JCI The Journal of Clinical Investigation

Variations in circulating catecholamines fail to alter human platelet alpha-2-adrenergic receptor number or affinity for [3H]yohimbine or [3H]dihydroergocryptine.

M A Pfeifer, … , L A Harker, D Porte Jr

J Clin Invest. 1984;74(3):1063-1072. https://doi.org/10.1172/JCI111473.

Research Article

A series of studies were performed to determine the relationship between physiologic levels of circulating plasma norepinephrine and epinephrine and human platelet alpha-2 binding site number and the affinity (KD) of these sites for antagonist radioligands. In one study, alpha-2-adrenergic binding site number and affinity were compared using both [3H]yohimbine and [3H]dihydroergocryptine as radioligands. There was good absolute and relative comparison for binding site number, but only a relative relationship for KD. In 46 normal subjects, there was no significant relationship between site number or KD and age, plasma epinephrine, or plasma norepinephrine concentration. Even after plasma epinephrine was raised nearly 20-fold by means of an intravenous infusion for 4 h in seven normal subjects, neither sites (608 +/- 68 vs. 567 +/- 120 sites/platelet) nor KD (2.01 +/- 0.94 vs. 2.14 +/- 1.15 nM) were significantly changed. Similarly, neither sites (445 +/- 55 vs. 421 +/- 53 sites/platelet) nor KD (1.44 +/- 0.29 vs. 2.10 +/- 0.75 nM) were significantly changed in six normal subjects when plasma norepinephrine levels increased during oral administration of prazosin for 1 wk. Thus, in a cross-sectional analysis and after a change in plasma catecholamine concentrations, there was no relationship in normal subjects between platelet alpha-2 binding site number or affinity of these sites for antagonist radioligands and the circulating catecholamine levels to which […]

Variations in Circulating Catecholamines Fail to Alter Human Platelet Alpha-2-Adrenergic Receptor Number or Affinity for [^{3}H]Yohimbine or [^{3}H]Dihydroergocryptine

M. A. Pfeifer, K. Ward, T. Malpass, J. Stratton, J. Halter, M. Evans, H. Beiter, L. A. Harker, and D. Porte, Jr.
University of Washington, Seattle, Washington

Abstract. A series of studies were performed to determine the relationship between physiologic levels of circulating plasma norepinephrine and epinephrine and human platelet alpha-2 binding site number and the affinity (K_D) of these sites for antagonist radioligands. In one study, alpha-2-adrenergic binding site number and affinity were compared using both [^{3}H]yohimbine and [^{3}H]dihydroergocryptine as radioligands. There was good absolute and relative comparison for binding site number, but only a relative relationship for K_D. In 46 normal subjects, there was no significant relationship between site number or K_D and age, plasma epinephrine, or plasma norepinephrine concentration. Even after plasma epinephrine was raised nearly 20-fold by means of an intravenous infusion for 4 h in seven normal subjects, neither sites (608±68 vs. 567±120 sites/platelet) nor K_D (2.01±0.94 vs. 2.14±1.15 nM) were significantly changed. Similarly, neither sites (445±55 vs. 421±53 sites/platelet) nor K_D (1.44±0.29 vs. 2.10±0.75 nM) were significantly changed in six normal subjects when plasma norepinephrine levels increased during oral administration of prazosin for 1 wk. Thus, in a cross-sectional analysis and after a change in plasma catecholamine concentrations, there was no relationship in normal subjects between platelet alpha-2 binding site number or affinity of these sites for antagonist radioligands and the circulating catecholamine levels to which the platelets were exposed.

In a group (n = 7) of patients who lack epinephrine-induced platelet aggregation due to abnormal thrombopoiesis, binding site number was decreased (304±36 vs. 572±29 sites/platelet, $P < 0.001$) and K_D tended to be greater (8.69±2.44 vs. 5.40±0.31 nM, P = NS) than in normal subjects (n = 46), despite having similar plasma catecholamine levels. There was no difference in binding site number (491±116 sites/platelet) and K_D (5.61±0.84 nM) in patients (n = 5) with autonomic insufficiency and low levels of upright plasma norepinephrine when compared with the normal subjects. Two patients were examined before and after the removal of a pheochromocytoma. Their binding site number and K_D were normal before the operation and essentially unchanged after the tumor removal and fall of plasma catecholamines.

Thus, this study demonstrates that within the physiologic and pathophysiologic range of plasma catecholamines (in men), there is no relationship between the circulating catecholamine concentration and either platelet alpha-2 adrenergic binding site number or the affinity of these sites for antagonist radioligands.

Address reprint requests to Dr. Pfeifer, Medical Research Service (151A), Veterans Administration Medical Center, 800 Zorn Avenue, Louisville, KY 40202. Dr. Pfeifer's present address is Division of Endocrinology/Metabolism, University of Louisville, KY 40202. Dr. Harker's present address is Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Received for publication 14 December 1982 and in revised form 10 May 1984.

The Journal of Clinical Investigation, Inc.
Volume 74, September 1984, 1063–1072

Introduction

The human platelet responds to alpha adrenergic stimulation by altering its aggregation characteristics. Low levels of the alpha adrenergic agonists, epinephrine and clonidine, potentiate platelet aggregation to other agents such as ADP and collagen (1, 2), whereas higher levels of alpha adrenergic agonists such as epinephrine and norepinephrine directly elicit platelet aggregation (3–5). Furthermore, in patients with platelet diseases and a lack of epinephrine-induced aggregation, the number of binding sites of alpha-2-adrenergic receptors per platelet are decreased (6). Thus, it appears that the alpha adrenergic receptor on the human platelet is important in both normal platelet physiology and platelet disease-related entities.

The number of binding sites and the affinity of the platelet alpha receptor have been studied using a variety of ligands, including the antagonists dihydroergocryptine (7–9), yohimbine

(10–13), phentolamine (14), or dihydroergonine (15), and the agonists clonidine (13, 16) or norepinephrine (14). These studies (7–13) have revealed that the binding sites are saturable, stereo-specific, readily reversible, have appropriately rapid kinetics, demonstrate the rank order of potency of alpha adrenergic receptor sites (epinephrine > norepinephrine ≫ isoproterenol), and are associated with inhibition of adenyl-cyclase activity. Other studies have demonstrated that this is a single class of sites which have the characteristics of alpha-2 rather than alpha-1 receptors (17–19).

