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Adrenergic Receptor Function in Panic Disorder

I. Platelet α_2 Receptors: G_i Protein Coupling, Effects of Imipramine, and Relationship to Treatment Outcome

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Various studies suggest α_2-adrenergic receptor (α_2AR) dysregulation in panic disorder (PD). Platelet α_2-AR exist in high- and low-conformational states as a function of their coupling to G_i protein. α_2AR coupling is important in signal transduction and is modulated by antidepressants. α_2AR density in the high- and low-conformational states, agonist affinity, and coupling efficiency were investigated in 21 healthy controls, 21 drug-free PD patients, and eight imipramine-treated patients using norepinephrine displacement of ^{3}H-yohimbine binding. Percentage of receptors in the high-conformational state (%R_H) and the ratio of the agonist dissociation constant to the receptor in the low-/high-conformational state (K_L/K_H), calculated from displacement experiments, were used as coupling indices. Patients had high α_2AR density in both conformational states. %R_H and K_L/K_H ratio were significantly different, particularly in patients with Hamilton scale for depression (HAMD) scores ⩾15. Imipramine treatment (29 weeks) had no effect on α_2AR density or coupling, despite improvement in anxiety ratings. High pretreatment α_2AR density and coupling predicted low severity of anxiety after treatment. Increased α_2AR density and abnormal coupling may represent an adaptive mechanism or trait marker in PD. ***[Neuropsychopharmacology 20:162–176, 1999]***

KEY WORDS: *Alpha$_2$-adrenergic receptors; G Protein; Coupling; Panic disorder; Anxiety; Depression*

Evidence for abnormal α_2-adrenergic receptor (α_2AR) function in panic disorder (PD) derives from cardiovascular, neuroendocrine, and neurochemical challenges, and from direct receptor binding studies. Cardiovascular responses to an acute intravenous clonidine challenge, a partial α_2AR agonist, were exaggerated in PD (Nutt 1986), consistent with postsynaptic AR supersensitivity. 3-Methoxy-4-hydroxyphenylethylene glycol (MHPG) and cardiovascular responses to acute yohimbine administration were higher in PD patients than in controls (Charney and Heninger 1986; Gurguis and Uhde 1990), despite normal norepinephrine (NE) responses (Albus et al. 1992; Gurguis et al. 1997c). Collectively, enhanced cardiovascular responses, despite normal NE responses, suggest increased postsynaptic end-organ vascular α_2AR function.

Neuroendocrine challenge studies in PD used clonidine as a probe of hypothalamic α_2AR function. Growth hormone response to clonidine in PD was blunted in

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virtually all studies (Charney and Heninger 1986; Uhde et al. 1986; Nutt 1989; Abelson et al. 1992), suggesting decreased hypothalamic α_2AR function (Siever et al. 1982; Siever and Uhde 1984). However, blunted growth hormone response to growth hormone-releasing factor in PD suggests alternative etiologies to decreased α_2AR function (Rapaport et al. 1989; Uhde et al. 1992; Tancer et al. 1993).

Platelet α_2AR receptor binding studies in PD yield inconsistent results. Low platelet α_2AR density was found using ^{3}H-yohimbine, but not ^{3}H-clonidine, in PD patients, and imipramine treatment increased or normalized receptor density (Cameron et al. 1984). Low ^{3}H-yohimbine and/or ^{3}H-clonidine maximum binding capacity was later replicated in PD and in generalized anxiety disorder (Albus et al. 1986; Cameron et al. 1990, 1996). Other studies found normal α_2AR density in PD (Norman et al. 1987; Nutt and Fraser 1987; Charney et al. 1989). However, low EC_{50} for epinephrine inhibition of adenylyl cyclase (AC) and low PGE_1- and NaFl-stimulated AC activity was found (Charney et al. 1989). The latter observations suggest either increased receptor density, high α_2AR coupling to G_i protein, abnormal G_i protein function, or abnormal $G_{i\alpha}$–AC interaction. Finally, high α_2AR density and the lack of antidepressant treatment effect on α_2AR density in PD was also reported (Butler et al. 1992). Therefore, evidence suggests abnormal α_2AR regulation in PD, although the nature of this dysregulation is unclear.

Platelet α_2AR, similar to brain α_2AR, are of the C10 subtype (previously classified as α_{2A}) (Lorenz et al. 1990; Bylund et al. 1992; Ordway et al. 1993). Platelet membrane α_2AR belong to a superfamily of G protein-coupled receptors. The receptor molecule (450 amino acids) has seven transmembrane spanning domains. The fourth transmembrane spanning domain is involved in ligand binding (Matsui et al. 1989), and the third intracytoplasmic loop is the site for G protein coupling and receptor phosphorylation. Platelet α_2AR are coupled to G_i protein (Simonds et al. 1989). They exist in high- and low-conformational states as a function of their coupling to G_i protein upon agonist binding (Kim and Neubig 1987; Neubig et al. 1988). Inhibition of AC is mediated by the high-conformational state (Hoffman et al. 1980; Thomsen et al. 1988). Coupling to G_i protein is an important step in the signal transduction cascade.

The ternary receptor model (DeLean et al. 1980, 1982; Kent et al. 1980) suggests that the formation of the high-conformational state is a transitory (intermediate) agonist–receptor-G protein complex, which precedes the activation of G_i protein and the dissociation of the $G_{i\alpha}$ from the inactive $\alpha\beta\gamma$ holotrimer G_i molecule. The $G_{i\alpha}$ subunit subsequently inhibits AC. Consistent with this model, the percentage of receptors in the high-conformational state ($\%R_H$) and the ratio of the agonist dissociation constant from the receptor in the low-/high-conformational states (K_L/K_H ratio) correlate with the agonist's intrinsic activity (DeLean et al. 1980, 1982; Kent et al. 1980; Hoffman et al. 1982). These two measures have been proposed as putative measures of receptor coupling to G protein.

Virtually all α_2AR binding studies in PD have measured the maximum binding capacity of the ligand (B_{max}). They have not examined α_2AR coupling, the relative distribution of receptors in the high- and low-conformational states, or agonist affinity to the receptor in either conformational state. In addition to downregulating receptor density (Salama et al. 1982; Smith et al. 1983; Keith et al. 1986; Giralt and García-Sevilla 1989; Lacroix et al. 1991; Kovachich et al. 1993; Ribas et al. 1993; Moret and Briley 1994), tricyclic antidepressants have been shown to modulate AR coupling through mechanisms directly influencing postreceptor signal transduction; specifically, G protein function and G protein–AC interaction (Okada et al. 1986; Fishman and Finberg 1987; Tsuchiya et al. 1988; Yamamoto et al. 1990). The efficacy of tricyclic antidepressants in PD also raises the possibility of abnormal α_2AR coupling, and, secondarily, of whether antidepressant modulation of α_2AR coupling by antidepressants is related to treatment outcome.

In this study we investigated platelet α_2AR function in 21 PD patients and 21 healthy controls. We conducted both antagonist-saturation and agonist (NE)-displacement experiments on platelet membranes from all subjects. Coupling indices were derived from agonist displacement curves. Radioreceptor binding experiments were repeated in eight of the 21 PD patients after treatment with imipramine at therapeutically effective doses, as ascertained by plasma imipramine levels. Severity of anxiety and depressive symptoms was assessed before and after treatment to determine whether treatment outcome was influenced by the nature of dysregulation in pretreatment α_2AR. We hypothesized that α_2AR density and coupling are abnormal in PD. We further hypothesized that treatment with antidepressants induces downregulatoin of α_2AR density and modulates α_2AR coupling to G_i protein.

METHODS

Subjects

Platelet α_2AR were investigated in 21 male patients with PD, with or without agoraphobia, and in 21 healthy controls. Patients were recruited from the Mental Health Clinic at the Dallas VA Medical Center. Patients met DSM-III-R (APA 1987) diagnostic criteria for PD with or without agoraphobia. None of the patients met diagnostic criteria for concurrent major depression or had a history of a major depressive episode within the previ-

ous 6 months. Diagnosis was made using a comprehensive semistructured clinical interview (GNMG).

Frequency of unexpected and situational panic attacks per week and severity of anticipatory anxiety were obtained from patients using a daily diary. Severity of anticipatory anxiety was defined as the percent of time awake that the patient is preoccupied with fear of having a panic attack or fear of entering a situation likely to trigger an attack, multiplied by the severity (on a 0 to 10 scale) of that fear. The product of these two factors was divided by 10 to obtain a final score on a 0 to 100 scale.

Healthy controls were gender- and age-matched with patients and had no personal or familial psychiatric history. Patients and controls were drug-free for at least 2 weeks (6 weeks in the case of serotonin reuptake inhibitors) before the study. Subjects had no current medical complaints and were physically healthy, as ascertained by medical history, physical examination, and laboratory work-up. Subjects observed a low-monoamine and restricted-caffeine diet for 3 days before blood sample collection. The study was approved by the Human Subjects Committee at the Dallas VA Medical Center, and all subjects signed an informed written consent form before participating in the study.

After baseline study, PD patients were treated with imipramine. Imipramine dosage was gradually increased to therapeutic levels, as verified by imipramine plasma levels. α_2AR were assayed after imipramine treatment in eight of the 21 patients.

Procedure

Subjects came to our laboratory at 7:30 A.M. on the procedure day, having observed an overnight fast and assumed a supine position in a hospital bed in a quiet room. An intravenous (IV) line was established (antecubital vein, nondominant arm). Subjects rested for 60 min following IV line placement. Sixty ml of blood were then drawn for receptor assay, and the IV line was discontinued.

Symptom ratings, including the Hamilton Scale for Anxiety (HAMA) Hamilton 1959), the Hamilton Scale for Depression (HAMD, 24-item) (Hamilton 1967), the Zung Anxiety Scale (both clinician- and self-rated; ZungC and ZungS, respectively) (Zung 1971), the Spielberger-State Anxiety Inventory (SpS), the Spielberger-Trait) Anxiety Inventory (SpT) (Spielberger et al. 1970; Spielberger 1983) and 100-mm Visual Analog Scales (VAS) of mood (anxiety, tension, irritability, fearfulness, depression, and anger) and somatic autonomic anxiety symptoms, were completed by patients and controls after the procedure (Aitken and Gedye 1968; Aitken 1969; Zealley and Aitken 1969; Folstein and Luria 1973; Luria 1975).

