar to those reported previously (42) with the exception of receptor density. The B_{max} of 46 ± 10 fmol/mg protein we observed is similar to that reported for guinea pig (37) and canine (43) lung, but substantially lower than that of 600 fmol/mg protein reported by Barnes and colleagues (42) in human lung from six patients. The latter authors commented that this unexpectedly high apparent α_1 -adrenergic receptor density might have been the result of uptake of ^3H]PRZ by contaminating carbon particles deposited in the lungs from cigarette smoke. Indeed, in their one patient without obstructive lung disease the B_{max} was 62 fmol/mg protein. In two of our patients the final membrane preparation was contaminated heavily with carbonaceous material which could not be removed by repeated washings. In these preparations the Scatchard plots were biphasic (concave upward) suggesting binding of ^3H]PRZ to more than one class of sites. Use of computer modeling, as described above, re-

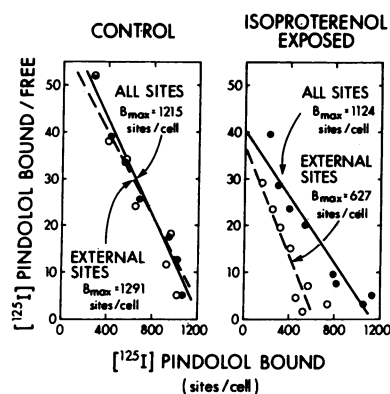


Figure 3. Scatchard plots of ^{125}I -pindolol binding at 4°C to intact human MNL illustrating internalization of β -adrenergic receptors (β AR) by preincubation with ISO in vitro. In the absence of preincubation with ISO (left) the total number of β -adrenergic receptors (all sites), measured with PRP as the competing ligand, was virtually identical to the number of external β -adrenergic receptors, measured with CGP as the competing ligand. In contrast, following preincubation with 10^{-5} M isoproterenol for 10 min at 37°C (right) the total number of β -adrenergic receptors was altered little if at all, but the number of external β -adrenergic receptors was reduced, implying agonist-induced internalization of β -adrenergic receptors. See Methods for details.

vealed that the data best fit a single class of receptors and that binding at the higher concentrations of ^3H]PRZ represented nonreceptor binding. It should be noted that in preliminary studies with canine lung (which were free of carbonaceous material) Scatchard plots were invariably linear (data not shown) even when ^3H]PRZ concentrations as high as 6.0 nM were used.

Mean (\pm SE) apparent dissociation constants (K_d) were 10 ± 2 pmol/liter for intact MNL β -adrenergic receptors, 72 ± 19 pmol/liter for MNL membrane β -adrenergic receptors, 46 ± 5 pmol/liter for lung β -adrenergic receptors, 4.1 ± 0.8 nmol/liter for platelet α_2 -adrenergic receptors and 1.3 ± 0.4 nmol/liter for lung α_1 -adrenergic receptors (data not shown).

Mean (\pm SE) plasma norepinephrine and epinephrine concentrations prior to induction of anesthesia (preinduction), 10 min after induction of anesthesia (post-induction) and at the time of lung resection are listed in Table II. Mean norepinephrine levels were approximately fivefold above resting normal values and mean epinephrine levels were approximately sevenfold above resting normal values (44). However, there was considerable scatter in the catecholamine levels.

Mean mononuclear leukocyte, platelet, and lung adrenergic receptor densities are listed in Table I. As with the plasma catecholamine levels, there was considerable scatter in the data. Notably, despite markedly elevated plasma catecholamine concentrations, more than 90% of β -adrenergic receptors measured in intact MNL were external, i.e., on the cell surface.

Because long-term propranolol or metaproterenol ingestion might conceivably alter adrenergic receptors, adrenergic receptor characteristics (Table I), relationships among adrenergic receptors (Table III) and between adrenergic receptors and plasma catecholamine levels (Tables IV and V) were determined both for the entire data set and after exclusion of data from patients taking these drugs.

