Regulation of Human Leukocyte Beta Receptors by Endogenous Catecholamines

RELATIONSHIP OF LEUKOCYTE BETA RECEPTOR DENSITY TO THE CARDIAC SENSITIVITY TO ISOPROTERENOL

J. FRASER, J. NADEAU, D. ROBERTSON, and A. J. J. WOOD, *Division of Clinical Pharmacology, Vanderbilt University Medical School, Nashville, Tennessee 37232*

ABSTRACT High levels of beta receptor agonist have previously been shown to down-regulate beta receptor density on circulating leukocytes in man; however, the factors controlling receptor density under physiological conditions have not previously been defined. To determine whether beta receptor density is normally down-regulated by circulating, physiological levels of catecholamines we have examined the relationship between receptor density and catecholamine levels. Urinary epinephrine and norepinephrine were significantly reciprocally correlated to lymphocyte receptor density. A similar relationship existed between beta receptor density and supine plasma epinephrine, norepinephrine, upright epinephrine, and norepinephrine levels. Change in sodium intake from 10 to 400 meq/d caused a 52% increase in lymphocyte and a 48% increase in polymorphonuclear beta receptor density. The changes in receptor density were accompanied by an increase in the sensitivity to isoproterenol measured as a fall in the dose of isoproterenol required to raise the heart rate by 25 beats per minute. Beta receptor density on both lymphocyte and polymorphonuclear cells was significantly correlated to the cardiac sensitivity to isoproterenol. Propranolol administration resulted in an increase in the density of beta receptors on lymphocyte and polymorphonuclear cells that correlated with the subject's pretreatment catecholamine levels.

These findings, therefore, suggest that physiological levels of catecholamines normally down-regulate beta receptors in man and that blockade of this down-regulation by propranolol allows receptor density to increase.

INTRODUCTION

The use of radiolabeled agonists and antagonists of high specific activity has allowed the development of radioligand binding assays for the detailed study of receptor function (1–9). The recent application of this technique to the beta adrenergic receptor on human leukocytes has allowed this to be extended to man. High levels of beta adrenergic agonists have been shown in vitro (10, 11) to result in a reduction, or down-regulation of beta adrenergic receptor density, whereas the in vivo administration of high doses of adrenergic agonists, for example in the treatment of asthma (12–15), or the high levels of catecholamines in patients with pheochromocytoma (15) result in reduced beta receptor density (16) and diminished cyclic AMP generation by leukocytes in response to isoproterenol. It has, therefore, been suggested that pharmacological doses of catecholamines will result in down-regulation of receptor density with consequent reduction in the sensitivity to adrenergic agents (17).

It is also possible that changes in beta receptor density account for the considerable interindividual variability in the response to both beta receptor agonists and antagonists (18). For example Schocken and Roth (19) found that leukocyte beta receptor density was reduced in elderly patients, while Vestal et al. (20) found that elderly subjects had a reduced cardiac chronotropic response to both beta receptor agonist and antagonist. This raises the question of whether

receptor density, measured on human leukocytes, might reflect receptor density in other less accessible tissues such as the heart and hence whether changes in leukocyte receptor density are reflected in changes in the cardiac chronotropic response to isoproterenol.

In spite of the evidence that pharmacological doses of catecholamines can reduce receptor function in man, the factors controlling receptor function in normal man have not been defined. We postulated that receptor density is normally regulated by the degree of sympathetic tone, thus increased sympathetic tone should result in increased production of catecholamines and down-regulation of beta adrenergic receptors. If beta adrenergic receptors are normally down-regulated by physiological levels of catecholamines then blockade of these receptors by administration of a beta receptor antagonist, such as propranolol, should result in release of this down-regulation and a consequent increase in receptor density. If this increase in receptor density is due to blockade of down-regulation then the higher the catecholamines and the greater the down-regulation prior to propranolol, the larger will be the rise in receptor density following propranolol treatment. The possibility of such an increase in receptor density during propranolol treatment has led some (17, 18) to suggest this as an explanation for the propranolol withdrawal syndrome that has been described following abrupt propranolol withdrawal and has been associated with increased frequency of angina and even myocardial infarction (21). This syndrome has many of the features of a hyperadrenergic state and it has been suggested that hypersensitivity to beta receptor agonists can occur during the period following propranolol withdrawal (21, 22, 23). In a previous study after 8 d of propranolol treatment beta receptor density remained elevated for 48 h after stopping propranolol suggesting that this might explain the previously described hyperadrenergic state (24). However, the relevance of 8 d of propranolol treatment to the usual therapeutic situation when propranolol is administered chronically, is unclear.

