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Research Article

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Acute Reduction in Human Platelet α_2-Adrenoreceptor Affinity for Agonist by Endogenous and Exogenous Catecholamines

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ABSTRACT The binding characteristics of *l*-epinephrine to intact human platelets were assessed under conditions of physiological and pharmacological variations in plasma catecholamine concentration. In competition with the α_2-adrenoreceptor antagonist yohimbine, mean platelet receptor affinity for *l*-epinephrine was decreased 3.4-fold after 2 h of upright posture and exercise. This change in agonist affinity correlated significantly with the increases in plasma epinephrine and norepinephrine that were stimulated by upright posture and exercise. Supine subjects infused with *l*-norepinephrine or *l*-epinephrine for 2 h also averaged a 3.3- and 2.7-fold decrease in platelet α_2-adrenoreceptor affinity for agonist with no change in receptor number or antagonist affinity. The α_2-adrenoreceptor agonist affinity changes were specific for α-agonists since they were blocked by phentolamine, and incubation with 10^{-5} M isoproterenol produced no change in α_2-adrenoreceptor affinity for *l*-epinephrine. In vitro exposure of intact human platelets to 10^{-6} to 10^{-10} M *l*-epinephrine for 2 h produced a concentration-related decrease in α_2-adrenoreceptor affinity for agonist. In all three paradigms, average slope factors approached 1.0 as affinity decreased, which is consistent with a heterogeneous receptor population that becomes more homogeneous after agonist exposure.

Incubation of platelet-rich plasma with 10^{-6} to 10^{-8} M *l*-epinephrine resulted in a dose- and time-related loss of aggregatory response to *l*-epinephrine; this demonstrates that agonist affinity changes are correlated with changes in receptor sensitivity.

These observations demonstrate that physiological variations in plasma catecholamines acutely modulate the intact human platelet α_2-adrenoreceptor's affinity for agonist, and can thereby alter the sensitivity of platelets to α_2-adrenergic agonist.

INTRODUCTION

Epinephrine acts via α_2-adrenoreceptors to initiate human platelet aggregation and secretion, and to inhibit platelet adenylate cyclase activity (1–5). Recently, the selective antagonist radioligand, [^{3}H]yohimbine, has been used to characterize α_2-adrenoreceptors in both intact and membrane preparations of human platelets (6–10). These studies have confirmed the α_2-adrenergic classification of these platelet receptors. Further studies have identified influences of sodium and magnesium on α_2-adrenoreceptor agonist binding affinity and have demonstrated high and low agonist affinity states of the receptor, which are modulated by guanine nucleotides (11–15).

Exposure of platelets to high concentrations of catecholamines in vitro has been reported to decrease the number of assayable platelet α_2-adrenoreceptors, either through a reduction in receptor number or by retention of agonist in the preparation (16, 17). Ex-

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posure to catecholamines at concentrations below 10^{-5} M has not been found to alter the number of platelet α_2-adrenoreceptors (18, 19). In vivo studies in which catecholamine concentrations range from 10^{-7} to 10^{-10} M have yielded contradictory results. It has been reported that patients with pheochromocytoma have low or normal numbers of receptors, and patients with idiopathic orthostatic hypotension have high or normal receptor density (20–22). No assessment of platelet α_2-adrenoreceptor agonist affinity has been reported under conditions of high or low plasma catecholamines.

Recent work in our laboratory has demonstrated that variations of plasma catecholamines within the physiological range are inversely related to β-adrenoreceptor density on human lymphocytes (23, 24). In chronic situations, receptor density was inversely correlated with plasma catecholamine concentration. In addition, we have recently described alterations in lymphocyte β-receptor affinity for agonist by short-term variations in plasma catecholamines (25). This has been associated with parallel changes in the ability of isoproterenol to stimulate lymphocyte adenylate cyclase activity. Thus, changes in plasma catecholamines within the physiological range appear to modulate β-receptor number, agonist affinity, and sensitivity.