The number of adrenergic binding sites in some organ systems has been observed to be inversely related to ambient catecholamine agonist concentrations under some conditions. Thus, in the presence of high catecholamine levels, the number of beta adrenergic receptors on human lymphocytes and frog erythrocytes is diminished. Conversely, the number of such B-receptors is increased when ambient catecholamine concentrations are low (20–22). When dihydroergocryptine (DHE)[1] was used as the ligand, a decreased number of platelet alpha-2-adrenergic binding sites was observed in vitro in the presence of supraphysiologic levels of epinephrine (23). However, in similar in vitro studies by other investigators, such down regulation of platelet alpha-2-adrenergic binding sites was not observed when yohimbine was used as the ligand (24). The authors of the latter study suggested that an "apparent down regulation" can be due to retained agonist. One group of investigators have also shown alteration in intact platelet alpha-2-adrenergic binding site numbers in patients with diseases that result in chronically low or high levels of plasma norepinephrine (25). However, the presence and relevance of agonist regulation of platelet alpha-2-adrenergic receptor binding site number and affinity during physiologic changes in plasma levels of the alpha-adrenergic agonists norepinephrine and epinephrine in vivo has not been established. Therefore, we assessed the possibility that variations of plasma catecholamine levels in man would be reflected by similar variations in the number of platelet alpha-2-adrenergic binding sites or the affinity of these sites for the antagonist radioligands [^{3}H]DHE and [^{3}H]yohimbine.

Methods

Subjects

Four groups of subjects were examined in these studies: normal subjects, subjects with abnormal platelet function manifested by a lack of epinephrine-induced aggregation, subjects with postural hypotension and no change in the levels of plasma norepinephrine upon standing, and subjects with documented pheochromocytoma.

Normal subjects

46 normal subjects participated in these studies. Not all the subjects participated in every procedure. Their average age was 38±2 yr (mean±SEM) (range: 19–79 yr). According to the 1959 Metropolitan Life Insurance Company tables, the percentage of ideal body weight in these subjects was 113±2% (range: 89–147%). 45 subjects were male. None had any clinical evidence of systemic disease nor were any taking medication, including aspirin, during these studies.

Patients with abnormal platelet function

Seven patients who lack epinephrine-induced aggregation were evaluated in these studies. Two of these patients had essential thrombocythemia. Four of the patients had polycythemia vera. One patient had a-beta-lipoproteinemia. None had any previous therapy for their corresponding diseases. None demonstrated epinephrine-induced aggregation; however, all manifested ADP- and collagen-induced platelet aggregation. Platelet aggregation was assessed by methods previously described (26). Possible epinephrine-induced aggregation was evaluated at increasing concentrations from 10^{-7} M to 10^{-3} M epinephrine. All seven patients demonstrated irreversible platelet aggregation to 2.0×10^{-6} M ADP and 1.0 μg/ml collagen or less. The average age of these individuals was 60±9 yr with a range of 22–87 yr. Their percentage of ideal body weight was 95±6% (range: 86–129%). Six subjects were male.

Patients with autonomic insufficiency and postural hypotension

Five patients were in this category. Three patients had insulin-dependent diabetes with the duration of disease of >10 yr. One patient had Shy-Drager syndrome (also known as multiple system atrophy), and one subject had idiopathic orthostatic hypotension. With the exception of insulin, none were on previous therapy. All patients had postural hypotension of >25 mmHg diastolic blood pressure. None of the patients demonstrated a change or rise in plasma norepinephrine levels upon standing. Their average age was 44±7 yr (range: 29–62 yr); percentage of ideal body weight was 105±8% (range: 77–125%). All five subjects were male.

Pheochromocytoma

Two patients were studied before and 3 mo after the removal of a pheochromocytoma. They were on no medications during either of these study periods. Neither patient was evaluated during an episode of adrenergic discharge. Their ages were 34 and 32 yr, and their ideal body weights were 116 and 111%. One subject was male.

Analytical methods

Measurement of alpha-2-adrenergic binding number and affinity for an antagonist radioligand was done in all the subjects. 250 ml of blood were withdrawn and anticoagulated with 3.2% sodium citrate in ratio of 9:1. Platelet lysates were prepared using the method described by Newman et al. (9). The platelet lysates were divided into two lots for two separate assays and frozen at −70°C. Analysis was performed within 1 wk after the preparation of platelet lysates. Platelet counts were performed on these lysates before and after the binding assays. Adjustments were made so that each incubation tube contained ~500,000 platelets/μl. Total binding and nonspecific binding were each determined by triplicate measurements via a filter assay technique (8, 9, 27) using Whitman FG-C glass fiber filters. Total binding was determined as that amount of ligand on the platelets following incubation for 30 min at 25°C. Nonspecific binding was the amount of ligand bound to the platelets under the same conditions in the presence of 0.95×10^{-5} M phentolamine. Specific binding was the difference in the average of total and nonspecific binding. The glass fiber filters were presoaked in 0.5% bovine serum albumin (BSA). In preliminary studies, it was found that presoaking the filters with BSA reduced nonspecific binding by 80% when [^{3}H]DHE was used as the radioligand.

Two adrenergic antagonists, DHE and yohimbine, were used as

1. *Abbreviations used in this paper:* DHE, dihydroergocryptine.

labeled ligands in the studies described in this manuscript. In preliminary studies using these ligands, the platelet preparation demonstrated the properties of saturation, rapid kinetics, reversibility, stereospecificity, and had the appropriate rank order of affinity characteristic of alpha-2-adrenergic binding sites. Two six-point Scatchard plots were performed for each individual to determine number of binding sites and the affinity for the antagonist radioligand. Thus, extrapolation of the regression line to the x-coordinate was necessary to estimate the number of binding sites. The concentrations of the labeled antagonist used were 2, 3, 4, 6, 8, and 10 nM. To eliminate inaccurate assays, only those studies in which the linear regression coefficient for the Scatchard plot were −0.80 or better were used for final analysis. If both assays for an individual sample generated acceptable Scatchard plots, then the average value of binding site number and affinity for the two plots was used. If only one Scatchard plot yielded a regression coefficient of −0.80 or better, then that single study was used to characterize the patient's platelet alpha-2 receptors. Approximately one-third of the [^{3}H]DHE studies and none of the [^{3}H]yohimbine studies were excluded.

In preliminary studies, the sites per platelet were compared with sites per mg of protein in 12 normal individuals. The same data were analyzed and expressed both ways. There was good agreement between these two measurements with linear coefficient of $r = 0.81$, $P < 0.01$. In our final platelet preparation, we found that 1.55×10^9 platelets yield 1 mg of lysate protein. Therefore, all subsequent analyses were expressed in terms of binding sites per platelet.

The intra-assay coefficient of variation of binding site number from the Scatchard plots using [^{3}H]DHE was 24.8%, and for [^{3}H]yohimbine, the coefficient of variation was 11.3%. The intra-assay coefficient of variation of affinity for antagonists was 28.5% for [^{3}H]DHE and 15.7% for [^{3}H]yohimbine. These values are the means of the intra-assay coefficients of variation calculated for each pair of measurements of alpha-2-adrenergic binding site number and affinity for antagonists performed on a given individual. A comparison of the results for DHE and yohimbine was performed and is described below.

Plasma norepinephrine and epinephrine were also measured in all subjects using a single isotope enzymatic assay (28).