Circulating levels of catecholamines can be altered by stimuli which activate the sympathetic nervous system (25). By means of such stimuli, it should be possible to increase endogenous norepinephrine and epinephrine levels and thus determine if significant down-regulation of beta receptor density is achieved by physiological levels of catecholamines. For purposes of this study it seemed prudent to utilize a stimulus of prolonged duration in order that there would be sufficient time for regulation of receptor density to occur. Restriction of dietary sodium increases plasma norepinephrine (25, 26, 27) while there is a trend toward reduced plasma norepinephrine following excessive sodium intake (26, 27). Parallel changes in plasma epinephrine probably occur. These changes in catecholamine levels are likely due to reduction and expansion, respectively, of blood volume. The volume reduction of low sodium intake results in baroreceptor-mediated activation of both the sympathetic and renin systems. Volume expansion results in the opposite effects although they are less marked in degree (26). Dietary sodium contents of 10, 150, and 400 meq were selected as low, medium, and high salt intakes for purposes of this study.

We therefore studied the relationship between beta receptor density on circulating leukocytes and sympathetic activity as measured by catecholamine levels in a group of normal volunteers. The effect of beta blockade and altered sodium balance were assessed in terms of three variables determining or reflecting sympathetic activation: (a) plasma catecholamine levels, (b) leukocyte beta receptor density, and (c) cardiac sensitivity to isoproterenol.

METHODS

Subjects. A total of 22 normotensive male volunteers aged 22–38 yr and 2 patients with pheochromocytoma participated in the study. The protocol had previously been approved by the Vanderbilt University Committee for the Protection of Human Subjects. All of the subjects, with the exception of the two with pheochromocytoma, had no abnormalities on an admission screening that included physical examination, electrocardiogram, chest x-ray, hematology profile, liver, and renal function tests. None of them had taken any medication for at least a month preceding the study and none received medication except propranolol during the period of the study.

Seven of the subjects received each of three diets containing 10, 150, and 400 meq of sodium per day for 10 d. The order in which the diets were administered was determined by chance and the studies were separated by 21.7 ± 1.1 d. On the last 2 d of each diet between 8:30 and 9.30 a.m. a blood sample was taken after the subject had been supine for at least 30 min, through an indwelling cannula that had been in place for at least 20 min for the measurement of leukocyte beta receptor density as described below and for epinephrine and norepinephrine by radioenzymatic assay (28). A further blood sample was taken after the subject had been standing for 10 min and the concentration of epinephrine and norepinephrine determined. On the last 2 d of the diet urine was collected for the measurement of catecholamines and sodium. The subjects were weighed and their blood pressure and pulse measured both supine and after they had been upright for 2 min at the end of each diet. Mean arterial pressure was determined as the diastolic pressure plus one third of the pulse pressure.

On the last day of each diet the cardiac sensitivity to isoproterenol was determined. Increasing doses of the beta agonist isoproterenol were administered and the rise in heart rate produced by each dose plotted against the logarithm of the dose of isoproterenol. At least five points on the log-linear portion of the dose-response curve were examined for each subject and the best-fit line determined by linear regression analysis. The dose of isoproterenol required to raise the heart rate by 25 beats per minute (I_{25})[1] was deter-

---

[1] *Abbreviations used in this paper:* B_{max}, beta receptor density; DHA, [³H]dihydroalprenolol; I_{25}, isoproterenol required to raise heart beat rate by 25 beats per minute.

mined from this line. The I_{25} is a measure of cardiac resistance to isoproterenol with increasing values implying increased resistance and lower values implying increased sensitivity to isoproterenol.

16 of the subjects received propranolol 80 mg three times daily for 4 wk. Two of the subjects withdrew after 1 wk of propranolol therapy because of insomnia, but their data is included. Blood samples were taken prior to propranolol treatment, weekly during propranolol treatment, and at 24, 48, 72, and 96 h after stopping propranolol for the measurement of leukocyte beta receptor density, plasma norepinephrine, epinephrine (28), and plasma propranolol (29). The subjects' weight, blood pressure, and heart rates both standing and supine were determined on each day as described above. 24-h urine collections were also made at these times and urinary catecholamine excretion measured.

The lymphocytes and polymorphonuclear cells were isolated from 60 ml of fresh blood anticoagulated with EDTA by centrifugation on Ficoll-Hypaque density gradients (30). The blood was centrifuged at $200 g$ for 15 min at room temperature and the platelet-rich plasma pipetted off (31). The buffy coat plus 7 ml of erythrocytes was then washed with 40 ml 0.01 M phosphate-buffered saline (pH 7.4) at 37°C and processed to isolate and separate the lymphocytes and polymorphonuclear cells. Contaminating erythrocytes were removed from the polymorphonuclears by a brief exposure to 0.2% saline (16). To prepare the particulate fraction, the cells were lysed for 30 min in 0.2% saline at 4°C, centrifuged for 15 min at $400 g$ at 4°C, resuspended in 5% glycerol, frozen for 1 min in a dry-ice acetone bath, and quickly thawed at room temperature. The particulate fraction was recovered by centrifugation at $1,000 g$ for 20 min at 4°C and washed with Tris buffer pH 7.4 at 4°C. Particulate fractions were resuspended in Tris buffer (Tris-HCl 75 mM, $MgCl_2$ 15 mM, pH 7.4) at 37°C at a final protein concentration of 1–3 mg/ml by gently homogenizing in a glass Teflon homogenizer.