Our purpose in this study was to investigate the effects of physiological changes in plasma catecholamines on α_2-adrenoreceptor number and agonist affinity in intact human platelets. We report here that short-term variations in plasma catecholamines within the physiological range modulate the platelet α_2-adrenoreceptor's affinity for and sensitivity to agonist without changes in receptor number.

METHODS

Materials. [^{3}H-Methyl]yohimbine (sp. act. 80–90 Ci/mmol, New England Nuclear, Boston, MA) was stored in ethanol under N_2 at −20°C until use. Phentolamine hydrochloride (Ciba-Geigy Corp., Summit, NJ), clonidine hydrochloride (Boehringer Ingelheim Ltd., Elmsford, NY), *l*-propranolol hydrochloride (Ayerst Laboratories, New York), prazosin hydrochloride (Pfizer, Inc., Groton, CT), *d*-epinephrine bitartrate, and *d*-norepinephrine bitartrate (Winthrop Laboratories, Rensselaer, NY) were gifts.

l-Epinephrine bitartrate, *l*-norepinephrine bitartrate, *l*-isoproterenol bitartrate, and *dl*-normetanephrine hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO; yohimbine hydrochloride from Aldrich Chemical Co., Inc., Milwaukee, WI, and rauwolscine hydrochloride from Carl Roth, Karlsruhe, Federal Republic of Germany.

Platelet isolation. Blood was drawn by using the double-syringe technique into 9:1 (vol/vol) 3.8% trisodium citrate or acid-citrate-dextrose (8 g citric acid, 22 g trisodium citrate, and 25 g dextrose per liter) and centrifuged at 200 *g* at room temperature for 14 min. The upper three-fourths of the platelet-rich plasma (PRP)[1] was placed over a 50% bovine serum albumin cushion (26) and then, the platelets were pelleted by centrifugation at 1,000 *g* at room temperature for 12 min. The platelets were gently resuspended in 12.7 mM citrate-100 mM NaCl, pH 6.7, recentrifuged over an albumin cushion, and resuspended in pH 7.5 buffer that consisted of 50 mM Tris, 100 mM NaCl, 0.5 mM Na_2 EDTA, 8 mM $MgCl_2$, and 0.8 mM ascorbic acid. Platelet yield was 85–95% of the platelets in PRP, and platelet size distribution was not altered by this preparative technique. Platelet counts were performed on a model ZBI Coulter Counter and size distribution analysis determined with a Coulter Channelyzer II (Coulter Electronics, Inc., Hialeah, FL).

In some experiments, the platelets resuspended in buffer were incubated for 2 h with varying cncentrations of *l*-epinephrine. The incubations were terminated by 15-fold dilution with citrate-saline and platelets were pelleted onto an albumin cushion as above. The platelets were washed by resuspension and recentrifugation in the citrate-saline solution, and then resuspended in the Tris-saline-EDTA-$MgCl_2$ buffer. 80–95% of the platelets were recovered after these washing procedures with no change in size distribution. The incubation with epinephrine also did not affect the platelet yield when compared wth control incubations.

Binding assay. Freshly prepared platelets suspended in Tris-saline-EDTA-$MgCl_2$ buffer were incubated for 30 min in a total volume of 250 μl at 25°C with slow shaking, 6–10 nM of [^{3}H]yohimbine and varying concentrations of competing ligands. The incubation was terminated by dilution with 5 ml of buffer and rapid filtration at reduced pressure through Whatman GF/C glass filters (Whatman Laboratory Products, Inc., Clifton, NJ). The incubation tube was rinsed with two 5-ml aliquots of buffer and an additional two 5-ml washes of the filter were performed. The filters were air dried and counted in 5 ml of 2:1 toluene/Triton X-100 (scintillation grade, Research Products International Corp., Mt. Prospect, IL) containing 30 ml/liter Liquifluor (New England Nuclear) at 39–43% counting efficiency. Nonspecific binding was defined as the radioactivity bound in the presence of 10 μM of phentolamine and it represented 15–40% of the total bound [^{3}H]yohimbine. Nonspecific binding was not affected by the addition of *l*-epinephrine to assay tubes containing phentolamine. Total counts bound were 1–2% of the added radioactivity with filter blanks <0.2%.

