Delayed Desensitization of α2-Adrenoceptor-Mediated Platelet Aggregation in Depressed Patients

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After prolonged exposure to epinephrine, platelets are observed to desensitize α2-adrenoceptor–mediated aggregation responses in vitro. Herein, this phenomenon was studied as a possible in vitro model for α2-adrenoceptor dysregulation in depression. Platelet-rich plasmas obtained from 22 unipolar depressed patients and 25 healthy subjects were preincubated with 20 μmol/L of epinephrine for various lengths of time prior to stirring. By comparing the subsequent extents of aggregation, we observed significantly less desensitization at 4, 20, 30, or 60 minutes postepinephrine exposure ($p \leq .05$) in depressed patients as compared to healthy controls. This blunted desensitization appeared to be due to a delayed onset of desensitization during the first 0.5 to 2 minutes after epinephrine exposure, since thereafter, the monoexponential desensitization rate did not differ in depressed patients, but the extent of desensitization remained less as compared to healthy subjects. The extent of desensitization was correlated ($r = -0.48$, $p = .02$) with the density (B_{max}) of the α2-adrenoceptor high-affinity state, as detected in undesensitized platelet membranes by p^{125}I-clonidine binding. An elevation was also observed in the density of nonadrenergic p^{125}I-clonidine–binding sites (putative imidazoline I_1 sites) in platelet membranes from depressed patients compared to healthy control subjects. Following treatment with desipramine, the patients ($n = 15$) displayed more normal (nonblunted) extents of desensitization of aggregation, and the B_{max} values for the putative I_1 sites were at the levels of healthy controls. If similar aberrations exist in neurons of depressed patients, this may explain a dysregulation of the noradrenergic system believed to underlie depression. **[Neuropsychopharmacology 9:55–66, 1993]**

KEY WORDS: *Depression; α2-adrenoceptor; Desensitization; Imidazoline; G-proteins*

Inhibitory α2-adrenoceptors play an important physiologic role in regulating monoamine release (both norepinephrine and serotonin) in the central nervous system (Langer 1981; Raiteri et al. 1983, 1990; Starke 1981, 1987). An autoinhibitory role for presynaptic α2-adrenoceptors is of particular interest to the study of depression because reduced monoaminergic function has been postulated to underlie depression (Bunney and Davis 1965; Schildkraut 1965; Meana et al. 1992). Idazoxan, an α2-adrenoceptor antagonist is an effective antidepressant (Osman et al. 1989), suggesting the presence of supersensitive α2-adrenoceptors in depression. Meana and coworkers (Meana et al. 1992) have also shown that both the density and affinity of high-affinity state α2-adrenoceptors are elevated in certain brain regions of suicide victims with histories of depression. Furthermore, most current concepts regarding mechanism(s) of action of antidepressant treatments invoke dysfunction of central noradrenergic receptors (Char-

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ney et al. 1981; Sulser 1984). Downregulation of α2-adrenoceptors has been implicated during chronic antidepressant therapy (Vetulani and Antkiewicz-Michaluk 1985; Finberg 1987).

Regarding peripheral measurements, platelet radioligand–binding density (B_{max}) for high-affinity state α2-adrenoceptors has been reported by us (Piletz et al. 1990, 1991) and others (Garcia-Sevilla et al. 1981; reviewed in Werstiuk et al. 1992) to be elevated in depressed patients. Several laboratories (reviewed in Piletz et al., 1991) have additionally reported that the B_{max} of high-affinity state radioligand binding to platelet α2-adrenoceptors declines following successful antidepressant treatment of depressed patients (Garcia-Sevilla et al. 1981, 1990; Healy et al. 1983; Pandey et al., 1989). Other groups have failed to detect a decline in α2-adrenoceptor binding with treatment (reviewed in Werstiuk et al. 1992). Platelet α2-adrenoceptors have further been reported to be supersensitive in depressed patients with regard to epinephrine-induced aggregation (Garcia-Sevilla et al. 1990) and epinephrine-induced phosphoinositide responses (Mori et al. 1991), but not with epinephrine-mediated inhibition of adenylate cyclase (Garcia-Sevilla et al. 1990). Thus, a considerable body of literature implicates platelet α2-adrenoceptor supersensitivity in depression.

Most previous positive reports of increased α2-adrenoceptor density in depression have utilized agonist radioligand–binding assays. ^{3}H-Agonists label predominantly the high-affinity state of α2-adrenoceptors that are coupled to guanine nucleotide binding regulatory proteins (G-proteins) (Hoffman and Lefkowitz 1980; Thomsen et al. 1988; reviewed in Kafka and Paul 1986; Piletz et al. 1991). ^{3}H-Antagonists, on the other hand, have not shown differences at the α2-adrenoceptor in depression, and these agents label both high- and low-affinity states of the α2-adrenoceptor (Asakura et al. 1985). Based on these radioligand binding differences, Garcia-Sevilla et al. (1986) have hypothesized that enhanced coupling of the high-affinity α2-adrenoceptor state might underlie receptor supersensitivity in depression.

Dysregulation of the α2-adrenoceptor was proposed as the dominant factor in the "Dysregulation Theory of Depression" as put forward by Siever and Davis (1985). Under this theory it was postulated that altered α2-adrenoceptor regulation might manifest as an erratic or unstable noradrenergic system, for which there is considerable evidence in depressed patients (Siever and Davis 1985; Gwirtsman et al. 1989). One important level of receptor regulation is desensitization. After prolonged exposure of target cells to hormones or neurotransmitters, the subsequent receptor-mediated responses evoked by agonists are often observed to be attenuated or desensitized. In the best studied example of this, the desensitization of β-adrenoceptors was shown to initially involve uncoupling of an active receptor state formed between the receptor and the G-protein (Sibley and Lefkowitz 1985). Subsequently, receptor uncoupling is followed by receptor phosphorylation and receptor internalization leading to downregulation (Sibley and Lefkowitz 1985; Bouvier et al. 1988).

Previous studies have demonstrated that preincubation of unstirred platelets with an α2-adrenoceptor agonist (i.e., epinephrine or clonidine) will desensitize in vitro the subsequent aggregation when platelets are rechallenged and stirred (Cooper et al. 1978; Brodde et al. 1982; Hollister et al. 1983; Motulsky et al. 1986). With intact platelets, this form of desensitization is homologous, that is, specific for only α2-adrenoceptors (Motulsky et al. 1986; Pitcher et al. 1992); it does not attenuate the ability of α2-adrenoceptors to inhibit cyclic adenosine monophosphate accumulation (Motulsky et al. 1986); and after short incubation times (1 to 60 minutes), this desensitization does not downregulate α2-adrenoceptor number, but instead results in a twofold reduction of affinity for the agonist (Hollister et al. 1983). Furthermore, desensitization of epinephrine-initiated platelet aggregation may be physiologically important because this process has been demonstrated in vivo to exist in response to acute infusions of norepinephrine (Jones et al. 1986; Hamilton et al. 1987).

In the present study, we have sought to determine if epinephrine-initiated desensitization might differ in depressed patients relative to healthy controls in vitro. Specifically, we have utilized the well-documented phenomena of desensitization of platelet α2-adrenoceptor–mediated aggregation in vitro (Mutulsky et al. 1986) to monitor platelet α2-adrenoceptor desensitization in patients and controls. Thereby, we provide, for the first time, evidence that depressed patients might differ from healthy subjects in regard to their intrinsic ability to desensitize an α2-adrenoceptor-mediated response.

