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Neutrophil β_2-adrenoceptor function in major depression: G_s coupling, effects of imipramine and relationship to treatment outcome

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Abstract

Abnormal β_2-adrenoceptor density and β_2-adrenoceptor-mediated cyclic adenosine monophosphate (cAMP) responses were inconsistently reported in major depressive disorder. Tricyclic antidepressants downregulate β-adrenoceptor density and decrease coupling to G_s protein. Abnormal β-adrenoceptor coupling may exist in major depressive disorder and may relate to treatment response. We investigated β_2-adrenoceptor coupling to G_s protein in 25 controls, 23 major depressive disorder drug-free patients and 16 major depressive disorder patients after chronic imipramine treatment using agonist displacement experiments. Pretreatment β_2-adrenoceptor coupling and density were normal in patients as a whole. Chronic imipramine induced β_2-adrenoceptor uncoupling. This effect was observed in treatment responders who had increased β_2-adrenoceptor density in the high-conformational state and supercoupling prior to treatment. β_2-adrenoceptor density decreased after imipramine treatment. Treatment non-responders had seemingly normal pretreatment β_2-adrenoceptor function, which was not changed by imipramine. Differences in β_2-adrenoceptor regulation in major depressive disorder may underlie treatment response. The results indirectly implicate abnormal agonist-mediated β_2-adrenoceptor gene expression, protein kinase A, and protein kinase C in major depressive disorder.

Keywords: β_2-Adrenoceptor; G_s protein, coupling; Neutrophil; Depression, major; Anxiety; Antidepressant, tricyclic

1. Introduction

Lymphocyte or neutrophil β-adrenoceptors are of the β_2 subtype. They have been used as a peripheral model to investigate β-adrenoceptor function in major depressive disorder. β_2-adrenoceptor binding studies in major depressive disorder have been inconsistent. Decreased lymphocyte or leukocyte β_2-adrenoceptor density has been reported in some (Extein et al., 1979; Wood et al., 1986; Carstens et al., 1987; Pandey et al., 1990; Jeanningros et al., 1991) but not other studies which found normal lymphocyte β_2-adrenoceptor density (Ebstein et al., 1988; Mann et al., 1990; Mazzola et al., 1991; Werstiuk et al., 1996). Similarly, brain β-adrenoceptor binding studies have been inconsistent. High β-adrenoceptor density in frontal cortex of suicide victims and/or depressed subjects (Arango et al., 1992) was not replicated by other investigators who reported normal β-adrenoceptor density (Meyerson et al., 1982; Stockmeier and Meltzer, 1991; Gurguis et al., 1999b), while still others found region-specific decreases in β-adrenoceptor density (De Paermentier et al., 1992; Little et al., 1993). In contrast to this inconsistency, the majority of studies reported decreased isoproterenol- or β_2-adrenoceptor-mediated cyclic adenosine monophosphate (cAMP) in major depressive disorder (Pandey et al., 1979; Ebstein et al., 1988; Halper et al., 1988; Mann et al., 1990, 1997). Consistent with the decreased β_2-adrenoceptor-mediated cAMP responses, heart rate response to isoproterenol infusion (reflecting cardiac β_2-adrenoceptor function) was also decreased in major depressive disorder patients (Bertschy et al., 1989). Finally, agonist-induced Guanylyl-imidotriphosphate (Gpp(NH)p) binding was decreased in major depressive disorder and normalized after treatment (Avissar et al., 1998). Collectively, decreased β-adrenoceptor-mediated cAMP response despite normal β-adrenoceptor density and decreased Gpp(NH)p binding

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suggest uncoupling of β-adrenoceptors from G_s protein, decreased formation of high-conformational state, and/or decreased agonist affinity in major depressive disorder. These measures have not been investigated in major depressive disorder.

Changes in lymphocyte β_2-adrenoceptor density and/or function were also observed after various antidepressants. Increases in leukocyte β_2-adrenoceptor density and potentiation of β_2-adrenoceptor-mediated cAMP responses were found following chronic iprindol, desipramine or electroconvulsive therapy and were correlated with improvement of depression (Pandey et al., 1990; Werstiuk et al., 1996). Normalization of blunted lymphocyte β_2-adrenoceptor-mediated cAMP response was also found after electroconvulsive therapy, although it was not related to treatment response (Mann et al., 1990). However, others reported decreases in β_2-adrenoceptor density after chronic treatment with the antidepressant amineptine, which was also correlated with clinical improvement (Mazzola et al., 1991). Similarly, high pretreatment β_2-adrenoceptor density decreased after chronic trazodone or amitriptyline treatment in treatment responders, but similar changes in β_2-adrenoceptors were not observed in treatment non-responders (Healy et al., 1985). Thus, changes in peripheral β_2-adrenoceptor function have been observed after antidepressant treatment although the direction of change is not clearly established. Previous studies have not investigated the effects of antidepressant treatment on β_2-adrenoceptor coupling or whether changes in β_2-adrenoceptor function are related to treatment outcome.