Platelet α_2-Adrenergic Receptor Assay

Platelet α_2AR binding assays were conducted according to García-Sevilla et al. (1981a), with modifications. Briefly, blood (60 ml) was drawn on acid citrate dextrose (ACD-NIH formula) and centrifuged (160 $\times$ *g* for 10 min) at room temperature. Platelet-rich plasma was buffered using ACD to pH 6.5 and centrifuged at 5,100 $\times$ *g* for 15 minutes. The platelet pellet was washed twice with Tyrodes buffer and recentrifuged at 5,100 $\times$ *g* for 15 min. Platelets were then resuspended and homogenized using a Brinkman polytron at setting 5 for 15 s. Platelet membranes were obtained using differential centrifugation, and the membrane pellet was resuspended in the Tris-HCl incubation buffer (50 mM Tris-HCl; 10 mM $MgCl_2$, pH 7.5). Fresh membrane preparations were used in the receptor binding experiments in all subjects.

Tritiated yohimbine (SA 70–90 Ci/mmol) was used as a ligand. Saturation experiments were conducted using seven ^{3}H-yohimbine concentrations (0.25–16.0 nM). Nonspecific binding was defined in the presence of norepinephrine (0.1 mM). Specific binding, defined as total binding minus nonspecific binding, was approximately 90%. Displacement experiments were conducted using 18 concentrations of "cold" NE (0–1.0 mM) to displace ^{3}H-yohimbine (2 nM). Samples were incubated at 25°C for 30 min. Incubation was terminated by rapid filtration over glassfiber filters #32 (Schleicher and Schuell, Keene, NH, USA) using a Brandel M-24R cell harvester (Brandel, Gaithersburg, MD, USA). Filters were air-dried and placed in glass scintillation vials with 20 ml of scintillation cocktail for counting in a Tri-Carb CA2200 (Packard, Downer's Grove, Ill, USA), with 55 to 66% efficiency. Protein concentrations were measured (Lowry et al. 1951).

Binding Data Analysis

Binding data were analyzed using LIGAND program (Munson and Rodbard 1983, 1984). LIGAND uses iterative curve-fitting methods based on the law of mass action. Scatchard analysis was used to analyze saturation binding experiments, using weighted curvilinear fitting techniques, with the dissociation constant equal to $-1/$slope, and the maximum binding capacity representing the x-intercept. Analysis of displacement experiments employed nonlinear iterative curve-modeling methods that test for the presence of more than one binding state. An F-test was used to compare the goodness-of-fit between the two models. A two-site model was accepted only if the goodness-of-fit was statistically significantly better than the one-site model ($p < .05$).

The maximum binding capacity (B_{max}) and the antagonist dissociation constant from the receptor (K_d) were measured from saturation experiments. Receptor density in the high- (R_H) and low- (R_L) conformational

states, and the agonist dissociation constant from the receptor in the high- (K_H) and low- (K_L) conformational states were measured from displacement curves. The total agonist-measured receptor density (R_T, $R_T = R_H + R_L$), $\%R_H$, and K_L/K_H ratio were determined. $\%R_H$ and the K_L/K_H ratio were used as putative indices of α_2AR coupling to G_i protein. These receptor coupling measures correlate with the agonist's intrinsic activity (DeLean et al. 1980, 1982; Kent et al. 1980). See Figure 1.

Statistical Analysis

Between-group differences (PD patients vs. healthy controls) in coupling measures ($\%R_H$ and K_L/K_H) and other receptor binding parameters were tested using two-tailed independent *t*-tests. One-way analysis of variance (ANOVA) was used in three-group comparisons. Two-tailed paired *t*-tests were used to compare pre- to post-treatment measures in patients. Finally, the relationships among symptom ratings and receptor binding parameters were tested before and after treatment, using Pearson's product moment analysis. To test if pretreatment receptor-binding parameters predicted symptom severity after treatment, we conducted regression analysis using pretreatment receptor-binding parameters as dependent variables and anxiety and depression severity ratings after treatment as independent variables. All data are presented as mean ± SEM.

RESULTS

There was no significant difference in age between PD patients and healthy controls (PD: 42.05 ± 1.82 years vs. NC: 41.33 ± 2.35 years, t = 0.240; p = NS). Patients had an average of 4.8 unexpected panic attacks and 3.00 situational panic attacks per week, with mean anticipatory anxiety severity of 20 (0–100 scale). Table 1 shows characteristics of patients and controls.

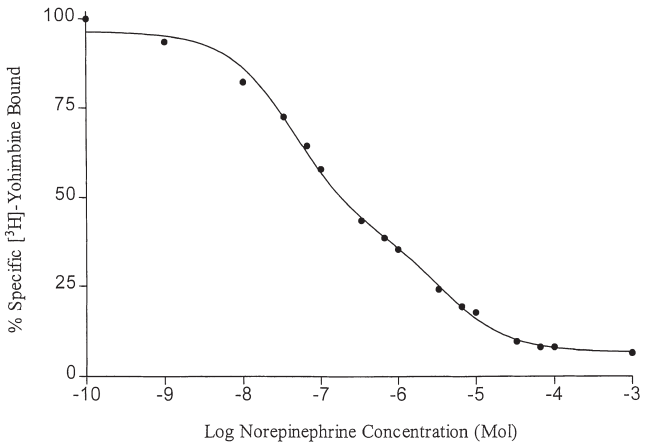

Figure 1. A representative norepinephrine displacement curve of ^{3}H-yohimbine binding to platelet membrane α_2-adrenergic receptors in a panic disorder patient. Displacement curves were flat and were resolved into a two-site model. Estimates of receptor density in the high- and low-conformational states as well as agonist affinity to each conformational state were derived.

Several measures of α_2AR density were higher in PD patients than in controls: B_{max} (t = 3.512, p = .001), R_H (t = 3.648, p = .001), R_L (t = 2.732, p = .009), and R_T (t = 3.518, p = .001). There was a trend for higher $\%R_H$ in patients (t = 1.740, p = .09) and lower K_L/K_H ratio (t = −1.804, p = .08). The trend for lower K_L/K_H ratio appeared secondary to a moderately lower K_H. There were no significant differences, however, between patients and controls in K_H or K_L.

To examine the effect of coexistence of depressed mood (albeit in absence of concurrent major depressive disorder), PD patients were subdivided into two groups based on their HAMD scores: "nondepressed" (HAMD score <15) and "depressed" (HAMD score ⩾15). Table 2 shows results of the one-way ANOVA comparing each of the two groups to controls. Patients with HAMD scores ⩾15 had significantly higher $\%R_H$ (t = 2.280, p = .03) and lower K_L/K_H ratio (t = 2.172, p = .03) than controls. No significant differences, however, were observed between "nondepressed" PD patients and controls.

Eight PD patients received treatment with imipramine for an average length of 203 ± 45 days. All eight patients were treated with imipramine at therapeutic plasma levels (imipramine: 125.5 ± 16.65 ng/ml; desipramine: 124.13 ± 13.99 ng/ml; total: 249.63 ± 26.30 ng/ml). Paired analysis showed that treatment with imipramine had no effect on α_2AR binding parameters. A numeric increase in the K_L/K_H ratio was found, but it did not reach statistical significance. There was a significant decrease in the frequency of panic attacks at the end of treatment. However, the decrease in HAMA and HAMD scores was less than the conventionally acknowledged limit for a positive outcome, defined as a 50% decrease in severity of pretreatment symptoms. Finally, some sporadic somatic symptoms that seem to have worsened are likely side effects of imipramine (Table 3).

The following correlations between psychological ratings and α_2AR binding parameters were found.

Healthy Controls (n = 21)

Age was negatively correlated with B_{max} (r = −0.375, p = .09) and R_H (r = −0.380, p = .09). K_L/K_H ratio was positively correlated with SpT (r = 0.500, p = .02). $\%R_H$ was positively correlated with SpS (r = 0.443, p = .04). R_H was positively correlated with VAS of tension (r = 0.433, p = .05) and anxiety (r = 0.415, p = .06), and with SpT (r = 0.373, p = .09). Both coupling measures, $\%R_H$ and K_L/K_H, were positively correlated with severity of