METHODS

Blood Collections

All patients and healthy control subjects gave written informed consent prior to entry into the study. Subjects were required to observe restrictions on diet and activity for 3 days preceding the blood collections (Piletz et al. 1990). The use of medication(s) was limited to items not known to affect platelet function and was permitted only if the subject had been receiving that particular medication for at least 3 months without interruption. Aside from the specified desipramine-treatment study (described below), the following "extra medications" were allowed. Two subjects were receiving antihypertensive medications (one was receiving

captopril and another was receiving enalapril maleate plus diazoxide). Three subjects were using antihistamines (one used cimetidine and two used seldane). Three subjects were using progesterones. One subject was using ibuprofen along with sudafed. One subject was using prednisone. One subject was using phenylpropanolamine. Another subject was using low-dose antibiotics. Blood samples were collected throughout all seasons of the year in an effort to minimize possible seasonal variation between groups. Subjects reported to the outpatient clinic at 8:30 AM. An indwelling 18-gauge angiocatheter was inserted into the anticubital vein with a heparin lock and subjects then rested in a supine position for at least 10 minutes prior to blood drawing which occurred between 9:00 and 10:00 AM.

In most cases, the blood drawing procedure occurred in four steps, with the exception that a subset of subjects (described below) had blood drawn only for the aggregation measurements. (1) The first 3 ml of blood was discarded to avoid tissue thromboplastin. (2) An additional 10 ml of blood was then collected into a vacutainer with heparin (20 U/ml) on ice for analysis of plasma catecholamines. (3) An additional 100 ml of blood was then collected for radioligand-binding measurements into two plastic 50-cc syringes and mixed with heparin (20 U/ml blood) to prevent coagulation. (4) An additional 27 ml of blood was collected for aggregation measurements and diluted with 3 ml of CPD (2.63% sodium citrate, 0.33% citric acid, 0.22% sodium phosphate, 2.55% dextrose) to prevent coagulation. Because platelet aggregation is influenced by the release of citrate from red blood cells during centrifugation (Ts'ao et al. 1976), we compensated for differences in citrate concentration that would be expected based on varying hematocrits between samples. This was accomplished by obtaining the hematocrit, and then further adjusting the sodium citrate concentration of each blood sample to that found in blood with a hematocrit of 50, prior to the aggregation measurements according to the formulations of Ts'ao et al. (1976) and Kelton et al. (1980). All platelet preparatory procedures were conducted in plastic tubes kept at room temperature. Plasma obtained from each subject was stored at −80°C and later thawed for the assay of catecholamine concentrations using high-performance liquid chromatography with a Coulochem detector (ESA Corp., Bedford, MA), as described by Mefford (1981).

Control Subjects

Prospective healthy subjects underwent an interview with a psychiatric nurse and a psychiatrist to establish physical and mental health (Piletz et al. 1990). Healthy subjects reported no personal or family history of psychiatric illness among first-degree relatives, and none indicated a history of alcohol or drug abuse. Healthy subjects were paid for their participation. For the aggregation measurements, there were 17 females and eight males in our healthy subject group, with an overall age of 36.6 ± 8.5 (SD) years. Fifteen of those healthy subjects (11 females, four males) had additional platelets collected at the same time for measurements of radioligand binding and plasma catecholamine concentrations.

Patients

Patients were recruited through hospital referrals and treated as outpatients. Depressed patients (n = 22) met Research Diagnostic Criteria (RDC) for primary major depression, unipolar subtype. Diagnoses were made by one of two experienced psychiatrists during a comprehensive intake interview. The kappa coefficient for interrater reliability was 0.82. The severity of depression was evaluated using the Hamilton Depression Rating Scale (HDRS) (Hamilton 1967). For epinephrine-initiated platelet aggregation measurements, the depressed patient group was composed of 15 females and seven males, with an overall age of 42.5 ± 11.4 years. Among the depressed patients a subset of 10 females and six males had additional platelets collected for the additional radioligand binding and plasma catecholamine measurements. To study the effects of treatment, 15 depressed patients were restudied for platelet aggregation after 6 weeks of treatment with desipramine (composed of eight females and seven males). Four other patients dropped out of the study before completing treatment, and three other patients were discontinued early due to adverse side effects of desipramine. The overall age of the patients at posttreatment was 43.2 ± 8.6 years. Patients were deemed responders to treatment if their posttreatment score on the HDRS was less than 10 or at least 50% reduced from the baseline score. Four of the 15 depressed patients did not respond to treatment by these criteria. An additional group (n = 4) of patients meeting RDC for generalized anxiety disorder were also recruited into the study, but because of their small number, they were not used for statistical comparisons. Among the anxiety patients there were three females and one male with an overall age 36.3 ± 8.4 years. Patients with generalized anxiety disorders were studied prior to treatment and, in addition to anxiety, they displayed a slight level of depression (HDRS = 11.0 ± 2.9).

Aggregation Measurements

Platelet-rich plasma (PRP) was prepared by centrifugation at 280 × g for 10 minutes at room temperature. The red cell pellet was recentrifuged at 2,000 × g for 15 minutes to obtain platelet-poor plasma (PPP). Platelets were counted with a Coulter ZM counter and a

model 256 channelizer (Coulter Electronics, Hialeah, FL) and the PRP was adjusted to 1.8×10^8 platelets/ml with PPP. Aliquots of PRP (0.25 ml) were placed in glass reaction tubes, gently gassed for 10 seconds with 5% CO_2/95% O_2, and sealed with parafilm. The samples remained sealed, except briefly during additions, until the stirring was initiated in the aggregometer. A platelet aggregometer (model PICA; Chrono-log Corp., Havertown, PA) was used to measure aggregation (Feinman et al. 1985). Prior to the addition of epinephrine, the PRP had been preincubated with 1 mmol/L aspirin for 20 minutes at 37°C to inhibit platelet cyclooxygenase and the second wave of aggregation (Motulsky et al. 1986).

Unstirred PRP was preincubated for 0 to 60 minutes at 37°C with 20 μmol/L epinephrine. Aggregations were initiated by adding a stir bar to each cuvette and stirring at setting No. 12 on the aggregometer. Light transmittance through untreated PRP was set at 0% and that through PPP at 100%. Measurements of the extent and rate of aggregation were obtained by measuring "primary" aggregations, defined by the point where the aggregatory response to 20 μmol/L epinephrine (in the presence of aspirin) deviated from a straight line on the chart recorder. Only primary aggregations are directly linked to the α2-adrenoceptor (Motulsky et al. 1986). A drop of 100 chart units indicated maximal aggregation (chart speed = 1 cm/min). The time from preparation of each PRP sample until the first addition of epinephrine was in no case less than 40 minutes, and the whole experiment (in duplicate) was accomplished within 3 hours of preparing the PRP. During this period, epinephrine-initiated aggregation measurements were made without preincubations to control for possible changes in the response during this period. In no case was there any change in the epinephrine-initiated platelet aggregation parameters during the first 110 minutes after preparation of the PRP, unless the platelets had been preincubated with epinephrine. However, with about half the samples there was a noticeable decline during longer storage times, even in the absence of epinephrine preincubation. This decline had been previously reported (Rossi and Louis 1975) and indicates a decline in platelet viability during long storage times. In such cases where this was noted, we utilized only the values from the first, unaffected, experimental period for the data analysis. Otherwise all values were taken from the average of repeated measures.