Increases in low pretreatment β_2-adrenoceptor density or β_2-adrenoceptor-mediated cAMP responses in major depressive disorder after antidepressant treatment is inconsistent with the mechanisms of action of tricyclic antidepressants. Decreases in β-adrenoceptor density or β-adrenoceptor-mediated cAMP responses by tricyclic antidepressants in rat brain or cell cultures is a conditio sine que non which has been replicated by multiple laboratories (Vetulani and Sulser, 1975; Banerjee et al., 1977; Honegger et al., 1986; Okada et al., 1988; Sulser, 1990; Hosoda and Duman, 1993). This decrease was limited to density in the high-conformational state (Manier et al., 1989; Gurguis et al., 1998) indicating uncoupling from G_s protein. Furthermore, evidence from cell cultures indicates that tricyclic antidepressants may act directly on post-synaptic β-adrenoceptors and that the decrease may not be merely an adaptive response to blockade of norepinephrine reuptake. Finally, similar effects by tricyclic antidepressants on β_2-adrenoceptors in peripheral non-neuronal tissues (e.g., blood cells, salivary gland) (Scarpace et al., 1992), suggests perhaps that β_2-adrenoceptors in peripheral tissues lacking presynaptic input, as in tissue cultures, may be used as a model to investigate the effects of antidepressants in pathophysiological conditions.

The literature indicates that only 50% of depressed patients respond favorably after the first antidepressant treatment trial (Depression Guideline Panel, 1993). While some patients respond to tricyclic antidepressants others may respond to serotonin specific reuptake inhibitors. We propose that heterogeneity in the pathophysiology of depression may underlie this phenomenon such that consistency between the mechanisms of action of antidepressants and the pathophysiology may be necessary for treatment response. For example, if antidepressants downregulate β-adrenoceptors or decease coupling to G_s protein, then upregulation of β-adrenoceptors and supercoupling may exist specifically in treatment responders.

In this study, we employed neutrophil membrane β_2-adrenoceptors as a peripheral model to investigate coupling to G_s protein in 23 major depressive disorder patients and 25 healthy controls. We also investigated the effects of chronic imipramine treatment on β_2-adrenoceptor coupling in 16 of the 23 patients in relation to treatment response. We conducted both antagonist-saturation and isoproterenol-displacement experiments. Coupling indices were derived from agonist-displacement curves. According to the ternary model for G protein-coupled receptors (DeLean et al., 1980, 1982), agonist binding to the β-adrenoceptors induces the formation of a transitory high-conformational state which comprises the agonist-receptor-G protein complex. This is reflected in agonist displacement curves which best fit a two-site model, revealing that β-adrenoceptors exist in high- and low-affinity states regulated by G protein. The percentage of receptors that exist in the high-conformational state and the ratio of the agonist dissociation constant from the receptor in the low-/high-conformational state have been shown to correlate with the agonist's intrinsic activity or capacity to induce the formation of cAMP and have been proposed as measures of β-adrenoceptor coupling to G_s protein.

We hypothesized that abnormal β_2-adrenoceptor function in major depressive disorder exists in their coupling to G_s protein. We further hypothesized that (1) pretreatment coupling measures would relate to treatment outcome (i.e., would differentiate between treatment responders and non-responders), and (2) that decreased coupling would be associated with positive treatment outcome. We explored the relationship between pre-treatment β_2-adrenoceptor binding parameters and severity of anxiety and depression after treatment.

2. Methods

2.1. Subjects

Neutrophil β_2-adrenoceptors were investigated in 25 male healthy controls and 23 male outpatients with recurrent unipolar major depressive disorder. Patients were recruited from the Mental Health Clinic at the Dallas VA Medical Center. Patients met DSM-IV (American Psychiatric Association, 1994) diagnostic criteria for major de-

pressive disorder. Diagnosis was made using a comprehensive semi-structured clinical interview by one of the investigators (GG).

All subjects signed an informed written consent form prior to participating in the study. Gender- and age-matched healthy controls had no personal or family psychiatric history. Patients and controls were drug-free for at least 2 weeks (6 weeks for serotonin reuptake inhibitors) before the study. All subjects were physically healthy, as ascertained by medical history, physical examination, and laboratory work-up. Subjects observed a low-monoamine and mildly restricted caffeine diet for 3 days prior to the procedure.

2.2. Procedure

Fasting subjects reported to the laboratory at 7:30 AM. Subjects assumed a supine position in a hospital bed, and an intravenous (i.v.) line was started. Subjects rested for 60 min, after which, 60 ml of blood was then drawn for radioreceptor assays and plasma catecholamine levels and the i.v. line was discontinued.

Symptom ratings, including the Hamilton Scale for Anxiety, the Hamilton Scale for Depression (HAM-D, 24-item), the Zung Anxiety Scale (both clinician- and self-rated), the Spielberger-State Anxiety Inventory, the Spielberger-Trait Anxiety Inventory and 100-mm Visual Analogue Scales of mood (anxiety, tension, irritability, fearfulness, depression, and anger) and somatic autonomic anxiety symptoms, were completed by all subjects after the procedure. Symptom ratings were assessed as detailed in Gurguis, et al. (1999a).

