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Research Article

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Plasma Catecholamine Modulation of Alpha$_2$ Adrenoreceptor Agonist Affinity and Sensitivity in Normotensive and Hypertensive Human Platelets

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Abstract

We measured α_2-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 correlated with reduced α_2-adrenoreceptor agonist affinity and fewer high affinity state receptors. Platelet aggregation and inhibition of adenylate cyclase by L-epinephrine also was 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 α_2-adrenoreceptor number occurred in these studies. In vitro incubation with L-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 α_2-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 α_2-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 α_2-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 α_2-adrenoreceptor and lymphocyte β_2-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 α_2-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 α_2-adrenoreceptors in vivo.

Methods

Materials. ([^{3}H]methyl)yohimbine (sp. act. 75–90 Ci/mol, New England Nuclear, Boston, MA, or Amersham Corp., Arlington Heights, IL) was stored in ethanol under N_2 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)[1] 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-[^{32}P]adenosine-5′-triphosphate from New England Nuclear; and prostaglandin E_1 (PgE_1) 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

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1. *Abbreviations used in this paper:* EC_{50}, concentration of epinephrine producing half-maximal aggregation; $EC_{0.5}$, concentration of epinephrine producing half-maximal inhibition of prostaglandin E_1-stimulated adenylate cyclase; $\Delta G°$, Gibbs free energy change; GTP, guanosine triphosphate; $\Delta H°$, enthalpy change; K_a, equilibrium association constant; K_d, equilibrium dissociation constant; ONS, overnight bedrest; PgE_1, prostaglandin E_1; PMSF, phenylmethylsulfonyl fluoride; R, gas constant; $\Delta S°$, 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 (EC_{50}) 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 [^{3}H]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 adenylate cyclase assay. Adenylate 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, allowed to thaw, then homogenized twice in a Brinkmann Polytron at setting 5 for 20 s at 4°C. Adenylate cyclase activity was determined in 10-min incubations at 37°C containing 50 mM Hepes, pH 7.5, 2 mM $MgCl_2$, 0.1 mM cAMP, 12.5 μM ATP, 5 mM creatine phosphate, 40 mU creatine kinase, 1×10^{-5} M timolol, 1×10^{-6} M prostaglandin E_1, and $0–1 \times 10^{-3}$ M L-epinephrine, containing 300,000 cpm α-[^{32}P]ATP in 200 μl final volume. Reactions were stopped by the addition of 600 μl 120 mM Zn acetate plus 500 μl 144 mM Na_2CO_3, centrifuged, and 1-ml aliquots were placed over 1-ml alumina columns. [^{32}P]cAMP was eluted with 4 ml 0.1 M Tris, pH 7.5, and counted by Cherenkov radiation. PgE_1-stimulated adenylate 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 PgE_1-stimulated cyclase ($EC_{0.5}$).

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

Subjects. 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 adenylate 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 α_2-adrenoreceptor 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 normal 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

Subjects (*n*)	Age	Blood pressure	Heart rate
Normotensives (10)	29±3	112±4 / 73±2	69±4
Hypertensives (16)	31±3	136±3* / 97±2*	75±3

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

Competition radioligand binding of L-epinephrine for [^{3}H]yohimbine-labeled α_2-adrenoreceptors on intact platelets after ONS and 3 h Up ambulation is illustrated for a normotensive (Fig. 1 *A*) and a hypertensive (Fig. 1 *B*) subject. After ONS, the platelets of both the normotensive and hypertensive subjects exhibited shallow competition binding curves for the agonist epinephrine. After 3 h Up however, the epinephrine competition binding curve for the normotensive subject was shifted to the right and was steeper. The hypertensive subject showed no change in agonist competition binding after 3 h Up.