Binding of [^{3}H]yohimbine to intact platelets was saturable and revealed a homogenous affinity of 4.1±0.9 nM (n = 41, mean±SD) with a number of platelet α_2-adrenoreceptors of 215±61 binding sites per platelet. Kinetic analysis of association and dissociation phases of binding revealed an association rate constant (k_1) of 0.016 $nM^{-1}min^{-1}$ and a dissociation rate constant (k_2) of 0.070 min^{-1} which resulted in a kinetically derived dissociation constant for [^{3}H]yohimbine of 4.4 nM. Binding of [^{3}H]yohimbine to intact platelets also was linear with platelet number from 20 × 10^6 to 160 × 10^6 platelets; the affinity constant is in good agreement with the potency of yohimbine in antagonizing *l*-epinephrine-induced platelet aggregation.

The ability of agonists to compete with [^{3}H]yohimbine binding to intact platelets was stereospecific and character-

[1] *Abbreviations used in this paper:* EC_{50}, concentration of competing ligand that dissociates 50% of the specifically bound [^{3}H]yohimbine; k_1, association rate constant; k_2, dissociation rate constant; PRP, platelet-rich plasma.

istic for α_2-adrenoreceptors with potency order of clonidine > *l*-epinephrine > *l*-norepinephrine > *d*-epinephrine > *d*-norepinephrine ≫ *l*-isoproterenol. Similarly, antagonists competed for [^{3}H]yohimbine binding in an order typical of α_2-adrenoreceptors: yohimbine = rauwolscine > phentolamine ≫ prazosin > propranolol. Hill coefficients for agonist competition binding ranged from 0.6 to 0.9, while antagonist binding Hill coefficients ranged from 0.9 to 1.1.

Calculations. The binding of [^{3}H]yohimbine to intact platelets was analyzed by the method of Scatchard (for equilibrium binding experiments) and by kinetic analysis using the first order rate equation for dissociation, $k_2 = \text{Ln}[RL_0]/[RL])/t$, where RL is ligand-receptor complex and RL_0 is receptor-ligand complex at equilibrium, and the second order equation for association kinetics $k_1 = (k_{obs} - k_2)/[\text{yohimbine}]$, where k_{obs} is the observed rate constant (30, 31). Epinephrine competition binding data analysis was performed by Logit or Hill transformation of data that was generated from 10-point competition curves determined in triplicate. Least square linear analysis of data between 19 and 81% specific binding determined the slope factor or "pseudo-Hill coefficient"; the abscissa intercept defined the concentration of competing ligand that dissociates 50% of the specifically bound [^{3}H]yohimbine (EC_{50}). Alternatively, competition binding data was analyzed by a nonlinear, weighted, curve-fitting computer program for determination of affinity constants and receptor number (32).

Aggregation. Aggregation was performed on PRP by the nephelometric technique of Born (27). Blood was drawn by the double-syringe technique into (9:1 vol) acid-citrate-dextrose and centrifuged in sealed tubes at 200 *g* for 20 min at room temperature. The PRP was aspirated and the remaining blood was centrifuged at 1,000 *g* for 10 min to obtain platelet-poor plasma. The PRP was incubated with and without various concentrations of *l*-epinephrine with no agitation at 25°C. Aliquots were removed at 30, 60, and 120 min and aggregatory response to *l*-epinephrine was determined simultaneously in epinephrine-incubated and control samples. Aggregatory response was determined graphically from the slope of the first phase of epinephrine-induced aggregation and normalized by dividing the epinephrine-incubated sample response by the control-incubated response. Variability in aggregatory response was minimized by using sealed siliconized tubes and syringes, by using each PRP sample as its own control, and by performing simultaneous control and *l*-epinephrine-treated aggregations. Values were tested by the pooled Chi-square test by using $P < 0.01$ as indication of a significant difference (28).