2.3. Neutrophil β_2-adrenoceptor assay

Fresh membrane preparations were used in all receptor binding experiments. Neutrophil β_2-adrenoceptor binding assays were conducted according to Davies and Lefkowitz, (1980). Briefly, blood was drawn through a 19-ga needle and was mixed immediately with 0.9% sodium chloride-dextran solution and allowed to settle for 40 min. The supernatant was then aspirated and layered over Ficoll-paque solution and was centrifuged at $500 \times g$ at 25°C for 40 min. The supernatant was discarded and the polymorphonuclear cells were washed in cold deionized water for 20 s. Polymorphonuclear cells were suspended in normal saline and centrifuged for 10 min at 4°C, then were resuspended in incubation buffer (Tris–HCl 50 mM, $MgCl_2$ 10 mM, pH 7.65) and homogenized using a polytron for 15 s. Polymorphonuclear cell membranes were obtained using differential centrifugation at 4°C. The membrane pellet was suspended in the Tris–HCl incubation buffer. Protein concentrations were measured (Lowry et al., 1951).

[^{125}I]Iodocyanopindolol (S.A. 2200 Ci/mmol) was used as a ligand in saturation experiments using seven concentrations (3.0–25.0 pM). Non-specific binding, defined in the presence of isoproterenol (0.1 mM), was approximately 10–15% of total binding at 12 pM [^{125}I]Iodocyanopindolol. Displacement experiments were conducted using 18 varying concentrations of unlabeled isoproterenol (0–1.0 mM) to displace 12.0 pM [^{125}I]Iodocyanopindolol. Samples were incubated at 37°C for 45 min. Incubation was terminated by rapid filtration over Whatman GF/C glass fiber filters and radioactivity was counted using a gamma counter (ICN 4/600 plus, USA) with 76% counting efficiency.

Plasma epinephrine and norepinephrine levels were measured using a radioimmunoassay (Katcombi-RIA, IBL, Hamburg, Germany) with 0.0164 and 0.591 pM/ml sensitivity for epinephrine and norepinephrine, respectively. Intra- and interassay coefficients of variation were 5.2% and 13.4%, respectively for epinephrine, and 6.2% and 9.3%, respectively for norepinephrine.

2.4. Binding data analysis

Binding data were analyzed using LIGAND program (Munson and Rodbard, 1984). The maximum binding capacity and the antagonist dissociation constant, B_{max} and K_d, respectively, were derived from weighted, curvilinear Scatchard analysis of saturation experiments, with the B_{max} representing the x-intercept and K_d being equal to $-1/\text{slope}$. Analysis of displacement experiments employed least square non-linear iterative curve-modeling methods that test for the presence of more than one affinity state. An F-test was used to compare the goodness-of-fit of one- vs. two-site models. A two-site model was accepted only if the goodness-of-fit was statistically significantly better than the one-site model ($P < 0.05$).

Receptor density in the high (R_H)- and low (R_L)-conformational states, and isoproterenol dissociation constant from the receptor in the high (K_H)- and low (K_L)-conformational states were measured from displacement curves. The total isoproterenol-measured receptor density ($R_T = R_H + R_L$), $\%R_H$, and K_L/K_H ratio were calculated. $\%R_H$ and the K_L/K_H ratio were used as indices of β_2-adrenoceptor coupling to G_s.

2.5. Treatment phase

After baseline (pre-treatment) assay of β_2-adrenoceptors, patients were treated with imipramine. A group of 16 patients completed an 8-week imipramine treatment course (59 ± 5.6 days). Imipramine dosage was initiated and titrated gradually to therapeutic levels (150–250 μg/l), which were verified by plasma imipramine levels. At the end of the treatment course, subjects were categorized into treatment responders and treatment non-responders. Treatment responders were operationally defined as patients whose post-treatment HAM-A and HAM-D scores decreased by 50% from pretreatment ratings. Similar criteria have been previously used (Elkin et al., 1989). β_2-adrenoceptors and plasma epinephrine and norepinephrine levels

were reassayed in these patients after the 8 weeks while they were still on imipramine.

2.6. Statistical analysis

Group differences (major depressive disorder patients vs. healthy controls) in coupling measures ($\%R_H$ and K_L/K_H) among other binding parameters were tested using two-tailed independent *t*-tests. One-tailed paired *t*-tests were used to compare pre- to post-treatment changes in binding measures in patients. To test if pre-treatment receptor binding parameters predicted symptom severity after treatment, we conducted regression analysis using pre-treatment receptor binding parameters as dependent variables and anxiety and depression severity ratings after treatment as independent variables. Data are presented as means ± S.E.M.

3. Results

There were no significant differences in age between patients (44.7 ± 10.0 years) and healthy controls (41.9 ± 11.6 years), ($t = 0.898$, P = NS), nor between treatment responders (45.0 ± 10.8 years) and non-responders (46.8 ± 10.6 years) ($t = 0.345$, P = NS). Five patients met DSM-IV diagnostic criteria for comorbid panic disorder with mean number of panic attacks of 3.3 per week.

3.1. Major depressive disorder patients vs. controls prior to treatment

Patients had average HAM-D and HAM-A scores of 27.24 ± 1.36 and 21.37 ± 1.06, respectively. In addition, they had a Zung-C score of 37.7 ± 1.3, Zung-S of 42.52 ± 2.5, Sp-S score of 52.95 ± 2.05, and a Sp-T score of 62.32 ± 1.45. These scores and other VAS of mood and autonomic anxiety symptoms were all significantly higher in patients than in controls.