Table 1. Psychological Measures in Healthy Controls and Panic Disorder Patients

Psychological Measures	Healthy Controls (n = 21)	PD pre-tx (n = 21)	t-Statistic (Independent)[a]	p-Value	PD pre-tx (n = 8)	PD post-tx (n = 8)	t-Statistic (Paired)[b]	p-Value
Severity of Panic Attacks:								
Number of unexpected PA*/week	0.00	4.81 ± 1.27			5.75 ± 1.79	1.38 ± 0.91	2.483	.04
Number of situational PA/week	0.00	3.00 ± 0.88			5.38 ± 1.91	0.75 ± 0.31	2.235	.06
Number of total PA/week	0.00	7.81 ± 1.37			11.13 ± 1.46	2.13 ± 0.91	4.648	.002
Severity of anticipatory anxiety	0.00	31.14 ± 4.67			20.75 ± 3.43	6.89 ± 3.07	3.926	.006
Anxiety & Depression Ratings:								
HAM-Anxiety	1.71 ± 0.30	21.45 ± 1.14	16.396	.00	18.75 ± 2.41	12.44 ± 2.28[c]	2.878	.02
HAM-Depression	1.24 ± 0.23	16.36 ± 0.97	15.249	.00	16.00 ± 1.74	10.31 ± 2.19[c]	3.195	.01
Zung-Clinician	21.50 ± 0.42	56.14 ± 9.17	4.054	.001				
Zung-Self Report	31.90 ± 1.60	49.79 ± 1.38	8.369	.00	51.33 ± 2.62	47.25 ± 1.50[c]	3.288	.02
Spielberger-State	27.52 ± 0.95	49.57 ± 2.48	8.296	.00	48.75 ± 5.48	40.25 ± 3.39[c]	1.295	NS
Spielberger-Trait	27.62 ± 0.92	56.43 ± 2.32	11.543	.00	55.63 ± 3.63	45.63 ± 3.57[c]	2.420	.04
Mood VAS**:								
Depression	9.19 ± 2.88	23.00 ± 4.32	2.659	.01	23.13 ± 7.81	22.88 ± 6.75[c]	0.054	NS
Anxiety	20.10 ± 3.25	41.05 ± 4.01	4.055	.00	36.13 ± 7.04	34.75 ± 6.74[c]	0.359	NS
Tension	18.29 ± 3.38	38.43 ± 4.06	3.814	.00	39.13 ± 8.25	34.63 ± 6.90[c]	0.684	NS
Fearfulness	8.29 ± 2.59	19.19 ± 3.75	2.394	.02	15.13 ± 7.72	15.50 ± 4.44	−0.072	NS
Anger	9.10 ± 2.90	11.71 ± 3.03	0.625	NS	8.75 ± 5.17	10.38 ± 6.30	−0.248	NS
Irritability	13.33 ± 3.50	28.25 ± 4.93	2.484	.02	22.75 ± 8.80	26.63 ± 9.26	−0.675	NS
Somatic Anxiety VAS:								
Dry mouth	10.24 ± 2.67	26.90 ± 5.89	2.578	.01	15.88 ± 8.52	31.00 ± 8.16[c]	−1.326	NS
Shaking or trembling	5.71 ± 1.70	15.48 ± 3.14	2.729	.009	7.75 ± 4.81	14.38 ± 6.01	−0.967	NS
Rapid heart	5.33 ± 1.43	19.10 ± 4.63	2.840	.007	11.25 ± 6.17	11.50 ± 3.75	−0.039	NS
Palpitations	5.14 ± 1.40	12.71 ± 2.69	2.499	.02	7.25 ± 3.26	6.63 ± 2.82	0.126	NS
Difficulty breathing	5.10 ± 1.36	17.00 ± 4.36	2.604	.01	7.00 ± 3.64	7.75 ± 3.23	−0.150	NS
Rapid breathing	5.29 ± 1.41	15.57 ± 4.38	2.234	.03	8.00 ± 5.48	7.63 ± 3.03	0.056	NS
Feeling dizzy	8.57 ± 3.57	22.90 ± 5.46	2.196	.03	8.50 ± 5.32	6.88 ± 3.27	0.241	NS
Weakness in muscles	7.76 ± 2.29	28.95 ± 5.45	3.583	.001	20.13 ± 7.31	9.00 ± 3.77	1.442	NS
Sweating	5.00 ± 1.39	19.81 ± 4.38	3.222	.003	7.38 ± 2.25	17.50 ± 8.33[c]	−1.106	NS
Numbness or tingling	4.90 ± 1.37	14.24 ± 2.98	2.841	.007	10.00 ± 3.90	15.63 ± 5.48[c]	−2.423	.04
Tightness in chest	4.62 ± 1.37	12.95 ± 3.89	2.021	.05	1.88 ± 0.61	4.75 ± 1.87	−1.941	.09
Urinary urgency	4.86 ± 1.37	22.24 ± 4.67	3.572	.001	16.38 ± 7.05	17.00 ± 7.88[c]	−0.123	NS
Chest pain	4.05 ± 1.18	11.20 ± 2.44	2.674	.01	4.63 ± 2.05	9.13 ± 4.07	−1.154	NS
Hot or cold flashes	4.38 ± 1.23	20.43 ± 5.41	2.891	.006	1.63 ± 0.73	5.25 ± 2.10	−1.683	NS
Nausea	4.76 ± 1.35	21.76 ± 4.71	3.469	.001	8.38 ± 3.90	6.38 ± 2.34	0.624	NS

Values are mean ± SEM.
*PA: panic attacks
**VAS: Visual Analog Scales of mood and somatic anxiety symptoms
[a]healthy controls vs. panic disorder patients pre-treatment (PD pre-tx, n = 21)
[b]PD pre-tx (n = 8) vs. PD post-tx
[c]$p \leq .05$ for independent t-tests comparing healthy controls to panic disorder patients post-treatment (PD post-tx)

anxiety. There were no correlations between α_2AR binding parameters and VAS of somatic autonomic anxiety symptoms.

PD Patients Before Treatment (n = 21)

Age was correlated with various measures of α_2AR density: B_{max} (r = 0.515, p = .02), R_H (r = 0.449, p = .04), R_L (r = 0.447, p = .03) and R_T (r = 0.464, p = .03). The K_L/K_H ratio correlated positively with the total number of panic attacks during the previous week (r = 0.455, p = .04) and negatively with HAMD score (r = −0.603, p = .004), SpS (r = −0.458, p = .04), and ZungC (r = −0.796, p = .03). $\%R_H$ was positively correlated with HAMA score (r = 0.443, p = .04), HAMD score (r = 0.509, p = .02) and VAS of fearfulness (r = 0.400, p = .07), racing heart (r = 0.596, p = .004), and palpitations (r = 0.411, p = .04).

R_H correlated negatively with severity of anticipatory anxiety (r = −0.426, p = .05) and positively with HAMD score (r = 0.424, p = .05), SpS (r = 0.470, p = .03), ZungC (r = 0.881, p = .009), and VAS of depression (r = 0.433, p = .05). R_L was negatively correlated with severity of anticipatory anxiety (r = − 0.410, p = .06)

Table 2. Platelet α_2-Adrenergic Receptor Binding Parameters in Healthy Controls and Patients with Panic Disorder

ALPHA	K_d (nmol)	B_{max} (fmol/mg protein)	R_H (fmol/mg protein)	R_L (fmol/mg protein)	R_T (fmol/mg protein)	$\%R_H$	K_H (nM)	K_L (μM)	K_L/K_H
Healthy Controls ($n = 21$)	2.24 ± 0.14	213.6 ± 15.60	193.7 ± 15.29	124.3 ± 10.64	318.0 ± 21.86	60.73 ± 1.98	28.49 ± 5.08	2.59 ± 0.63	107.6 ± 16.83
PD pre-tx[a] <15 ($n = 7$)	2.22 ± 0.13	338.4 ± 73.60	299.0 ± 66.61	183.6 ± 31.41	482.6 ± 95.39	60.60 ± 2.33	20.59 ± 2.42	1.80 ± 0.25	89.18 ± 8.47
PD pre-tx ⩾15 ($n = 14$)	2.11 ± 0.13	380.0 ± 49.97[c]	367.9 ± 47.75[e]	179.8 ± 22.56[e]	547.8 ± 68.99[c]	67.07 ± 1.40[d]	39.40 ± 7.39	2.63 ± 0.41	69.55 ± 4.81[d]
Total PD pre-tx ($n = 21$)	2.15 ± 0.10	366.1 ± 40.53	345.0 ± 38.53	181.1 ± 17.87	526.1 ± 54.95	64.91 ± 1.36	33.13 ± 5.31	2.35 ± 0.29	76.09 ± 4.63
F-Statistic[b]	0.238	6.275	7.314	3.647	6.383	3.311	1.744	0.367	1.886
p-Value	NS	.004	.002	.03	.004	.04	NS	NS	NS

Values are mean ± SEM.
[a]PD pre-tx: panic disorder patients pre-treatment
[b]comparing healthy controls vs. PD < 15 vs. PD ⩾ 15
[c]$p < .001$ vs. healthy controls (Bonferroni-corrected)
[d]$p < .03$ vs. healthy controls
[e]$p < .06$ vs. healthy controls

Table 3. Platelet α_2-Adrenergic Receptor Binding Parameters in Patients with Panic Disorder Before and After Treatment

ALPHA	K_d (nmol)	B_{max} (fmol/mg protein)	R_H (fmol/mg protein)	R_L (fmol/mg protein)	R_T (fmol/mg protein)	$\%R_H$	K_H (nM)	K_L (μM)	K_L/K_H
PD pre-tx[a] ($n = 8$)	2.36 ± 0.13	466.4 ± 57.11	419.7 ± 55.08	221.2 ± 18.85	641.0 ± 70.19	64.17 ± 2.51	31.03 ± 6.12	2.38 ± 0.52	76.10 ± 5.20
PD post-tx[b] ($n = 8$)	2.24 ± 0.11	379.0 ± 82.17	343.0 ± 52.49	192.5 ± 54.58	535.5 ± 100.2	66.26 ± 4.17	40.41 ± 13.13	3.47 ± 1.25	94.00 ± 18.70
t-Statistic (paired)	1.123	1.142	1.768	0.671	1.327	−0.620	−0.876	−1.076	−0.824
p-Value	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values are mean ± SEM.
[a]PD pre-tx: panic disorder patients pre-treatment
[b]PD post-tx: panic disorder patients post-treatment

and VAS of tension (r = −0.439, p = .05). R_T correlated negatively with severity of anticipatory anxiety (r = −0.432, p = .05) and positively with SpT (r = 0.444, p = .04), ZungC (r = 0.853, p = .01), and VAS of depression (r = 0.396, p = .08). B_{max} correlated negatively with the severity of anticipatory anxiety (r = −0.456, p = .04), and positively with HAMD score (r = 0.382, p = .09), SpT (r = 0.480, p = .03), and ZungC (r = 0.931, p = .002).

K_d correlated negatively with severity of anticipatory anxiety (r = −0.519, p = .01) and positively with VAS of tension (r = 0.525, p = .01). K_H was positively correlated with SpS (r = 0.489, p = .02), SpT (r = 0.430, p = .05), ZungC (r = 0.918, p = .004), and VAS of anxiety (r = 0.411, p = .06), and tension (r = 0.433, p = .04) (see Figure 2 and Figure 3).

Thus, the severity of anticipatory anxiety was negatively correlated with several measures of α_2AR binding density. Both coupling measures of α_2AR were correlated with several ratings of anxiety and depression, although in opposite directions. Psychological ratings were negatively correlated with the K_L/K_H ratio, but they were positively correlated with $\%R_H$. α_2AR parameters showed only scattered correlations with somatic autonomic anxiety symptoms, which did not suggest a consistent discernible pattern.