Beta receptor density of particulate fractions was then measured using [³H]dihydroalprenolol (DHA) (51 Ci/mmol, New England Nuclear, Boston, Mass.) as described by Williams and Galant (7, 8). The incubation included 0.1 mM phentolamine, 0.3 mM catechol, and 0.1 mM ascorbic acid to reduce nonspecific binding (8). Specific binding was defined as total DHA bound minus DHA bound in the presence of 20 μM DL-propranolol or 1 mM L-isoproterenol and was in the range of 50 to 70% of total binding. DHA binding was assayed at four concentrations from 1 to 5 nM for each determination of beta receptor density (B_{max}) of DHA bound. B_{max} and dissociation constant (K_d) of DHA binding were calculated from Scatchard plots of specific binding data (Fig. 1) (32). Protein was determined by the method of Lowry et al. (33). The standard deviation for B_{max} in samples from the same donor run concurrently was ±10.2% for lymphocytes and ±6.5% for polymorphonuclear cells. When samples were taken weekly for 4 wk from subjects in whom diet, posture, and degree of activity were uncontrolled the standard deviation of B_{max} was ±29.0% for lymphocytes and ±19.6% for polymorphonuclear cells. B_{max} measured in subjects on the controlled sodium diet averaged 40.9±3.3 and 40.1±2.8 fmol/mg protein in polymorphonuclear cells and 30.5±3.2 and 31.2±2.7 fmol/mg protein in lymphocytes on the second to last and last days of the diets, respectively, thus showing excellent reproducibility. The results were analyzed using Student's t test for paired values and in the case of the propranolol treatment an analysis of variance was used to determine the statistical significance of the changes in receptor density following propranolol treatment.

RESULTS

Urinary sodium output on the three diets is shown in Table I and was close to the calculated intake demonstrating the subject's compliance with the diet. There was a significant fall in weight (Table I) between the 150- and 10-meq sodium diet. This was accompanied by a significant rise in the heart rate response to standing and a fall in both supine and upright systolic and mean upright blood pressure (Table I).

The effect of changes in sodium intake on the urinary excretion of epinephrine and norepinephrine is shown in Table II. Urinary norepinephrine increased by 31% on the 10-meq sodium diet compared with the 400-meq sodium diet. Urinary epinephrine was significantly higher when the subjects were taking 150 compared with 400 meq of sodium per day. Plasma norepinephrine and epinephrine also increased as sodium intake decreased (Table II).

Beta receptor density (B_{max} of DHA binding) on lymphocytes was 52% higher on the 400-meq sodium intake compared with the 10-meq diet (Table II). B_{max} on polymorphonuclear cells increased 48% from 37.0±3.6 (SEM) to 54.3±4.7 fmol/mg protein on the 400-meq Na/d diet compared to the 10-meq Na/d diet (Table II).

A significant relationship was found between the logarithm of urinary epinephrine ($r = -0.527, P < 0.001$) norepinephrine ($r = -0.327, P < 0.02$) and B_{max} on lymphocytes. Lymphocyte receptor density was also significantly correlated to supine ($r = -0.482, P < 0.001$) and upright ($r = -0.460, P < 0.001$) epinephrine and more weakly to supine ($r = -0.306, P < 0.05$) and upright norepinephrine ($r = -0.364, P < 0.02$). The B_{max} on polymorphonuclear cells was significantly correlated
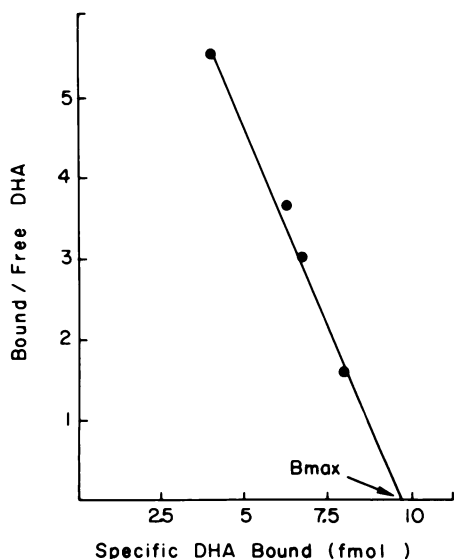

FIGURE 1   Scatchard plot of specific [³H]DHA binding.