Protocol

General. Studies were performed in the Special Studies Unit at the Veterans Administration Medical Center, Seattle, WA. All patients and subjects were fasting from midnight the night before, and the studies were performed at ~8:00 a.m. on the morning of the study. No over-the-counter medications, including aspirin and antihistamines, were allowed for 1 wk prior to the study. With the exception of insulin ($n = 3$), none of the subjects were on any prescription medication at the time of the study. These three subjects did not take their insulin on the day of the study until after the studies were completed. Patients were not allowed to smoke cigarettes on the day of the study. Patients assumed the recumbent position, and a 19-gauge butterfly needle was inserted into one antecubital vein and kept patent by a slow infusion of 0.9% sodium chloride. After 30 min, two 2.5-ml samples for the measurement of plasma catecholamines (norepinephrine and epinephrine) were withdrawn. The results from these two samples were averaged to represent basal plasma epinephrine and norepinephrine in the subjects. Then, 250 ml of blood was withdrawn for the platelet alpha-2-adrenergic receptor assay. In studies where an infusion was necessary, a second intravenous line was then begun and used as an infusion site for the drugs. Samples for plasma catecholamines and platelets were always withdrawn from the original site.

Comparison of [^{3}H]DHE and [^{3}H]yohimbine. 12 normal subjects were studied. 500 ml of blood were withdrawn from each subject and the platelet lysates prepared. The platelet lysates were then divided into four lots and subsequently frozen. Two assays, using [^{3}H]DHE and two assays using [^{3}H]yohimbine were performed on the platelet lysates from each patient. The number of alpha-2-adrenergic binding sites and their affinity for the antagonists were then compared.

Cross-sectional studies of normal subjects. The effect of aging on binding site number and affinity for radiolabeled antagonists was evaluated in the 46 normal subjects. Furthermore, the relationship between the plasma level of norepinephrine and epinephrine and binding site number or antagonist affinity was determined. [^{3}H]DHE was used for determination of binding site number and antagonist affinity in all these subjects.

Intergroup comparisons. Comparisons were made of binding site number and antagonist affinity between normal subjects and patients who lacked epinephrine-induced platelet aggregation, patients with pheochromocytoma before and after removal of their tumor, and patients with postural hypotension due to autonomic insufficiency. Binding site number and antagonist affinity were determined by using [^{3}H]DHE in all subjects.

Epinephrine infusions. Seven normal individuals had alpha-2-adrenergic binding site number and antagonist affinity (K_D) determined before and after a 4-h infusion of 75 ng/kg per minute epinephrine. The infusion mixture also had ascorbic acid (1 mg/ml) to prevent oxidation of the epinephrine. [^{3}H]yohimbine was used to analyze binding site number and antagonist affinity in all these studies.

Prazosin studies. In six normal individuals, plasma catecholamines and binding site number and antagonist affinity (K_D) were determined before and after administration of 2 mg of prazosin three times a day for 1 wk. After 30 min of rest, plasma catecholamines were measured at −5, 0, 2, 5, and 10 min after assuming the upright position. Other studies have shown that prazosin results in an elevation of plasma norepinephrine in patients with congestive heart failure and hypertension (29–31). [^{3}H]yohimbine was used as the labeled ligand to analyze all these studies.

Statistical analysis. Statistical techniques included both paired and non-paired t tests and analysis with linear regression.

Results

Comparison of [^{3}H]DHE to [^{3}H]yohimbine. 12 normal subjects had binding assays done in duplicate on the separate lots of platelet lysates prepared from a single blood sample using either [^{3}H]DHE or [^{3}H]yohimbine as the labeled ligand. The average number of binding sites per platelet was 584±103 for DHE and 589±97 for yohimbine. These results were not significantly different. Furthermore, the relationship between binding site number in these two studies was highly significant ($r = 0.88$, $P < 0.001$, Fig. 1). Thus, individuals with the highest sites per platelet using [^{3}H]DHE also had the highest number of sites per platelet using [^{3}H]yohimbine. In contrast to the agreement in the number of binding sites using the two ligands, [^{3}H]DHE generated a much higher K_D than [^{3}H]yohimbine (4.43±0.76 vs. 0.94±0.05 nM, $P < 0.001$). However, there still was a significant relationship between the two assays, $r = 0.61$, $P < 0.05$. Thus, even though the lysates had a much lower affinity (i.e., higher K_D) for [^{3}H]DHE than for [^{3}H]yohimbine, the subjects with the highest affinity (lowest K_D) using one

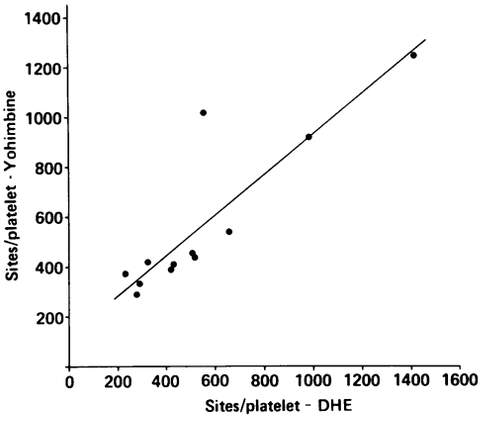

Figure 1. Relationship between the number of alpha-2-adrenergic binding sites per platelet determined by Scatchard analysis using [^{3}H]DHE or [^{3}H]yohimbine as the labeled ligand antagonist. Duplicate measurements were made for each of the 12 subjects (n = 12) from blood drawn in a single sample. Patients with greater sites per platelet using one ligand tended to have the greatest sites per platelet with the other ligand. $y = 126 + 0.80x$; $r = 0.88$; $p < 0.001$.

ligand tended to have the highest affinity using the other ligand.

Effect of aging in normal subjects. There was no relationship between age and either binding site number (r = 0.04, P = NS) or binding site K_D for radiolabeled antagonists (r = 0.05, P = NS) in the 46 normal subjects.

Intergroup comparison. The intergroup comparisons are summarized in Table I. A wide range of values was observed for normal subjects: the K_D for radiolabeled antagonist and number of binding sites were 5.40±0.31 nM and 572±29 sites/platelet, respectively. This represents a fivefold range in normal subjects. As a group, the patients with abnormal platelet aggregation properties had a smaller number of binding sites than the normal subjects (304±36 sites/platelet, n = 7, P < 0.001). The average K_D for antagonists was not significantly different than that of the normal subjects (8.69±2.44 nM, n = 7, P = NS), although two subjects had a K_D that was higher than the normal range. The average age of these patients was higher than that of the normal subjects (P < 0.05, Table I), but there was no significant difference in either plasma norepinephrine or epinephrine (Table I).