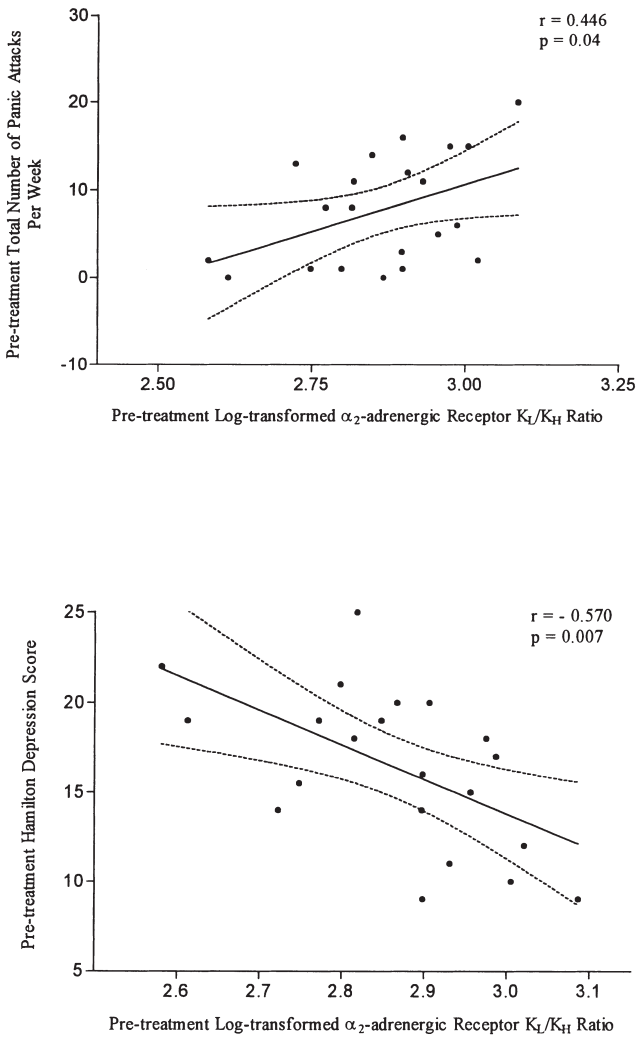

Figure 2. The relationship between pretreatment coupling measure K_L/K_H ratio and both frequency of panic attacks and Hamilton depression score in PD patients before treatment.

PD Patients After Treatment (n = 8)

$\%R_H$ was negatively correlated with VAS of shakiness (r = −0.786, p = .02) and palpitations (r = −0.623, p = .10). R_H was negatively correlated with ZungS (r = −0.694, p = .05), SpT (r = −0.676, p = .07), and VAS of tension (r = −0.652, p = .08). R_L correlated negatively with SpT (r = −0.735, p = .04) and ZungS (r = −0.901, p = .002) and positively with VAS of shakiness (r = 0.642, p = .08). R_T was negatively correlated with SpT (r = −0.754, p = .03) and ZungS (r = −0.855, p = .007). B_{max} was negatively correlated with SpT (r = −0.777, p = .02) and Zung S (r = −0.872, p = .005).

K_d correlated negatively with VAS of anger (r = −0.705, p = .05). K_H was negatively correlated with HAMD score (r = −0.686, p = .06). Post-treatment log K_L was negatively correlated with VAS of shakiness (r = −0.656, p = .08), racing heart (r = −0.875, p = .004), palpitations (r = −0.753, p = .03), difficulty breathing (r = −0.751, p = .03), rapid breathing (r = −0.771, p = .02), dizziness (r = −0.672, p = .07), weakness in the muscles (r = −0.706, p = .05), numbness (r = −0.727, p = .04), choking (r = −0.656, p = .08), chest pain (r = −0.681, p = .06), hot flashes (r = −0.727, p = .04), nausea (r = −0.756, p = .03), and dry mouth (r = −0.748, p = .03).

Post-treatment correlations between α_2AR binding parameters and psychological ratings, particularly coupling measures, were only sporadic. In contrast to pretreatment correlations, where α_2AR binding parameters were not correlated with somatic anxiety symptoms, post-treatment K_L showed moderately strong correlations with multiple measures of severity of somatic anxiety symptoms.

Results of the regression analysis (n = 8) examining the relationship between pretreatment α_2AR binding parameters and severity of symptoms after treatment revealed the following.

Pretreatment K_L/K_H ratio was negatively correlated with post-treatment number of unexpected panic attacks (r = −0.688, p = .06), number of total panic attacks (r = −0.657, p = .07), severity of anticipatory anxiety (r = −0.754, p = .03), HAMD score (r = −0.733, p = .04), and VAS of depression (r = −0.674, p = .07).

Pretreatment R_H was negatively correlated with post-treatment VAS of anxiety (r = −0.711, p = .05), tension (r = −0.826, p = .01), palpitations (r = −0.637, p = .09), difficulty breathing (r = −0.626, p = .10), rapid

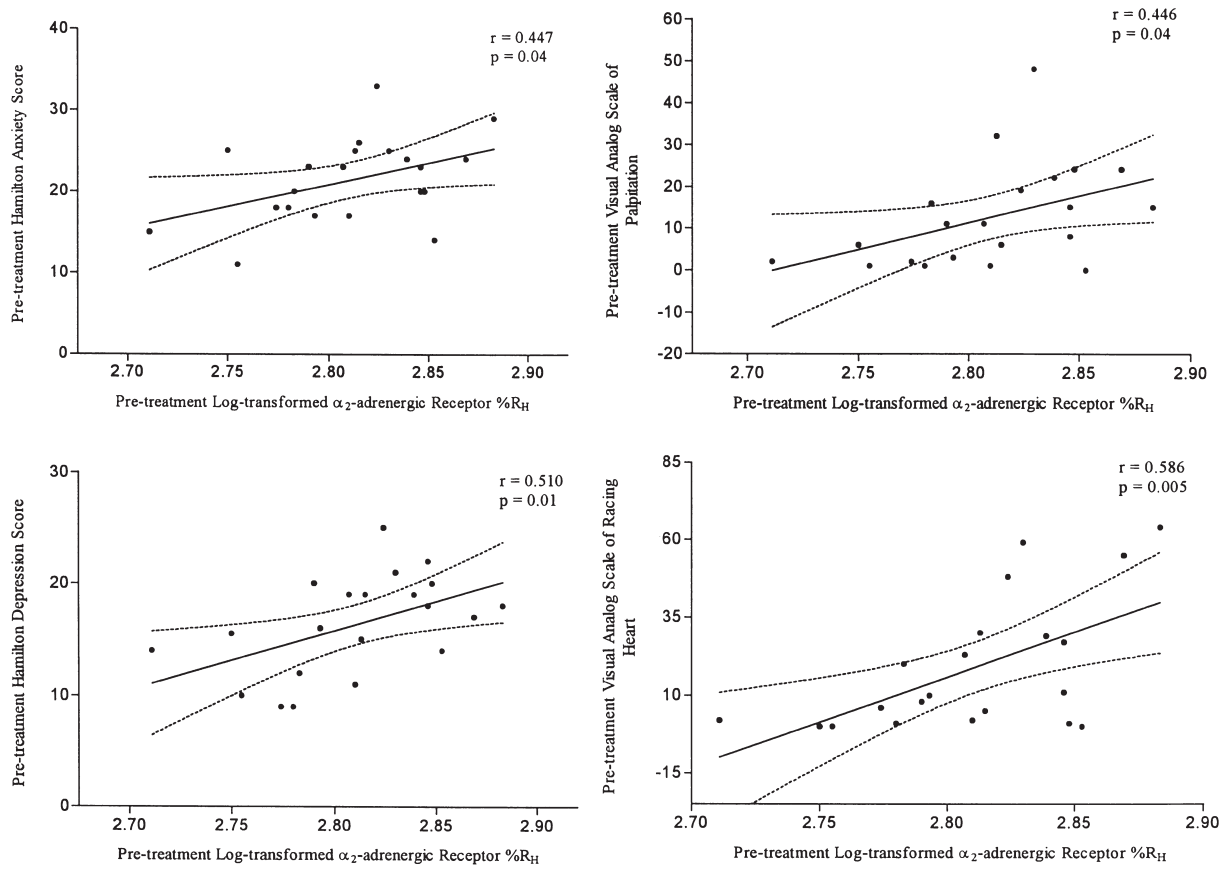

Figure 3. The pretreatment coupling measure % R_H correlated with severity of depressive and anxiety, as well as somatic anxiety, symptoms prior to treatment in PD patients.

breathing (r = −0.623, p = .10), and hot flashes (r = −0.645, p = .08). Pretreatment R_L was negatively correlated with post-treatment SpS (r = −0.782, p = .02), SpT (r = −0.911, p = .002), ZungS (r = −0.706, p = .05), and VAS of anxiety (r = −0.749, p = .03), and tension (r = −0.827, p = .01). Pretreatment R_T was negatively correlated with post-treatment SpS (r = −0.651, p = .08), SpT (r = −0.712, p = .05), and VAS of anxiety (r = −0.759, p = .03), and tension (r = −0.870, p = .005). Pretreatment B_{max} was negatively correlated with post-treatment SpS (r = −0.713, p = .04), SpT (r = −0.640, p = .09), and VAS of anxiety (r = −0.721, p = .04), tension (r = −0.852, p = .007), and fearfulness (r = −0.648, p = .08).

Pretreatment K_d was positively correlated with post-treatment number of unexpected panic attacks during the previous week (r = 0.686, p = .06), and with SpS (r = 0.773, p = .02). Pretreatment K_H was negatively correlated with post-treatment VAS of shakiness (r = −0.629, p = .09) (See Figure 4, Figure 5, and Figure 6).

Thus, the higher the K_L/K_H ratio before treatment, the lower the number of panic attacks and severity of anxiety and depression after treatment. Also, the higher the receptor density before treatment, the lower the severity of anxiety and depression after treatment.

DISCUSSION

To our knowledge, this is the first study in PD to examine parameters of α_2AR coupling and density in the high- and low-conformational states and agonist affinity to both states. Present results demonstrate upregulation of α_2AR density in PD, with trends for higher %R_H and lower K_L/K_H ratio, putative indices of coupling to G_i protein. Upregulation of receptor density in the high- and low-conformational states contributed equally to this upregulation in total receptor density. When patients were subdivided based on their HAMD scores, upregulation in receptor density was more pronounced in patients with HAMD scores ⩾15. Furthermore, these patients had significantly higher %R_H and lower K_L/K_H ratio than healthy controls. There were no differences in agonist affinity to the receptor in either conformational state, although patients with HAMD scores ⩾15 seemed to have lower agonist affinity to the receptor in the high-conformational state. Imipramine treatment had no effect on α_2AR binding parameters; however, high pretreatment α_2AR density and coupling were associated with low post-treatment symptom severity of anxiety and depression.

