Published in final edited form as:

J Psychiatr Res. 2008 January ; 42(1): 41–49.

PLATELET IMIDAZOLINE RECEPTORS AS STATE MARKER OF DEPRESSIVE SYMPTOMATOLOGY

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Abstract

Objective—Previous studies have shown that imidazoline receptors (IR-1) are increased in platelets and frontal cortex of depressed patients, and this up-regulation is normalized (down-regulated) after antidepressant drug treatments. It has been hypothesized that IR-1 up-regulation during the depressive episode may be a state marker for depressive symptomatology. The goal of the present study was to address the state versus trait question.

Method—Twelve healthy subjects (6 males and 6 females) met stringent inclusion and exclusion criteria for physical and mental health. They received desipramine for six weeks in order to simulate the length of time and dosing used previously to obtain an IR-1 down-regulation and a therapeutic response in depressed patients. Outcome and safety measures included clinical, psychological, and cardiovascular assessments obtained throughout the study. Plasma concentrations of desipramine were measured throughout the six weeks of treatment and again after 2 weeks following tapered discontinuation of desipramine. Platelet receptors were assessed by Western blotting and radioligand binding assays.

Results—Healthy subjects taking desipramine experienced mild dysphoric effects but there were no adverse events. The binding of 8 nM p-[¹²⁵I] clonidine to IR-1 and α_2 -adrenoceptors in healthy subjects did not change during desipramine treatment. The immunodensity of the 33 kD band associated with IR-1 gradually increased to a maximum, by week-6, of 26% higher than baseline ($p < 0.01$ compared to baseline). Two weeks after desipramine discontinuation, there was a decline in α_2 -adrenoceptor binding and 33 kD band's immunodensity ($p = 0.04$).

Conclusions—The findings support the hypothesis that platelet IR-1 binding sites are a marker of mood state rather than of antidepressant-induced pharmacological regulation. By comparison, platelet α_2 -adrenoceptors appear to be regulated by desipramine as a pharmacological effect independent of mood state.

Keywords

Imidazoline receptors; α_2 adrenoceptors; Depression; Healthy subjects; Desipramine; State Marker

1. Introduction

Imidazoline receptors (IR) were first identified in the brainstem where their activation by clonidine lowers sympathetic outflow (Bousquet, 1997), blood pressure (Chan, Burke, Zhu, Piletz, & Head, 2005; Hamilton, 1992), and intraocular pressure (Campbell & Potter, 1994). Although clonidine and structurally related compounds have high affinity for α_2 -adrenoceptors, these imidazoline compounds also have high affinity for the IR-1 subtype of IR (Piletz, Zhu, & Chikkala, 1996c). Several endogenous compounds have been proposed as putative neurotransmitters for IR: agmatine, harmaline, and imidazole-ribotide (Halaris & Piletz, 2003; Musgrave & Badoer, 2000; Prell et al., 2004). None of the classical monoamines have substantial affinity for IR (Bousquet, 1997). Imidazoline receptors have been linked to neuroplasticity (Piletz, Wang, & Zhu, 2003b), have been postulated to be a possible state marker for depression (Halaris et al., 2003), and may be a target for anxiolytic drug action (Zhu, Paul, Sec, Peeler, & Piletz, 2003b).

At least two pharmacological subtypes of imidazoline-selective binding sites are known (Ernsberger et al., 1995a). The first is the IR-1 binding site which has preferential affinity for clonidine and structurally-related imidazoline compounds, is encoded by a non-G-protein coupled protein called IRAS (Piletz et al., 2003b; Piletz et al., 2000a; Wu et al., 2005), and is associated with plasma membranes (Ernsberger et al., 1995a). The second is the IR-2 binding site which has preference for idazoxan-like imidazoline compounds, is encoded by monoamine oxidases, and is located mostly on the outer membrane of mitochondria (Nutt et al., 1995).

