Plasma Catecholamine Modulation of Alpha2 Adrenoreceptor Agonist Affinity and Sensitivity in Normotensive and Hypertensive Human Platelets

Alan S. Hollister, Jack Onrot, Suzanna Lonce, John H. J. Nadeau, and David Robertson
Departments of Medicine and Pharmacology, Division of Clinical Pharmacology, Vanderbilt University, Nashville, Tennessee 37232

Abstract

We measured α2-adrenoreceptor density as well as affinity for and sensitivity to agonist on intact platelets of normotensive and hypertensive subjects before and after physiological increases in plasma catecholamines. In normotensives, posture-induced rises in plasma catecholamines were accompanied by increased α2-adrenoreceptor agonist affinity and fewer high affinity state receptors. Platelet aggregation and inhibition of adenylate cyclase by 1-epinephrine was also reduced. Hypertensive subjects had similar rises in plasma catecholamines with upright posture, but showed no change in receptor affinity or sensitivity. No change in platelet α2-adrenoreceptor number occurred in the studies. In vitro incubation with 1-epinephrine revealed that platelets from hypertensives had slower desensitization than those from normotensives. Binding studies at different temperatures and with varying sodium concentrations found no thermodynamic or sodium-dependent differences between normotensive and hypertensive groups.

These studies demonstrate that platelets from hypertensive subjects exhibit a defect in the ability of physiological concentrations of agonist to desensitize the α2-adrenoreceptor.

Introduction

The influence of the sympathetic nervous system on blood pressure is mediated predominantly by the catecholamines norepinephrine and epinephrine acting at alpha and beta adrenoreceptors. Many prior investigations of the sympathetic nervous system in human hypertension have concentrated on attempts to detect elevated plasma catecholamines in hypertensive subjects. Although these studies varied greatly in design, the data do not support the hypothesis that catecholamine excess is the sole cause of hypertension (1, 2). However, recent studies have shown that hypertensive subjects have enhanced pressor responsiveness to catecholamine and sympathomimetic amine infusions (3–7). These findings are consistent with the proposal that vasoconstrictor alpha adrenoreceptors of hypertensive subjects are more sensitive to catecholamines than are those of normotensives.

Increased responsiveness of adrenergic receptors may be secondary to an increase in the number of receptors and/or an increase in receptor-effector coupling efficiency. An increase in platelet α2-adrenoreceptor density combined with pressor, cardioacceleratory and proaggregatory hypersensitivity to catecholamines has been reported in orthostatic hypotensive subjects with chronically low circulating catecholamines (8–10). However, several reports have found normal numbers of platelet α2-adrenoreceptors in hypertensive subjects (11–13), suggesting that the enhanced sensitivity of hypertensives to catecholamines is not associated with an increase in adrenoreceptor number.

We have demonstrated recently that plasma catecholamines inversely regulate platelet α2-adrenoreceptor and lymphocyte β2-adrenoreceptor affinity for and sensitivity to agonists over short time periods in normotensive subjects (14–16). Since enhanced pressor sensitivity of hypertensives cannot be attributed to increased α-adrenoreceptor density, we studied agonist regulation of α2-adrenoreceptor agonist affinity and sensitivity in order to determine whether abnormal affinity state regulation is associated with enhanced receptor sensitivity found in hypertension. We report here that hypertensives exhibit a defect in agonist-mediated desensitization of platelet α2-adrenoreceptors in vivo.

Methods

Materials. ([3H]methyl)homobine (sp. act. 75–90 Ci/mol, New England Nuclear, Boston, MA, or Amersham Corp., Arlington Heights, IL) was stored in ethanol under N2 at −20°C until use. L-Epinephrine bitartrate, adenosine-3',5'-cyclic monophosphate (cAMP), adenosine-5'-diphosphate (ADP), adenosine-5'-triphosphate (ATP), creatine phosphate and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co., Milwaukee, WI; Hepes from U. S. Biochemical Corp., Cleveland, OH; creatine kinase from Boehringer Mannheim GmbH, Federal Republic of Germany, α-[3H]adenosine-5'-triphosphate from New England Nuclear, and prostaglandin E1 (PGE1) from Upjohn Diagnostics, Kalamazoo, MI. Timolol was a gift from Merck, Sharpe and Dohme, West Point, PA.