Radioligand Binding

The preparation of purified plasma membranes from platelets has been previously described (Piletz et al. 1990). Saturation binding assays (20 points each) were performed in duplicate using p^{125}I-clonidine (New England Nuclear, Boston, MA) in concentrations from 0.01 to 10 nmol/L. The reactions consisted of 0.25 ml volumes of 0.015 mg plasma membrane protein, 5 mmol Hepes, 0.5 mmol $MgCl_2$, 0.5 mmol EGTA, pH 7.4 (adjusted with 0.1 mmol NaOH). The p^{125}I-clonidine (New England Nuclear) was isotopically diluted with unlabeled p-iodoclonidine (Research Biochemicals, Natick, MA) to 440 Ci/mmole. Specific binding at α2-adrenoceptors was determined by subtracting total binding from that observed in the presence of 10 μmol/L norepinephrine. Specific binding to nonadrenergic-binding sites was determined as the additional binding displaced by an imidazoline compound, either 10 μmol/L clonidine or 10 μmol/L moxonidine. Moxonidine was the gift of Dr. Siegfried Schafer (Solvay Pharma Corporation, Hannover, Germany). Reactions were conducted for 60 minutes at 21°C and terminated by rapid filtration. Radioactivity trapped on the filters was counted for 10 minutes per sample in a liquid scintillation counter with 71% efficiency (Piletz et al. 1991b). The α2-adrenoceptor binding (specific binding ± norepinephrine) and the nonadrenergic-binding (additional specific binding ± the imidazoline compound) sites were quantified using a LIGAND computer-assisted two-site fit (McPherson 1985; Piletz et al. 1992).

Data Analysis

Data from the initial epinephrine-initated platelet aggregations (initial extent and rate without desensitization) were directly used for statistical comparisons between subject groups, without transformation. However, the desensitization of aggregation data was first expressed as a percent of the maximal extent or maximal rate of epinephrine-intiated aggregation obtained in platelets prior to preincubation with epinephrine (i.e., the percent of pre-desensitized aggregation). A time course for the desensitization of each subject was constructed and modeled by a one-component exponential decay algorithm (GraphPAD Software, San Diego, CA). Thus, the half-time ($t½$) and $extent_{max}$ (after 60 min) of desensitization were derived from both of the aggregation parameters (rate and extent) for each subject. Additional parameters that were analyzed included the density (B_{max}) and affinity (K_d) values for p^{125}I-clonidine binding sites, the plasma catecholamine concentrations, and the clinical rating scores. Student's *t* tests, along with repeated-measures analysis of variance, were used to test for statistical differences and correlations (SAS/STAT, 1989). For each test, we examined whether the variables were normally distributed, and then we determined whether target variables were related to possible confounders (age, sex). Bonferroni *p* values were used to adjust for multiple comparisons. Multiple regression analyses were performed for each target variable with subject's age, sex, and plasma noradrenaline concentration as covariates.

because there were only four nonresponders to treatment, we did not perform separate analyses on those patients. However, statistical comparisons were performed by excluding the nonresponders from the total patient group, as well as the selected patients taking certain medications, and in no case did this alter the significance of any of the findings; therefore, only the results from the overall depressed patients group are reported. The data are expressed as the mean plus or minus standard deviation.

RESULTS

Ages of the healthy subject group (36.6 ± 8.5 years) were slightly younger than those of the depressed patients (42.5 ± 11.4 years) ($p \leq .05$). However, males and females were of approximately equal age among the subject groups (see under Methods). Moreover, there was no effect of age or gender on any of the variables measured. Among the depressed patients, the severity of depression as measured by the HDRS was 25.6 ± 4.4. After treatment with desipramine, the HDRS rating scale for depressed patients was 11.4 ± 9.4. This overall improvement in HDRS scores following treatment was statistically significant ($p = .0001$).

In the absence of epinephrine preincubation (i.e., undesensitized), we observed no significant differences in either the extent or the rate of platelet aggregation between depressed patients, healthy subjects, and anxiety patients (Table 1). In keeping with this, neither the K_d nor the B_{max} of p^{125}I-clonidine binding at the platelet α2-adrenoceptor were significantly different between the untreated diagnostic categories (Table 1). Furthermore, there were no statistically significant effects of the 6- to 8- week treatment with desipramine on undesensitized epinephrine-initiated aggregation parameters. There was, however, a marginal decline ($p = .08$) in the B_{max} for α2-adrenoceptor binding in depressed patients following treatment (Table 1). No significant changes were noted in the α2-adrenoceptor K_d following treatment. These results thus indicate no changes in the high-affinity state of the platelet α2-adrenoceptor in depression, at least in undesensitized platelets.

We next measured desensitized platelet aggregatory responses to epinephrine throughout a 0.5- to 60-minute preincubation period, in vitro. A typical pattern of the responses to epinephrine desensitization of aggregation from a healthy subject is shown in Figure 1. Desensitization parameters for both the rate and extent of aggregation were computed. Because the two measures were highly concordant, in the interest of space we present analyses on only the desensitization of extent of aggregation data.

Whereas the aggregation of nonpreincubated platelets was comparable in depressed patients and healthy controls (Table 1) after epinephrine desensitization, the subsequent aggregations were higher in depressed pa-

Table 1. Undesensitized Epinephrine-Initiated Platelet Aggregation and Related Factors in Patients and Healthy Subjects

Parameter	Depressed Pre-DMI	Depressed Post-DMI	Healthy Subjects	Anxiety Pre-TX
Aggregation extent (chart units)	18.3 ± 8.7	21.6 ± 8.4	20.5 ± 5.9	18.7 ± 4.5
Aggregation rate (U/sec)	0.57 ± 0.27	0.57 ± 0.15	0.56 ± 0.20	0.55 ± 0.14
α2-AR_H K_d (nMol)	0.07 ± 0.06	0.07 ± 0.07	0.07 ± 0.05	0.07 ± 0.06
α2-AR_H B_{max} (fmol/mg)	59.9 ± 37.2	37.0 ± 30.8*	56.4 ± 28.0	58.8 ± 14.5
I_1 Site K_d (nMol)	2.58 ± 2.16	1.16 ± 0.65*	1.26 ± 0.77	0.78 ± 0.27
I_1 Site B_{max} (fmol/mg)	828 ± 342§	367 ± 33§	352 ± 249	285 ± 139
Plasma NE (pg/mL)	282 ± 140	438 ± 25‡	195 ± 72	339 ± 72
Plasma EPI (pg/ml)	65.9 ± 34.3	67.8 ± 39.4	63.7 ± 28.3	63.0 ± 20.0
Plasma DA (pg/ml)	69.9 ± 30.9	70.9 ± 53.2	85.4 ± 28.6	61.0 ± 19.4

Aggregation values were obtained from chart recordings as shown in Figure 1 prior to desensitization (i.e., 0 min.).

Abbreviations: α2-AR_H = high-affinity state of the α2-adrenoceptor detected by p^{125}I-clonidine saturation binding; I_1 = the putative Imidazoline$_1$ receptor detected by p^{125}I-clonidine saturation binding (see Methods Section); NE = norepinephrine; EPI = epinephrine; and DA = dopamine.

* $p = .08$; ‡ $p < .03$; § $p < .005$.