Relationships among adrenergic receptor densities on MNL, PLT, and lung are listed in Table III. Circulating MNL and lung membrane β -adrenergic receptor densities were correlated highly ($r = 0.845$, $P < 0.001$) as shown in Fig. 4. This

Table I. Adrenergic Receptor Densities on MNL, PLT, and Lung*

	n	Mean±SE	Range
MNL β AR			
MNL membranes (fmol/mg)	15 (11)	53±8 (56±9)	15–133 (29–133)
Intact MNL, external (sites/cell)	15 (11)	1,190±139 (1,270±178)	488–2,850 (745–2,850)
Intact MNL, internal (sites/cell)	15 (11)	53±9 (42±11)	12–115 (12–115)
Intact MNL, total (sites/cell)	15 (11)	1,240±144 (1,310±185)	542–2,960 (762–2,960)
Lung β AR (fmol/mg)	12 (11)	81±9 (83±10)	32–150 (32–150)
PLT α ₂ AR (fmol/mg)	14 (11)	357±28 (398±22)	183–521 (301–521)
Lung α ₁ AR (fmol/mg)	10 (9)	46±10 (50±10)	10–103 (16–103)

* Values in parentheses are after exclusion of data from patients taking propranolol or metaproterenol.

relationship was less apparent when lung membrane β-adrenergic receptors were compared with those of intact MNL, and was unaffected by exclusion of data from patients taking relevant drugs. No relationship was apparent between PLT α₂-adrenergic and lung α₁-adrenergic receptors. MNL membrane β-adrenergic receptor density was correlated, albeit rather weakly, with total ($r = 0.552$, $P < 0.05$) and external ($r = 0.577$, $P < 0.05$) β-adrenergic receptor density on intact MNL (data not shown). We have no simple explanation for the weakness of this relationship but, since both determinations were performed on the same samples, suggest that it reflects analytical rather than biological variation. It may have been due to differences in assay conditions (temperature, time, displacing agents), variations in cell survival during the lengthy incubation of intact cells, or to loss of receptors during the centrifugation steps of the preparation of membranes. MNL β-adrenergic and PLT α₂-adrenergic receptor densities were not correlated nor were lung β-adrenergic and α₁-adrenergic receptor densities (data not shown).

Relationships between adrenergic receptor densities on MNL, PLT, and lung and plasma norepinephrine concentrations and plasma epinephrine concentrations are listed in Tables IV and V, respectively. Lung α₁-adrenergic receptor densities were directly related to plasma norepinephrine and epinephrine concentrations as shown in Fig. 5. Lung β-adrenergic receptor densities appeared to be inversely related to preinduction plasma levels of norepinephrine and epinephrine concentrations, as shown in Fig. 5, although this relationship was significant statistically only for epinephrine for the entire

data set, was not significant statistically after exclusion of data from patients taking propranolol or metaproterenol, and did not hold for post-induction and resection plasma catecholamine values (Tables IV and V). In contrast, neither MNL β-adrenergic receptor densities nor PLT α₂-adrenergic receptor densities were correlated with plasma catecholamine levels.

Discussion

This comparison of adrenergic receptors on circulating and extravascular tissues provides two new findings in humans. First, there is a direct relationship between β-adrenergic receptor densities on circulating MNL and lung tissue. Second, sympathochromaffin activity, as reflected by plasma catecholamine levels (44, 45), is directly related to α₁-adrenergic receptor density (and perhaps inversely related to β-adrenergic receptor density) on this extravascular tissue, but not related to adrenergic receptor densities on circulating cells.

β-adrenergic receptor density on MNL membranes was correlated highly ($r = 0.845$, $P < 0.001$) with that on lung membranes obtained simultaneously. This relationship was less apparent for total and cell surface β-adrenergic receptors on intact MNL and those on lung membranes. Thus, although it is often appropriate to study intact MNL (3, 15, 34), our findings suggest that use of MNL membrane preparations is preferable when MNL β-adrenergic receptors are to be compared with those in membrane preparations from extravascular tissues. The present findings are consistent with those of Aarons and Molinoff (46) in rats. They found parallel increments in lymphocyte, lung and heart β-adrenergic receptor densities during administration of the antagonist propranolol. Parenthetically, the relationship between MNL and lung β-adrenergic receptor density was unaltered when data from patients taking propranolol or metaproterenol were excluded in the present study. Thus, these data provide further support for the use of measurements of MNL β-adrenergic receptor density as an index of that in human lung, as well as in human heart (12). It should be noted, however, that tissue-specific responses to some stimuli, such as hypoxia (47) and hypertension (48), have been reported in some animal studies. Furthermore, as discussed shortly, adrenergic receptor status on circulating cells did not reflect clearly the relationships with sympathochromaffin activity found with adrenergic receptor status on an extravascular tissue in the present study.