There were no differences in coupling measures or in other receptor binding parameters between healthy controls and depressed patients as a whole, i.e., not categorized into responders and non-responders (Table 1). There were also no differences between healthy controls and patients in supine basal plasma epinephrine (Controls: 0.09 ± 0.01 vs. Patients: 0.086 ± 0.01 pmol/ml, $t = 0.282$, P = NS) or in norepinephrine levels (Controls: 0.85 ± 0.05 vs. Patients: 0.81 ± 0.07 pmol/ml, $t = 0.471$, P = NS).

3.2. Effects of chronic imipramine treatment

Sixteen of the 23 depressed patients finished an 8-week imipramine treatment course. There was a significant drop in anxiety and depression scores such that patients had an average HAM-D score of 11.56 ± 1.8 ($P < 0.00$), HAM-A score of 10.84 ± 1.78 ($P < 0.00$). There were also statistically significant decreases in other anxiety and depression ratings after treatment.

Imipramine treatment induced a decrease in β_2-adrenoceptor coupling and density as reflected in a significant decrease in $\%R_H$. This was primarily due to a significant decrease in R_H, as there was no change in R_L. Consequently, there was only a statistical trend towards significance for a decrease in R_T (Table 2).

In conjunction with these receptor changes, imipramine significantly increased basal supine plasma norepinephrine levels (Pre-treatment: 0.81 ± 0.07 vs. Post-treatment: 1.40 ± 0.12 pmol/ml, $t = -5.710$, $P = 0.00$) but had no effects on epinephrine levels (Pre-treatment: 0.086 ± 0.01 vs. Post-treatment: 0.103 ± 0.02 pmol/ml, $t = -1.386$, P = NS).

3.3. Pre-treatment β_2-adrenoceptor regulation in treatment responders vs. non-responders

According to the operationally defined criteria, there were nine treatment responders and seven treatment non-responders. There was no difference between treatment responders and non-responders in severity of depression or

Table 1
Neutrophil β-adrenoceptor binding parameters in normal controls and patients with major depressive disorder
Values are means ± S.E.M.

	K_d (pmol)	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
Normal controls ($n = 25$)	24.00 ± 2.07	44.20 ± 6.04	36.64 ± 4.29	13.30 ± 1.79	49.94 ± 5.77	72.71 ± 1.87	18.90 ± 3.69	3.70 ± 1.10	197.88 ± 32.33
Major depression ($n = 23$)	26.11 ± 3.44	61.25 ± 9.91	44.77 ± 4.75	14.42 ± 1.99	59.19 ± 6.44	75.85 ± 1.93	27.38 ± 9.05	6.06 ± 2.25	265.52 ± 93.62

Table 2
Neutrophil β-adrenoceptor binding parameters in patients with major depressive disorder pre- and post-treatment
Values are means ± S.E.M.

	K_d (pmol)	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
Pre-treatment ($n = 16$)	29.43 ± 4.62	68.30 ± 12.85	47.47 ± 5.40	13.27 ± 1.71	60.74 ± 6.90	77.80 ± 1.48	29.12 ± 12.85	3.70 ± 2.66	216.83 ± 51.88
Post-treatment ($n = 16$)	23.16 ± 2.52	45.16 ± 7.01[a]	37.39 ± 5.81[b]	14.24 ± 2.25	51.64 ± 7.86[c]	72.64 ± 2.11[d]	19.34 ± 4.55	3.82 ± 1.85[c]	137.28 ± 27.55[c]

[a] $P \leq 0.03$, paired one-tailed t-test, $t = 2.038$.
[b] $P \leq 0.04$, paired one-tailed t-test, $t = 1.928$.
[c] $P \leq 0.08$–0.09, paired one-tailed t-test, $t = 1.471$–1.401.
[d] $P \leq 0.02$, paired one-tailed t-test, $t = 2.154$.

anxiety ratings prior to treatment. However, after treatment, there were statistically significant differences between responders and non-responders in HAM-D scores (Responders: 6.44 ± 4.1 vs. Non-responders: 18.1 ± 4.3, $t = 5.577$, $P = 0.00$), HAM-Anxiety scores (Responders: 6.44 ± 5.3 vs. Non-responders: 16.5 ± 4.7, $t = -3.942$, $P = 0.001$), among other anxiety/depression ratings.