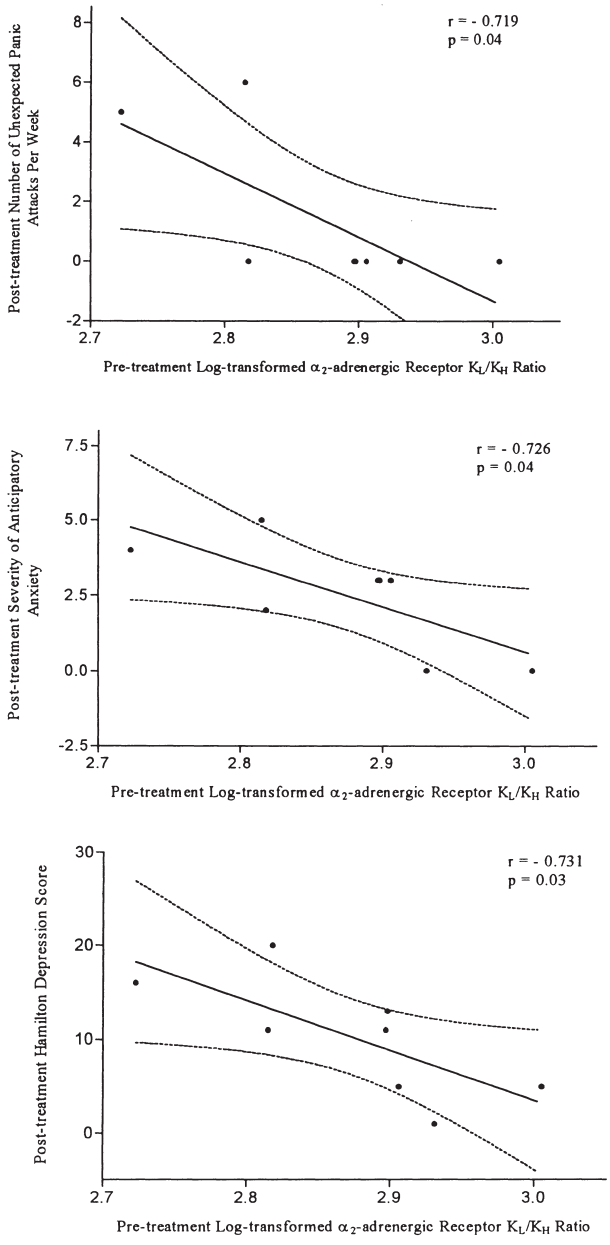

Figure 4. The K_L/K_H ratio prior to treatment predicted severity of anxiety and depressive symptoms after treatment in PD patients, so that the higher the K_L/K_H ratio the lower the symptom severity after treatment.

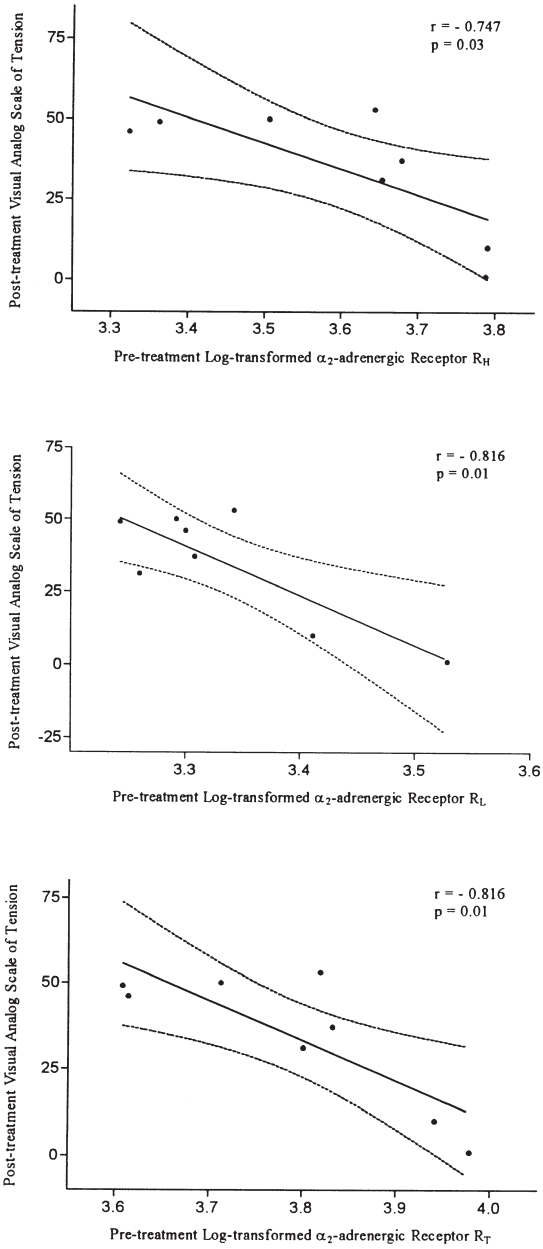

Figure 5. High pretreatment α_2-adrenergic receptor density predicted low severity of tension after treatment, suggesting the compensatory nature of upregulation in α_2-adrenergic receptor density in PD.

Several interpretations may underlie α_2AR upregulation in PD. Upregulation in α_2AR density may be secondary to low NE levels or NE turnover rate, which is consistent with accumulating reports of low NE and/or MHPG levels in PD and other anxiety disorders (Hamlin et al. 1983; Edlund et al. 1987; Middleton et al. 1994; Murburg et al. 1994, 1995; Gurguis and Uhde 1998).

However, given that the majority of studies reported normal plasma NE levels in PD (Cameron et al. 1984, 1987, 1990, 1996; Albus et al. 1986; Schneider et al. 1987; Stein et al. 1992; Gurguis and Uhde 1998), failure in mechanisms involving agonist-mediated regulation of gene expression of adrenergic receptors (Hadcock and Malbon 1993, and references therein) may provide alternate, more plausible explanations for the present findings. Protein kinase A (PKA) is of particular interest, because it mediates agonist-induced receptor phosphorylation and downregulation. Upregulation of α_2AR density, therefore, may be attributable to abnormal PKA activity. Also, protein kinase C (PKC) has been shown to phosphorylate G_i protein, to interfere with α_2AR–G_i protein interaction, and to attenuate α_2AR-

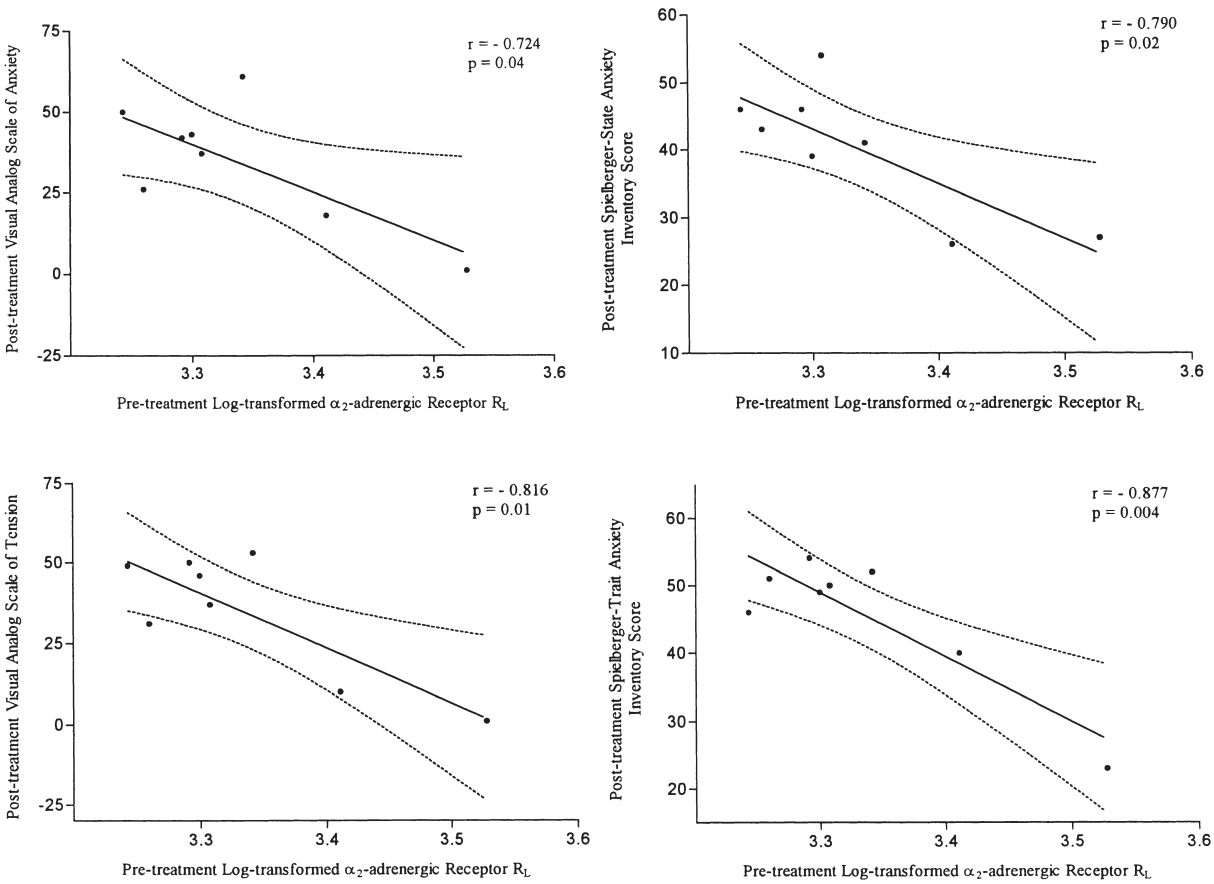

Figure 6. Pretreatment α_2-adrenergic receptor density in the low-conformational state (R_L) was correlated with post-treatment severity of anxiety and tension on several scales.

mediated inhibition of AC or cyclic adenosine monophosphate (cAMP) responses (Jakobs et al. 1985; Katada et al. 1985; García-Sáinz and Gutiérrez-Venegas 1989). Under normal conditions, both $\%R_H$ and the K_L/K_H ratio correlate positively with the agonist's intrinsic activity. Abnormal PKA and/or PKC activity also may explain the intriguing discrepancy found in PD patients in this study, wherein $\%R_H$ was increased but the K_L/K_H ratio was decreased.