In the group of normotensive subjects, the mean affinity of platelet α_2-adrenoreceptors for L-epinephrine fell from 0.93±0.15 μM at ONS to 1.84±0.38 μM ($P < 0.01$) after 3 h Up. In contrast, the hypertensive subjects as a group showed no overall change (ONS, 1.11±0.21 μM, vs. 3 h Up, 0.94±0.13 μM; not significant). At 3 h Up, platelet α_2-adrenoreceptors from hypertensive subjects exhibited a significantly higher affinity for agonist than those of normotensive subjects ($P < 0.05$). In normotensives, the change in circulating norepinephrine was significantly correlated with the fall in platelet α_2-adrenoreceptor affinity for the agonist, L-epinephrine ($n = 10$, $r = 0.86$, $P < 0.001$). Fig. 2 *A* depicts the relationship between plasma norepinephrine and the affinity of platelet α_2-adrenoreceptors for agonist in each subject after ONS and 3 h Up. In contrast, despite similar rises in plasma catecholamines in the hypertensive subjects, there was no consistent reduction in platelet α_2-adrenoreceptor affinity for agonist ($n = 16$, $r = -0.0040$, $P > 0.2$, Fig. 2 *B*).

Analysis of receptor high and low affinity state. Computerized weighted, curvefitting analysis of agonist competition binding revealed two agonist affinity states for the platelet α_2-adrenoreceptors in the ONS samples from normotensive and hypertensive

Table II. Plasma Catecholamines

Subjects (*n*)	Norepinephrine	Epinephrine
	ng/liter	*ng/liter*
Normotensives (10)		
ONS	233±37	40±10
3 h Up	548±57*	68±11
Hypertensives (16)		
ONS	292±25	52±10
3 h Up	622±79*	71±10

* $P < 0.001$ vs. ONS.

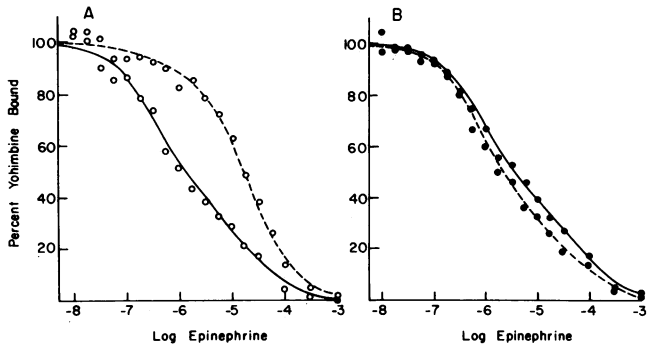

Figure 1. Competition radioligand binding of L-epinephrine for [^{3}H]yohimbine-labeled α_2-adrenoreceptors on intact platelets after ONS (solid lines) and 3 h Up (dashed lines). (*A*) Normotensive subject. (*B*) Hypertensive subject. Lines are constructed from the weighted, nonlinear curve-fitting parameters calculated from each data set.

subjects. 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 (K_d) (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 K_d'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 ($P < 0.001$).

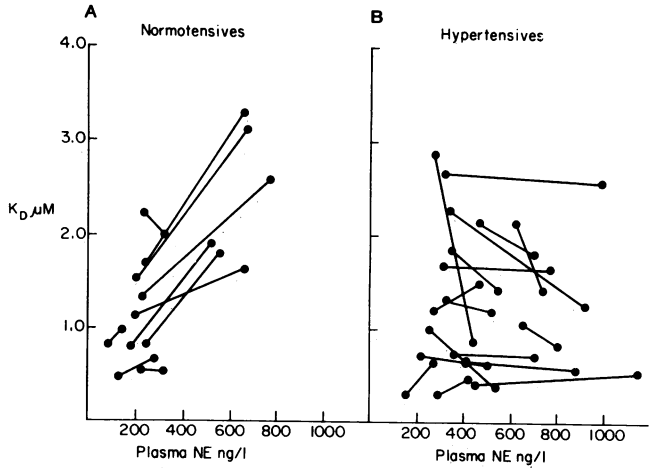

Figure 2. Platelet α_2-adrenoreceptor affinity for agonist plotted against plasma norepinephrine concentration. (*A*) Normotensives. (*B*) Hypertensives. Affinity for agonist and plasma norepinephrine were measured after 8 h ONS and again after 3 h Up. Each line connects the two determinations in a single subject.