Platelet isolation. Platelets were isolated from freshly drawn blood for radioligand binding studies as previously described (14). In binding studies on the effect of varying extracellular sodium concentrations, N-methyl-D-glucamine was substituted for the sodium in the wash solutions and incubation buffer. For aggregation studies, platelet-rich plasma was diluted with platelet-poor plasma to approximately 300,000 platelets/μl and aggregatory response to 1 × 10−4 to 1 × 10−8 M l-epinephrine and 3 × 10−5 to 3 × 10−7 M ADP was determined in a Payton dual channel aggregometer between 45 and 75 min after blood was drawn. Sensitivity


Address all correspondence to Dr. Hollister, Division of Clinical Pharmacology, Department of Medicine, Vanderbilt University, Nashville, Tennessee 37232.

Received for publication 19 April 1985 and in revised form 9 January 1986.

1. Abbreviations used in this paper: EC50, concentration of epinephrine producing half-maximal aggregation; CA, concentration of epinephrine producing half-maximal inhibition of prostaglandin E1-stimulated adenylate cyclase; ΔG°, Gibbs free energy change; GTP, guanosine triphosphate; ΔH°, enthalpy change; K<sub>a</sub>, equilibrium association constant; K<sub>d</sub>, equilibrium dissociation constant; ONS, overnight bedrest; PGE1, prostaglandin E1; PMSF, phenylmethylsulfonyl fluoride; R, gas constant; ΔS°, entropy change; T, temperature; 3 h Up, 3 hours of upright posture.
to ADP-induced aggregation was expressed as the concentration of ADP necessary to cause biphasic platelet aggregation (17). Sensitivity to L-epinephrine–induced aggregation was assessed by plotting the slope of the first phase of aggregation against the log of the L-epinephrine concentration from which the concentration of epinephrine producing half-maximal aggregation (EC50) was determined graphically. In vitro epinephrine desensitization experiments involved incubation of platelet-rich plasma from hypertensives and normotensives with vehicle, 1 × 10−6 M and 1 × 10−7 M L-epinephrine at 25°C without agitation. At 30, 60, and 120 min, aliquots were tested for aggregatory responsiveness to 1 × 10−5 M L-epinephrine and the slope of the first phase of aggregation was expressed as a percentage of the simultaneous vehicle-incubated sample.

**Binding assay.** Competition binding using 19 concentrations of L-epinephrine against 6–10 nM [3H]yohimbine was performed as described previously (14). Data were analyzed by the weighted, nonlinear, curve-fitting procedure based on the law of mass action described by DeLean et al. (18) for average agonist affinity, high and low agonist affinities (one and two binding site curve-fitting), the proportion of receptors in each affinity state, and the total number of receptors. Since the calculated affinity constants are log-normally distributed, mean affinities expressed in the text are the antilog of the mean log affinity constants. Statistical comparisons of affinity constants were made between means and standard deviations of the logarithmic transformations.