Plasma catecholamine determinations were performed by a radioenzymatic assay as previously described (29).

The Vanderbilt University Committee for the Protection of Human Subjects reviewed and approved the procedures in this study. Human subjects abstained from all medications for 14 d before these studies. All were normotensive healthy males between 20 and 38 yr of age. Posture/exercise and infusion study protocols were carried out on the Elliot V. Newman Clinical Research Center Ward.

RESULTS

Effect of posture and exercise on platelet α_2-adrenoreceptor affinity and number. Table I represents the effect of overnight bedrest, 30 min of treadmill exercise, and 120 min of upright posture on plasma catecholamines, the EC_{50}, and the Hill coefficient or slope for *l*-epinephrine binding in competition with [^{3}H]yohimbine for the α_2-adrenoreceptor on intact platelets. After 8 h of bedrest, plasma catecholamines, epinephrine EC_{50}, and the slope were all low. 30 min of treadmill exercise at 75% of maximal predicted heart rate (modified Bruce protocol) caused an increase in plasma catecholamines without a significant change in binding characteristics. However, with 2 h of upright posture, there was an average 3.4-fold increase in EC_{50}, which was accompanied by a significant increase in the mean slope toward 1.0. There was a significant correlation between the increase in EC_{50} at 120 min and the increase in plasma norepinephrine ($n = 20$, $r = 0.65$, $P < 0.01$) or the increase in plasma epinephrine ($n = 20$, $r = 0.59$, $P < 0.01$). Despite higher catecholamine levels at 30 min, no significant changes in agonist affinity or Hill coefficient occurred immediately after exercise. This lag in the appearance of agonist affinity change suggests a time dependency for affinity change at physiological catecholamine concentrations and rules out the possibility that retained agonist is the source of the affinity change at 120 min. In another group of subjects, Scatchard analysis of [^{3}H]yohimbine binding revealed no change in the number of binding sites per platelet (227±23 vs. 213±24, mean±SEM) nor in the affinity of [^{3}H]yohimbine for binding (4.54±0.49 vs. 5.10±0.28

TABLE I

Effect of Posture and Exercise on Agonist Binding to Intact Platelet α_2-Adrenoreceptors and on Plasma Catecholamines

	Epinephrine competition binding		Plasma catecholamines	
Time	EC_{50}	Slope	Norepinephrine	Epinephrine
min	*μM*		*nM*	
0	1.34±0.12	0.74±0.04	1.21±0.12	0.14±0.02
30	1.82±0.32	0.84±0.03	3.00±0.24‡	0.28±0.05*
120	4.54±0.67‡	0.98±0.08*	2.59±0.44*	0.21±0.03

12 normal subjects remained fasting at bedrest overnight in hospital before blood sampling (time 0). They then exercised on a treadmill at 75% maximal predicted heart rate (modified Bruce protocol) for 30 min before a second blood sample. The subjects remained upright and ambulatory for the ensuing 90 min for a total of 120 min of upright posture before the last blood sample. The EC_{50} for *l*-epinephrine was determined by Hill plot analysis of ten point competition binding curves run in triplicate. Values are expressed as mean±SEM.

* $P < 0.05$ when compared with time 0.

‡ $P < 0.01$ when compared with time 0.

nM, mean±SEM) between the overnight and 2-h upright samples. There was no difference in platelet size distribution between the overnight supine and 2-h upright samples obtained from six of these subjects. Thus, the agonist affinity change at 120 min is not due to a change in binding site number, affinity for antagonist, or "selection" of a population of platelets with lower agonist affinity.