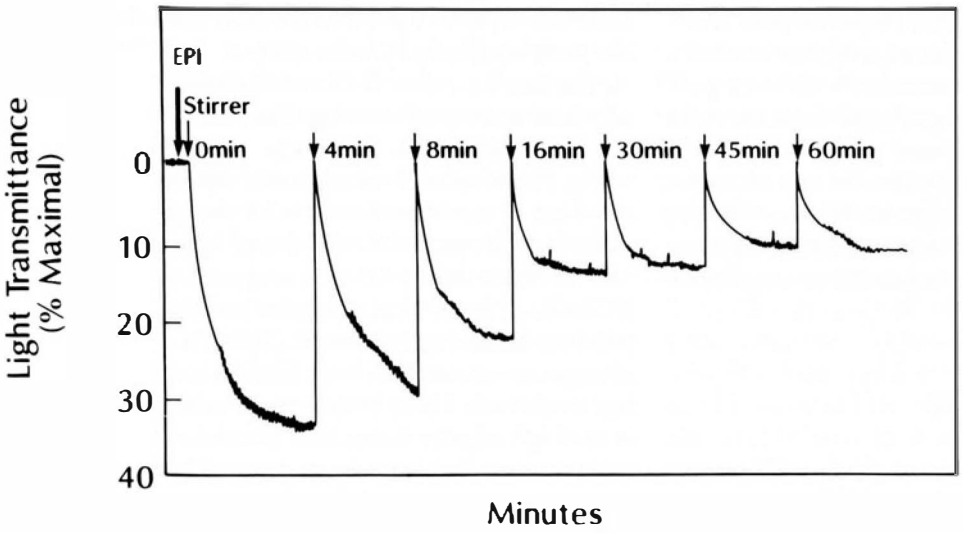

Figure 1. Tracings from one healthy subject's platelets showing the successively smaller platelet aggregations in response to progressively longer periods of preincubation with 20 μmol epinephrine (EPI) prior to stirring. Each of the tracings is from a fresh tube of PRP and the time scale is not continuous.

tients than in healthy subjects (Fig. 2). The higher aggregations for patients were statistically significant at 4, 20, 30, and 60 minutes after preincubation with epinephrine (60 minutes = E_{max}) (Fig. 3). As can be seen in Figure 2, after only 4 minutes of preincubation with epinephrine, the aggregation values from the healthy controls were clustered around 60% of the pre-desensitized aggregation extents. With depressed patients the values were clustered around 75% of the pre-desensitized aggregation extents. Interestingly, four nonresponders as well as three patients who were subsequently discontinued from desipramine treatment due to adverse treatment side effects were among those patients with the values closest to control levels (Fig. 2). Following treatment of patients with desipramine, desensitization still tended to be less in patients than in healthy subjects, but it was not statistically significant (Fig. 2). Thus epinephrine appeared to desensitize platelet aggregation less in depressed patients than in healthy subjects.

A blunted desensitization of aggregation in depressed patients appeared to result from a delayed onset of desensitization during the first 0.5 to 2 minutes after epinephrine preincubation (Fig. 3 insert). Thereafter, in the 2- to 60-minute preincubation cases, the overall desensitization rate was not different in depressed patients compared to controls. Desensitization over the entire 2- to 60-minute preincubation periods yielded $t½ = 11.3 \pm 8.0$ minutes in untreated depressed patients, versus $t½ = 18.9 \pm 16.6$ minutes in healthy subjects (NS, $p > .05$).

Concentrations of plasma catecholamines were found to exist within normal ranges among our untreated subject groups (Table 1). The only significant finding with catecholamines was that norepinephrine concentrations were elevated during desipramine treatment (Table 1). However, none of the plasma catecholamine concentrations were found to be correlated with any of the aggregation or radioligand binding values.

Although the emphasis of this study was primarily on epinephrine-initiated aggregation, we also measured the B_{max} of nonadrenergic Imidazoline-binding sites (putative I_1) on platelets. The radioligand-binding assay could readily discriminate I_1 and α2-adrenoceptor sites. Surprisingly, we observed an elevation in p^{125}I-clonidine binding to the nonadrenergic Imidazoline I_1 site in depressed patients despite no change in

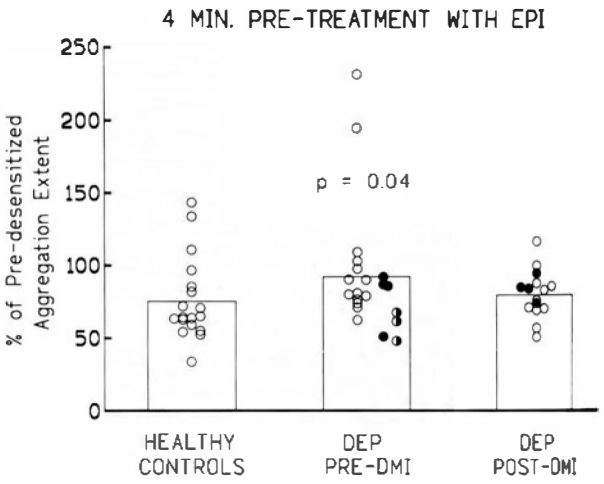

Figure 2. Scatter-plot of individual subject values for the extent of epinephrine-initiated platelet aggregations after a 4-minute preincubation (without stirring) with 20 μmol epinephrine. Values are expressed as a percent of the epinephrine-initiated aggregations observed without preincubations (i.e., percent of pre-desensitized aggregation extents). Closed circles represent the depressed patients who did not respond to 6- to 8-week treatment with desipramine (DMI nonresponders). Half-filled circles represent those patients who discontinued desipramine treatment prior to the post-treatment blood drawing because of medication side effects.