**Platelet adenylyl cyclase assay.** Adenylyl cyclase activity was measured in lysed platelet preparations by a modification of the method of Johnson (19). Washed platelets were pelleted on a 50% albumin cushion and resuspended in ice-cold 50 mM Hepes, pH 7.5, 4 mM dithiothreitol, 0.4 mM EGTA and 0.04 mM PMSF. Samples were frozen, thawed, and then homogenized twice in a Brinkmann Polytron at setting 5 for 20 s at 4°C. Adenylyl cyclase activity was determined in 10-min incubations at 37°C containing 50 mM Hepes, pH 7.5, 2 mM MgCl2, 0.1 mM cAMP, 12.5 mM ATP, 5 mM creatine phosphate, 40 μM creatine kinase, 0.1 M EDTA, 1 × 10−3 M ICI 118, 551, 710, 1 × 10−4 M prostaglandin E1, and 0–1 × 10−3 M L-epinephrine, containing 300,000 cpm [32P]ATP in 200 μl final volume. Reactions were stopped by the addition of 600 μl 120 mM Zn acetate plus 500 μl 144 mM Na2CO3, centrifuged, and 1-ml aliquots were placed over 1-ml alumina columns. [32P]cAMP was eluted with 4 ml 0.1 M Tris, pH 7.5, and counted by Cherenkov radiation. PyEl-stimulated adenylyl cyclase activity was linear with respect to amount of platelet lysate added and to time for at least 15 min and was inhibited 40–80% by L-epinephrine in a dose-related manner. Values are reported as the concentration of L-epinephrine necessary to half-maximally inhibit the PyEl-stimulated cyclase (EC50).

**Platelet catecholamine concentrations** were determined by a radioenzymatic assay as previously described (20).

**Subjects** for this study were normotensive and hypertensive males, 20–41 yr of age, who abstained from all medications for at least 2 wk before the study. All procedures in this study were reviewed and approved by the Vanderbilt University Committee for the Protection of Human Subjects. Subjects were admitted to the Elliot V. Newman Clinical Research Center and after 8 h of overnight bedrest (ONS) a supine blood sample was drawn for plasma catecholamine concentrations and platelet studies. The subjects then ambulated about the ward for 3 h prior to a second blood sample. Platelets isolated from each blood sample were divided into three parts for simultaneous determination of radioligand binding, platelet aggregation and adenylyl cyclase assay. Mean age, blood pressure, and heart rate for the subjects are shown in Table I.

### Results

**Effect of ONS and 3 h of upright posture (3 h Up) on plasma catecholamine concentrations and average platelet α2-adrenoceptor agonist affinity.** In comparison with ONS, 3 h Up produced a 135% increase in plasma norepinephrine (P < 0.01) and a 70% rise in plasma epinephrine in normotensive subjects (Table II). Similarly, hypertensives showed increases of 113% in plasma norepinephrine (P < 0.01) and 37% in plasma epinephrine after 3 h Up. There were no significant differences between normotensives and hypertensives in plasma catecholamine concentrations in either posture.

### Table I. Age, Resting Blood Pressure and Heart Rate of Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Blood Pressure</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives (10)</td>
<td>29±3</td>
<td>112±4</td>
<td>69±4</td>
</tr>
<tr>
<td>Hypertensives (16)</td>
<td>31±3</td>
<td>136±3*</td>
<td>75±3</td>
</tr>
</tbody>
</table>

Mean±SEM. * P < 0.001 vs. normotensives.

### Table II. Plasma Catecholamines

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Norepinephrine (ng/liter)</th>
<th>Epinephrine (ng/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives (10)</td>
<td>233±37</td>
<td>40±10</td>
</tr>
<tr>
<td>3 h Up</td>
<td>548±57*</td>
<td>68±11</td>
</tr>
<tr>
<td>Hypertensives (16)</td>
<td>292±25</td>
<td>52±10</td>
</tr>
<tr>
<td>3 h Up</td>
<td>622±79*</td>
<td>71±10</td>
</tr>
</tbody>
</table>

* P < 0.001 vs. ONS.
The mean affinity constant for the high affinity state was not significantly different in hypertensives vs. normotensives (0.37±0.09 μM vs. 0.28±0.08 μM, respectively). Similarly, there was no significant difference in the low affinity state equilibrium dissociation constant (Kd) (9.1±4.9 μM vs. 8.9±4.1 μM, respectively). There were no differences in the number of receptor sites per platelet between groups (hypertensives, 210±17; normotensives, 183±11).