Effect of catecholamine infusions on platelet α_2-adrenoreceptor agonist binding. To ascertain whether the changes in platelet α_2-adrenoreceptor agonist affinity were due to increased catecholamines, we infused *l*-norepinephrine or *l*-epinephrine intravenously into groups of seven normal subjects in the supine position after overnight bedrest. The effects of these infusions on the mean EC_{50}, slope, and plasma catecholamines are shown in Table II.

Infusion of *l*-norepinephrine at 50 ng/kg per minute produced no change in EC_{50} or slope after 30 min when compared with overnight bedrest. Like the exercise protocol, however, 2 h of *l*-norepinephrine infusion caused a significant increase in the EC_{50}. This decrease in affinity for agonist was accompanied by a shift in the slope factor toward 1.0. The *l*-norepinephrine infusion caused a 10–12 mmHg increase in mean blood pressure and a 5–8 beats/min decrease in heart rate.

TABLE II

Effect of 50 ng/kg per minute l-Norepinephrine or l-Epinephrine Infusions on Platelet α_2-Adrenoreceptor Agonist Binding and Plasma Catecholamines

	Epinephrine competition binding		Plasma catecholamines	
Time	EC_{50}	Slope	Norepinephrine	Epinephrine
min	*μM*		*nM*	
Norepinephrine infusion				
0	1.77±0.21	0.80±0.05	1.17±0.11	0.16±0.02
30	2.23±0.41	0.87±0.05	5.42±1.16‡	0.26±0.03*
120	5.88±0.86‡	0.98±0.08	5.72±0.60‡	0.29±0.06
Epinephrine infusion				
0	1.60±0.23	0.77±0.05	1.44±0.39	0.20±0.04
30	1.55±0.31	0.82±0.08	2.76±0.97	1.90±0.58*
120	4.24±0.58‡	0.94±0.05*	1.62±0.26	2.28±0.55‡

Seven normal subjects remained at bedrest and fasting overnight; a blood sample was obtained (time 0) and intravenous catecholamine infusions were begun while subjects remained supine. Additional blood samples were obtained from the opposite arm at 30 and 120 min during the infusion for plasma catecholamines and *l*-epinephrine competition binding determinations as described in Methods and Table I. Values are expressed as mean±SEM.

* $P < 0.05$ when compared with time 0.

‡ $P < 0.01$ when compared with time 0.

With these catecholamine infusions, the time course and extent of receptor agonist affinity change was similar to that obtained in the posture and exercise study. In addition, they suggest that either norepinephrine or epinephrine exposure is adequate to alter α_2-adrenoreceptor agonist affinity.

Effect of in vitro exposure of intact human platelets to l-epinephrine on α_2-adrenoreceptor binding. In order to elucidate the dose-response relationship between catecholamines and change in α_2-receptor agonist affinity, we incubated isolated, intact human platelets (obtained at 8:00 a.m. from ambulatory subjects) with varying concentrations of *l*-epinephrine for 2 h at 25°C. The platelets were then washed in isotonic media and competition and Scatchard binding were performed on the intact platelets in comparison with simultaneous control incubations. Table III shows the effects of 10^{-6}–10^{-10} M *l*-epinephrine incubation on the affinity for agonist and the Hill coefficient, or slope of the Logit plot Incubation with 10^{-6}–10^{-8} M *l*-epinephrine caused significant increases in the platelet α_2-adrenoreceptor EC_{50} for epinephrine binding. There was also a dose-related effect on the slope factor; higher *l*-epinephrine concentrations caused larger shifts in the slope toward 1.0 and reached the 0.01 level of significance at 10^{-7} M epinephrine. Platelet size distribution was not affected by 2-h *l*-epinephrine incubations at concentrations up to 10^{-6} M. In additional incubations at 10^{-7} and 10^{-8} M *l*-epinephrine, there was no decline in the epinephrine concentration of the medium during the 2-h incubation.