Previous studies have shown that IR-1 are increased in frontal cortex and platelets of depressed patients (Garcia-Sevilla et al., 1996; Piletz et al., 2003a; Piletz, Halaris, Nelson, Qu, & Bari, 1996a; Piletz, Halaris, Saran, & Marler, 1991; Piletz, Halaris, Chikkala, & Qu, 1996b; Piletz, Schubert, & Halaris, 1986), and this up-regulation is normalized (down-regulated) after antidepressant drug treatments (Garcia-Sevilla et al., 1996; Piletz et al., 1991; Piletz et al., 1996b; Zhu et al., 1999; Zhu, Halaris, & Piletz, 1997). In two western blotting studies, the immunoreactivity of IRAS in 33 kD and 45 kD bands (peptides of IRAS (Zhu, Hayes, Chen, Baldwin, & Piletz, 2003a) was increased in platelets and prefrontal cortical membranes (Garcia-Sevilla et al., 1996) but decreased in hippocampal homogenates (Piletz et al., 2000b) from depressed patients relative to matched controls. In another study of postmortem brain tissue, the binding ratio of p-[¹²⁵I]clonidine to IR-1 over α_2 -adrenoceptors was markedly increased in all orbital frontal cortical layers from depressed patients compared to the same layers from age/sex-matched control subjects with no history of depression (Piletz et al., 2003a). Thus, the density of platelet and brain IR-1 appears to be altered in depression.

In the present study we have addressed the state versus trait question of platelet IR-1 up-regulation in depression, and of antidepressant-induced changes in platelet IR-1 and α_2 -adrenoceptors. Desipramine, a norepinephrine (NE) reuptake inhibitor type antidepressant, was administered for six weeks to 12 healthy human subjects in order to simulate the length of time and dosing used to obtain a therapeutic response in depressed patients (Piletz et al., 1996b). Plasma concentrations of desipramine were measured throughout the six weeks of treatment and again after 2 weeks following discontinuation of treatment. Detailed clinical, psychological, and cardiovascular assessments were obtained throughout the study. The obtained results are consistent with platelet IR-1 being a mood-state marker for depression, different than a pharmacological state marker for platelet α_2 -adrenoceptors. The present findings clarify previous reports that showed antidepressant treatment effects on these platelet receptors in depressed patients.

2. Method

2.1 Subjects

Twelve healthy paid volunteers were recruited by advertising. All gave written informed consent using a form approved by the University's Institutional Review Board (IRB). Inclusion criteria specified the subjects should have blood pressures between 100–140 mm Hg systolic and 60–90 mm Hg diastolic, with additional findings in the normal range on the following: medical and psychiatric history examination, structured clinical interview for mood disorders (SCID-Major Depression), complete blood count, chemistry panel, thyroid panel, urinalysis, electrocardiogram, and urine screen for psychotropic compounds and drugs of abuse. Subjects were excluded if they had a lifetime history of any psychiatric disorder or drug dependence; drug or alcohol abuse within the preceding year; family history of depression or psychosis; indeterminate family psychiatric history; current significant abnormality on physical, mental status, or laboratory examination; current medication use; bleeding diatheses; or, for females, a positive pregnancy test, admission of sexual activity without contraception, or evidence of late luteal phase dysphoria. The study design called for six males and six females, which was met. Eight subjects were self-identified as Caucasians, three African-Americans, and one Asian. The mean age of the subjects was 29.2 ± 4.7 (SD) years.

2.2 Clinical Procedures

Each subject was assessed once at baseline (just prior to treatment), repeatedly during six weeks of desipramine treatment, and after two weeks following tapered discontinuation (over seven days) of desipramine. Desipramine was dispensed in weekly doses, starting immediately after the baseline platelet collection. The starting dose was 50 mg/day and was adjusted as high as 200 mg/day based on plasma desipramine concentrations. Plasma desipramine concentrations were obtained at 1, 2, 4, 6, and 8 weeks of the study. We aimed to achieve a maintenance plasma concentration (between weeks 4–6) of 150 ng/ml plasma, unless significant side effects precluded such a level. To obtain accurate desipramine concentrations, subjects were instructed to take their prescribed desipramine pill in a single nighttime dose between 21:00 and 23:00 hours the night before blood drawing days. On blood drawing days, they adhered to restrictions of food intake, coffee, and exercise, and reported to the clinic between 9:00 and 10:00 hours. No other medications, such as over-the-counter analgesics, were allowed from 2 days prior to the blood drawings and a record of such over-the-counter medication use was obtained. Clinical questionnaires and rating scales were administered at 1–2 week intervals. These included a semi-structured assessment of adverse medication effects, vital signs, and the self-rating Beck Depression Inventory. Ratings at baseline and endpoints (weeks 6 and 8) also included the Hamilton Depression Rating Scale (HAM-D, 21 item), the COVI anxiety scale, and a Premenstrual Dysphoric Disorder checklist for women derived from research criteria in the DSM-IV “further study” section (American Psychiatric Association).