Composite competition binding curves generated by computer from the high and low affinity Kd’s (Fig. 3 A) demonstrate a shift to the right in the binding displacement by agonist when the 3 h Up platelet samples of normotensive subjects are compared with ONS. Platelets of hypertensive subjects exhibited a nonsignificant shift to the left over the same time period. In normotensive subjects, the mean proportion of receptors in the high affinity state for agonist fell from 71 to 38% after 3 h Up (P < 0.001) (Fig. 3 B). In contrast, hypertensive subjects exhibited no change in the proportion of high affinity state receptors despite similar increases in circulating plasma catecholamines. After 3 h Up, platelets from hypertensives had a significantly higher proportion of their receptors in the high affinity state than did normotensives (P < 0.001).

The threshold for biphasic aggregation stimulated by ADP was 2.2±0.3 μM for normotensives and 2.8±0.4 μM for hypertensives. These did not change significantly in either group after 3 h Up (2.3±0.3 μM and 2.9±0.6 μM, respectively). Thus, the change in aggregatory sensitivity to epinephrine in normal subjects did not generalize to another type of receptor.

Inhibition of PGE1-stimulated platelet membrane adenylate cyclase activity by L-epinephrine. Another measure of platelet α2-adrenoceptor sensitivity is the ability of L-epinephrine to inhibit adenylate cyclase. Theoretically, a decrease in receptor affinity for agonist should result in a shift to the right of the dose-response curve for α2-adrenoceptor–mediated inhibition of the cyclase. Using the same platelet samples obtained after

**Figure 1.** Competition radioligand binding of L-epinephrine for [3H]yohimbine-labeled α2-adrenoreceptors on intact platelets after ONS (solid lines) and 3 h Up (dashed lines). (A) Normotensive subject. (B) Hypertensive subject. Lines are constructed from the weighted, nonlinear curve-fitting parameters calculated from each data set.

**Figure 2.** Platelet α2-adrenoreceptor affinity for agonist plotted against plasma norepinephrine concentration. (A) Normotensives. (B) Hypertensives. Affinity for agonist and plasma norepinephrine were measured after 8 h ONS and again after 3 h Up. Each line connects the two determinations in a single subject.

**Table III.** EC50 for Epinephrine-induced Aggregation

<table>
<thead>
<tr>
<th>Subjects(n)</th>
<th>ONS</th>
<th>3 h Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives (10)</td>
<td>1.2±0.2</td>
<td>2.1±0.4*</td>
</tr>
<tr>
<td>Hypertensives (10)</td>
<td>1.1±0.2</td>
<td>1.0±0.1‡</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. ONS.
‡ P < 0.02 vs. normotensives 3 h Up.
Table IV. Platelet Adenylate Cyclase Inhibition:
L-Epinephrine Concentration for Half-Maximal Inhibition

<table>
<thead>
<tr>
<th></th>
<th>ONS 3 h Up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM</td>
</tr>
<tr>
<td>Normotensives</td>
<td>0.54±0.10</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>0.61±0.08</td>
</tr>
</tbody>
</table>

* P < 0.02 vs. ONS.
† P < 0.02 vs. normotensives 3 h Up.

ONS and 3 h Up from normotensive and hypertensive subjects, we determined the EC50 (Table IV).

After 3 h Up, platelets from normotensive subjects showed a significant increase in the EC50. In contrast, platelets from hypertensives exhibited no change in the ability of L-epinephrine to inhibit adenylate cyclase and, at the 3 h Up sampling time, were significantly more sensitive to L-epinephrine than normotensives' platelets. The magnitude and direction of changes in L-epinephrine-inhibited adenylate cyclase are consistent with the changes measured by the mean receptor affinity for L-epinephrine and by the EC50 for L-epinephrine-induced aggregation.