The effects of 2 h of incubation with each concentration of *l*-epinephrine on antagonist binding by the α_2-adrenoreceptor is shown on the right side of Table III. Control and *l*-epinephrine incubations were performed simultaneously on platelets from the same individual; then, the samples were washed in isotonic media and competition or Scatchard binding was performed. Data were analyzed by the nonlinear curve-fitting program of DeLean et al. (32) for the yohimbine affinity constant and receptor number; values are expressed as the mean±SEM of five to seven experiments each. No change in either antagonist binding or num-

TABLE III

Effect of l-Epinephrine Incubation in vitro on Agonist and Antagonist Binding to α_2-Adrenoreceptors on Intact Platelets

Incubation conditions	Agonist binding		Antagonist binding	
	Epinephrine EC_{50}	Slope	Yohimbine equilibrium dissociation constant	Platelet α_2-adrenoreceptors
	μM		*nM*	*sites/platelet*
Control	2.93±0.23	0.77±0.07	3.77±0.27	227±25
10^{-6} M epinephrine	5.53±0.59‡	1.08±0.08‡	3.93±0.24	235±16
Control	2.80±0.42	0.75±0.05	3.73±0.32	186±22
10^{-7} M epinephrine	5.91±0.97*	0.97±0.04*	3.58±0.34	199±26
Control	2.86±0.42	0.74±0.06	4.34±0.46	280±49
10^{-8} M epinephrine	5.97±0.68*	0.91±0.08	4.63±0.56	276±40
Control	2.74±0.51	0.68±0.07	5.10±1.51	262±44
10^{-9} M epinephrine	3.63±0.47	0.83±0.09	3.92±0.50	268±40
Control	2.71±0.05	0.72±0.14	3.92±0.50	203±33
10^{-10} M epinephrine	3.44±0.47	0.78±0.17	4.29±0.43	203±33

Platelets were isolated as described in Methods, divided into two samples, and incubated with and without (Control) *l*-epinephrine for 2 h at 25°C. The platelets were then washed repeatedly, resuspended, and ten point *l*-epinephrine competition or yohimbine binding were performed. Values presented are the mean±SEM of five to nine experiments. Yohimbine equilibrium dissociation constant and number of platelet α_2-adrenoreceptors per platelet were calculated by the nonlinear, curve-fitting program of DeLean et al. (32).

* $P > 0.05$ when compared with control sample.

‡ $P > 0.01$ when compared with control sample.

ber of receptors per platelet was found, despite the wide range of *l*-epinephrine concentrations. These findings demonstrate that the changes in agonist binding were not secondary either to altered receptor affinity for antagonist or to a change in receptor number.

We also incubated platelets with the β-agonist isoproterenol, and added an α-antagonist to the *l*-epinephrine incubations to determine the selectivity of agonist-induced affinity changes that were measured above. 2-h incubation with 10^{-5} M isoproterenol produced no change in *l*-epinephrine EC_{50} or slope. (EC_{50}, 2.93±0.63 vs. 2.80±0.65; slope, 0.81±0.07 vs 0.81±0.04, for control vs. isoproterenol-incubated, respectively. Mean±SEM of three experiments.) Furthermore, inclusion of 10^{-5} M phentolamine in the 2-h incubations with 10^{-6} M *l*-epinephrine prevented the agonist-induced reduction in α_2-adrenoreceptor affinity. (EC_{50}, 5.45±0.29 vs. 2.81±0.20 for epinephrine-incubated vs. epinephrine plus phentolamine-incubated. Mean±SEM of four experiments.)