Five subjects with postural hypotension secondary to autonomic insufficiency were also evaluated. Neither the average number of binding sites (491±116 sites/platelet), nor the average K_D for antagonists (5.61±0.84 nM) were significantly different from the normal values. Furthermore, all the values were within the normal range. Supine plasma norepinephrine was within the normal range with the exception of patient 57 who had idiopathic orthostatic hypotension. Normal supine norepinephrine levels have been observed in patients with the varied etiologies of the autonomic insufficiency in these subjects (32, 33). However, the lack of a rise in plasma norepinephrine levels in these subjects would imply that they had chronically low upright levels of norepinephrine. Neither age nor plasma epinephrine were significantly different in these subjects (Table I).

Two patients were evaluated before and after the removal of a pheochromocytoma. Samples for the binding studies were taken during a quiescent period, whereas samples for plasma levels of norepinephrine and epinephrine were measured during both quiescent and episodic "attack" periods. During an episodic adrenergic "attack", patient 59 had norepinephrine levels as high as 920 pg/ml and epinephrine levels as high as 770 pg/ml. Patient 60 had norepinephrine levels of 1420 pg/ml and epinephrine levels of 2520 pg/ml during these episodic "attacks." The quiescent levels of plasma catecholamines are reported in Table I; these levels were drawn at the same time the blood for platelet adrenergic receptor assay was obtained. Plasma epinephrine levels decreased in both patients after the removal of the pheochromocytoma. In contrast, before the operation, norepinephrine was normal in patient 59 during quiescent periods and rose slightly after removal of the tumor; however, there was a decrease in plasma norepinephrine in patient 60 after tumor removal. In both patients, before the tumor was removed, the number of binding sites per platelet and K_D for antagonists were within the normal range, and there was not a consistent change in either measurement after the tumor removal.

Cross-sectional analysis of binding parameters and plasma catecholamines. The left panel of Fig. 2 demonstrates that there was not a significant relationship in normal subjects between the number of sites per platelet and plasma epinephrine levels (r = −0.18, P = NS). The right panel of Fig. 2 illustrates the lack of relationship between K_D for antagonist, and plasma epinephrine levels in normal subjects (r = −0.14, P = NS). Fig. 3 demonstrates the lack of these relationships in normal subjects for plasma norepinephrine level. For comparison, the other patient groups are also illustrated in these figures.

Epinephrine infusion. In seven normal subjects, alpha-adrenergic binding sites and K_D for antagonists were measured before and after a 75 ng/kg per minute epinephrine infusion. Before the epinephrine infusions, plasma epinephrine values were 50±5 pg/ml and plasma norepinephrine values were 278±37 pg/ml. There was a significant increase in plasma epinephrine levels (914±93 pg/ml, P < 0.001), but no significant change in norepinephrine values (303±40 pg/ml, P = NS). Heart rate increased from 66±2 beats/min to 94±4 (P < 0.001) during the epinephrine infusion. Systolic blood pressure increased from 113±3 mmHg to 131±3 mmHg (P < 0.005) and diastolic blood pressure decreased from 76±4 to 65±7 mmHg, but this was not a significant effect (P < 0.1). In spite of these biochemical and physiologic changes during the epinephrine infusion, neither number of binding sites per platelet (608±68 vs. 567±120 sites/platelet, P = NS) nor the K_D for antagonists (2.01±0.94 vs. 2.14±1.15 nM, P = NS) were significantly changed (Fig. 4).

Prazosin study. Six patients were studied before and after

Table I. Catecholamine Levels and Binding Characteristics in Normals, Patients Who Lack Epinephrine-induced Aggregation, and Patients with Chronic Hypo- or Hyper-Adrenergic Activity

Subject types and subject number	Age	Norepinephrine	Epinephrine	Sites/ platelet*	K_D*	Diagnosis
	yr	*pg/ml*	*pg/ml*		*nM*	
Normal subjects (n = 46)						
$\bar{x}$±SEM	38±2	277±20	49±6	572±29	5.40±0.31	
Range	19–79	70–640	0–160	225–1096	2.04–10.10	
Patients with abnormal platelet aggregation						
47	60	930	40	296	6.81	Polycythemia vera
48	32	320	30	140	15.40	Essential thrombocythemia
49	87	300	40	344	3.40	Polycythemia vera
50	59	260	80	403	5.00	a-beta-lipoproteinemia
51	22	320	40	245	4.70	Essential thrombocythemia
52	78	320	210	287	20.20	Polycythemia vera
53	79	220	50	412	5.30	Polycythemia vera
$\bar{x}$±SEM	60±9	381±93	70±24	304±36	8.69±2.44	
P (vs. normal patients)	0.05	NS	NS	0.001	NS	
Patients with autonomic dysfunction						
54	36	190	70	355	5.42	Diabetes mellitus
55	29	120	80	242	4.12	Diabetes mellitus
56	34	240	30	647	5.29	Diabetes mellitus
57	57	50	10	871	8.84	Idiopathic orthostatic hypotension
58	62	345	25	342	4.40	Shy-Drager's disease
$\bar{x}$±SEM	44±7	189±51	43±14	491±116	5.61±0.84	
P (vs. normal patients)	NS	NS	NS	NS	NS	
Patients with pheochromocytoma						
59 Before removal	34	230	305	406	4.40	
After removal	34	300	40	463	5.70	
60 Before removal	32	860	1655	627	8.69	
After removal	32	300	40	581	5.54	

* Determined by DHE binding.

1 wk of prazosin (2 mg three times a day). As illustrated in Fig. 5, both supine and upright plasma levels of norepinephrine were elevated during the prazosin. Plasma levels of epinephrine, however, were not significantly changed during the prazosin. As shown in Fig. 6, prazosin treatment resulted in no change in either the number of binding sites (445±55 vs. 421±53 sites/platelet, P = NS) or the K_D for antagonists (1.44±0.29 vs. 2.10±0.75 nM, P = NS).

Discussion

We have found that there is no relationship in number of platelet alpha-adrenergic binding sites or affinity (K_D) for antagonists and plasma levels of catecholamines within the physiologic range in man. Furthermore, this paper uniquely evaluated paired conditions which acutely or chronically changed the plasma levels of either norepinephrine or epinephrine; there were no consistent changes in the number of binding sites or their affinity for radiolabeled antagonists. These findings may be applicable only to men, since we did not study a sufficient number of female subjects to reach any conclusions concerning sex.