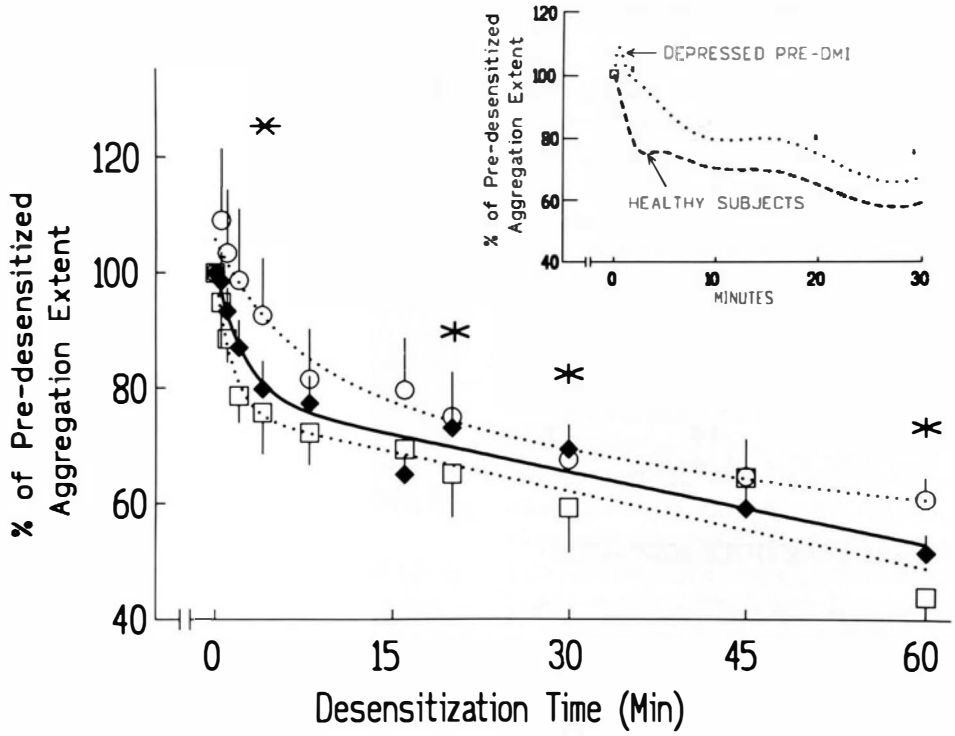

Figure 3. Comparison of the desensitization time courses observed in depressed patients and healthy subjects. Data from tracings of each subject were obtained as in Figure 1 and quantitated as described in Table 2 and Methods. Values represent the percent of the initial aggregation extent (i.e., at 0 minutes of preincubation with epinephrine) from depressed patients prior to treatment (O—O), depressed patients after treatment for 6 weeks with desipramine (◆—◆), and healthy control subjects (□- - -□). Asterisks indicate the times of preincubation at which statistical differences ($p \leq .05$) were observed between untreated patients and healthy subjects. Curves were fit by a monoexponential decay algorithm. Insert: Smooth lines were fit to the same data as shown in the larger figure to indicate differences in the shape of desensitization between depressed patients and healthy subjects; emphasizing an apparent delay in depressed patients at the outset of desensitization during short-time (0 to 2 minute) preincubations with epinephrine.

α2-adrenoceptor sites (Table 1). Moreover, the B_{max} for the nonadrenergic I_1 site declined following treatment of the patient group (Table 1). This rather robust increase in I_1 sites in depression ($p < .005$) might explain our previous positive findings of increased ^{3}H-amino-clonidine binding in depression (Piletz et al. 1990, 1991) compared to no change in α2-adrenoceptors observed in this study. Our earlier reports lacked the sensitivity to completely distinguish α2-adrenoceptors from I_1 sites (discussed in Piletz et al. 1990, 1991); therefore, an upregulation of binding in depression may have been solely due to I_1 sites misidentified as α2-adrenoceptors. However, the nonadrenergic measurement of ^{125}I-clonidine binding to I_1 sites (K_d or B_{max}) did not correlate with epinephrine-initiated platelet aggregation, in keeping with the reported low affinity of I_1 sites for epinephrine (Piletz et al. 1992).

Next we attempted to determine whether correlations existed between the measured variables. Surprisingly, with undesensitized platelets, we failed to obtain any significant correlations between epinephrine-initiated aggregation and α2-adrenoceptor density; only a weak but nonsignificant correlation existed between the extent of aggregation and the α2-adrenoceptor B_{max} (for all subjects $r = 0.203$, $p = .241$). A similar nonsignificant correlation was also found between the extent of undesensitized aggregation and the I_1 B_{max} (for all subjects $r = 0.24$, $p = .19$). No other trends were observed among the undesensitized aggregation parameters in any diagnostic groups. Furthermore, the undesensitized aggregation parameters were not correlated with any of the subsequent desensitization parameters. However, a significant correlation was observed between the maximum extent of desensitization in response to epinephrine and the B_{max} for the high-affinity state platelet α2-adrenoceptor. Figure 3A demonstrates this correlation for all subjects ($r = 0.479$, $p = .02$). This correlation remained statistically significant ($p \leq .05$) among just the depressed patients (as seen in the open circles of Fig. 3A). Because of the large number of variables in this study, any single correlation might represent a type I statistical error. However, no correlations were found between any of the other desensitization of aggregation parameters and the other

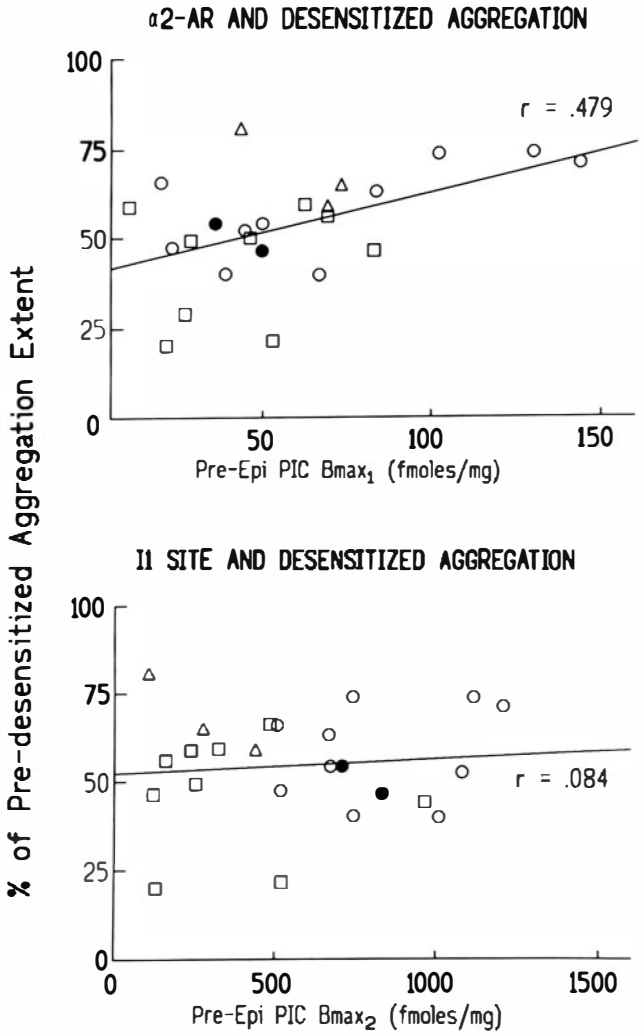

Figure 4. Correlations between platelet p^{125}I-clonidine (PIC) B_{max} values and the maximal extent of desensitization of epinephrine-initiated platelet aggregation. The B_{max} values were obtained from each subject using platelets prior to epinephrine desensitization (PRE-EPI) (see Methods). The B_{max1} corresponds to the density of α2-adrenoceptors, and B_{max2} corresponds to the density of I_1 receptors. Maximal extents of desensitization were determined by modeling the monoexponential decay of the data from each subject as shown in Figures 1 and 2 throughout the 60-minute preincubations. The data are from untreated subjects: depressed patients prior to treatment with desipramine who responded clinically after 6 weeks (O), depressed patients prior to treatment with desipramine who did not respond clinically after 6 weeks (●), patients with generalized anxiety disorder (△), and healthy subjects (□). The lines were derived by linear regression analysis.

variables, including the nonadrenergic I_1 site radioligand-binding parameters (Fig. 3B).

Following 6 to 8 weeks of treatment with desipramine, platelets were recollected from a subgroup of 15 depressed patients and reanalyzed for desensitization in vitro. The results were equivocal regarding a possible normalization in the desensitization of aggregation parameters following treatment. In treated patients, the extent of desensitization was no longer different in patients compared to healthy subjects, suggesting a normalization (Figs. 2 and 3, closed diamonds). However, with regard to the rate of desensitization, in medicated patients the rates were still higher than in healthy subjects ($p \leq .5$ at 8 and 20 minutes), as had been observed in the untreated patients (Fig. 2). Moreover, we observed no association between any of the biochemical measurements and the clinical rating scales (HDRS-A or HDRS-D scores). Therefore, these results are more consistent with the possibility that blunted desensitization is a trait marker, rather than an index of severity for depression.