Effect of in vitro exposure of platelets to l-epinephrine on platelet aggregation induced by l-epinephrine. To determine the physiological effects of the agonist affinity changes caused by prior agonist exposure, we incubated PRP with 10^{-6}–10^{-8} M *l*-epinephrine at 25°C and determined *l*-epinephrine-induced platelet aggregation after 30, 60, and 120 min of incubation. Fig. 1 illustrates the progressive loss of aggregatory sensitivity to *l*-epinephrine when the first phase of aggregation is normalized to simultaneous control response. There was a time- and dose-dependent effect at both the higher *l*-epinephrine concentrations, while 10^{-8} M *l*-epinephrine was not significantly different from control. This agonist-mediated desensitization of the platelet α_2-adrenoreceptor demonstrates that the physiological responsiveness of the platelet α_2-adrenoreceptor is correlated with the receptor affinity for agonist.

DISCUSSION

Our studies demonstrate that elevations in plasma catecholamines within the physiological range can alter

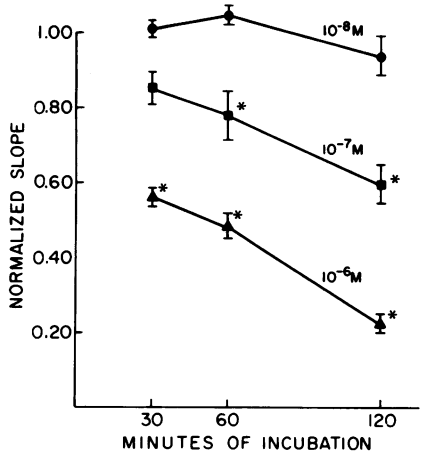

FIGURE 1 Effect of incubation with 10^{-6}, 10^{-7}, and 10^{-8} M *l*-epinephrine on subsequent aggregatory response to *l*-epinephrine. Duplicate PRP samples were incubated with and without *l*-epinephrine at 25°C without agitation, then, aliquots were removed and aggregation was performed in a dual channel aggregometer. The slope of the first phase of *l*-epinephrine-induced aggregation was determined graphically and normalized by division with the value obtained from the sample incubated without epinephrine. Values are the mean±SEM for six to eight subjects determined in quadruplicate at each time point. $^*P < 0.01$ by the grouped Chi-squared test.

the agonist binding characteristics of platelet α_2-adrenoreceptors without changing the total number of receptors. The affinity changes were similar in magnitude to the changes found in lymphocyte β-adrenoreceptor agonist affinity after the same physiological stimuli (25). The change in the slope factor, and the positive correlation between the increase in plasma catecholamines and the increase in EC_{50} support the concept of an agonist-induced decrease in receptor affinity without change in number. Since 15–18 concentrations of competitive ligand are necessary to define two affinity states of receptors (33), we have confined our data analysis of ten point agonist competition binding curves to changes in EC_{50} and the slope factor. These represent conservative estimates of the heterogeneity in receptor agonist affinity and changes therein.

The infusion of catecholamines into supine subjects also resulted in a decrease in agonist affinity, this further supported the conclusion that catecholamine exposure causes the change in receptor affinity. These data also reduce the possibility that the changes in EC_{50} during the posture and exercise protocol were due to in vivo factors other than adrenergic agonist exposure.

An unexpected finding was the apparent time lag between agonist exposure and affinity change. In vitro studies of turkey erythrocyte and cultured astrocytoma cells (34, 35), and in vivo studies of lymphocytes (25, 36) indicate that the β-adrenoreceptor shows rapid changes in affinity and sensitivity to agonist after exposure to agonists. We found little or no change in agonist binding after 30 min of in vivo exposure to levels of catecholamines that eventually resulted in affinity changes after 120 min. This suggests that there is either a concentration-time dependency for this affinity change, or that a series of events which take longer than 30 min must occur to effect changes in affinity in the platelet α_2-adrenoreceptor system. Our data do not permit us to differentiate between these or other explanations for the time delay in affinity change.