Axis labels: Bound / Free DHA (vertical), Specific DHA Bound (fmol) (horizontal), Bmax

TABLE I

Effect of Alterations in Sodium Intake on Weight, Heart Rate, Blood Pressure, and Urine Sodium Excretion (Mean±SEM)

	Diet		
meq Na⁺/d	10	150	400
Urine Na⁺, *meq/24 h*	8.6±1.8	119.9±7.7	330.6±10.9
Weight, *kg*	79.7±2.0*	82.2±1.8	82.6±1.8
Heart rate supine, *bpm*	65±2	68±2	65±2
Heart rate upright, *bpm*	93±4‡	85±5	82±5
Increase in heart rate on standing, *bpm*	28±5*	15±3	17±3
Systolic BP supine, *mm Hg*	117±3‡	123±5	123±4
Systolic BP upright, *mm Hg*	110±3§	126±3	123±4
Mean blood pressure supine, *mm Hg*	78.8±2.3	80.5±2.2	81.4±3.9
Mean blood pressure upright, *mm Hg*	90.4±2.4§	95.2±1.9	92.3±2.4

Compared to 150 meq Na⁺/d.
* $P < 0.01$, ‡ $P < 0.05$, § $P < 0.001$.
bpm, beats per minute.
BP, blood pressure.

with supine epinephrine ($r = -0.348, P < .01$), upright epinephrine ($r = -0.349, P < .01$), supine norepinephrine ($r = -0.341, P < 0.02$), and upright norepinephrine ($r = -0.277, P < 0.05$). The correlations of B_{max} of DHA binding on lymphocytes with upright norepinephrine, supine epinephrine, and upright epinephrine were significant with or without the patients with pheochromocytoma.

The propranolol concentrations in subjects during treatment were 130±25.3 ng/ml. That these concentrations achieved beta blockade was shown by the reduction in the rise in pulse rate on standing (Table III) which was 21±3.0 beats/min before propranolol and 12.4±1.2 on propranolol therapy. There was also the expected fall in supine and upright pulse rate and in the rate pressure product (Table III). Urinary norepinephrine was significantly elevated 1 wk after starting propranolol therapy, whereas urinary epinephrine

was significantly increased in the 24 h after stopping propranolol treatment (Table IV).

Propranolol administration resulted in a 27% increase in lymphocyte beta receptor density (Table IV) after 1 wk of treatment. Similar changes were seen in polymorphonuclear beta receptor density, which increased 29% after 1 wk of propranolol treatment (Table IV). This increase persisted over the 4 wk of treatment and averaged 44% for both lymphocytes and polymorphonuclear cells. No relationship was found between propranolol concentrations and change in B_{max}. The dissociation constant of DHA binding did not change significantly during treatment being 1.78±0.36 nM in lymphocytes and 1.44±0.15 nM in polymorphonuclear cells prior to propranolol and 2.41±0.7 nM in lymphocytes and 3.16±1.12 nM in polymorphonuclear cells after 4 wk of propranolol.

The rise in beta receptor density (B_{max} of DHA bind-

TABLE II

Leukocyte Receptor Density, Catecholamine Levels, and Isoproterenol Sensitivity (I_{25}) on 10-, 150-, and 400-meq/d Sodium Diets (Mean±SEM)

Diet	10 meq/d	150 meq/d	400 meq/d
B_{max} lymphocytes, *fmol/mg protein*	29.1±4.4*	32.7±4.9	44.3±7.7
B_{max} polymorphonuclear cells, *fmol/mg protein*	37.0±3.6*	38.1±5.1*	54.3±4.7
Supine plasma norepinephrine, *pg/ml*	282±67	221±53	178±32
Upright plasma norepinephrine, *pg/ml*	613±162*	381±63	330±65
Supine plasma epinephrine, *pg/ml*	53±21*	25±7	15±6
Upright plasma epinephrine, *pg/ml*	73±36	33±7	25±9
24 h urinary norepinephrine, *μg/g creatinine*	24±2‡	21±1	18±2
24 h urinary epinephrine, *μg/g creatinine*	16.8±2.4	16.5±1.8*	12.3±1.3
I_{25}, *μg*	1.76±0.35*	1.64±0.32*	1.10±0.27

* $P < 0.05$. Differences refer to 400 meq diet.
‡ $P < 0.005$.

TABLE III
Weight, Blood Pressure, Heart Rate, and Rate Pressure Product (RPP) during and following Propranolol Treatment

	Propranolol 240 mg/d			Hours off propranolol				
	Pre	1 wk	4 wk	24 h	48	72	96	168
Weight, kg	74.1±3.2	74.7±3.2	75.1±3.4*	76.7±4.3	75.1±4.1	74.9±4.2	74.6±3.7	74.8±3.4
Mean blood pressure supine, mm Hg	83.1±2.6	79.4±2.4*	76.8±3.6‡	76.0±2.8*	75.4±3.2‡	79.2±3*	79.0±2.7	75.8±2.1§
Mean blood pressure upright, mm Hg	94.8±2.0	92.1±2.8	91.2±2.5	91.3±2.5	93.5±2.0	92.0±2.5	91.4±2.4	90.5±2.4*
Heart rate supine, bpm	65±3	56±3"	52±3"	61±3*	64±3	67±3	66±2	68±4
Heart rate upright, bpm	85±3	65±4§	65±2§	77±4	85±4	85±5	83±5	87±4
RPP supine	7,378±480	6,320±410‡	5,703±433"	6,958±455	6,206±424	7,716±493	7,272±226	7,644±424
Increase in RPP on standing	2,402±382	886±148"	1,630±294	1,641±238	2,786±234	2,260±351	2,000±406	2,154±217