Prior to treatment, $\%R_H$ was significantly higher in treatment responders in comparison to controls (Controls: 72.71 ± 1.87 vs. Responders: 80.67 ± 1.58 vs. Non-responders: 74.10 ± 2.05, $F = 3.274$, $P = 0.05$) providing evidence for β_2-adrenoceptor supercoupling to G_s protein in this patient subgroup. This was due to significantly higher R_H in treatment responders than controls (Controls: 36.64 ± 4.29 vs. Responders: 57.21 ± 7.09 vs. Non-responders: 34.40 ± 10.05 fmol/mg protein, $F = 3.662$, $P = 0.03$, Bonferroni-corrected $P = 0.04$). There were no group differences in R_L. Treatment responders had higher R_T than treatment non-responders ($t = 1.994$, $P = 0.06$), and the overall ANOVA showed a similar trend (Controls: 49.94 ± 5.77 vs. Responders: 71.82 ± 9.65 vs. Non-responders: 46.49 ± 7.23 fmol/mg protein, $F = 2.413$, $P = 0.10$). Finally, there was a trend for lower K_L in treatment responders (Controls: 3.70 ± 1.10 vs. Responders: 9.26 ± 4.46 vs. Non-responders: 1.22 ± 0.25 μM, $F = 2.591$, $P = 0.09$) (Table 3). These differences in β_2-adrenoceptor function were observed despite the lack of significant differences in pretreatment basal supine epinephrine levels between controls, treatment responders or treatment non-responders (Controls: 0.090 ± 0.01 vs. Responders: 0.085 ± 0.01 vs. Non-responders: 0.80 ± 0.009 pmol/ml, $F = 0.216$, $P =$ NS), or in norepinephrine levels (Controls:

Table 3
Neutrophil β-adrenoceptor binding parameters in patients with major depressive disorder
Values are mean ± S.E.M.

	Treatment responders ($n = 9$)		Treatment non-responders ($n = 7$)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
K_d (pmol)	25.56 ± 2.79	22.01 ± 4.00	34.40 ± 10.05	24.88 ± 2.25
B_{max} (fmol/mg protein)	65.85 ± 9.49[a]	45.58 ± 11.05	71.46 ± 28.05	44.54 ± 7.12
R_H (fmol/mg protein)	57.21 ± 7.09[bc]	40.76 ± 9.35	34.94 ± 5.80	33.06 ± 6.16
R_L (fmol/mg protein)	14.61 ± 2.77	15.11 ± 3.46	11.55 ± 1.63	13.13 ± 2.86
R_T (fmol/mg protein)	71.82 ± 9.65[de]	55.87 ± 12.60	46.49 ± 7.23	46.19 ± 8.48
$\%R_H$	80.67 ± 1.58[fg]	73.20 ± 2.87	74.10 ± 2.05	71.91 ± 3.36
K_H (nM)	38.95 ± 22.59	20.46 ± 7.57	16.48 ± 4.67	17.89 ± 4.43
K_L (μM)	9.26 ± 4.46[h]	5.01 ± 3.26	1.22 ± 0.25[i]	2.28 ± 0.79
K_L/K_H	267.11 ± 71.04[j]	142.25 ± 40.47	152.18 ± 74.10	130.90 ± 38.96

[a] $P \leq 0.007$, paired one-tailed, t-tests, $t = 3.132$, vs. treatment responders, post-treatment.
[b] $P \leq 0.03$, two-tailed independent t-tests, $t = 2.330$, vs. treatment non-responders, pre-treatment.
[c] $P \leq 0.02$, paired one-tailed, t-tests, $t = 2.547$, vs. treatment responders, post-treatment.
[d] $P \leq 0.06$, two-tailed independent t-tests, $t = 1.994$, vs. treatment non-responders, pre-treatment.
[e] $P \leq 0.03$, paired one-tailed, t-tests, $t = 2.138$, vs. treatment responders, post-treatment.
[f] $P \leq 0.02$, two-tailed independent t-tests, $t = 2.584$, vs. treatment non-responders, pre-treatment.
[g] $P \leq 0.03$, paired one-tailed, t-tests, $t = 2.226$, vs. treatment responders, post-treatment.
[h] $P \leq 0.03$, paired one-tailed, t-tests, $t = 2.172$, vs. treatment responders, post-treatment.
[i] $P \leq 0.10$, paired one-tailed, t-tests, $t = -1.430$, vs. treatment non-responders, post-treatment.
[j] $P \leq 0.06$, paired one-tailed, t-tests, $t = 1.692$, vs. treatment responders, post-treatment.

0.85 ± 0.05 vs. Responders: 0.84 ± 0.10 vs. Non-responders: 0.77 ± 0.13 pmol/ml, $F = 0.206$, $P =$ NS).

3.4. Pre- to post-treatment changes in β_2-adrenoceptor function based on treatment outcome

Imipramine induced β_2-adrenoceptor uncoupling in treatment responders as reflected in the significant decrease in both $\%R_H$ and in the K_L/K_H ratio. Imipramine significantly decreased β_2-adrenoceptor total density in treatment responders. This decrease was primarily due to significant decrease in R_H as there was no change in R_L. By contrast, imipramine had no effect on any of β_2-adrenoceptor binding parameters in treatment non-responders (Table 3).

This differential effect of imipramine on β_2-adrenoceptors between treatment responders and treatment non-responders occurred despite the fact that the increase in basal supine norepinephrine levels after imipramine treatment was equally observed in responders and non-responders (Responders: 1.55 ± 0.155 vs. Non-responders: 1.214 ± 0.168 pmol/ml, $t = 1.448$, $P =$ NS). Imipramine had no effect on supine plasma epinephrine levels either in treatment responders or treatment non-responders.