A third possible explanation is that upregulation of α_2AR density represents a compensatory mechanism for enhanced stimulatory input to the AC system through increased βAR function. α_2AR density correlates positively with βAR density (Falkay et al. 1994). High intracellular cAMP levels increase the transcription rates of α_2AR, an effect that is mediated through the 5′ promoter region of the α_2AR gene (Hadcock and Malbon 1993). Also, stimulation of AC increases the expression of the inhibitory $G_{i\alpha 2}$ protein (Hadcock et al. 1990). Conversely, activation of the inhibitory input to AC is associated with increased β_2AR responsiveness and expression (Hadcock et al. 1991; Port et al. 1992). Increased α_2AR density, therefore, is consistent with results showing upregulation of βAR density and higher coupling to G_s protein in PD (Albus et al. 1986; Gurguis et al. 1994, 1997b).

The compensatory nature of upregulation of α_2AR density is consistent with findings in the present investigation showing that: (1) high anticipatory anxiety was associated with low α_2AR density; (2) high anxiety and depression ratings were associated with low K_L/K_H ratio; (3) high pretreatment K_L/K_H ratio was associated with a low number of panic attacks and severity of anxiety and depression at the end of treatment; and (4) high pretreatment receptor density was associated with low severity of anxiety and depression at the end of treatment.

High α_2AR density in PD is consistent with findings by Butler and colleagues (1992), who found increased ^{3}H-rauwolscine maximum binding capacity in platelet membranes from PD patients. Vascular α_2AR mediate vasoconstriction and lack receptor reserve (Bolli et al. 1985; Nichols and Ruffolo 1991). Increased α_2AR density, therefore, is consistent with enhanced α_2AR-mediated cardiovascular responses in PD patients (Nutt 1986, 1989). Increased α_2AR density is not consistent with decreased ^{3}H-clonidine and ^{3}H-yohimbine binding to platelet α_2AR (Cameron et al. 1984, 1990, 1996; Albus et al. 1986) or with normal α_2AR density in PD mea-

sured with ^{3}H-yohimbine or ^{3}H-rauwolscine (Norman et al. 1987; Nutt and Fraser 1987; Charney et al. 1989).

It seems that methodologic differences, other than the type of ligand used, may account for these discrepancies. Differences in the length of the drug wash-out period (which could not be ruled out in some studies), the blood collection procedure (such as the length of resting period before blood sampling), and gender (female PD patients may have higher catecholamine turnover rates and metabolite levels than males [Gurguis et al. 1991]) could have contributed to the inconsistent results.

Electrophysiologic and receptor binding studies in rat brain have shown that various antidepressant treatment modalities (ECT, antidepressants, lithium) induce downregulation of α_2AR density. Some studies reported an increase in R_H despite downregulation of total receptor density, suggesting that antidepressants enhance α_2AR-mediated inhibition of AC (Salama et al. 1982; Smith et al. 1983; Keith et al. 1986; Giralt and García-Sevilla 1989; Lacroix et al. 1991; Kovachich et al. 1993; Ribas et al. 1993; Moret and Briley 1994). In this investigation, chronic treatment with imipramine at therapeutic doses was not associated with downregulation of α_2AR density either in the high- or low-conformational state. More importantly, chronic imipramine treatment had no effect on measures of α_2AR coupling to G_i protein. This is consistent with the lack of effect of treatment with clomipramine and lofepramine on α_2AR density (Butler et al. 1992), with the persistence of blunted growth hormone response to clonidine following prozac treatment in PD patients (Coplan et al. 1995) and following clorgyline, desipramine, or amitriptyline treatment in depressed patients (Charney et al. 1982; Siever et al. 1982).

Clinical studies on the effects of antidepressants on platelet α_2AR in PD and depression have been inconsistent. Upregulation in α_2AR density was observed following imipramine treatment in PD (Cameron et al. 1984). Downregulation in α_2AR density following antidepressant treatment in depression was observed only in some studies (García-Sevilla et al. 1981a,b, 1986, 1987, 1990; Piletz and Halaris 1988; Piletz et al. 1991, 1993), but not the majority (Lenox et al. 1983; Pimoule et al. 1983; Siever et al. 1983; Stahl et al. 1983; Campbell et al. 1985; Cooper et al. 1985; Katona et al. 1989; Pandey et al. 1989; Wolfe et al. 1989; Bhatia et al. 1991; Healy et al. 1991; Kaneko et al. 1992; Werstiuk et al. 1992). Hence, the majority of studies on depression and two studies in PD did not find changes in α_2AR density in response to antidepressant treatment. It is reasonable, therefore, to conclude that upregulation of platelet α_2AR density may represent a link between the pathophysiology of PD and depression, which remains unchanged after antidepressant treatment. Increased platelet α_2AR density, therefore, may be a trait marker common to these related antidepressant-responsive disorders. Upregulation of α_2AR density in PD may also be related to the susceptibility of PD patients to major depression and to the notion that increased α_2AR density in PD is a trait marker.

It is worth noting that, despite the significant decrease in the total number of panic attacks after imipramine treatment, the decreases in post-treatment HAMA and HAMD scores were less than the conventionally acknowledged cut-off level of 50% decrease in severity of symptoms used to define successful treatment outcome. The small number of treated subjects in this study underscores the need to investigate antidepressant effects in a larger patient cohort. Our results do not address the likelihood of a decrease in post-treatment α_2AR density or change in coupling, had the decrease in HAMD scores been clinically significant. It is hypothetically likely that coupling parameters are different between treatment responders and nonresponders vis à vis healthy controls and that the effects of treatment on α_2AR coupling are different between treatment responders and nonresponders. Recent data from our laboratory have shown that depressed treatment nonresponsive patients have significantly lower α_2AR coupling, as compared to treatment-responsive patients (Gurguis et al. 1997a). Alternatively, it is possible that the therapeutic effect (downregulation of receptor density or changes in receptor coupling) occurred in early phases of treatment (3–6 weeks) but readjusted later on. Also, in this study, imipramine had pronounced effects on mood, although it lacked a noticeable effect on somatic anxiety symptoms. The lack of effect on somatic anxiety symptoms may be consistent with the lack of effect on platelet α_2AR binding measures, further suggesting that upregulation in α_2AR density may be a trait marker in PD.

In both controls and PD patients, pretreatment % R_H was positively correlated with severity of anxiety. However, the K_L/K_H ratio was positively correlated with severity of anxiety in healthy controls; whereas, it was negatively correlated with analogous measures in PD. This suggests a qualitative difference in the relationship between the K_L/K_H ratio and anxiety ratings in PD. In this study, we hypothesized abnormal α_2AR coupling to G_i protein in PD, anticipating that both coupling indices would be either higher or lower than normal values. The finding in this study of significantly higher % R_H, but lower K_L/K_H ratio, combined with the fact that the relationship between the K_L/K_H ratio and severity of anxiety was in an opposite direction to what was observed in controls, provide indirect evidence for abnormal PKC activity.

In summary, results of the present investigation demonstrate upregulation of α_2AR density and abnormal coupling to G_i protein in PD. Abnormal coupling was more pronounced in symptomatically "depressed" patients. α_2AR upregulation suggests abnormal agonist

regulation of α_2AR gene expression, possibly abnormal PKA or PKC activity. These results also demonstrate the lack of effect of antidepressant treatment on α_2AR and suggest that upregulation of α_2AR may be a trait marker in PD. Future studies should investigate the effect of antidepressant treatment on α_2AR coupling in relationship to treatment outcome. These future studies may identify possible predictors of treatment response and the effects of antidepressants on α_2AR coupling as related to treatment outcome. The role of PKA and PKC should also be subjects of future investigations in PD.

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REFERENCES

Abelson JL, Glitz D, Cameron OG, Lee MA, Bronzo M, Curtis GC (1992): Endocrine, cardiovascular, and behavioral responses to clonidine in patients with panic disorder. Biol Psychiatry 32:18–25

Aitken RCB (1969): Measurement of feelings using visual analogue scales. Proc Roy Soc Med 62:989–993

Aitken RCB, Gedye (1968): A study of two factors which affect arousal level and the apparent duration of a ten-minute interval. Br J Psychol 59:253–263

Albus M, Bondy B, Ackenheil M (1986): Adrenergic receptors on blood cells: Relation to the pathophysiology of anxiety. Clin Neuropharmacol 9:359–361

Albus M, Zahn TP, Breier A (1992): Anxiogenic properties of yohimbine: I. Behavioral, physiological, and biochemical measures. Eur Arch Psychiatr Clin Neurosci 241: 337–344

American Psychiatric Association (APA) (1987): Diagnostic and Statistical Manual of Mental Disorders, 3rd ed., rev. Washington, DC, American Psychiatric Press

Bhatia SC, Hsieh HH, Theesen KA, Townley RG, Andersen JM, Weiss S, Agrawal DK (1991): Platelet alpha-2 adrenoreceptor activity pretreatment and post-treatment in major depressive disorder with melancholia. Res Comm Chem Path Pharm 74:47–57

Bolli P, Erne P, Kiowski W, Amman FW, Bühler FR (1985): The adrenaline α_2-adrenoceptor-mediated vasoconstrictor axis. Clin Sci 68:141s–146s

Butler J, O'Halloran A, Leonard BE (1992): The Galway study of panic disorder II: Changes in some peripheral markers of noradrenergic and serotonergic function in DSM III-R Panic Disorder. J Aff Dis 26:89–100

Bylund DB, Blaxall HS, Iversen LJ, Caron MG, Lefkowitz RJ, Lomasney JW (1992): Pharmacological characteristics of α_2-adrenergic receptors: Comparison of pharmacologically defined subtypes with subtypes identified by molecular cloning. Molec Pharm 42:1–5

Cameron OG, Smith CB, Lee MA, Hollingsworth PJ, Nesse RM, Curtis GC (1984): Platelet α_2-adrenergic receptor binding and plasma catecholamines: Before and during imipramine treatment in patients with panic anxiety. Arch Gen Psychiatr 41:1144–1148