In contrast to the findings with β-adrenergic receptors, α-

Table II. Plasma Norepinephrine and Epinephrine Concentrations before and after Induction of Anesthesia and at the Time of Lung Resection

	n	Mean±SE	Range
Norepinephrine (pg/ml)			
Preinduction	12	864±192	183–2,040
Postinduction	15	1,030±259	51–3,330
Resection	15	1,050±208	154–2,990
Epinephrine (pg/ml)			
Preinduction	12	388±91	21–810
Postinduction	15	353±133	21–1,770
Resection	15	447±125	36–1,590

Table III. Relationships among Adrenergic Receptor Densities on MNL, PLT and Lung*

	No. of comparisons	Correlations coefficient (r)	P	
MNL β AR vs. lung β AR				
MNL Membranes vs. lung membranes	12 (11)	0.845 (0.838)	<0.001	(<0.001)
Intact MNL, external vs. lung membranes	12 (11)	0.570 (0.573)	NS	(NS)
Intact MNL, total vs. lung membranes	12 (11)	0.562 (0.573)	NS	(NS)
PLT α_2 AR vs. lung α_1 AR	10 (9)	0.280 (0.025)	NS	(NS)

* Values in parentheses are after exclusion of data from patients taking propranolol or metaproterenol.

adrenergic receptor density on platelet membranes was not correlated with that on lung membranes. This is perhaps not surprising since the receptors measured are of different subtypes, α_2 -adrenergic receptors on platelets and α_1 -adrenergic receptors on lung membranes. Parenthetically, human lung β -adrenergic receptors, like MNL β -adrenergic receptors, are predominantly of the β_2 subtype (41, 49, 50) although β_1 -adrenergic receptors are also present in lung (41, 50).

It should be emphasized that the use of tissue homogenates precluded precise anatomic localization of the receptors measured. In lung, α - and β -adrenergic receptors are distributed in alveolar walls, blood vessels and airways (50, 51). In homogenates from peripheral lung, analogous to the samples used in the present study, the β -adrenergic receptors are largely from alveolar walls (50) and the α -adrenergic receptors from vascular smooth muscle (51).

Despite elevated plasma norepinephrine and epinephrine concentrations during surgery, the vast majority (> 90%) of β -adrenergic receptors were found to be on the surface of intact MNL in these patients. These data provide further evidence that physiologic activation of the sympathochromaffin system does not result in substantial sequestration of β -adrenergic receptors on circulating cells in humans (15). However, the con-

centration of epinephrine required to produce a modest degree (20–30%) of internalization of MNL β -adrenergic receptors in vitro is ~ 5,000 pg/ml (15), much higher than the circulating levels in our patients. Since postjunctional adrenergic receptors in innervated tissues are exposed to norepinephrine concentrations higher than those in the circulation, and the actual neurally derived norepinephrine concentrations at such receptors are not known with certainty, these data do not exclude categorically receptor sequestration away from the cell surface by sympathetic neural activation in innervated tissues.

These data also disclosed highly significant positive relationships between lung α_1 -adrenergic receptor density and plasma norepinephrine and epinephrine concentrations after induction of anesthesia and at the time of lung resection in vivo in humans. This finding appears to be at variance with a body of published in vitro and animal studies. For example, in vitro studies with a variety of cultured cell lines have generally demonstrated a decrease in α_1 -adrenergic receptor density during exposure to agonist (52). Furthermore, Insel and colleagues (10) found a 76% reduction in lung α_1 -adrenergic receptor density in rats with implanted pheochromocytomas and markedly elevated plasma catecholamine levels. Thus, our finding of apparent up-regulation of lung α_1 -adrenergic recep-

Table IV. Relationships between AR Densities on Mononuclear MNL, PLT, and Lung and Plasma Norepinephrine*