* $P < 0.05$.
‡ $P < 0.02$.
" $P < 0.01$.
§ $P < 0.001$.
Compared with pretreatment values.
BPM, beats per minute.

ing) over the 4-wk period of propranolol treatment was correlated with the concentrations of plasma and urinary epinephrine and norepinephrine present before propranolol treatment was started. On polymorphonuclear cells there was a significant positive correlation between supine plasma norepinephrine ($r = 0.839$, $P < 0.001$; Fig. 2) epinephrine ($r = 0.735$, $P < 0.05$) measured before propranolol treatment and the increase in B_{max} of DHA binding on polymorphonuclear cells during the 4 wk of propranolol treatment. The rise in B_{max} on lymphocytes in response to propranolol treatment also correlated with initial supine plasma

norepinephrine ($r = 0.627$, $P < 0.05$) and 24 h urinary norepinephrine ($r = 0.735$, $P < 0.05$).

B_{max} following propranolol withdrawal is shown in Table IV. Receptor density had returned to base line or pretreatment levels by 48 h. At 24 h after withdrawal there was considerable variability in the degree of increase in B_{max} still present. This variation was related to the magnitude of the increase in B_{max} while on propranolol. There was a significant positive correlation between the B_{max} on polymorphonuclear cells at 24 h after propranolol withdrawal and the average B_{max} during propranolol treatment relative to the B_{max} before

TABLE IV
Leukocyte Receptor Density and Urinary Catecholamines prior to, during, and following Propranolol Treatment (Mean ±SEM)(Differences Refer to Pretreatment Levels)

|	Weeks on propranolol					Hours off propranolol				
	0	1	2	3	4	24	48	72	96	168
B_{max} lymphocytes, fmol/mg protein	34.4 ±2.7	43.6 ±3.0*	47.6 ±3.7‡	39.8 ±1.9§	46.4 ±2.5‡	38.0 ±4.1	33.3 ±4.2	39.5 ±2.5	32.5 ±2.6	35.7 ±2.6
B_{max} polymorphonuclear cells, fmol/mg protein	38.1 ±1.5	49.5 ±2.6‡	54.5 ±3.5‡	57.0 ±6.5*	59.9 ±6.1*	54.2 ±7.8	38.9 ±3.7	41.3 ±3.2	34 ±3.5	41.4 ±3.0
24 h urinary norepinephrine, μg/g creatinine	15.6 ±2.2	21.0 ±2.7*	15.4 ±1.8	17.8 ±2.3	20.3 ±4.0	21.7 ±4.7	17.8 ±3.5	23.2 ±6.6	19.3 ±4.7	19.8 ±3.7
24 h urinary epinephrine, μg/g creatinine	13.5 ±1.4	13.0 ±1.2	13.5 ±1.5	13.2 ±1.6	14.9 ±2.2	21.0 ±3.5§	16.0 ±3.1	20.2 ±5.8	16.8 ±3.8	19.6 ±3.8

* $P < 0.01$.
‡ $P < 0.005$.
§ $P < 0.05$.
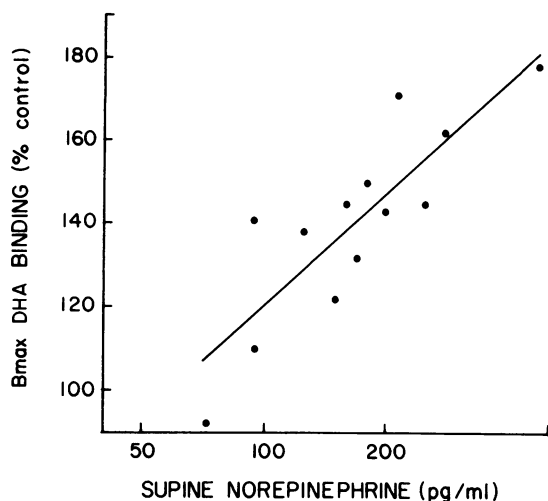
FIGURE 2 Mean beta receptor density on polymorphonuclear cells during the 4 wk of propranolol treatment (expressed as percentage of pretreatment density) related to pretreatment supine plasma norepinephrine level. ($r = 0.839$, $P < 0.001$).

FIGURE 3 Relationship between beta receptor density on lymphocytes and I_{25}. ($r = -0.573$, $P < 0.01$).

propranolol treatment ($r = 0.812$, $P < 0.005$). There was no evidence of overshoot or elevation of receptor density above pretreatment receptor density nor was there evidence of either heart rate or blood pressure elevation above pretreatment levels.