DISCUSSION

In previous studies (Piletz et al. 1990, 1991), we reported that platelets from depressed patients displayed elevated ^{3}H-p-aminoclonidine binding to high-affinity state α2-adrenoceptors, as compared to healthy subjects. Therefore, it was anticipated that epinephrine-initiated platelet aggregations might also be abnormally robust in depressed patients. Our first attempts to demonstrate this utilized undesensitized platelets (Fig. 1, "0 min"), similar in preparation to those platelets used in our earlier binding studies (Piletz et al. 1990, 1991). Nevertheless, in undesensitized platelets we were unable to detect any differences in aggregation between depressed patients and healthy subjects (Table 1). However, as short a time as 4 minutes of desensitization of aggregation by epinephrine revealed that the subsequent aggregation of platelets in depressed patients was indeed more robust than in healthy subjects (Fig. 2). A 1-hour time course of epinephrine preincubations further revealed that the extent of in vitro desensitization of platelet aggregation in depressed patients was blunted (Fig. 3). Moreover, that blunted desensitization of aggregation appeared to be consequential to a delayed onset of desensitization during the first 0 to 1 minutes of desensitization (Fig. 3 insert). Therefore, our data emphasize α2-adrenoceptor dysregulatory phenomena in depression, rather than a simple steady-state alteration.

Our failure to observe steady-state differences in aggregation is at odds with Garcia-Sevilla et al. (1990) who reported potentiation of epinephrine-initiated platelet aggregation in depressed patients using undesensitized platelets. One difference between our studies is that Garcia-Sevilla et al. (1990) used lower epinephrine concentrations (i.e., 1 μmol) to initiate aggregation rather than the 20-μmol concentration that

we used. However, another difference is that our subjects rested in the supine position prior to the blood drawing, whereas the subjects reported by Garcia-Sevilla et al. (1990) did not. Since desensitization of platelet epinephrine-initiated aggregation has been shown to occur in vivo after short-time infusions of epinephrine (Jones et al. 1986; Hamilton et al. 1987), sympathetic or adrenal output into the blood might desensitize the platelet α2-adrenoceptor. Elevations in plasma catecholamines occur in some individuals as a consequence of anticipatory stress prior to blood giving. Therefore, orthostatic changes in circulating catecholamines might be considered as another factor in the study of Garcia-Sevilla et al. (1990), which could have actually desensitized their subjects' platelets in vivo. If that were the case, then our data (Fig. 2) would be entirely consistent with their data.

Presynaptic α2-adrenoceptors provide feedback inhibitory control over the vesicular release of norepinephrine (Langer 1981). Siever and Davis (1985) have put forward the "dysregulation hypothesis of depression" based on evidence that the regulation of noradrenaline release is "inadequately buffered" in depression. Briefly summarized, that evidence is twofold: (A) plasma noradrenergic metabolite concentrations have been shown to be typically more variable in depressed patients than in healthy subjects under basal conditions (Gwirtsman et al. 1989), and (B) changes in diet or activity appear to cause alterations in the excretion of norepinephrine metabolites in depressed patients that do not occur in healthy individuals (Muscettola et al. 1984; Goode et al. 1973; Beckmann et al. 1979). Thus, Siever and Davis (1985) have further suggested that a dysregulated α2-adrenoceptor might underlie these findings such that "depressed patients display reduced responsiveness to discrete, appropriate stimuli." In fact, such statements might have been used to accurately describe our finding of blunted desensitization to epinephrine stimulation of the platelet α2-adrenoceptor. In this context, our data therefore extend the contention put forth by Siever et al. (1987) that α2-adrenoceptor dysregulation, such as blunted desensitization, might underlie depression.

Our aggregation measurements were performed in the unique plasma obtained from each individual, which contained endogenous catecholamines. Therefore, we sought to determine whether a blunted desensitization of the α2-adrenoceptor in depression might be correlated with, and therefore of secondary importance to, changes in concentrations of plasma catecholamines. However, we found no difference between the untreated diagnostic groups in their plasma catecholamine concentrations (Table 1). Similarly, three earlier studies have also failed to detect differences in plasma norepinephrine concentrations in depressed patients (Sevy et al. 1989; Siever et al. 1984; De Villiers et al. 1989). Therefore, it appears unlikely that the blunted desensitization observed in depressed patients at pretreatment could be explained by lowered catecholamine concentrations in plasma. However, since posttreatment plasma norepinephrine concentrations were elevated (Table 1), a possibility exists that the partial normalization of the blunting observed after desipramine treatment could relate to norepinephrine. Further investigations will need to be performed to determine if blunted desensitization is a state or trait marker for depression.

An intrinsic factor within the platelet should also be considered to possibly underlie the blunted α2-adrenoceptor desensitization in depression at pretreatment. One candidate for that intrinsic factor might be G-proteins, because these regulatory proteins are involved in β-adrenoceptor desensitization (Sibley and Lefkowitz 1985). Evidence supporting altered G-protein concentrations in depression has been reported by Young et al. (1991), who demonstrated that postmortem cerebral cortex G_s α subunits are elevated in bipolar affective disorder. Based on such evidence, Schreiber and Avissar (1991) have further suggested that hyperfunction of G-proteins might lead to an "unstable catastrophic system" characteristic of a manic or depressive state. Therefore, alterations in G-proteins in depression have precedent.

In the present study, we were unable to replicate our earlier results (Piletz et al. 1990, 1991), which demonstrated an elevation in α2-adrenergic binding of ^{3}H-p-aminoclonidine on platelets in depression. In the present study, the binding of p^{125}I-clonidine to platelet α2-adrenoceptors was not different in depressed patients compared to healthy controls (Table 1). It is unlikely that this discrepancy is due to differences between these radioligands because in numerous experiments designed to compare these radioligands, we have found no difference in the nature of the sites that these two analogues label (unpublished results). Moreover, present results confirm a decline in α2-adrenergic binding of p^{125}I-clonidine after desipramine treatment of patients (Table 1), which is comparable in magnitude to that which we previously reported with ^{3}H-p-aminoclonidine (Piletz et al. 1991). However, although not confirming our earlier pretreatment findings for elevated α2-adrenoceptor binding in undesensitized platelets from depressed patients, we observed that the density of radioligand binding to nonadrenergic Imidazoline sites (putative Imidazoline I_1 sites) was increased in depressed patients (Table 1). Our earlier data (Piletz et al. 1990, 1991) were compatible with two binding sites, but we did not perform two-site Scatchard plot analyses due to the lower specific activity of ^{3}H-p-aminoclonidine. It therefore seems conceivable that

we had previously misidentified I_1 sites for α2-adrenoceptor-binding sites. Therefore, the pretreatment elevation in the density of previously unidentified I_1 sites could explain our earlier findings of an elevation in α2-adrenoceptor binding in depression.

Thus, postdesensitization α2-adrenoceptor-mediated platelet aggregations (Fig. 2) as well as the density of nonadrenergic putative-I_1-binding sites (Table 1) were both elevated in depressed patients compared to healthy subjects. Obviously, this suggests that there might be some link between these phenomena. However, we found no correlations between the I_1-binding parameters and any of the aggregation desensitization parameters. Indeed, a review of the literature published at the First International Symposium on Imidazoline-preferred Receptors (In: *Fundamental and Clinical Pharmacology*, Vol. 6/supplement 1, 1992), indicated that aside from the high affinity that both I_1 and α2-adrenoceptors share for imidazoline ligands (i.e., clonidine), these receptors appear to be physically distinct entities. Additionally, our unpublished studies have failed to find any role for an I_1 receptor in platelet aggregation (after selectively blocking α2-adrenoceptors with concentrations of α2-antagonists that saturate α2- but not I_1-receptors, the further treatment of platelets with imidazolines such as moxonidine, did not modify platelet aggregation). Therefore, we have detected no obvious link between the higher density of I_1 sites and the blunted α2-adrenoceptor-mediated aggregations that were both observed in depression. Nevertheless, because the desensitization of epinephrine-initiated aggregation is a complex, multistep process, we cannot rule out that there may be some interaction between I_1-binding sites and epinephrine-initiated aggregation, perhaps at the level of G-proteins.