2 h of in vitro exposure to *l*-epinephrine produced a shift in the affinity for agonists and was accompanied by proportional increases in the slope factor toward 1.0. The absolute values for EC_{50} in the control samples were higher than in the in vivo protocols; this was probably because the samples were obtained from volunteers who had been erect for 60–90 min. Although this reduced the fold shift obtained on incubation with *l*-epinephrine, the effect of in vitro exposure to agonist is still evident. Since in vivo plasma epinephrine concentrations are in the range of 0.15–0.30 nM, our in vitro conditions required somewhat higher concentrations of *l*-epinephrine to cause a change in EC_{50}. This may be due, in part, to the incubation conditions, including artificial buffer, 25°C, and no stirring. However, these data still identify changes in platelet α_2-adrenoreceptors at concentrations of *l*-epinephrine that are three orders of magnitude lower than previously reported.

Incubation of PRP with 10^{-6} to 10^{-8} M *l*-epinephrine illustrates the functional effect of the agonist-mediated affinity changes on subsequent aggregatory response. The higher *l*-epinephrine concentrations resulted in a dose- and time-dependent desensitization of aggregatory response when challenged with the same agonist. Since these aggregatory studies were carried out with simultaneous control incubations by using the same platelet sample, variations due to time-dependent pH changes, ADP, platelet factor 4, thromboxane accumulation, different platelet concentrations, and concurrent plasma catecholamine concentrations were all controlled.

Our results indicate that the time of day and the circumstances under which samples are obtained may influence the results in adrenergic agonist binding studies on circulating cellular elements. The relatively short-term changes in agonist affinity without change in receptor number are in contrast to the alterations in receptor number reported in pheochromocytoma

and orthostatic hypotension (20, 21). These discrepancies raise the possibility that short-term regulation of platelet sensitivity occurs through modulation of receptor affinity for agonist, whereas long-term sensitivity changes are mediated by alterations in receptor number.

We also considered the possibility that endogenous or exogenous catecholamines or in vitro exposure to catecholamines might involve the selection of a population of platelets which were less sensitive to adrenergic agonists (37). This might produce the changes in agonist affinity found in our experiments. If this occurred, however, we would have expected to find fewer platelets after in vitro exposure to *l*-epinephrine, and/or a change in size distribution of the platelets after in vivo or in vitro exposure to catecholamines. Neither of these changes occurred.

Prior studies found reductions in platelet α_2-adrenoreceptor number and function after in vitro exposure to *l*-epinephrine concentrations of 10^{-5} M or greater (16, 17). These results were interpreted as showing "down-regulation" of receptor number or agonist retention by the platelet α_2-adrenoreceptor. However, unlike our studies, these studies incubated platelets with high concentrations of *l*-epinephrine for long periods of time; yet, no data concerning platelet count or size distribution was presented. Furthermore, no significant change in receptor number or function occurred after incubation with concentrations of *l*-epinephrine below 10^{-5} M or for less than 4 h (16, 17). In contrast, we found a maximal effect on platelet α_2-adrenoreceptor agonist affinity after in vitro incubation with 10^{-7} M *l*-epinephrine for 2 h. Our antagonist binding studies further demonstrate that no change in number of receptors per platelet nor in receptor affinity for antagonist occurs after exposure to more physiological concentrations of catecholamines. In addition, our in vivo protocols found no change in platelet α_2-adrenoreceptor agonist affinity after 30 min of exposure to elevated catecholamines. Since plasma catecholamines were highest at 30 min, our results are not compatible with the concept of a retained agonist that causes changes in receptor number or affinity for agonist.

In summary, we have found [^{3}H]yohimbine to be a specific, rapid, saturable, and reversible ligand for the identification of α_2-adrenoreceptors on intact human platelets. Endogenous and exogenous catecholamines can reduce the affinity of the intact platelet α_2-adrenoreceptor for agonist without a change in receptor number. In vitro exposure of intact platelets to epinephrine results in a rapid loss of sensitivity to epinephrine-induced aggregation and a dose-related reduction in α_2-adrenoreceptor affinity for agonist. These observations demonstrate acute regulation of intact human platelet α_2-adrenoreceptor's affinity for and sensitivity to agonist by endogenous and exogenous catecholamines.

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