Cameron OG, Lee MA, Curtis GC (1987): Endocrine and physiological changes during "spontaneous" panic attacks. Psychoneuroendocrinology 12:321–331

Cameron OG, Smith CB, Lee MA, Hollingsworth PJ, Hill EM, Curtis GC (1990): Adrenergic status in anxiety disorders: Platelet alpha$_2$-adrenergic receptor binding, blood pressure, pulse, and plasma catecholamines in panic and generalized anxiety disorder patients and in normal subjects. Biol Psychiatry 28:3–20

Cameron OG, Smith CB, Nesse RM, Hill EM, Hollingsworth PJ, Abelson JA, Hariharan M, Curtis GC (1996): Platelet α_a-adrenoreceptors, catecholamines, hemodynamic variables, and anxiety in panic patients and their asymptomatic relatives. Psychosom Med 58:289–301

Campbell IC, McKernan RM, Checkley SA, Glass IB, Thompson C, Shur E (1985): Characterization of platelet alpha$_2$ adrenoceptors and measurement in control and depressed subjects. Psychiatry Res 14:17–31

Charney DS, Heninger GR (1986): Abnormal regulation of noradrenergic function in panic disorder. Arch Gen Psychiatr 43:1042–1054

Charney DS, Heninger GR, Sternberg DE (1982): Failure of chronic antidepressant treatment to alter growth hormone response to clonidine. Psych Res 7:135–138

Charney DS, Innis RB, Duman RS, Woods SW, Heninger GR (1989): Platelet alpha-2-receptor binding and adenylate cyclase activity in panic disorder. Psychopharmacology 98:102–107

Cooper SJ, Kelley JG, King DJ (1985): Adrenergic receptors in depression effects of electroconvulsive therapy. Br J Psychiatry 147:23–29

Coplan JD, Papp LA, Martinez J, Pine D, Rosenblum LA, Cooper T, Liebowitz MR, Gorman JM (1995): Persistence of blunted human growth hormone response to clonidine in fluoxetine-treated patients with panic disorder. Am J Psychiatry 152:619–622

DeLean A, Stadel JM, Lefkowitz RJ (1980): A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. J Biol Chem 255:7108–7117

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

Edlund MJ, Swann AC, Davis CM (1987): Plasma MHPG in untreated PD. Biol Psychiatry 22:1488–1491

Falkay G, Melis K, Kovács L (1994): Correlation between beta- and alpha-adrenergic receptor concentrations in human placenta. J Rec Res 14:187–195

Fishman PH, Finberg, JPM (1987): Effect of the tricyclic antidepressant desipramine on β-adrenergic receptors in cultured rat glioma C_6 cells. J Neurochem 49:282–289

Folstein MF, Luria R (1973): Reliability, validity, and clinical application of the visual analogue mood scale. Psychol Med 3:479–486

García-Sáinz JA, Gutiérrez-Venegas G (1989): Activation of protein kinase C alters the interaction of the α_2-adrenoceptors and the inhibitory GTP-binding protein (G_i) in human platelets. FEBS 257:427–430

García-Sevilla JA, Hollingsworth PJ, Smith CB (1981a): Alpha-2 adrenoreceptors on human platelets: Selective labeling by [^{3}H] clonidine and [^{3}H] yohimbine and competitive inhibition by antidepressant drugs. Euro J Pharmacol 74:329–341

García-Sevilla JA, Zis AP, Hollingsworth PJ, Greden JF, Smith CB (1981b): Platelet α_2-adrenergic receptors in major depressive disorder: Binding of tritiated clonidine before and after tricyclic antidepressant drug treatment. Arch Gen Psychiatr 38:1327–1333

García-Sevilla JA, Guimón J, García-Vallejo P, Fuster MJ (1986): Biochemical and functional evidence of supersensitive platelet α_2-adrenoceptors in major affective disorder: Effect of long-term lithium carbonate treatment. Arch Gen Psychiatr 43:51–57

García-Sevilla JA, Udina C, Fuster MJ, Alvarez E, Casas M (1987): Enhanced binding of [^{3}H] (-) adrenaline to platelets of depressed patients with melancholia: Effect of long-term clomipramine treatment. Acta Psychiatr Scand 75:150–157

García-Sevilla JA, Padró D, Giralt T, Guimón J, Areso P (1990): α_2-Adrenoceptor-mediated inhibition of platelet adenylate cyclase and induction of aggregation in major depression. Arch Gen Psychiatr 47:125–132

Giralt MT, García-Sevilla JA (1989): Acute and long-term regulation of brain α_2-adrenoceptors after manipulation of noradrenergic transmission in the rat. Eur J Pharmacol 164:455–466

Gurguis GNM, Uhde TW (1990): Plasma 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) and growth hormone responses to yohimbine in panic disorder patients and normal controls. Psychoneuroendrocrinology 15:217–224

Gurguis GNM, Mefford IN, Uhde TW (1991): Hypothalamic–pituitary-adrenocortical activity in panic disorder: Relationship to plasma catecholamine metabolites. Biol Psychiatry 30:502–506

Gurguis GNM, Choate JC, Antai-Otong D, Petty F, Rush AJ (1994): Higher beta adrenoreceptor density and supercoupling to G_s protein in panic disorder patients. Biol Psychiatry, Abstract #448

Gurguis GNM, Phan S, O'Neill J, Griffith J, Petty F, Davis L, Rush AJ (1997a): $Alpha_2$ and $Beta_2$ adrenergic receptor coupling to G_s and G_i proteins in major depression: Relationship to treatment outcome. Abstract #396. San Diego, Society for Biological Psychiatry

Gurguis GNM, Turkka J, George DT, Linnoila M (1997b): β-Adrenoreceptor coupling to Gs protein in alcohol dependence, panic disorder, and patients with both conditions. Neuropsychopharmacology 16:69–76

Gurguis GNM, Vitton BJ, Uhde TW (1997c): Behavioral, sympathetic, and adrenocortical responses to yohimbine in panic disorder patients and normal controls. Psychiatry Res 71:27–39

Gurguis GNM, Uhde TW (1998): The relationship between plasma MHPG and norepinephrine: Employing regression models in estimating centrally derived MHPG and peripheral norepinephrine turnover rate in panic disorder. J Psych Res, 32:11–17

Hadcock, JR, Malbon, CC (1993): Agonist regulation of gene expression of adrenergic receptors and G proteins. J Neurochemistry 60:1–9

Hadcock JR, Ros M, Watkins DC, Malbon CC (1990): Cross-regulation between G-protein-mediated pathways: Stimulation of adenylyl cyclase increases expression of the inhibitory G-protein, $G_{i\alpha 2}$. J Biol Chem 265:14784–14790

Hadcock JR, Port JD, Malbon CC (1991): Cross-regulation between G-protein-mediated pathways: Activation of the inhibitory pathway of adenylylcyclase increases the expression of β_2-adrenergic receptors. J Biol Chem 266:11915–11922

Hamilton M (1959): The assessment of anxiety states by rating. Br J Med Psychol 32:50–55

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

Hamlin CL, Lydiard RB, Martin D, Dackis CA, Pottash AC, Sweeney D, Gold MS (1983): Urinary excretion of noradrenaline metabolite decreased in panic disorder. Lancet 2:740–741

Healy DT, Paykel ES, Whitehouse AM, White W, Wilton-Cox H, Johnson AL, Theodorou AE, Lawrence K, Horton RW (1991): Platelet [^{3}H]imipramine and α_2-adrenoceptor binding in normal subjects during desipramine administration and withdrawal. Neuropsychopharmacology 4:117–124

Hoffman BB, Michel T, Kilpatrick DM, Lefkowitz RJ, Tolbert MEM, Gilman H, Fain JN (1980): Agonist versus antagonist binding to α-adrenergic receptors. Proc Natl Acad Sci 77:4569–4573

Hoffman BB, Michel T, Brenneman TB, Lefkowitz RJ (1982): Interactions of agonists with platelet α_2-adrenergic receptors. Endocrinology 110:926–932

Jakobs KH, Bauer S, Watanabe Y (1985): Modulation of adenylate cyclase of human platelets by phorbol ester: Impairment of the hormone-sensitive inhibitory pathway. FEBS 151:425–430

Kaneko M, Kanno T, Honda K, Mashiko H, Oosuga N, Watanabe A, Kumashiro H (1992): Platelet alpha-2 adrenergic receptor binding and plasma free 3-methoxy-4-hydroxyphenyl-ethylene glycol in depressed patients before and after treatment with mianserin. Neuropsychobiology 25:14–19

Katada T, Gilman AG, Watanabe Y, Bauer S, Jakobs KH (1985): Protein kinase C phosphorylates the inhibitory guanine-nucleotide-binding regulatory component and apparently suppresses its function in hormonal inhibition of adenylate cyclase. FEBS 151:431–437

Katona CLE, Theodorou AE, Davies SL, Hale AS, Kerry SM, Horton RW, Kelly JS, Paykel ES (1989): [^{3}H]Yohimbine binding to platelet α_2-adrenoceptors in depression. J Aff Dis 17:219–228

Keith RA, Howe BB, Salama AI (1986): Modulation of peripheral beta-1 and alpha-2 receptor sensitivities by the administration of the tricyclic antidepressant, imi-

pramine, alone and in combination with alpha-2 antagonists to rats. J Pharm Exper Ther 236:356–363

Kent RS, DeLean A, Lefkowitz RJ (1980): A quantitative analysis of beta-adrenergic receptor interactions: Resolution of high- and low-affinity states of the receptor by computer modeling of ligand binding data. Mol Pharmacol 17:14–23

Kim MH, Neubig RR (1987): Membrane reconstitution of high-affinity α2 adrenergic agonist binding with guanine nucleotide regulatory proteins. Biochem 26:3664–3672

Kovachich GB, Frazer A, Aronson CE (1993): Effect of chronic administration of antidepressants on α_2-adrenoceptors in the locus coeruleus and its projection fields in rat brain determined by quantitative autoradiography. Neuropsychopharmacology 8:57–65

Lacroix D, Blier P, Curet O, Montigny C (1991): Effects of long-term desipramine administration on noradrenergic neurotransmission: Electrophysiological studies in the rat brain. J Pharm Exper Ther 257:1081–1090