In vitro desensitization of normotensive's and hypertensive's platelet α2-adrenoceptors. In order to examine more closely the differences in agonist desensitization of platelet α2-adrenoceptors between normotensives and hypertensives, we measured the effect of in vitro exposure of platelets to L-epinephrine on L-epinephrine–stimulated aggregation (Table V). Platelet-rich plasma from normotensive and hypertensive subjects was incubated at 25°C without agitation with 1 × 10⁻⁶ M or 1 × 10⁻⁷ M L-epinephrine or vehicle, then tested for aggregatory response to 10⁻⁵ M L-epinephrine. When expressed as a percentage of the vehicle-incubated response, in vitro incubation with L-epinephrine resulted in a significant time- and concentration-dependent loss in aggregatory response to 10⁻⁵ M L-epinephrine in platelet samples obtained from normotensive and hypertensive subjects (pooled χ² test; time, χ² = 63.9, d.f. = 4, P < 0.001; concentration, χ² = 116.8, d.f. = 4, P < 0.001). However, platelets from hypertensive subjects showed significantly less desensitization after L-epinephrine exposure than did normotensives (pooled χ² = 14.0, d.f. = 4, P < 0.01). At high L-epinephrine concentrations, this difference was most apparent at the 30-min time point, whereas after 10⁻⁷ M L-epinephrine incubations the differences persisted throughout the 2 h of incubation. Thus, in vitro incubation with supraph ysiological concentrations of α2-adrenergic agonist also demonstrates a deficiency in agonist-mediated receptor desensitization in platelets from hypertensives.

Thermodynamic analysis of agonist and antagonist binding to platelet α2-adrenoceptors of normotensive and hypertensive subjects. If the differences between normotensive and hypertensive in agonist-mediated α2-adrenoceptor desensitization are due to either altered recognition of agonist or an abnormality in the receptor-G-protein complex, analysis of agonist and antagonist binding at different temperatures may differentiate between subject groups. We determined the platelet α2-adrenoceptor affinity constants at 15°C, 25°C and 37°C for L-epinephrine and yohimbine for five hypertensive and five normotensive subjects. The Gibbs free energy change (ΔG*) was calculated from the mean receptor affinity for L-epinephrine and by the EC50 for L-epinephrine-induced aggregation.

In Table VI, the equilibrium thermodynamic parameters derived from normotensive and hypertensive subjects' platelet α2-adrenoceptor agonist and antagonist binding.

These data indicate that the thermodynamic driving forces for agonist binding are the ΔH*, which is opposed by thermodynamically unfavorable decreases in entropy. In contrast, antagonist binding is driven by entropy. The absence of a large difference in thermodynamic driving forces provides evidence against the existence of marked differences in receptor-ligand and/or receptor-G-protein interaction between platelet α2-adrenoceptors from normotensive and hypertensive subjects.

Influence of sodium concentration on the agonist affinity of platelet α2-adrenoceptors. Platelet α2-adrenoceptor affinity for agonist has been shown to be inversely proportional to the sodium concentration (21, 22). Since the effect of sodium appears to be exerted on the internal side of the membrane (22) and hypertensive subjects have been shown to exhibit altered membrane sodium fluxes (23, 24), we examined the influence of varying sodium concentration on the agonist-mediated receptor desensitization in platelets from hypertensives.

Table V. Effect of L-Epinephrine Incubation on Aggregatory Response to L-Epinephrine

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Epinephrine concentration</th>
<th>Percentage of vehicle response incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10⁻⁶ M</td>
<td>30</td>
</tr>
<tr>
<td>Normotensives</td>
<td></td>
<td>55±2</td>
</tr>
<tr>
<td>Hypertensives</td>
<td></td>
<td>77±7</td>
</tr>
<tr>
<td>Normotensives</td>
<td>10⁻⁷ M</td>
<td>86±2</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>10⁻⁷ M</td>
<td>96±6</td>
</tr>
</tbody>
</table>

Table VI. Equilibrium Thermodynamic Parameters for Agonist and Antagonist Binding to Intact Platelets from Normotensives and Hypertensives

<table>
<thead>
<tr>
<th></th>
<th>ΔG*</th>
<th>ΔH*</th>
<th>ΔS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist (epinephrine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antagonist (yohimbine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensives</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