	No. of comparisons	Preinduction r	Postinduction r	Resection r
MNL β AR				
MNL membranes	12–15 (8–11)	-0.266 (-0.344)	-0.157 (-0.020)	0.029 (0.084)
Intact MNL, external	12–15 (8–11)	-0.457 (-0.390)	-0.433 (-0.300)	-0.347 (-0.304)
Intact MNL, total	12–15 (8–11)	-0.438 (-0.370)	-0.426 (-0.296)	-0.350 (-0.303)
Lung β AR	9–12 (8–11)	-0.455 (-0.449)	-0.004 (0.040)	-0.054 (-0.072)
PLT α_2 AR	11–14 (8–11)	-0.446 (-0.378)	0.165 (0.126)	0.201 (0.219)
Lung α_1 AR	7–10 (6–9)	0.629 (0.874)*	0.878 [‡] (0.901) [‡]	0.840 [§] (0.900) [‡]

* Values in parentheses are after exclusion of data from patients taking propranolol or metaproterenol.

* $P < 0.05$; [‡] $P < 0.001$; [§] $P < 0.002$.

Table V. Relationships between Adrenergic receptor (AR) Densities on MNL, PLT and Lung and Plasma Epinephrine

	No. of comparisons	Preinduction r	Postinduction r	Resection r
MNL β AR				
MNL Membranes	12–15 (8–11)	-0.497 (-0.596)	-0.037 (0.100)	0.079 (-0.055)
Intact MNL, external	12–15 (8–11)	-0.515 (-0.522)	-0.376 (-0.284)	-0.338 (-0.383)
Intact MNL, total	12–15 (8–11)	-0.504 (-0.513)	-0.380 (-0.284)	-0.349 (-0.391)
Lung β AR	9–12 (8–11)	-0.693* (-0.676)	0.015 (-0.019)	-0.179 (-0.243)
PLT α_2 AR	11–14 (8–11)	-0.112 (-0.118)	0.315 (0.297)	0.026 (0.182)
Lung α_1 AR	7–10 (6–9)	0.640 (0.904) [§]	0.809 [‡] (0.833)	0.860 [‡] (0.856) [‡]

Values in parentheses are after exclusion of data from patients taking propranolol or metaproterenol.

* $P < 0.05$; [‡] $P < 0.005$; [§] $P < 0.02$; ^{||} $P < 0.01$.

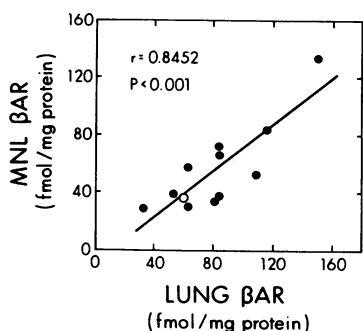


Figure 4. Relationship between mononuclear leukocyte (MNL) membrane β -adrenergic receptor (β BAR) densities and lung membrane β -adrenergic receptor densities. The open symbol denotes data from a patient taking propranolol.

tor density during short-term sympathochromaffin activation *in vivo* in humans is unprecedented, and probably the result of complex regulatory events rather than simple agonist-receptor interaction.

Evidence that catecholamines might up-regulate adrenergic receptors *in vivo* in humans was first reported by Tohmeh and Cryer (25) who observed an initial increase followed by a late decrease in MNL β -adrenergic receptor density during infusions of isoproterenol and of epinephrine. These observations have been confirmed (26). Furthermore, increments in MNL β -adrenergic receptor density have been found to occur during physical exercise (27, 28), which is known to activate the sympathochromaffin system, in humans. The interpretation of these findings is limited by the possibility that the interventions might have altered the population of MNL studied. Although there is not universal agreement (53), there is evidence that adenylate cyclase responsiveness to agonists (54–56) and β -adrenergic receptor density (57) differ among lymphocyte populations. However, the present findings in a tissue with a fixed cell population, the lung, provide further support for the concept of short-term endogenous agonist up-regulation of some adrenergic receptors in humans. The pattern is not simple, however, since lung β -adrenergic receptor density was not positively related to plasma catecholamine levels (indeed, it appeared to be inversely related to antecedent