3.5. Results of the regression analysis ($n = 16$) examining the relationship between pre-treatment β_2-adrenoceptor binding parameters and severity of symptoms after treatment revealed the following

Pre-treatment R_H correlated negatively with post-treatment HAM-A ($r = -0.662$, $P = 0.005$), HAM-D ($r = -0.672$, $P = 0.004$), Sp-S ($r = -0.476$, $P = 0.06$), Zung-C ($r = -0.549$, $P = 0.03$), and Zung-S ($r = -0.606$, $P = 0.01$). Pre-treatment R_L was negatively correlated with post-treatment HAM-A ($r = -0.535$, $P = 0.03$), HAM-D ($r = -0.534$, $P = 0.03$), Sp-S ($r = -0.677$, $P = 0.004$), Zung-S ($r = -0.802$, $P = 0.00$), and VAS of depression ($r = -0.570$, $P = 0.02$), anxiety ($r = -0.508$, $P = 0.04$). Pre-treatment R_T correlated negatively with post-treatment HAM-A ($r = -0.650$, $P = 0.006$), HAM-D ($r = -0.658$, $P = 0.006$), Sp-S ($r = -0.541$, $P = 0.03$), Zung-C ($r = -0.522$, $P = 0.04$), Zung-S ($r = -0.673$, $P = 0.004$).

Pre-treatment K_H correlated positively with post-treatment VAS of irritability ($r = 0.657$, $P = 0.006$), dry mouth ($r = 0.443$, $P = 0.08$), shakiness ($r = 0.757$, $P = 0.001$), rapid heart ($r = 0.889$, $P = 0.00$), palpitations ($r = 0.598$, $P = 0.01$), difficulty breathing ($r = 0.860$, $P = 0.00$), rapid breathing ($r = 0.811$, $P = 0.00$), dizziness ($r = 0.774$, P

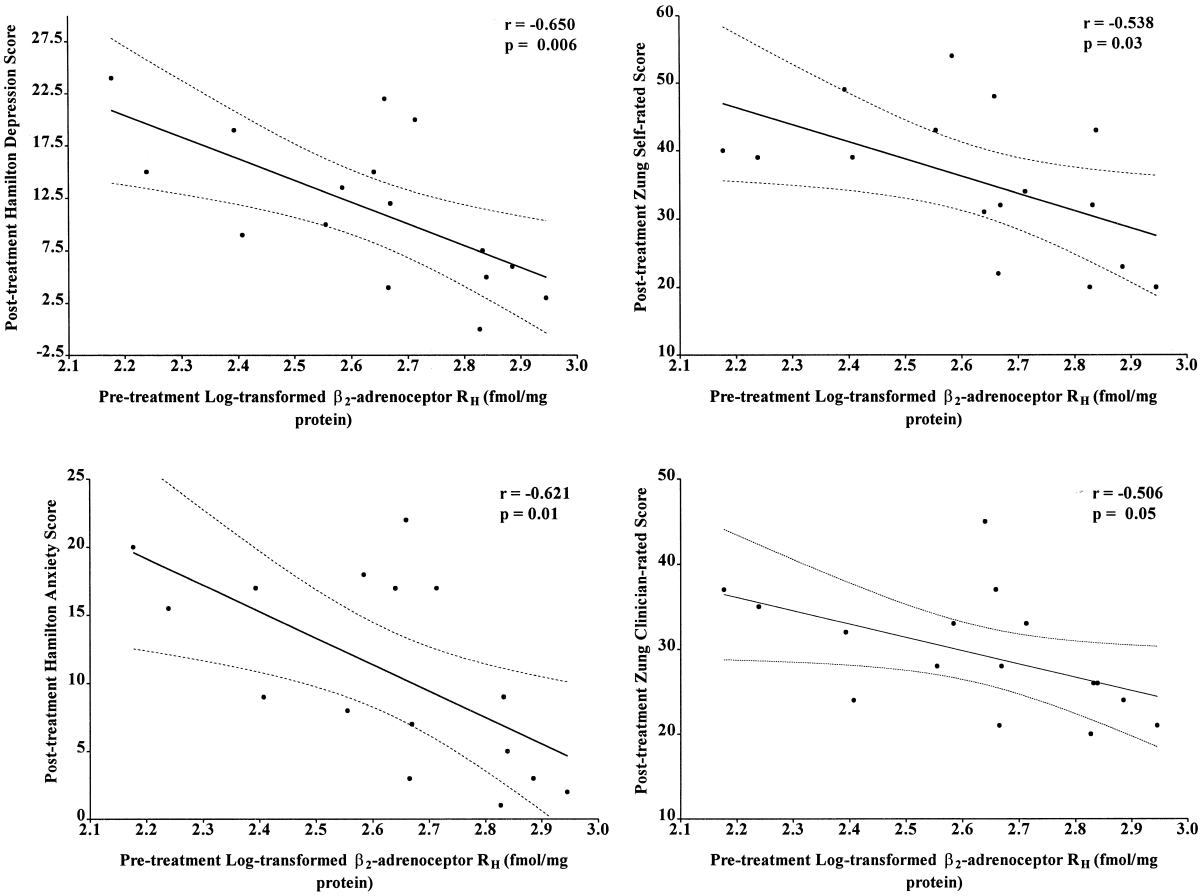

Fig. 1. The relationship between β-adrenoceptor density in the high-conformational state prior to treatment and severity of anxiety and depression after treatment of patients with major depressive disorder. Higher pre-treatment density predicted lower severity of anxiety and depression as a treatment outcome.