α2 Receptor Affinity in Hypertension

These studies demonstrate that, in normotensive subjects, physiologic increases in plasma catecholamines acutely regulate platelet α2-adrenoceptor affinity for and sensitivity to agonist.
These findings confirm our prior work (14) and extend it to the identification of a loss of high affinity state receptors as the source of the mean affinity change. Since no change in the total number of receptors occurred during the affinity change, we conclude that the loss of high affinity state receptors is most probably a result of the conversion of high affinity state receptors to low affinity state receptors. Although these data were collected in intact platelet binding studies, the results are consistent with the modulation of \( \alpha_2 \)-adrenoreceptor agonist affinity by guanine nucleotide regulatory protein and guanosine triphosphate (GTP), a model derived from platelet membrane studies (21, 22). The affinity change was accompanied by a loss of aggregatory sensitivity to agonist as well as the ability of agonist to inhibit adenylate cyclase. These data provide further evidence for the concept of the high affinity state of the \( \alpha_2 \)-adrenoreceptor being the "coupled" or sensitive state. In addition, since long-term exposure to adrenergic agonists results in a loss of adrenergic receptors in vivo (8, 25, 26), whereas short-term exposure results in mean affinity changes, our results support the principle of long-term regulation of sensitivity via receptor number changes and short-term regulation via affinity changes. Whether the molecular mechanisms for affinity regulation are identical to the model derived from membrane studies or are unique to the intact platelet was not determined by our studies. Nonetheless, both binding and sensitivity measurements indicate desensitization of intact platelet \( \alpha_2 \)-adrenoreceptors after in vivo or in vitro exposure to agonist.

In contrast to normotensives, hypertensive subjects did not exhibit changes in mean affinity, proportion of high affinity state receptors, or sensitivity to either epinephrine-induced aggregation or inhibition of adenylate cyclase after physiological increases in plasma catecholamines. This failure to desensitize after agonist exposure resulted in platelet \( \alpha_2 \)-adrenoreceptors of hypertensive subjects being significantly more sensitive than those of normotensive subjects after 3 h Up.

Similar findings of a defect in adrenoreceptor desensitization in hypertension have been observed in the \( \beta_2 \)-adrenoreceptor system of adipocytes and lymphocytes (27, 28). Lymphocyte \( \beta_2 \)-adrenoreceptors of supine hypertensives were less sensitive to agonist than those of normotensives and showed no alteration in agonist affinity after 3 h Up. In contrast, normotensives’ \( \beta_2 \)-adrenoreceptors desensitized with upright posture, ultimately to the same level of sensitivity as hypertensives. If the dynamics of adrenergic receptor sensitivity regulation of circulating blood elements can be generalized to vascular adrenoreceptors, these studies suggest a combined defect of reduced \( \beta_2 \)-adrenoreceptor vasodilatation and enhanced \( \alpha_2 \)-adrenoreceptor vasoconstriction in hypertension. This defect would be secondary to impaired agonist-mediated adrenoreceptor desensitization in hypertensive subjects. Direct studies of vascular receptor sensitivity are necessary to confirm these hypotheses.

Several membrane-limited mechanisms for altering agonist affinity of platelet \( \alpha_2 \)-adrenoreceptors have been demonstrated (21, 22, 24). In rabbit platelet membrane preparations, elevated GTP and sodium concentrations lower the affinity of platelet \( \alpha_2 \)-adrenoreceptors for agonists (29); GTP, by shifting high agonist affinity site binding to low affinity sites, and sodium by inducing a parallel rightward shift of the agonist binding without alterations in the proportion of receptors in the high affinity state. Using intact human platelets, our studies confirmed the shift in agonist binding affinity caused by increasing sodium concentrations and found no change in the proportion of receptors in the high affinity state in either normotensives or hypertensives. Because of the parallel shift in affinity and the lack of differences between normotensives and hypertensives, the possibility that an alteration in the \( \alpha_2 \)-adrenoreceptor "sodium site" occurs in hypertension is lessened.