Although radioligand-binding parameters for the putative I_1 site were not correlated with aggregation, a correlation was nevertheless observed between the density of high-affinity state α2-adrenoceptor sites (B_{max}) and the extent of epinephrine-initiated desensitization (Fig. 3). This correlation indicated that the greater the density of the high-affinity adrenoceptor state the less the desensitization. Unfortunately due to the relatively low number of subjects, this correlation needs to be substantiated. Nevertheless, because G-proteins are known to be coupled with high-affinity state platelet α2-adrenoceptors, a slower desensitization might also be consistent with a higher degree of coupling of G-proteins in the high-affinity adrenoceptor state.

Whether platelets provide a reliable model of the brain α2-adrenoceptor is still an open question. In human brain, the principal α2-adrenoceptor subtype ($\alpha 2_A$) is the same as that identified in human platelets (Kobilka et al. 1987; Bylund et al. 1988; Ordway et al. 1993). Moreover, the density of human α2-adrenoceptor-binding sites in platelets is under genetic control (Propping et al. 1986). Nevertheless, Nutt and Molyneux (1986) reported no correlation between platelet ^{3}H-yohimbine binding and the central nervous system response to a clonidine infusion test (blood pressure and growth hormone response) in healthy subjects or in subjects with panic disorder. Also, Hamilton et al. (1985) reported no correlation between platelet ^{3}H-yohimbine binding and brain ^{3}H-clonidine binding in rabbits that were treated under a variety of regulatory conditions. Unfortunately, due to the aforementioned distinctions between yohimbine- (α2-adrenergic) and clonidine- (imidazoline) binding sites, the significance of these reports remains uncertain. Future studies will therefore be required to determine whether the expression of brain α2-adrenoceptors is regulated similarly to that in platelets and whether desensitization is also altered in brains of depressed patients.

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REFERENCES

Asakura M, Tsukamoto T, Imafuku J, Matsui H, Ino M, Hasegawa K (1985): Quantitative analysis of rat brain alpha-2-receptors discriminated by ^{3}H-clonidine and ^{3}H-rauwolscine. Eur J Pharmacol 106:141–147

Beckmann H, Ebert MH, Post RM (1979): Effect of moderate exercise on urinary MHPG in depressed patients. Pharmacopsychiatria 12:351–356

Bouvier M, Hausdorff WP, De Bl A, O'Dowd BF, Kobilka BK, Caron MG, Lefkowitz RJ (1988): Removal of phosphorylation sites from the β2-adrenergic receptor delays onset of agonist-promoted desensitization. Nature 333:370–373

Brodde OE, Anlauf M, Graben N, Bock KD (1982): In vitro and in vivo down-regulation of human platelet α2-adrenoceptors by clonidine. Eur J Clin Pharmacol 23: 403–409

Bunney WE Jr, Davis JM (1965): Norepinephrine in depressive reactions: A review. Arch Gen Psychiatry 13:483–494

Bylund DB, Ray-Prenger C, Murphy TJ (1988): Alpha-2A and alpha-2B adrenergic receptor subtypes: Antagonist binding in tissues and cell lines containing only one subtype. J Pharmacol Exp Ther 245:600–607

Charney DS, Menkes DB, Heninger GR (1981): Receptor sensitivity and the mechanism of action of antidepressant treatment: Implications for the etiology and therapy of depression. Arch Gen Psychiatry 38:1160–1180

Cooper B, Handin RI, Young LH, Alexander RW (1978):

Agonist regulation of the human platelet α-adrenergic receptor. Nature 274:703–706

De Villiers AS, Russell VA, Carstens ME, Searson JA, van Zyl AM, Lombard CJ, Taljaard JJF (1989): Noradrenergic function and hypothalamic-pituitary-adrenal axis activity in adolescents with major depressive disorder. Psychiatry Res 27:101–109

Feinman RD, Detwiler TC, Ingerman-Wojenski C (1985): The lumi-aggregometer as a research and clinical tool. In Longenecker G (ed), The Platelets: Physiology and Pharmacology. New York, Academic Press, pp 429–440

Finberg JP (1987): Antidepressant drugs and down-regulation of presynaptic receptors. Biochem Pharmacol 36:3557–3562

Garcia-Sevilla JA, Zis AP, Hollingsworth PJ, Greden JF, Smith CB (1981): Platelet alpha-2 adrenergic receptors in major depressive disorder. Arch Gen Psychiatry 38:1327–1333

Garcia-Sevilla J, Padro D, Giralt T, Guimon J, Areso P (1990): α2-Adrenoceptor-mediated inhibition of platelet adenylate cyclase and induction of aggregation in major depression. Arch Gen Psychiatry 46:125–132

Goode DJ, Dekirmenjian H, Meltzer HY, Maas JW (1973): Relation of exercise to MHPG excretion in normal subjects. Arch Gen Psychiatry 29:391–396

Gwirtsman HE, Halaris AE, Wolf AW, DeMet E, Piletz JE, marler M (1989): Apparent phase advance in diurnal MHPG rhythm in depression. Am J Psychiatry 146: 1427–1433

Hamilton CA, Jones CR, Mishra N, Barr S, Reid JL (1985): A comparison of α2-adrenoceptor regulation in brain and platelets. Brain Res 347:350–353

Hamilton CA, Deighton NM, Reid JL (1987): Rapid and reversible desensitisation of vascular and platelet alpha2 adrenoceptors. Naunyn-Schmiedebergs Arch Pharmacol 335:534–540

Hamilton M (1967): Development of a rating scale for primary depression. Br J Soc Clin Psychol 6:278–296

Healy D, Carney PA, Leonard BE (1983): Monoamine-related markers of depression: Changes following treatment. J Psychiatr Res 17:251–260

Hoffman BB, Lefkowitz RJ (1980): Radioligand binding studies of adrenergic receptors: New insights into molecular and physiological regulation. Annu Rev Pharmacol Toxicol 20:581–608

Hollister AS, Fitzgerald GA, Nadeau JHJ, Robertson D (1983): Acute reduction in human platelet α2-adrenoceptor affinity of agonist by endogenous and exogenous catecholamines. J Clin Invest 72:1498–1505

Jones CR, Giembcyz M, Hamilton CA, Rodger IW, Whyte K, Deighton N, Elliott HL, Reid JL (1986): Desensitization of platelet α2-adrenoceptors after short term infusions of adrenoceptor agonist in man. Clin Sci 70:147–153

Kafka MS, Paul SM (1986): Platelet alpha-2 adrenergic receptors in depression. Arch Gen Psychiatry 43:91–95

Kelton JG, Powers P, Julian J, Boland V, Carter CJ, Gent M, Hirsh J (1980): Sex-related differences in platelet aggregation: Influence of the hematocrit. Blood 56:38–41