We have confirmed the work by other laboratories (6) that there is a decrease in number of platelet alpha-2-adrenergic binding sites in patients with essential thrombocythemia. It is possible that the decrease in binding site number is related to

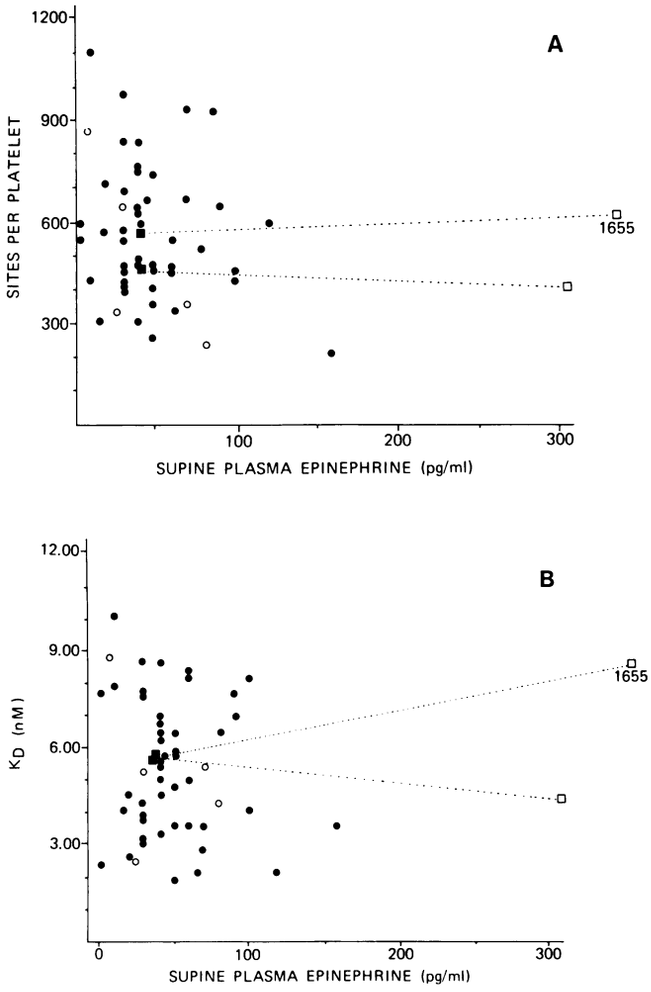

Figure 2. The relationship between supine plasma epinephrine level and number of alpha-2-adrenergic binding sites per platelet (*A*) or affinity for [^{3}H]DHE (K_D) (*B*). There was no relationship in the normal subjects in either of these binding parameters and the level of plasma epinephrine. There was no consistent change in either binding site number or K_D after the removal of a pheochromocytoma in two patients. Patients with autonomic insufficiency also tended to have normal binding site numbers and K_D. ●, normal patients, n = 46, r = −0.18 (*A*) and −0.14 (*B*), P = NS; ○, autonomic dysfunction, n = 5; □, pheochromocytoma (preoperation), n = 2; ■, pheochromocytoma (postoperative) n = 2.

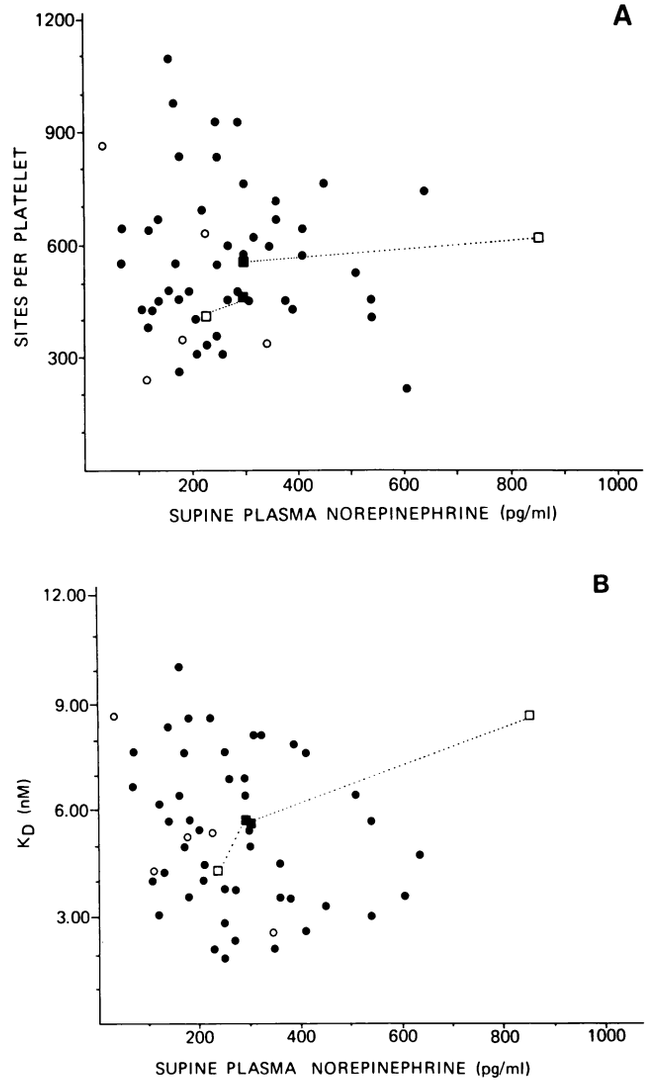

Figure 3. The relationship between supine plasma norepinephrine levels and platelet alpha-2-adrenergic binding site number (*A*) and affinity (K_D) (*B*). There was no relationship between plasma norepinephrine and either of these binding parameters. Similarly to Fig. 2, the patients with autonomic insufficiency tended to have normal binding site number and affinity for [^{3}H]DHE. The two patients with pheochromocytoma were essentially unchanged in regards to platelet alpha-2-adrenergic binding site number and affinity after tumor removal. ●, normal patients, n = 46, r = −0.07 (*A*) and −0.23 (*B*), P = NS; ○, autonomic dysfunction, n = 5; □, pheochromocytoma (preoperation), n = 2; ■, pheochromocytoma (postoperation), n = 2.

the decrease in platelet aggregation function, but it may also reflect a nonspecific defect in the platelet membrane related to abnormal thrombopoiesis. In future studies, the number and affinity of other receptors should also be measured on platelet membranes in these individuals to determine whether this is a specific platelet membrane problem or whether it is a specific alpha-2-adrenergic receptor abberation. Normal ADP- and collagen-induced aggregation shown in this other study and our study lends credence to the possibility that the effect may be related to alpha adrenergic receptor number or affinity.

Our studies did not demonstrate a difference in binding site number or K_D for antagonists between patients with orthostatic hypotension and normal subjects. This finding

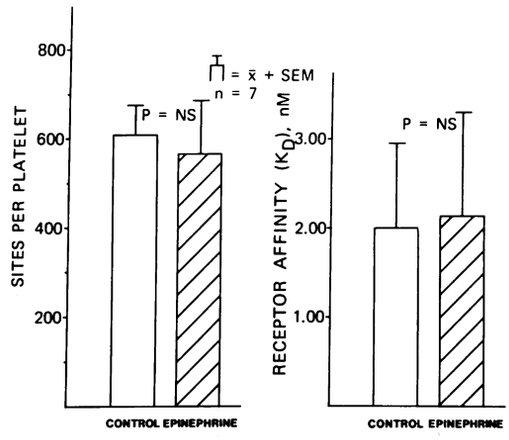

Figure 4. The effects of a 4-h 75 ng/kg per minute epinephrine infusion in seven normal subjects on platelet alpha-2-adrenergic binding characteristics. Neither binding site number of affinity for [^{3}H]yohimbine were changed after this infusion, despite a rise in plasma epinephrine from 50±5 to 914±93 pg/ml ($P < 0.001$).

contrasts with two reports from one group of investigators of an increase of platelet alpha-2-adrenergic binding sites in such patients (25, 34). These differences may be due to differences in patient populations. We studied patients with a mixed group of etiologies causing the orthostatic hypotension. Patients with the Shy-Drager Syndrome (25, 34) may be different from patients with other forms of orthostatic hypotension associated with chronically low levels of upright plasma norepinephrine. Our study included only one patient with the Shy-Drager Syndrome (patient 58); however, this patient's number of binding sites tended to be low and not high.