The cardiac sensitivity to isoproterenol was measured as the dose of isoproterenol required to raise the heart rate by 25 beats per minute on 10-, 150-, and 400-meq sodium diets and the results are shown in Table II. There was a significant increase in sensitivity to isoproterenol, that is a reduction in the dose of isoproterenol required to raise the heart rate by 25 beats per minute, when the subjects received 400-meq sodium diets compared with 150- and 10-meq diets. This increase in sensitivity to isoproterenol accompanied the increase in leukocyte beta receptor density on the high salt diet and resulted in a significant correlation between I_{25} and the B_{max} on lymphocytes (Fig. 3) and polymorphonuclear cells (Fig. 4).

DISCUSSION

Although leukocyte receptor density has been extensively studied the factors controlling receptor density and accounting for the large interindividual variation in receptor density have not been determined. We have shown that receptor density is reciprocally related to circulating catecholamine concentrations and also to 24-h urinary catecholamine levels, which reflect sympathetic function throughout the day. It is of interest to note that although the relationship existed for both epinephrine and norepinephrine it was stronger for epinephrine. This is
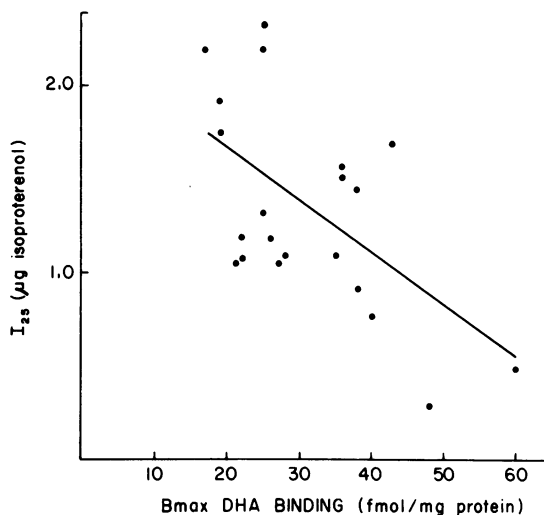
consistent with the greater affinity of epinephrine for the beta$_2$ receptor compared with norepinephrine. Lymphocytes as prepared here were a mixture of B and T lymphocytes and ~20% monocytes. Polymorphonuclear cells were more homogeneous but have a shorter life than lymphocytes. Results were similar with each cell type, except that B_{max} of DHA binding on polymorphonuclear cells although correlating with plasma catecholamines did not correlate with 24-h urinary catecholamines.

The relationship between catecholamines and leukocyte B_{max} therefore suggested that the levels of circulating catecholamines derived from their neuronal or adrenomedullary release down-regulate receptor den-
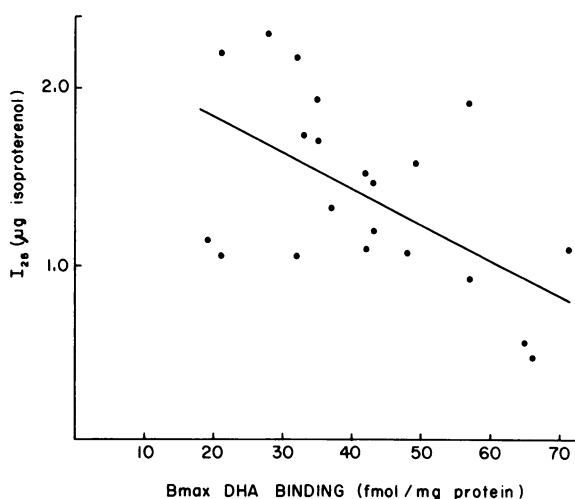

FIGURE 4 Relationship between beta receptor density on polymorphonuclear cells and I_{25}. ($r = -0.516$, $P < 0.02$).

sity in normal subjects so that the greater an individual's catecholamine levels, the greater would be the level of down-regulation produced. To test this hypothesis further we administered the beta adrenergic receptor blocker propranolol in an attempt to block the down-regulating effect of catecholamines. If the hypothesis that sympathetic tone normally down regulates B_{max} is correct then the greater the pretreatment catecholamine levels or sympathetic tone, the greater will be the degree of down regulation and hence, the greater will be the rise in receptor density during propranolol treatment. This was confirmed by the strong correlation between pretreatment catecholamine levels and rise in B_{max} on propranolol.