Another potential cause for the defect in agonist-mediated \( \alpha_2 \)-adrenoreceptor desensitization in hypertensives is an alteration in the receptor and/or receptor-guanine nucleotide regulatory protein interaction. Changes in structure of these molecules or alterations in their binding to each other might have resulted in thermodynamic differences in agonist and antagonist binding between hypertensives and normotensives. However, similar to the results found in the \( \beta \)-adrenergic system (30, 31), we determined that agonist binding by the \( \alpha_2 \)-adrenoreceptor of intact platelets was temperature sensitive and driven by large decreases in enthalpy, whereas antagonist binding showed little change with temperature and was driven by positive changes in entropy. We found no difference in the thermodynamic driving forces for agonist and antagonist binding between platelet \( \alpha_2 \)-adrenoreceptors from these subjects when the platelet samples were drawn after ONS. These results are consistent with, but do not prove, the hypothesis that no gross structural change in \( \alpha_2 \)-adrenoreceptors and/or their associated guanine nucleotide regulatory protein differentiates hypertensives from normotensives.

These studies have decreased the possibility that either sodium concentration differences or receptor-G-protein changes underlie the defect in agonist-mediated platelet \( \alpha_2 \)-adrenoreceptor desensitization found in hypertensive subjects. Another potential site for this defect is an alteration in membrane “microviscosity” which may secondarily affect receptor-modulating mechanisms. An increased platelet membrane microviscosity has been described in spontaneously hypertensive rats and ascribed either to protein (32) or to fatty acid (33) constituents of the membrane. The fatty acid contents of platelet and red cell membranes appear to be genetically determined and associated with either a family history or the development of hypertension in rats and men (34, 35). Exactly how these membrane alterations interact with agonist-mediated adrenergic receptor desensitization and whether they may be the source of the defect identified in human essential hypertension remains to be elucidated.

In summary, in normotensive human subjects we have identified an agonist-induced reduction in platelet \( \alpha_2 \)-adrenoreceptor affinity for agonist which is characterized by a reduction in the proportion of receptors in the high affinity state and is correlated with a fall in receptor sensitivity. In contrast, hypertensive subjects failed to exhibit changes in \( \alpha_2 \)-adrenoreceptor agonist affinity or sensitivity when exposed to the same in vivo stimuli. There were no significant differences between normo-

---

**Figure 4.** Affinity of intact platelet \( \alpha_2 \)-adrenoreceptors for L-epinephrine in isotonic assay media containing varying sodium concentrations. Mean±SEM for five normotensive (solid line) and five hypertensive (dashed line) subjects.
tensives and hypertensives in the number of $\alpha_2$-adrenoceptors per platelet, the rise in plasma catecholamines after 3 h Up, the thermodynamic driving forces for agonist and antagonist binding and the influence of sodium on platelet $\alpha_2$-adrenoceptor agonist affinity. The precise molecular basis for the defect in agonist-mediated $\alpha_2$-adrenoceptor desensitization in hypertension remains to be identified. We conclude that essential hypertension is characterized by a defect in agonist-mediated platelet $\alpha_2$-adrenoceptor desensitization and postulate that this defect may be related to the hyperresponsiveness of hypertensives to adrenergic pressor agents.

Acknowledgments
The authors express their appreciation for the excellent technical assistance of Loretta Speier, Natalie Johnson and Oscar Safeek, to Dr. Roger Johnson for the adenylate cyclase assay methodology, and to Dr. Joel Hardman for advice and review of the manuscript.

Dr. Hollister is the recipient of a Burroughs-Wellcome Postdoctoral Fellowship and is the Clinical Associate Physician of the Eliot V. Newman Clinical Research Center, RF-00095, and additionally supported by HL 31419. Dr. Robertson is the recipient of a Research Career Development Award, GM-00494, and is supported by HL-14192 and GM-31304.

References