$= 0.00$), weakness in the muscles ($r = 0.516$, $P = 0.04$), sweating ($r = 0.752$, $P = 0.001$), tightness in the chest ($r = 0.921$, $P = 0.00$), urinary urgency ($r = 0.725$, $P = 0.001$), chest pain ($r = 0.874$, $P = 0.00$), hot/cold flashes ($r = 0.654$, $P = 0.006$), and nausea ($r = 0.472$, $P = 0.06$).

Thus, the pattern that emerges from these correlations shows that the higher the receptor density measure before treatment, the lower the severity of anxiety and depression after treatment. Furthermore, the higher the agonist dissociation constant from the receptor in the high-conformational states prior to treatment, the higher the severity of autonomic anxiety symptoms after treatment (Fig. 1).

4. Discussion

This is the first study to investigate β_2-adrenoceptor coupling to G_s protein in major depressive disorder, specifically, in relation to treatment response. Results of the present investigation showed differences in β_2-adrenoceptor regulation exist among major depressive disorder patients as a function of treatment response. While β_2-adrenoceptor density and coupling appeared normal in major depressive disorder patients as a whole, abnormal β_2-adrenoceptor coupling and density existed only in imipramine treatment-responsive patients. Upregulation of β_2-adrenoceptor density was primarily observed in the high-conformational states. Supercoupling was reflected in high $\%R_H$ and in a similar trend for the K_L/K_H ratio. β_2-adrenoceptor density and coupling were normal in imipramine non-responsive patients. Clinical response to imipramine treatment was observed only in patients with evidence for β_2-adrenoceptor dysregulation, but this therapeutic effect was not observed in patients with seemingly normal β_2-adrenoceptor function. This supports our hypothesis that congruence between the mechanism of action of antidepressants and the pathophysiologic state may be required for a positive treatment outcome. Differences in β_2-adrenoceptor function between responders and non-responders suggest heterogeneity in the pathophysiology of depression and indicate that this heterogeneity may underlie treatment response.

In this investigation, imipramine induced downregulation of β_2-adrenoceptor density in the high-conformational state and uncoupling of β_2-adrenoceptors from G_s protein. These results are consistent with basic neuroscience studies in rat brain and cell cultures reporting downregulation and uncoupling of β-adrenoceptors by tricyclic antidepressants (Manier et al., 1989; Gurguis et al., 1998). Although these mechanisms have long been reported in basic science studies, it remained unclear whether these effects are mere pharmacological effects or are specifically related to their therapeutic efficacy. The fact that the effects of imipramine on β_2-adrenoceptors were only observed in treatment responders whereas no similar effects were observed in treatment non-responders provides, for the first time, evidence for the association between these specific mechanisms and therapeutic or clinical efficacy. Results of the regression analysis further confirmed the predictive capacity of β_2-adrenoceptor density in the high-conformational states such that high pre-treatment β_2-adrenoceptor density predicted low anxiety and depression ratings at the end of treatment.

It is intriguing why imipramine did not exert any pharmacological effects on any β_2-adrenoceptor binding parameters in treatment non-responders since tricyclic antidepressants decrease β-adrenoceptor density and coupling under non-pathological conditions in rat or mouse brain or in cell cultures. An interaction, therefore, between pathophysiologic processes and drug action can not be ruled out and may underlie the absence of imipramine's effects in non-responders. Protein kinase C has been shown to regulate β-adrenoceptor membrane expression. Inhibition of protein kinase C by 1-(5-isoquinolinylsulfonyl)-2-methylpiprazine (H-7) attenuated both isoproterenol-induced and desipramine-induced β-adrenoceptor downregulation (Asakura et al., 1989; Hui and Yu, 1989). These observations were replicated by others who also reported that treatment of C6 cells with H-7 alone induced upregulation of β-adrenoceptors (Manji et al., 1991). Abnormal protein kinase C activity may, therefore, exist in treatment non-responders and may have interfered with effects of imipramine. In this context, serotonin specific reuptake inhibitors, tricyclic antidepressants and electroconvulsive therapy have been shown to differentially modulate protein kinase C activity (Mann et al., 1995; Vetulani and Nalepa, 1996). Also, unlike tricyclic antidepressants, downregulation of β-adrenoceptor density by serotonin specific reuptake inhibitors have not been consistently shown (Garcha et al., 1985; Nelson et al., 1990, 1991; Nalepa and Vetulani, 1993; Goodnough and Baker, 1994; Pälvimäki et al., 1994; Sapena et al., 1994; Koe and Lebel, 1995). It is therefore likely that tricyclic antidepressant-non-responsive patients may be responsive to serotonin specific reuptake inhibitors. It would be of interest to investigate characteristics of β_2-adrenoceptor function in patients responsive to serotonin specific reuptake inhibitors and the effects of these agents on β-adrenoceptor coupling and density using a similar paradigm. These differences in mechanisms of action may give credence to the clinical practice of switching treatment non-responsive patients to an antidepressant that belongs to a group of antidepressants with differing mechanisms of action, i.e., serotonin reuptake inhibitors vs. norepinephrine reuptake inhibitors.