In a previous study, three of four patients with pheochromocytoma had platelet alpha-2-adrenergic binding site numbers within the normal range (25) although the group as a whole had less binding sites than normal controls using [^{3}H]DHE as the ligand. Another study (35) has published a small increase in platelet alpha-2-adrenergic binding sites after the removal of a pheochromocytoma in one patient. Initially, this patient was within one standard deviation of the quoted normal values and increased to the mean for normals. However, in a study

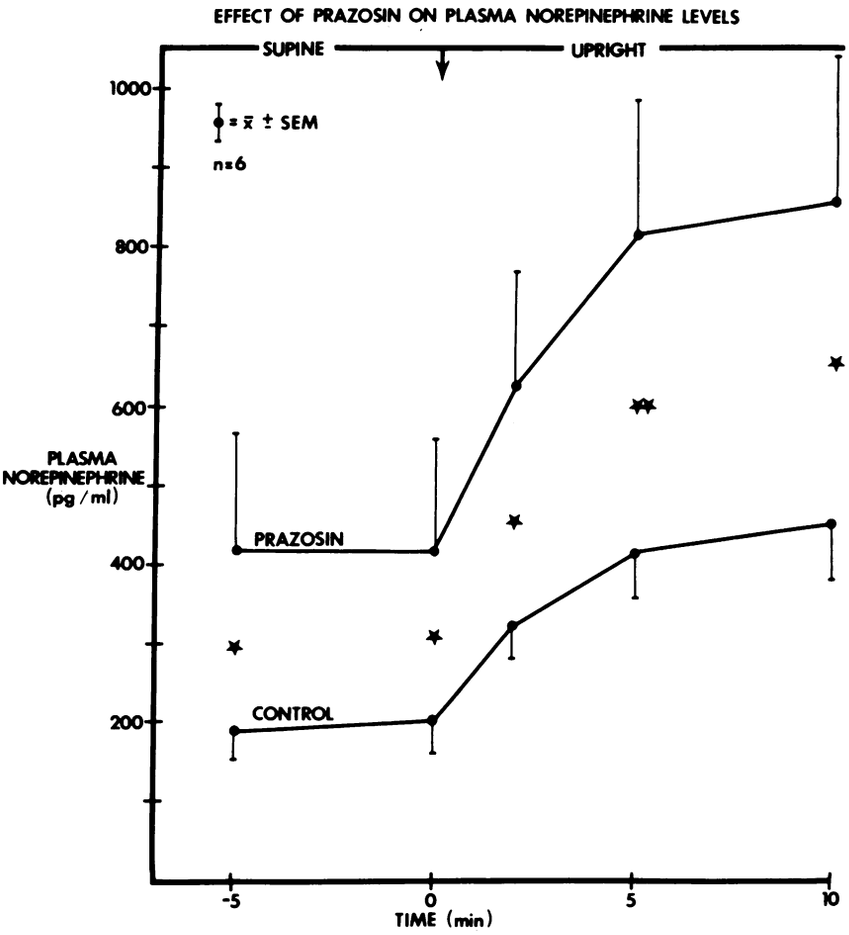

Figure 5. The effect of 2 mg three times a day for 7 d of oral prazosin on plasma norepinephrine level in six normal subjects. Both supine and upright plasma norepinephrine were elevated during prazosin in these normal subjects (*$P < 0.05$; **$P < 0.025$).

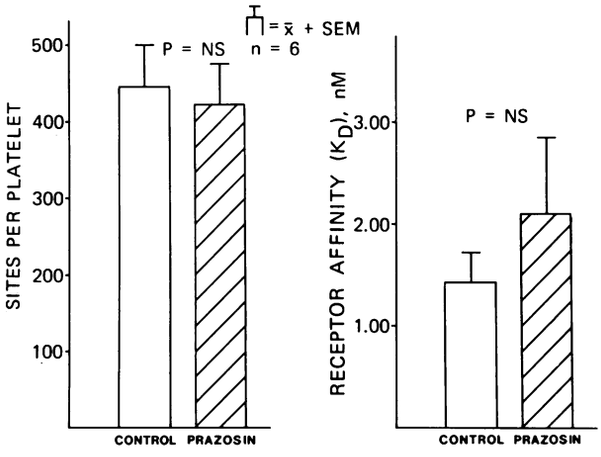

Figure 6. The effect of the elevation of plasma norepinephrine concentration due to prazosin in six normal subjects on the platelet alpha-2-adrenergic binding site number and affinity (K_D) for [^{3}H]yohimbine. Neither of the platelet binding parameters were significantly changed in these individuals during prazosin.

by another group of workers in which [^{3}H]yohimbine was used as the ligand, the platelet alpha receptor number in patients with pheochromocytoma was not different from that of normal subjects (36). Similarly, both of our patients had normal binding site number and affinity for antagonist before the removal of the tumor, and there was not a consistent change in number or affinity after the removal. These differences in pheochromocytoma patients could be from small patient population studied. Most of the patients in all the studies are within one standard deviation of the quoted normal values. The increase in platelet alpha-2-adrenergic binding sites seen in one study (35) after removal of the tumor is similar to the results seen in our patient 59. These small changes are consistent with the variability of the assay.

Comparison studies using the ligands of [^{3}H]DHE and [^{3}H]yohimbine demonstrated good absolute and relative agreement for binding site number, but only relative agreement for affinity. Although agreement between number of binding sites using the two radioligands is the expected result, in other studies (37, 38), the number of binding sites per platelet has appeared to be greater using [^{3}H]DHE. The difference in these studies may be due to small differences in technique (BSA blanks) and the number and type of subjects studied. However, we have observed great variability in studies using [^{3}H]DHE as the labeled ligand and a systematic overestimation of site number when Scatchard plots have low linear correlation coefficient of < -0.80. Similar to other studies (37, 38), the K_D for antagonists was higher for [^{3}H]DHE than for [^{3}H]yohimbine. This absolute difference in K_D represents the antagonist characteristic for the receptor affinity.