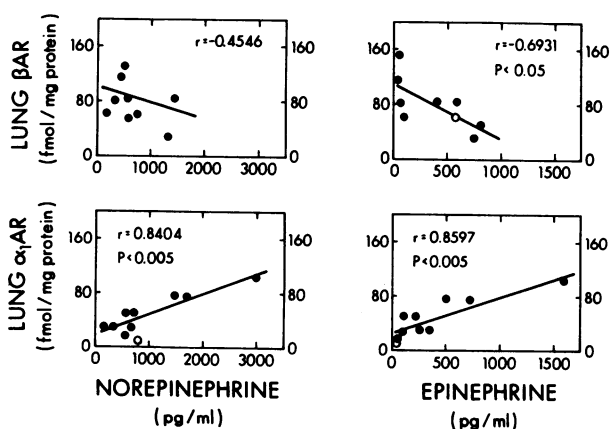


Figure 5. Relationships between lung membrane β -adrenergic receptor (β BAR) densities and pre-induction plasma norepinephrine and epinephrine concentrations and between lung membrane α_1 -adrenergic receptor (α_1 AR) densities and plasma norepinephrine and epinephrine concentrations at the time of lung resection. The open symbols denote data from patients taking propranolol or metaproterenol.

plasma catecholamine concentrations). Thus, the findings are most consistent with reciprocal regulation of extravascular tissue, at least lung, α - and β -adrenergic receptor densities by the sympathochromaffin system in humans. Clearly, the evidence that activation of the sympathochromaffin system results in up-regulation of some adrenergic receptors in some tissues remains fragmentary. However, in view of the present and previous (25–28) findings, this concept warrants further study.

Notably, this apparent reciprocal regulation of α - and β -adrenergic receptors by the sympathochromaffin system was only demonstrable with adrenergic receptor measurements in an extravascular catecholamine target tissue (lung). Neither MNL β -adrenergic receptor nor platelet α -adrenergic receptor densities were correlated with plasma catecholamine concentrations. It is, of course, possible that relatively weak relationships were missed since for our sample size statistical power exceeds 95% only with a true correlation coefficient of 0.770 or greater (39). Nonetheless, our findings in this regard are consistent with most (7, 17, 18, 21, 22), but not all (14, 16), previous data. Thus, although measurements of adrenergic receptors (at least β -adrenergic receptors) on circulating cells can be used as indices of extravascular target tissue adrenergic receptor densities (at least in lung and heart), it would appear that the actual extravascular tissues of interest should be used to study adrenergic receptor regulation by endogenous catecholamines in humans rather than relying on inferences from circulating cells.

Since extravascular tissue (lung) and circulating MNL β -adrenergic receptor densities were found to be correlated and tissue β - (and α -) adrenergic receptor densities were found to be related to sympathochromaffin activity, one might ask why the latter was not found to be related to MNL β - (or platelet α -) adrenergic receptor density? We would reconcile this apparent contradiction as follows. The level of sympathochromaffin activity is almost assuredly only one of many factors that modulate adrenergic receptor density. If so, one would expect this catecholamine-regulated modulation to be most apparent in tissues exposed to marked fluctuations in catecholamine levels, i.e., those adjacent to norepinephrine-releasing sympathetic nerve terminals. It would be expected to be least apparent in circulating tissues since fluctuations in circulating norepinephrine are damped relative to those near sympathetic nerve terminals in extravascular tissues (44).

Finally, a semantic issue warrants comment. In the preceding paragraphs we have referred to lung adrenergic receptor up- and down-regulation in relation to circulating catecholamine levels. These terms are used commonly to indicate increments and decrements, respectively, in the total number of cellular receptors (1–3). We measured plasma membrane, rather than whole cell, adrenergic receptor densities in lung tissue; any internalized receptors in the “light vesicles” would have been discarded in the preparation of the membrane fractions (58, 59). Thus, we cannot distinguish differences in receptor distribution away from the plasma membrane from differences in the total number of cellular receptors. Nonetheless, we note our inability to demonstrate β -adrenergic receptor internalization into intact MNL under *in vivo* conditions, as discussed earlier, and submit that it is plasma membrane receptors that are more relevant to the biologic responsiveness of the tissue.

In summary, the data presented demonstrate that MNL and lung β -adrenergic receptor densities are correlated in

humans. Thus, they support the use of measurements of β -adrenergic receptors on circulating MNL as indices of extravascular catecholamine target tissue β -adrenergic receptors. However, the data suggest that extravascular tissues should be used to study adrenergic receptor regulation by endogenous catecholamines. Finally, these data provide further support for the concept of up-regulation, as well as down-regulation, of some adrenergic receptor populations during short-term activation of the sympathochromaffin system in humans.

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