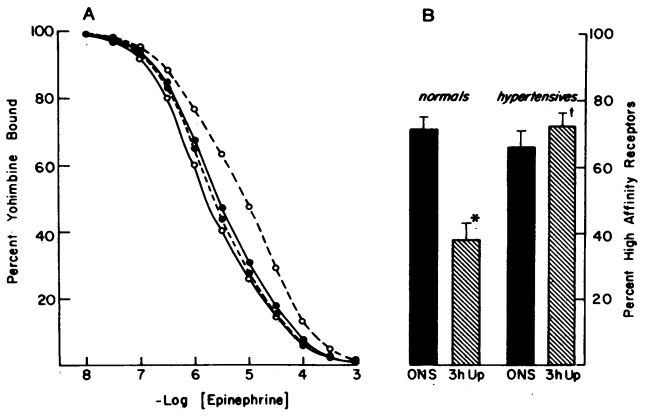

Figure 3. (*A*) Composite, computer-generated competition binding curves for L-epinephrine displacement of [^{3}H]yohimbine in normotensive subjects (open circles) and hypertensive subjects (closed circle) after ONS (solid lines) and 3 h Up (dashed lines). (*B*) Percentage of platelet α_2-adrenoreceptors exhibiting high affinity binding of agonist after ONS (solid bars) and after 3 h Up (striped bars). Normotensive subjects, $n = 10$; hypertensives, $n = 16$. *$P < 0.001$ vs. ONS; †$P < 0.001$ vs. normotensives 3 h Up.

Aggregatory sensitivity to L-epinephrine and ADP. In order to determine whether the failure of hypertensives' platelet α_2-adrenoreceptors to reduce affinity after agonist exposure was reflected in sensitivity changes, the same platelet samples obtained after ONS and 3 h Up were tested for in vitro aggregatory sensitivity to L-epinephrine and ADP. The EC_{50}'s from L-epinephrine dose-response curves at each time point are found in Table III. Aggregatory sensitivity of platelets to L-epinephrine decreased significantly in normotensives after 3 h Up ($P < 0.05$), while hypertensives did not. After 3 h Up, hypertensives' platelets were significantly more sensitive to L-epinephrine–induced aggregation than were normotensives ($P < 0.02$). These findings agree well by regression analysis with the changes in agonist affinity found in simultaneous binding experiments ($r = 0.70$, $P < 0.01$).

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 PgE_1-stimulated platelet membrane adenylate cyclase activity by L-epinephrine. Another measure of platelet α_2-adrenoreceptor 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 α_2-adrenoreceptor–mediated inhibition of the cyclase. Using the same platelet samples obtained after

Table III. EC_{50} for Epinephrine-induced Aggregation

Subjects (*n*)	ONS	3 h Up
	μM	μM
Normotensives (10)	1.2±0.2	2.1±0.4*
Hypertensives (10)	1.1±0.2	1.0±0.1‡

* $P < 0.05$ vs. ONS.
‡ $P < 0.02$ vs. normotensives 3 h Up.

Table IV. Platelet Adenylate Cyclase Inhibition: L-Epinephrine Concentration for Half-Maximal Inhibition

	ONS	3 h Up
	μM	*μM*
Normotensives	0.54±0.10	1.03±0.17*
Hypertensives	0.61±0.08	0.52±0.09‡

* $P < 0.02$ vs. ONS.
‡ $P < 0.02$ vs. normotensives 3 h Up.

ONS and 3 h Up from normotensive and hypertensive subjects, we determined the $EC_{0.5}$ (Table IV).

After 3 h Up, platelets from normotensive subjects showed a significant increase in the $EC_{0.5}$. 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 EC_{50} for L-epinephrine-induced aggregation.

In vitro desensitization of normotensive's and hypertensive's platelet α_2-adrenoreceptors. In order to examine more closely the differences in agonist desensitization of platelet α_2-adrenoreceptors 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 χ^2 test; time, $\chi^2 = 63.9$, d.f. = 4, $P < 0.001$; concentration, $\chi^2 = 116.8$, d.f. = 4, $P < 0.001$). However, platelets from hypertensive subjects showed significantly less desensitization after L-epinephrine exposure than did normotensives (pooled $\chi^2 = 14.0$, 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 supraphysiological concentrations of α_2-adrenergic agonist also demonstrates a deficiency in agonist-mediated receptor desensitization in platelets from hypertensives.