The 4-h epinephrine infusions in vivo resulted in no change in platelet alpha-2-adrenergic binding site number or affinity for antagonists. However, to demonstrate down-regulation in vivo it is possible that a longer period of time of elevation of the agonist may be necessary. On the other hand, our patients with pheochromocytoma did not have a decrease in platelet alpha-2-adrenergic binding sites and no change was observed after tumor removal. The studies with prazosin, an alpha-1-adrenergic receptor antagonist, allowed for a more chronic elevation in plasma norepinephrine in normal subjects. The expected plasma level of prazosin achieved (39, 40) with this dose of prazosin used would not be likely to compete for the alpha-adrenergic receptor sites on platelets (9), a finding confirmed by us in preliminary studies not reported here. Therefore, it is not likely that prazosin acted as a protective agent preventing a down regulation effect from the increased plasma norepinephrine. There was no change in the number of alpha-2-adrenergic binding sites after 1 wk of prazosin and chronically elevated levels of plasma norepinephrine in these individuals.

In other studies where [^{3}H]DHE was used as the ligand, it has been shown that intact platelets in vitro show a decrease in aggregation and a decrease in alpha-2-adrenergic sites per platelet (23) when incubated with 10^{-4} M epinephrine. In contrast, other workers (24) have shown in studies which utilize [^{3}H]yohimbine that there was only an "apparent down regulation" after incubation of intact platelets with supraphysiologic levels of epinephrine. Although the differences in these two studies may be due to inherent differences of the two ligands, it is more likely that the apparent down regulation of platelet alpha-2-adrenergic receptors described in both papers represents an artifact due to catecholamine uptake by intact platelets as was discussed in the latter study (24). The incubation concentrations used in both of these in vitro studies far exceeds the normal physiologic range or even disease-related range in human subjects.

Other studies have shown a change in platelet adrenergic receptor affinity for agonists but not receptor number after in vitro additions of GTP or agonists (18, 41, 42). Thus, there appear to be two agonist affinity states, a high and low affinity, but a single antagonist affinity. Separate studies have shown that 40% of normal subjects have a curvilinear clonidine (an alpha-2 agonist) ligand Scatchard plot and 60% have a straight line Scatchard plot (13). The explanation for this is not clear. However, these data suggest that agonists may not be the ideal method of analyzing alpha-2-adrenergic receptors, if they may both change affinity and may or may not result in a curved Scatchard plot.

We hypothesize that in this adrenergic receptor system at physiologic plasma catecholamine concentrations, there is no change in receptor number or affinity associated with acute or chronic changes in catecholamine levels. This lack of receptor regulation by circulating levels of catecholamines may be common to all alpha-2-receptors, as has been hypothesized by others (36). An alternative consideration would be the possible "reappearance" of the alpha-2-binding sites during the process of preparing the platelet particulate. In the rat parotid gland,

there is a rapid resensitization of alpha receptors that appears to be regulated by membrane voltage (43). Still another consideration could be the observed lack of platelet alpha-2-adrenergic receptor regulation, which may be due to the fact that the platelet is lacking a nucleus that may be necessary to regulate receptor number. It should be further noted that no change in binding site number or affinity for antagonists was observed even during chronic changes of circulating catecholamines, when new platelets are replaced by the megakaryocyte. Since other tissues such as the parotid gland (43) and the liver are subject to ligand-induced down regulation, the apparent lack of down regulation of alpha-2-adrenergic binding sites may be an exception rather than the general rule. However, the liver and probably the parotid gland have alpha-1-adrenergic receptors rather than alpha-2-adrenergic receptors.

Acknowledgments

We would like to acknowledge Pat Hagan and Connie Meyers, Veterans Administration Medical Center, for their secretarial skills. We would also like to thank Dr. R. J. Lefkowitz, Departments of Medicine and Biochemistry, Duke University, Durham, NC, and Dr. R. W. Alexander, Department of Medicine, Harvard Medical School, Peter Bent Brigham Hospital, Boston, MA, for helping establish this assay in Dr. Pfeifer's laboratory.

This investigation was supported in part by a U. S. Public Health Service Special Emphasis Research Career Award, AM 00738; by National Institutes of Health grants AM 12829, AG 01926, AM 17047, and HL 29036; by the Kentucky Heart Association; and by the Seattle and Louisville Veterans Administration Medical Centers.

References

1. Grant, J. A., and M. C. Scrutton. 1979. Novel alpha$_2$ adrenoreceptors primarily responsible for inducing human platelet aggregation. *Nature (Lond.).* 277:659–661.

2. Huang, E. M., and T. C. Detwiler. 1981. Characteristics of synergistic actions of platelet agonists. *Blood.* 57:685–691.

3. O'Brien, J. R. 1963. Some effects of adrenaline and anti-adrenaline compounds on platelets in vitro and in vivo. *Nature (Lond.).* 200:763–764.

4. Mills, D. C. B., and G. C. K. Roberts. 1967. Effects of adrenaline on human platelets. *J. Physiol.* 193:443–453.

5. Bygdeman, S., and O. Johnson. 1969. Studies on the effect of adrenergic blocking drugs on catecholamine-induced platelet aggregation and uptake by noradrenaline and 5-hydroxytryptamine. *Acta Physiol. Scand.* 75:129–138.

6. Kaywin, P., M. McDonough, P. A. Insel, and S. J. Shattil. 1978. Platelet function in essential thrombocythemia. Decreased epinephrine responsiveness associated with a deficiency of platelet alpha-adrenergic receptors. *N. Engl. J. Med.* 299:505–509.

7. Kafka, M. S., J. F. Tallman, C. C. Smith, and J. L. Costa. 1977. Alpha-adrenergic receptors on human platelets. *Life Sci.* 21:1429–1438.

8. Alexander, R. W., B. Cooper, and R. I. Handin. 1978. Characterization of the human platelet alpha-adrenergic receptor. Correlation of [^{3}H]dihydroergocryptine binding with aggregation and adenylate cyclase inhibition. *J. Clin. Invest.* 61:1136–1144.

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

10. Motulsky, H. J., S. J. Shattil, and P. A. Insel. 1980. Characterization of alpha-2-adrenergic receptors on human platelets using ^{3}H-yohimbine. *Biochem. Biophys. Res. Commun.* 97:1562–1570.

11. Daiguji, M., H. Y. Meltzer, and D. C. U'Prichard. 1981. Human platelet alpha-2-adrenergic receptors: labeling with ^{3}H-yohimbine, a selective antagonist ligand. *Life Sci.* 28:2705–2717.

12. Mukheyie, A. 1981. Characterization of alpha-2-adrenergic receptors in human platelets by binding of a radioactive ligand ^{3}H yohimbine. *Biochim. Biophys. Acta.* 676:148–154.