Both (1) high β_2-adrenoceptor density and supercoupling in treatment responders despite normal concurrently measured plasma epinephrine and norepinephrine levels, and (2) failure of β_2-adrenoceptor density to downregulate in treatment non-responders despite increase in plasma norepinephrine levels after imipramine treatment, provide evidence for abnormal agonist-mediated gene expression of β-adrenoceptors in major depressive disorder (Hadcock

and Malbon, 1993). The β-adrenoceptor kinase (βARK) phosphorylates the receptor at the serine–threonine-rich carboxyl terminus of the β-adrenoceptor molecule and mediates its uncoupling from G protein (Hausdorff et al., 1990). In addition, β-adrenoceptors undergo a biphasic response to excessive agonist stimulation, whereby, increased agonist stimulation results in an initial increase in expression of membrane receptors, followed by downregulation of membrane receptors. Protein kinase A plays an obligate role in this process. In this study, pretreatment super coupling of β_2-adrenoceptors, in the presence of normal epinephrine or norepinephrine levels indirectly suggests decreased βARK activity. The results also implicate abnormal protein kinase A in major depression. Recently, Shelton et al. (1996) reported decreased β-adrenoceptor-linked protein kinase A in small cohort of depressed patients, 30% of whom were on Sertraline. Therefore, the exact nature of protein kinase abnormality remains unclear.

Alternatively, hypercortisolemia which has been reported in a subgroup of depressed patients may have contributed to the high β_2-adrenoceptor density and coupling observed in this study (Carroll et al., 1976a,b; Kathol et al., 1989). Glucocorticoids have been shown to (1) increase β-adrenoceptor membrane expression and mRNA through the glucocorticoid response element located on the 5′ flank of the β-adrenoceptor gene promoter (Hadcock and Malbon 1988; Malbon and Hadcock, 1988), (2) to increase β-adrenoceptor coupling to G_s protein (Chang and Bourne, 1987), and (3) to increase G protein levels (Saito et al., 1989).

Imipramine raised plasma norepinephrine levels and induced downregulation of β_2-adrenoceptor density and uncoupling from G_s in treatment responders. While the downregulation and uncoupling of β_2-adrenoceptors in treatment responders can be most parsimoniously attributed to the increase in plasma norepinephrine levels, other mechanisms can not be ruled out. Similar effects were observed in cell cultures in absence of agonists. One mechanism involves the enhancement by imipramine of protein kinase A activity (Perez et al., 1991, 1992; Popoli et al., 1995; Mori et al., 1998; Tadokoro et al., 1998). Indeed, both mechanisms could have contributed to the results. It has been shown that agonist- and tricyclic antidepressant-mediated downregulatory effects on β-adrenoceptors were additive, suggesting that different mechanisms may be involved, despite some overlap (U'Prichard and Enna, 1979; O'Donnell and Frazer, 1985; Brunello et al., 1990; Zini et al., 1991). Finally, it is likely that β-adrenoceptor downregulation by imipramine was mediated through reducing the phosphorylation of the cyclase response element-binding protein and consequently the transcriptional activity of the cyclase response element (Schwaninger et al., 1995).

To the extent, we did not observe differences in β_2-adrenoceptor density in depressed patients as a whole, or in treatment non-responders, the results of this investigation are consistent with previous studies of normal β_2-adrenoceptor density in major depressive disorder (Ebstein et al., 1988; Mann et al., 1990; Mazzola et al., 1991; Werstiuk et al., 1996), and with similar findings in brain β-adrenoceptors from suicide victims and depressed subjects (Meyerson et al., 1982; Stockmeir and Meltzer, 1991; Gurguis et al., 1999b). Our results, however, are not consistent with studies which reported downregulation of β_2-adrenoceptor density in major depressive disorder (Extein et al., 1979; Wood et al., 1986; Carstens et al., 1987; Pandey et al., 1990; Jeanningros et al., 1991). Methodological differences such as gender, diagnostic criteria, treatment responsiveness and receptor binding methods may account for the discrepancy. It is worth noting that in our study we used pure membrane preparations and a long resting period that allowed for plasma catecholamines to reach basal kinetics prior to blood sampling to rule out possible confounding effects of transient changes in plasma catecholamine levels on β_2-adrenoceptor binding parameters. Finally, although all our subjects were males, gender does not have an effect on β_2-adrenoceptor function (Halper et al., 1984). Methodologically, the present results lend validity to the use of neutrophil membrane β_2-adrenoceptors as peripheral markers in depression and as a model to investigate the effects of various antidepressants on β_2-adrenoceptors.

In summary, results of the present investigation revealed differences in β_2-adrenoceptor regulation between depressed patients who are tricyclic antidepressant-responders and those who are non-responders. The results provide evidence for heterogeneity in the pathophysiology of depression that may underlie treatment response. The results also indirectly implicate abnormal protein kinase A and protein kinase C in major depression and should be pursued in future investigations.

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