Thermodynamic analysis of agonist and antagonist binding to platelet α_2-adrenoreceptors of normotensive and hypertensive subjects. If the differences between normotensives and hypertensives in agonist-mediated α_2-adrenoreceptor desensitization

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

Subjects	Epinephrine concentration	Percentage of vehicle response incubation time (min) 30	60	120
Normotensives	10^{-6} M	55±2	52±2	28±2
Hypertensives	10^{-6} M	77±7	59±2	30±1
Normotensives	10^{-7} M	86±2	76±3	59±2
Hypertensives	10^{-7} M	96±6	89±4	67±4

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

	ΔG°	ΔH°	ΔS°
	kcal/mol	*kcal/mol*	*cal/mol-deg*
Agonist (epinephrine)			
Normotensives	−8.45	−17.5	−30.4
Hypertensives	−8.49	−17.9	−31.7
Antagonist (yohimbine)			
Normotensives	−11.3	+.994	+41.3
Hypertensives	−11.1	+.568	+39.1

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 α_2-adrenoreceptor affinity constants at 15°C, 25°C and 37°C for L-epinephrine and yohimbine of five hypertensive and five normotensive subjects. The Gibbs free energy change (ΔG°) was calculated from the equation, $\Delta G° = -RT \ln K_a$, and a Van't Hoff plot of 1/T vs. ln K_a yielded a slope of (ΔH°)/R. The entropic change was then calculated from the equation; $\Delta G° = \Delta H° - T\Delta S°$. Table VI shows the thermodynamic parameters derived from normotensive and hypertensive subjects' platelet α_2-adrenoreceptor 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 α_2-adrenoreceptors from normotensive and hypertensive subjects.

Influence of sodium concentration on the agonist affinity of platelet α_2-adrenoreceptors. Platelet α_2-adrenoreceptor 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 hypertensives' and normotensives' platelet α_2-adrenoreceptor affinity for agonist (Fig. 4).

Lower assay sodium concentrations produced higher mean affinity constants for intact platelet α_2-adrenoreceptor agonist binding without changing the proportion of receptors in the high affinity state (analysis of variance: $F_{3,21} = 539.0$, $P < 0.0005$). The platelets from five hypertensive subjects exhibited no significant difference in agonist binding affinity at any assay sodium concentration when compared with five normotensive subjects (analysis of variance: $F_{1,8} = 0.756$, $P > 0.5$). The proportion of receptors with high affinity for agonist did not differ between groups with mean±SEM of 75±6% at 1 mM Na^+, 79±4% at 10 mM Na^+, 78±5% at 30 mM Na^+ and 76±6% at 100 mM Na^+. These data reduce the possibility that an altered sodium "site" on the α_2-adrenoreceptor characterizes essential hypertension and is responsible for the defect in agonist-mediated desensitization.

Discussion

These studies demonstrate that, in normotensive subjects, physiological increases in plasma catecholamines acutely regulate platelet α_2-adrenoreceptor affinity for and sensitivity to agonist.

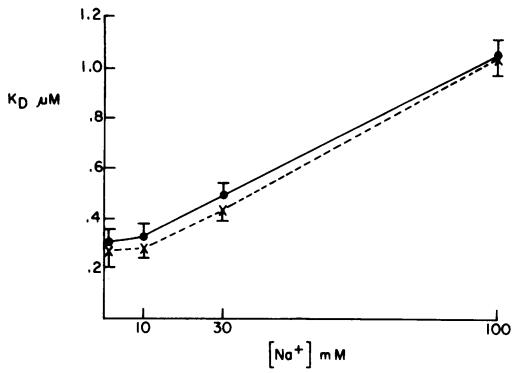

Figure 4. Affinity of intact platelet α_2-adrenoreceptors for L-epinephrine in isotonic assay media containing varying sodium concentrations. Mean±SEM for five normotensive (solid line) and five hypertensive (dashed line) subjects.

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 α_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 α_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 α_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 α_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 β_2-adrenoreceptor system of adipocytes and lymphocytes (27, 28). Lymphocyte β_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' β_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 β_2-adrenoreceptor vasodilatation and enhanced α_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 α_2-adrenoreceptors have been demonstrated (21, 22, 24). In rabbit platelet membrane preparations, elevated GTP and sodium concentrations lower the affinity of platelet α_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 α_2-adrenoreceptor "sodium site" occurs in hypertension is lessened.