13. Garcia-Sevilla, J. A., P. J. Hollingsworth, and C. B. Smith. 1981. Alpha-2-adrenoreceptors on human platelets: selective labelling by ^{3}H-clonidine and ^{3}H-yohimbine and competitive inhibition by antidepressant drugs. *Eur. J. Pharmacol.* 74:329–341.

14. Lynch, C. J., and M. L. Steer. 1981. Evidence for high and low affinity alpha-2-receptors. Comparison of ^{3}H-norepinephrine and ^{3}H-phentolamine finding to human platelet membranes. *J. Biol. Chem.* 256:3298–3303.

15. Jakobs, K. H., and R. Rauschek. 1978. ^{3}H-Dihydroergonine binding to alpha-adrenergic receptors in human platelets. *Klin. Wochenschr.* 56:139–145.

16. Shattil, S. J., M. McDonough, J. Turnbull, and P. A. Insel. 1981. Characterization of alpha-adrenergic receptors in human platelets using ^{3}H-clonidine. *Mol. Pharmacol.* 19:179–183.

17. Hoffman, B., and R. J. Lefkowitz. 1980. Alpha-adrenergic receptor subtypes. *N. Engl. J. Med.* 302:1390–1396.

18. Hoffman, B. B., D. Mullikin-Kilpatrick, and R. J. Lefkowtiz. 1980. Heterogeneity of radioligand binding to alpha-adrenergic receptors. Analysis of guanine nucleotide regulation of agonist binding in relation to receptor subtypes. *J. Biol. Chem.* 255:4645–4652.

19. Hoffman, B. B., A. DeLean, C. L. Wood, D. D. Schocken, and R. J. Lefkowitz. 1979. Alpha-adrenergic receptor subtypes: quantitative assessment by ligand binding. *Life Sci.* 24:1739–1746.

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

21. Tohmeh, J. F., and P. E. Cryer. 1980. Biphasic adrenergic modulation of beta-adrenergic receptors in man. *J. Clin. Invest.* 65:836–840.

22. Chuang, D.-M., and E. Costa. 1979. Evidence for internalization of the recognition site of beta-adrenergic receptors during receptor subsensitivity induced by (−)-isoproterenol. *Proc. Natl. Acad. Sci. USA.* 76:3024–3028.

23. Cooper, B., R. I. Handin, L. H. Young, and R. W. Alexander. 1978. Agonist regulation of the human platelet alpha-adrenergic receptor. *Nature (Lond.).* 274:703–706.

24. Karliner, J. S., H. J. Motulsky, and P. A. Insel. 1982. Apparent "down-regulation" of human platelet alpha$_2$-adrenergic receptors is due to retained agonist. *Mol. Pharmacol.* 21:36–43.

25. Davis, I. B., D. Sudera, and P. S. Sever. 1981. Endogenous agonist regulation of alpha-adrenoceptors in man. *Clin. Sci. (Lond.).* 61:207–210.

26. Born, G. V. R., and M. Lioss. 1963. The aggregation of blood platelet. *J. Physiol.* 168:178–195.

27. Santi, D. V., C. H. Sibley, E. R. Perriard, G. M. Tomkins, and J. D. Baxter. 1973. A filter assay for steroid hormone receptors. *Biochemistry.* 12:2412–2416.

28. Evans, M. I., J. B. Halter, and D. Porte, Jr. 1978. Comparison of double- and single-isotope enzymatic derivative methods for measuring catecholamines in human plasma. *Clin. Chem.* 24:567–570.

29. Colucci, W. S., G. H. Williams, and E. Braunwald. 1980. Increased plasma norepinephrine levels during prazosin therapy for severe congestive heart failure. *Ann. Intern. Med.* 93:452–453.

30. Stein, L., D. P. Henry, and M. H. Weinberger. 1981. Increase in plasma norepinephrine during prazosin therapy for chronic congestive heart failure. *Am. J. Med.* 70:825–832.

31. Izzo, J. L., D. Horwitz, and H. R. Keiser. 1981. Physiologic mechanisms opposing the hemodynamic effects of prazosin. *Clin. Pharmacol. Ther.* 29:7–11.

32. Cryer, P. E., A. B. Silverberg, J. V. Santiago, and S. D. Shad. 1978. Plasma catecholamines in diabetes: the syndromes of hypoadrenergic hyperadrenergic postural hypotension. *Am. J. Med.* 64:407–416.

33. Ziegler, M. G., C. R. Lake, and I. J. Kopin. 1977. The sympathetic-nervous-system defect in primary orthostatic hypotension. *N. Engl. J. Med.* 296:293–297.

34. Davis, B., D. Sudera, G. Sagnella, E. Marchesi-Saviottie, C. Mathias, R. Bannister, and P. Sever. 1982. Increased number of alpha receptors in sympathetic denervation supersensitivity in man. *J. Clin. Invest.* 69:779–784.

35. Chobanian, A. V., C. P. Tifft, H. Sackel, and A. Pitruzella. 1982. Alpha and beta adrenergic receptor activity on circulating blood cells of patients' idiopathic orthostatic hypotension and pheochromocytoma. *Clin. Exp. Hypertens.* A4:793–806.

36. Snavely, M. D., H. J. Motulsky, D. T. O'Connor, M. G. Ziegler, and P. A. Insel. 1982. Adrenergic receptors in human and experimental pheochromocytoma. *Clin. Exp. Hypertens.* A4:829–848.

37. Motulsky, H. J., and P. A. Insel. 1982. [^{3}H]Dihydroergocryptine binding to alpha-adrenergic receptors of human platelets. A reassessment using the selective radioligands [^{3}H]prazosin, [^{3}H]yohimbine, and [^{3}H]rauwolscine. *Biochem. Pharmacol.* 31:2591–2597.

38. Hoffman, B. B., T. Michel, T. B. Brenneman, and R. J. Lefkowitz. 1982. Interactions of agonists with platelet alpha-2-adrenergic receptors. *Endocrinology.* 110:926–932.

39. Jallion, P. 1980. Clinical pharmacokinetics of prazosin. *Clin. Pharmacokinet.* 5:365–376.

40. Hobbs, D. C., T. M. Twomey, and R. F. Palerm. 1978. Pharmacokinetics of prazosin in man. *J. Clin. Pharm.* 18:402–406.

41. Tsai, B. S., and R. J. Lefkowitz. 1979. Agonist-specific effects of guanine nucleotides on alpha-adrenergic receptors in human platelets. *Mol. Pharmacol.* 16:61–68.

42. Hollister, A. S., G. A. Fitzgerald, and D. Robertson. 1982. Catecholamines reduce alpha-2-adrenoreceptor agonist affinity in intact human platelets *in vivo* and *in vitro*. *Clin. Res.* 30:253.

43. Strittmatter, W. J., J. N. Davis, and R. J. Lefkowitz. 1977. Alpha-adrenergic receptors in rat parotid cells. II. Desensitization of receptor binding sites and potassium release. *J. Biol. Chem.* 252:5478–5482.