During the period of propranolol withdrawal return to pretreatment B_{max} had occurred by 48 h. Although the purpose of this study was not to investigate the so-called propranolol withdrawal syndrome it is interesting to note that after 4 wk of propranolol we did not observe the persistence of up regulation of B_{max} beyond disappearance of propranolol that others have seen (24) after shorter periods of propranolol treatment. Of particular interest was the demonstration that the greater the elevation in B_{max} produced by propranolol the higher the B_{max} at 24 h after propranolol withdrawal. In addition the patients with the highest pretreatment catecholamine levels had the greatest rise in B_{max}. It is possible, therefore, that a subgroup of patients exist, for example with impaired myocardial function and reduced ejection fraction, who have high levels of circulating catecholamines and hence experience an exaggerated increase in B_{max} on propranolol. Such patients might be especially vulnerable in the withdrawal period when their up-regulated beta receptors are exposed to their elevated circulating catecholamines.

These findings, therefore, suggest that plasma catecholamines regulate leukocyte adrenergic receptor number. The extent to which cardiac beta receptors mediating chronotropy are activated by direct sympathetic stimulation, as opposed to stimulation by circulating catecholamines is at present unknown. Because sympathetic activation and increased circulating norepinephrine and epinephrine are so closely correlated in our study, it is impossible to determine whether the altered isoproterenol sensitivity related more directly to synaptic or plasma levels of catecholamines. Nevertheless, there is a striking correlation between changes in leukocyte beta receptor numbers and isoproterenol sensitivity changes in a system at least partially dependent on synaptic catecholamine concentrations for activation.

Although leukocyte adrenergic receptors have been extensively studied in both man and animals (17) the assumption that changes in leukocyte receptor density reflects adrenergic function in other less accessible tissues has not been tested in man. By varying sodium intake within a group of individuals we were able to alter their catecholamines within the physiological range. This increase in sodium intake reduced catecholamine levels and by reducing down regulation allowed receptor density to increase with a resultant increase in sensitivity. The correlation between leukocyte beta receptor density and cardiac sensitivity to isoproterenol validates the use of this technique in studying adrenergic receptor function in man.

Although pharmacological doses of adrenergic agents have previously been shown to alter receptor function in man (12–16) this study demonstrates that changes in catecholamine levels within the physiological range are associated with alteration in receptor density and sensitivity to adrenergic agents.

By showing that B_{max} on leukocytes correlate with changes in cardiac responsiveness to isoproterenol, and by demonstrating that receptor density in vivo is controlled by catecholamine levels, we have confirmed the utility of measuring leukocyte receptor density. In the future it should be possible, by combining measurement of B_{max} and circulating agonist concentrations to define groups of patients with abnormal receptor regulation.

ACKNOWLEDGMENTS

REFERENCES

1. Lefkowitz, R. J., J. Roth, W. Pricer, and I. Pastan. 1970. ACTH receptors in the adrenal: specific binding of ACTH-^{125}I and its relation to adenyl cyclase. Proc. Natl. Acad. Sci. U. S. A. 65: 745–752.

2. Freychet, P., J. Roth, and D. M. Neville, Jr. 1971. Monoiodoinsulin: demonstration of its biological activity and binding to fat cells and liver membranes. Biochem. Biophys. Res. Commun. 43: 400–408.

3. Conolly, M. E., and J. K. Greenacre. 1977. The beta-adrenoceptor of the human lymphocyte and human lung parenchyma. Br. J. Pharmacol. 59: 17–23.

4. Lefkowitz, R. J., C. Mukherjee, M. Coverstone, and M. G. Caron. 1974. Stereospecific [^{3}H] (−)-alprenolol binding sites, beta-adrenergic receptors and adenylate cyclase. Biochem. Biophys. Res. Commun. 60: 703–709.

5. Aurbach, G. D., S. A. Fedak, C. J. Woodard, J. S. Palmer, D. Hauser, and F. Troxler. 1974. Beta-adrenergic receptor: stereospecific interaction of iodinated beta-blocking agent with high affinity site. Science (Wash. D. C.). 186: 1223–1224.

6. Lefkowitz, R. J., and L. T. Williams. 1977. Catecholamine binding to the beta-adrenergic receptor. Proc. Natl. Acad. Sci. U. S. A. 74: 515–519.

7. Williams, L. T., R. Snyderman, and R. J. Lefkowitz. 1976. Identification of beta-adrenergic receptors in human lymphocytes by (−) [^{3}H]alprenolol binding. J. Clin. Invest. 57: 149–155.

8. Galant, S.P., S. Underwood, L. Duriseti, and P. A. Insel. 1978. Characterization of high affinity beta$_2$-adrenergic

receptor binding of (−) [³H]-dihydroalprenolol to human polymorphonuclear cell particulates. *J. Lab. Clin. Med.* **92:** 613–618.

9. Newman, K. D., L. T. Williams, N. H. Bishopric, and R. J. Lefkowitz. 1978. Identification of alpha-adrenergic receptors in human platelets by [³H]dihydroergocryptine binding. *J. Clin. Invest.* **61:** 395–402.

10. Mukherjee, C., M. G. Caron, and R. J. Lefkowitz. 1975. Catecholamine induced subsensitivity of adenylate cyclase associated with loss of beta-adrenergic receptor binding sites. *Proc. Natl. Acad. Sci. U. S. A.* **72:** 1945–1949.