Another potential cause for the defect in agonist-mediated α_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 β-adrenergic system (30, 31), we determined that agonist binding by the α_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 α_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 α_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 α_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 α_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 α_2-adrenoreceptor agonist affinity or sensitivity when exposed to the same in vivo stimuli. There were no significant differences between normo-

tensives and hypertensives in the number of α_2-adrenoreceptors 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 α_2-adrenoreceptor agonist affinity. The precise molecular basis for the defect in agonist-mediated α_2-adrenoreceptor desensitization in hypertension remains to be identified. We conclude that essential hypertension is characterized by a defect in agonist-mediated platelet α_2-adrenoreceptor desensitization and postulate that this defect may be related to the hyperresponsiveness of hypertensives to adrenergic pressor agents.

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References

1. Goldstein, D. S. 1981. Plasma norepinephrine in essential hypertension. *Hypertension (Dallas).* 3:48–52.

2. Robertson, D., D. G. Shand, J. W. Hollifield, A. S. Nies, J. C. Frolich, and J. A. Oates. 1979. Alterations in the response of the sympathetic nervous system and renin in borderline hypertension. *Hypertension (Dallas).* 1:118–124.

3. Doyle, A. E., J. R. E. Fraser, and R. J. Marshall. 1959. Reactivity of forearm vessels to vasoconstrictor substances in hypertensive and normotensive subjects. *Clin. Sci. (Lond.).* 18:441–454.

4. Vlachakis, N. D. 1979. Blood pressure response to norepinephrine infusion in relation to plasma catecholamines and renin activity in man. *J. Clin. Pharmacol.* 19:654–661.

5. Grimm, M., P. Weidmann, G. Keusch, A. Meier, and Z. Gluck. 1980. Norepinephrine clearance and pressor effect in normal and hypertensive man. *Klin. Wochenschr.* 58:1175–1181.

6. Weidmann, P., M. Grimm, A. Meier, Z. Gluck, G. Keusch, I. Minder, and C. Beretta-Piccoli. 1980. Pathogenic and therapeutic significance of cardiovascular pressor reactivity as related to plasma catecholamines in borderline and established essential hypertension. *Clin. Exp. Hypertens.* 2:427–449.

7. Goldstein, D. S. 1983. Arterial baroreflex sensitivity in essential hypertension. *Circulation.* 68:234–240.

8. Davies, I. B., D. Sudera, and P. S. Sever. 1981. Endogenous agonist regulation of α-adrenoreceptors in man. *Clin. Sci. (Lond.).* 61:207S–210S.

9. Robertson, D., A. S. Hollister, E. L. Carey, C. S. Tung, and M. R. Goldberg. 1983. Vascular β_2-adrenoreceptor hypersensitivity in autonomic dysfunction. *J. Am. Coll. Cardiol.* 3:850–856: II-6.

10. Robertson, D., M. R. Goldberg, A. S. Hollister, D. Wade, and R. M. Robertson. 1983. Clonidine raises blood pressure in severe idiopathic orthostatic hypotension. *Am. J. Med.* 74:193–200.

11. Motulsky, H. J., D. J. O'Connor, and P. A. Insel. 1983. Platelet α_2-adrenergic receptors in treated and untreated essential hypertension. *Clin. Sci. (Lond.).* 64:265–272.

12. Boon, N. A., J. M. Elliott, C. L. Davies, F. J. Conway, J. V. Jones, D. G. Grahame-Smith, and P. Sleight. 1983. Platelet α_2-adrenoreceptors in borderline and established essential hypertension. *Clin. Sci. (Lond.).* 65:207–208.

13. Hollister, A. S., J. Nadeau, S. Lonce, and D. Robertson. 1983. Abnormality in catecholamine regulation of platelet α_2-adrenoreceptor agonist affinity in essential hypertension. *Clin. Res.* 31:331A.

14. Hollister, A. S., G. A. FitzGerald, J. H. J. Nadeau, and D. Robertson. 1983. Acute reduction in human platelet α_2-adrenoreceptor affinity for agonist by endogenous and exogenous catecholamines. *J. Clin. Invest.* 72:1498–1505.

15. Feldman, R., L. E. Limbird, J. H. J. Nadeau, G. A. FitzGerald, D. Robertson, and A. J. J. Wood. 1983. Dynamic regulation of leukocyte beta adrenergic receptor-agonist interactions by physiological changes in circulating catecholamines. *J. Clin. Invest.* 72:164–170.