Kobilka BK, Matsui H, Kobilka TS, Yang-Feng TL, Francke U, Caron MG, Lefkowitz RJ, Regan JW (1987): Cloning, sequencing, and expression of the gene coding for the human platelet α-adrenergic receptor. Science 238:650–656

Langer SZ (1981): Presynaptic regulation of the release of catecholamines. Pharmacol Rev 32:337–362

McPherson CA (1985): Analysis of radioligand binding experiments. A collection of computer programs for the IBM PC. J Pharmacol Meth 14:213–228

Meana JJ, Barturen F, Garcia-Sevilla JA (1992): α2-Adrenoceptors in the brain of suicide victims: Increased receptor density associated with major depression. Biol Psychiatry 31:471–490

Mefford IN (1981): Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: Measurement of catecholamines, serotonin and metabolites in rat brain. J Neurosci Meth 3:207–224

Mori H, Koyama T, Yamashita I (1991): Platelet alpha-2 adrenergic receptor-mediated phosphoinositide responses in endogenous depression. Life Sci 48:741–748

Motulsky HJ, Shattil SJ, Ferry N, Rozansky D, Insel PA (1986): Desensitization of epinephrine-initiated platelet aggregation does not alter binding to the α2-adrenoceptor receptor or receptor coupling to adenylate cyclase. Mol Pharmacol 29:1–6

Muscettola G, Potter WZ, Pickard D, Goodwin FK (1984): Urinary 3-methoxy-4-hydroxyphenylglycol and major affective disorders. Arch Gen Psychiatry 41:337–342

Nutt DJ, Molyneux SG (1986): The effect of clonidine on plasma MHPG: Evidence against tonic alpha-2 adrenoceptor control of noradrenergic function. Psychopharmacology 90:509–512

Ordway GA, Jaconetta SM, Halaris AE (1993): Characterization of subtypes of α2-adrenoceptors in the human brain. J Pharmacol Exp Ther 264:967–976

Osman OT, Rudorfer MV, Potter WZ (1989): Idazoxan: A selective α2-antagonist and effective sustained antidepressant in two bipolar depressed patients. Arch Gen Psychiatry 46:958–959

Pandey GN, Janicak PG, Javaid JI, Davis JM (1989): Increased 3H-clonidine binding in platelets of patients with depressive and schizophrenic disorders. Psychiatry Res 28:73–88

Piletz JE, Halaris A, Saran A, Marler M (1990): Elevated ^{3}H-para-aminoclonidine binding to platelet purified plasma membranes from depressed patients. Neuropsychopharmacology 3:201–210

Piletz JE, Andorn AC, Unnerstall JR, Halaris A (1991a): Binding of [^{3}H]-p-aminoclonidine to α2-adrenoceptor states plus a non-adrenergic site on human platelet plasma membranes. Biochem Pharmacol 42:569–584

Piletz JE, Halaris A, Saran A, Marler MR (1991b): Desipramine lowers ^{3}H-para-aminoclonidine binding in platelets of depressed patients. Arch Gen Psychiatry 48: 813–820

Piletz JE, Slatten KR, Halaris AE (1992): Imidazoline-preferring binding sites in human platelets: Plasma membrane subtype I1 is elevated in mood disorders. Fund Clin Pharmacol 6(Suppl 1):49s (Abstract)

Pitcher JA, Inglese J, Higgins JB, Arriza JL, Casey PJ, Kim C, Benovic JL, Kwatra MM, Caron MG, Lefkowitz RJ (1992): Role of β-gamma subunits of G proteins in targeting the β-adrenergic receptor kinase to membrane-bound receptors. Science 257:1264–1267

Propping P, Friedl W, Hebebrand J, Lentes K-U (1986):

Genetic studies at the receptor level: Investigations in human twins and experimental animals. In Porter R, Bock G, Clark S (eds), Antidepressants and Receptor Function. (Ciba Foundation Symposium 123) Wiley, Chichester, pp 42–56

Raiteri M, Maura G, Versace P (1983): Functional evidence for two stereochemically different alpha-2 adrenoceptors regulating central norepinephrine and serotonin release. J Pharmacol Exp Ther 224:679–684

Raiteri M, Maura G, Folghera S, Cavazzani P, Andrioli GC, Schlicker E, Schalnus R, Gothert M (1990): Modulation of 5-hydroxytryptamine release by presynaptic inhibitory α2-adrenoceptors in the human cerebral cortex. Naunyn-Schmiedebergs Arch Pharmacol 342:508–512

Rossi EC, Louis G (1975): A time-dependent increase in the responsiveness of platelet-rich plasma to epinephrine. J Lab Clin Med 85:300–306

Schildkraut JJ (1965): The catecholamine hypothesis of affective disorders: A review of the supporting evidence. Am J Psychiatry 122:509–522

Schreiber G, Avissar S (1991): Lithium sensitive G protein hyperfunction: A dynamic model for the pathogenesis of bipolar affective disorder. Med Hypotheses 35:237–243

Sevy S, Papadimitriou GN, Surmont DW, Goldman S, Mendlewicz J (1989): Noradrenergic function in generalized anxiety disorder, major depressive disorder and healthy subjects. Biol Psychiatry 25:141–152

Sibley DR, Lefkowitz RJ (1985): Molecular mechanisms of receptor desensitization using the β-adrenergic receptor-coupled adenylate cyclase system as a model. Nature 317:124–129

Siever LJ, Davis KL (1985): Overview: Toward a dysregulation hypothesis of depression. Am J Psychiatry 142:1017–1031

Siever LJ, Kafka MS, Targum S, Lake CR (1984): Platelet alpha-adrenergic binding and biochemical responsiveness in depressed patients and controls. Psychiatry Res 11:287–302

Siever LJ, Coccaro EF, Davis KL (1987): Chronobiologic instability of the noradrenergic system in depression. In Halaris A (ed), Chronobiology and Psychiatric Disorders. New York, Elsevier, pp 1–9

Starke K (1981): Presynaptic receptors. Annu Rev Pharmacol Toxicol 21:7–30

Starke K (1987): Presynaptic α-autoreceptors. Rev Physiol Biochem Pharmacol 107:73–146

Sulser F (1984): Antidepressant treatments and regulation of norepinephrine-receptor-coupled adenylate cyclase systems in brain. In Usdin E, Goldstein M, Friedhoff AJ, Georgotas A (eds): Frontiers in Biochemical and Pharmacological Research in Depression. New York, Raven Press, pp 249–261

Thomsen JW, Jacquez JA, Neubig RR (1988): Inhibition of adenylate cyclase is mediated by the high affinity conformation of the α2-adrenergic receptor. Mol Pharmacol 34:814–822

Ts'ao C, Lo R, Raymond J (1976): Critical importance of citrate-blood ratio in platelet aggregation studies. Am J Clin Pathol 65:518–522

Vetulani JL, Antkiewics-Michaluk A (1985): Alpha-adrenergic receptor changes during antidepressant treatment. Acta Pharmacol Toxicol 56 (Suppl 1):55–65

Werstiuk ES, Auffarth SE, Coote M, Gupta RN, Steiner M (1992): Platelet α2-adrenergic receptors in depressed patients and healthy volunteers: The effects of desipramine. Pharmacopsychiatry 25:199–206

Young LT, Li PP, Kish SJ, Siu KP, Warsh JJ (1991): Postmortem cerebral cortex G_s α-subunit levels are elevated in bipolar affective disorder. Brain Res 553:323–326