11. Johnson, G. L., B. B. Wolfe, T. K. Harden, P. B. Molinoff, and J. P. Perkins. 1978. Role of beta-adrenergic receptors in catecholamine-induced desensitization of adenylate cyclase in human astrocytoma cells. *J. Biol. Chem.* **253:** 1472–1480.

12. Morris, H. G., S. A. Rusnak, J. C. Selner, K. Barzens, and J. Barnes. 1977. Adrenergic desensitization in leukocytes of normal and asthmatic subjects. *J. Cyclic Nucleotide Res.* **3:** 439–446.

13. Conolly, M. E., and J. K. Greenacre. 1976. The lymphocyte beta-adrenoceptor in normal subjects and patients with bronchial asthma. *J. Clin. Invest.* **58:** 1307–1316.

14. Greenacre, J. K., P. Schofield, and M. E. Conolly. 1978. Desensitization of the beta-adrenoceptor of lymphocytes from normal subjects and asthmatic patients in vitro. *Br. J. Clin. Pharmacol.* **5:** 199–206.

15. Greenacre, J.K., and M. E. Conolly. 1978. Desensitization of the beta-adrenoceptor of lymphocytes from normal subjects and patients with pheochromocytoma: studies in vivo. *Br. J. Clin. Pharmacol.* **5:** 191–197.

16. Galant, S. P., L. Duriseti, S. Underwood, and P. A. Insel. 1978. Decreased beta-adrenergic receptors on polymorphonuclear leukocytes after adrenergic therapy. *N. Engl. J. Med.* **299:** 933–936.

17. Lefkowitz, R. J. 1979. Direct binding studies of adrenergic receptors: biochemical, physiologic and clinical implications. *Ann. Intern. Med.* **91:** 450–458.

18. Glaubiger, G., and R. J. Lefkowitz. 1977. Elevated beta-adrenergic receptor number after chronic propranolol treatment. *Biochem. Biophys. Res. Commun.* **78:** 720–725.

19. Schocken, D. D., and G. S. Roth. 1977. Reduced beta-adrenergic-receptor concentrations in aging man. *Nature (Lond.)* **267:** 856–858.

20. Vestal, R. E., A. J. J. Wood, and D. G. Shand. 1979. Reduced beta-adrenoceptor sensitivity in the elderly. *Clin. Pharmacol. Ther.* **26:** 181–186.

21. Shand, D. G., and A. J. J. Wood. 1978. Propranolol withdrawal syndrome—why? *Circulation.* **58:** 202–203.

22. Boudoulas, H., R. P. Lewis, R. E. Kates, and G. Dalamangas. 1977. Hypersensitivity to adrenergic stimulation after propranolol withdrawal in normal subjects. *Ann. Intern. Med.* **87:** 433–436.

23. Nattel, S., R. E. Rangno, and G. Van Loon. 1979. Mechanism of propranolol withdrawal phenomena. *Circulation.* **59:** 1158–1164.

24. Aarons, R. D., A. S. Nies, J. Gal, L. R. Hegstrand, and P. B. Molinoff. 1980. Elevation of beta-adrenergic receptor density in human lymphocytes after propranolol administration. *J. Clin. Invest.* **65:** 949–957.

25. Robertson, D., G. A. Johnson, R. M. Robertson, A. S. Nies, D. G. Shand, and J. A. Oates. 1979. Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. *Circulation.* **59:** 637–643.

26. Luft, F. C., L. I. Rankin, D. P. Henry, R. Bloch, C. E. Grim, A. E. Weyman, R. H. Murray, and M. H. Weinberger. 1979. Plasma and urinary norepinephrine values at extremes of sodium intake in normal man. *Hypertension.* **1:** 261–266.

27. Nicholls, M. G., W. Kiowski, A. J. Zweifler, S. Julius, M. A. Schork, and J. Greenhouse. 1980. Plasma norepinephrine variations with dietary sodium intake. *Hypertension.* **2:** 29–32.

28. Passon, P. G., and J. D. Peuler. 1973. A simplified radiometric assay for plasma norepinephrine and epinephrine. *Anal. Biochem.* **51:** 618–631.

29. Wood, A. J. J., K. Carr, R. E. Vestal, S. Belcher, G. R. Wilkinson, and D. G. Shand. 1978. Direct measurement of propranolol bioavailability during accumulation to steady-state. *Br. J. Clin. Pharmacol.* **6:** 345–350.

30. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand. J. Clin. Lab. Invest.* **21**(Suppl. 97): 77–89.

31. Steer, M. L., J. Khorana, and B. Galgoci. 1979. Quantitation and characterization of human platelet alpha-adrenergic receptors using [³H]phentolamine. *Mol. Pharmacol.* **16:** 719–728.

32. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51:** 660–672.

33. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.