16. Hollister, A. S., G. A. FitzGerald, and D. Robertson. 1981. Reduction in platelet α_2-receptor agonist affinity by endogenous and exogenous catecholamines in man. *Clin. Res.* 29:819A.

17. Vlachakis, N. D., and L. Aledort. 1979. Platelet aggregation in relationship to plasma catecholamines in patients with hypertension. *Atherosclerosis.* 32:451–460.

18. DeLean, A., A. A. Hancock, and R. J. Lefkowitz. 1982. Validation and statistical analysis of a computer modeling method for quantitative analysis of radioligand binding data for mixtures of pharmacological receptor subtypes. *Mol. Pharmacol.* 21:5–16.

19. Awad, J. A., R. A. Johnson, K. H. Jakobs, and G. Schultz. 1983. Interactions of forskolin and adenylate cyclase. Effects on substrate kinetics and protection against inactivation by heat and *N*-ethylmaleimide. *J. Biol. Chem.* 258:2960–2965.

20. 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.

21. Limbird, L. E., J. L. Speck, and S. K. Smith. 1982. Sodium ion modulates agonist and antagonist interactions with the human platelet alpha$_2$-adrenergic receptor in membrane and solubilized preparations. *Mol. Pharmacol.* 21:609–617.

22. Motulsky, H. J., and P. A. Insel. 1983. Influence of sodium on the α_2-adrenergic receptor system of human platelets. *J. Biol. Chem.* 258:3913–3919.

23. Tosteson, D. C., N. Adragna, I. Bize, H. Solomon, and M. Canessa. 1981. Membranes, ions and hypertension. *Clin. Sci. (Lond.).* 61(Suppl.) 7:55–105.

24. Postnov, Y. V., and S. N. Orlov. 1984. Essential hypertension as a disorder of cellular membranes. *Kardiologiya.* 24:5–12.

25. Brodde, O.-E., M. Anlauf, N. Graben, and K. D. Bock. 1982. *In vitro* and *in vivo* down-regulation of human platelet α_2-adrenoceptors by clonidine. *Eur. J. Clin. Pharmacol.* 23:403–409.

26. Weiss, R. J., and C. B. Smith. 1983. Altered platelet alpha$_2$-adrenoceptor in patients with angina pectoris. *J. Am. Coll. Cardiol.* 2: 631–637.

27. Postnov, Y. V. 1975. Essential hypertension as a membrane pathology. *Kardiologiya.* 15:18–23.

28. Feldman, R. D., L. E. Limbird, J. Nadeau, D. Robertson, and A. J. J. Wood. 1984. Leukocyte β-receptor alterations in hypertensive subjects. *J. Clin. Invest.* 73:648–653.

29. Michel, T., B. B. Hoffman, and R. J. Lefkowitz. 1980. Differential regulation of the α_2-adrenergic receptor by Na^+ and guanine nucleotides. *Nature (Lond.).* 288:709–711.

30. Weiland, G. A., K. P. Minneman, and P. B. Molinoff. 1980. Thermodynamics of agonist and antagonist interactions with mammalian β-adrenergic receptors. *Mol. Pharmacol.* 18:341–347.

31. Pittman, R. N., and P. B. Molinoff. 1980. Interactions of agonists and antagonists with β-adrenergic receptors on intact L6 muscle cells. *J. Cyclic Nucleotide Res.* 6:421–435.

32. Aragon-Birlouez, I., T. Montenay-Garestier, and M. A. Devynck. 1984. Further analysis of cell membrane changes in genetic hypertension in rats by diphenylhexatriene fluorescence polarization. *Clin. Sci.* 66: 717–723.

33. McGregor, L., R. Morazain, and S. Renaud. 1981. Platelet functions and fatty acid composition of platelet phospholipids in spontaneously hypertensive rats fed saturated or polyunsaturated fats. *Atherosclerosis.* 38:129–136.

34. Bianchi, G., P. Ferrari, D. Trizio, M. Ferrandi, L. Torielli, B. R. Barber, and E. Polli. 1985. Red blood cell abnormalities and spontaneous hypertension in the rat. A genetically determined link. *Hypertension.* 7: 319–325.

35. Nara, Y., M. Kihara, T. Nabika, M. Mano, R. Horie, and Y. Yamori. 1984. Dietary effect on platelet aggregation in men with and without a family history of essential hypertension. *Hypertension.* 6:339–343.