Lenox RH, Ellis JE, Van Riper DA, Ehrlich YH, Peyser JM, Shipley JE, Weaver LA (1983): Platelet α_2-adrenergic receptor activity in clinical studies of depression. In Usdin E, Goldstein M, Friedhoff AJ, Georgotas A (eds), Frontiers in Neuropsychiatric Research. London, MacMillan Press, pp 331–356

Lorenz W, Lomasney JW, Collins S, Regan JW, Caron MG, Lefkowitz RJ (1990): Expression of three α_2-adrenergic receptor subtypes in rat tissues: Implications for α_2 receptor classification. Mol Pharm 38:599–603

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951): Protein measurement with folin phenol reagent. J Biol Chem 193:265–275

Luria RE (1975): The validity and reliability of the visual analogue mood scale. J Psychiatric Res 12:51–57

Matsui H, Lefkowitz RJ, Caron MG, Regan JW (1989): Localization of the fourth membrane spanning domain as a ligand binding site in the human platelet α_2-adrenergic receptor. Biochemistry 28:4125–4130

Middleton HC, Ashby M, Robbins TW (1994): Reduced plasma noradrenaline and abnormal heart rate variability in resting panic disorder patients. Biol Psychiatry 36:847–849

Moret C, Briley M (1994): Effect of milnacipran and desipramine on noradrenergic α_2-autoreceptor sensitivity. Prog Neuro-Psychopharmacol Biol Psychiatry 18:1063–1072

Munson RJ, Rodbard D (1983): LIGAND: A versatile computerized approach for characterization of ligand binding systems. Anal Biochem 197:220–239

Munson, PJ, Rodbard, D (1984) Computerized analysis of ligand binding data: Basic principles and recent development. In Rodbard D, Fortig (eds), Computers in Endocrinology, New York, The Raven Press, pp 117–145

Murburg MM, McFall ME, Veith RC (1994): Basal sympathoadrenal function in patients with PTSD and depression. In Murburg MM (ed), Catecholamine Function in Posttraumatic Stress Disorder: Emerging Concepts. Washington, DC, American Psychiatric Press, pp 175–188

Murburg MM, McFall ME, Lewis N, Veith RC (1995): Plasma Norepinephrine Kinetics in Patients with Posttraumatic Stress Disorder. Biol Psychiatry 38:819–825

Neubig RR, Gantzos RD, Thomsen WJ (1988): Mechanism of agonist and antagonist binding to α_2-adrenergic receptors: Evidence for a precoupled receptor-guanine nucleotide protein complex. Biochemistry 27:2374–2384

Nichols AJ, Ruffolo RR (1991): Functions mediated by α-adrenoceptors. In Ruffolo RR (ed), α-adrenoceptors: Molecular biology, Biochemistry, and Pharmacology. New York, Karger, pp 105–179

Norman TR, Kimber NM, Judd FK, Burrows GD, McIntyre IM (1987): Platelet ^{3}H-rauwolscine binding in patients with panic attacks. Psychol Res 22:43–48

Nutt DJ (1986): Increased central alpha$_2$-adrenoceptor sensitivity in panic disorder. Psychopharmacology 90:268–269

Nutt DJ, Fraser S (1987): Platelet binding studies in panic disorder. J Aff Dis 12:7–11

Nutt DJ (1989): Altered central alpha-2-adrenoceptor sensitivity in panic disorder. Arch Gen Psychiatr 46:165–169

Okada F, Tokumitsu Y, Ui M (1986): Desensitization of beta-adrenergic receptor-coupled adenylate cyclase in cerebral cortex after in vivo treatment of rats with desipramine. J Neurochemistry 47:454–459

Ordway GA, Jaconetta SM, Halaris AE (1993): Characterization of subtypes of alpha-2 adrenoceptors in the human brain. J Pharmacology 264:967–976

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

Piletz JE, Halaris A (1988): Super high-affinity ^{3}H-para-aminoclonidine binding to platelet adrenoceptors in depression. Prog Neuro-Psychopharmacol Biol Psychiatr 12:541–553

Piletz JE, Halaris A, Saran A, Marler MR (1991): Desipramine lowers tritiated para-aminoclonidine binding in platelets of depressed patients. Arch Gen Psychiatr 48:813–820

Piletz JE, Chikkala D, Khaitan L, Jackson K, Qu Y (1993): Delayed desensitization of α_2-adrenoceptor-mediated platelet aggregation in depressed patients. Neuropsychopharmacology 9:55–66

Pimoule C, Briley MS, Gay C, Loo H, Sechter D, Zarifian E, Raisman R, Langer SZ (1983): ^{3}H-Rauwolscine binding in platelets from depressed patients and healthy volunteers. Psychopharmacology 79:308–312

Port JD, Hadcock JR, Malbon CC (1992): Cross-regulation between G-protein-mediated pathways: Acute activation of the inhibitory pathway of adenylylcyclase reduces β_2-adrenergic receptor phosphorylation and increases β-adrenergic responsiveness. J Biol Chem 267:8468–8472

Rapaport MH, Risch SC, Gillin JC, Golshan, Janowsky DS (1989): Blunted growth hormone response to peripheral infusion of human growth hormone-releasing factor in patients with panic disorder. Am J Psychiatry 146:92–95

Ribas C, Miralles A, García-Sevilla JA (1993): Acceleration by chronic treatment with clorgyline of the turnover of brain α_2-adrenoceptors in normotensive but not in spontaneously hypertensive rats. Br J Pharm 110:99–106

Salama AI, Garcia A, Wang CH, Lin L, Prichard DCU (1982): Relationship between adaptive changes in brain α_2- and β-adrenergic receptors following repeated imipramine, yohimbine, and electroconvulsive shock treatment to rats. In Usdin E, Goldstein M, Friedhoff AJ, Georgotas A (eds), Frontiers in Neuropsychiatric Research. London, MacMillian, pp 145–158

Schneider P, Evans L, Ross-Lee L, Wiltshire B, Eadie M, Hoey KH (1987): Plasma biogenic amine levels in agoraphobia with panic attacks. Pharmacopsychology 20:102–104

Siever LJ, Uhde TW, Silberman EK, Jimerson DC, Aloi JA, Post RM, Murphy DL (1982): Growth hormone response to clonidine as a probe of noradrenergic receptor responsiveness in affective disorder patients and controls. Psychiatry Res 6:171–183

Siever LJ, Kafka MS, Insel TR, Lake CR, Murphy DL (1983): Effect of long-term clorgyline administration on human platelet alpha-adrenergic receptor binding and platelet cyclic AMP responses. Psychiatry Res 9:37–44

Siever LJ, Uhde TW (1984): New studies and perspectives on the noradrenergic receptor system in depression: Effects of the α_2-adrenergic agonist clonidine. Biol Psychiatry 19:131–156

Simonds WF, Goldsmith PK, Codina J, Unson CG, Spiegel AM (1989): G_{i2} mediates α_2-adrenergic inhibition of adenylyl cyclase in platelet membranes: In situ identification with G_α C-terminal antibodies. Proc Natl Acad Sci 86:7809–7813

Smith CB, Hollingsworth PJ, García-Sevilla JA, Zis AP (1983): Platelet alpha$_2$ adrenoreceptors are decreased in number after antidepressant therapy. Prog Neuro-Psychopharmacol Biol Psychiatry 7:241–247

Spielberger CD (1983): Manual for the State–Trait Anxiety Inventory, rev. ed. Palo Alto, CA, Consulting Psychologists Press

Spielberger CD, Gorsuch RL, Luschene RE (1970): STAI Manual for the State–Trait Anxiety Inventory ("Self-Evaluation Questionnaire"). Palo Alto, CA, Consulting Psychologists Press

Stahl SM, Lemoine PM, Ciaranello RD, Berger PA (1983): Platelet alpha$_2$-adrenergic receptor sensitivity in major depressive disorder. Psychiatry Res 10:157–164

Stein MB, Tancer ME, Uhde TW (1992): Heart rate and plasma norepinephrine responsivity to orthostatic challenge in anxiety disorders: Comparison of panic disorders and social phobia and normal control subjects. Arch Gen Psychiatr 49:311–317

Tancer ME, Stein MB, Black B, Uhde TW (1993): Blunted growth hormone responses to growth hormone-releasing factor and to clonidine in panic disorder. Am J Psychiatry 150:336–337

Thomsen WJ, Jacquez JA, Neubig RR (1988): Inhibition of adenylate cyclase is mediated by the high affinity conformation of the α_2-adrenergic receptor. Mol Pharm 34:814–822

Tsuchiya F, Ikeda H, Hatta Y, Saito T (1988): Effects of desipramine administration on receptor adenylate cyclase coupling in rat cerebral cortex. Japan J Psych Neurol 42:858–859

Uhde TW, Vittone BJ, Siever LJ, Kaye WH, Post RM (1986): Blunted growth hormone response to clonidine in panic disorder patients. Biol Psychiatry 21:1077–1081

Uhde TW, Tancer ME, Rubinow DR, Roscow DB, Boulenger JP, Vittone B, Gurguis G, Geraci M, Black B, Post RM (1992): Evidence for hypothalamo-growth hormone dysfunction in panic disorder: Profile of growth hormone (GH) response to clonidine, yohimbine, caffeine, glucose, GRF and TRH in panic disorder patients versus healthy volunteers. Neuropsychopharmacology 6:101–118

Werstiuk ES, Auffarth SE, Coote M, Gupta RN, Steiner M (1992): Platelet α_2-adrenergic receptors in depressed patients and healthy volunteers: The effects of desipramine. Pharmacopsychology 25:199–206

Wolfe N, Gelenberg AJ, Lydiard RB (1989): Alpha$_2$-adrenergic receptor sensitivity in depressed patients: Relation between ^{3}H-yohimbine binding to platelet membranes and clonidine-induced hypotension. Biol Psychiatry 25:382–392

Yamamoto H, Kagaya A, Kuroda Y, Mikuni M, Takahashi K (1990): Effect of antidepressants on the GTP binding sites in rat brain homogenate. Japan J Psych Neurol 44:133–134

Zealley AK, Aitken RCB (1969): Measurement of mood. Proc Roy Soc Med 62:993–996

Zung WWK (1971): A rating instrument for anxiety disorders. Psychosomatics 12:371–379