Phlebotomy was performed in the morning after an overnight fast. A 19 gauge intravenous catheter was inserted in an antecubital vein and a heparin lock applied to maintain vein patency. Then, before drawing blood, the subject rested in a semi-supine position for 30 minutes. The first 5 ml of blood was placed on ice for later analysis of desipramine blood concentrations. Platelet samples were obtained from an additional 30 ml of blood using an OptiPrep one-step gradient procedure at room temperature (Accurate Chemical & Scientific Co., Westbury, NY) (Bagamery, Kvell, Landau, & Graham, 2005). Platelets were stored at -80° until use.

2.3 Platelet Assays

Preparation of samples for western blotting began with one-third of the platelets (from 10 ml blood) obtained from the one-step OptiPrep gradient (Bagamery et al., 2005). Each thawed sample was homogenized in 50 mM Tris-HCL, pH 7.5, with 1 mM Pefabloc protease inhibitor

(Sigma Chem Co.) at 9,000 rpm for 1 min (30 sec twice) on ice using a tissuemizer. This was centrifuged at $20,000 \times g$ for 10 min at 4°C and the pellet resuspended in 200 μl of 40 mM Tris-HCL, pH 6.8, 4% SDS with incubation at 75°C for 5 min to dissolve the membranes. According to each sample's protein concentration, which was subsequently determined in an aliquot by the Lowry method, dilutions were made to 1 $\mu\text{g}/\mu\text{l}$ and then mixed with an equal volume of Laemmli sample buffer with Cleland's reagent and tracking dye (Biorad Co.). Each mixture was denatured and exactly 7.5 μg protein (15 μl) loaded twice into different lanes of two slab electrophoresis gels (i.e., samples were repeated on different western blots). The unknowns in different lanes of a single slab gel were from the same subject obtained at 0, 1, 2, 4, and 6 weeks on desipramine, and after a two week discontinuation period thereafter (week-8). In addition, each slab gel included 5 lanes for a 5-point standard curve (2.5 – 12.5 μg protein) of platelet membranes from a Red Cross volunteer source, plus two lanes of molecular weight markers (Biorad Co.). The western blots were probed with Imidazoline Receptor Binding Protein (IRBP) antiserum (Wang, Regunathan, Ruggiero, & Reis, 1993). This procedure enhanced chemilluminescence to develop film (ECL type; Amersham), followed by densitometry of the major immunoreactive band at 33 kD size (Zhu et al., 1999).

For radioligand binding assays, the platelet preparation began with two-thirds of the thawed platelets (from 20 ml blood) from the OptiPrep one-step source (Bagamery et al., 2005). These were suspended in 3.0 ml ice-cold buffer containing 1 mM Pefabloc (Sigma) and homogenized at 9,000 rpm for 1 min (30 sec twice) with a tissue-mizer. The sample was then centrifuged at $100,000 \times g$ for 60 min at 4°C , washed in 6.0 ml of 5.0 mM Hepes, 0.5 mM MgCl_2 , 0.5 mM EGTA, pH 7.4 (HME) buffer with 1 mM Pefabloc, centrifuged again at $100,000 \times g$ for 60 min, followed by resuspension in 0.5 ml HME buffer (Ernsberger, Piletz, Graff, & Graves, 1995b; Piletz et al., 1996b; Piletz & Sletten, 1993). Using this preparation, the radioligand binding assays were done in batches on different days. Each batch consisted of at least: (a) membranes from a single subject obtained at baseline (week-0), after six weeks on desipramine (week-6), and after discontinuation (week-8), and (b) platelet membranes from two "depression controls" taken at baseline and 6 weeks on desipramine (the "depression controls" are described in more detail below). Protein concentrations varied slightly between subjects from 0.15 mg/ml to 0.21 mg/ml, within the range previously established for linearity of specific radioligand binding (Piletz et al., 1993).

Radioligand binding was performed according to a previously described glass fiber filtration procedure (Ernsberger et al., 1995b; Piletz et al., 1996b; Piletz et al., 1993). Binding assays were performed at a single concentration of 8 nM p-[^{125}I]clonidine (New England Nuclear). This choice was made due to the fact that the volume of blood required for multi-time-point/7-point radioligand saturations at two binding sites (α_2 -adrenoceptors and IR-1), plus the western blots, was deemed excessive by the IRB. To mitigate the use of one-point assays, we carefully selected the radioligand concentration (8 nM) and added precision with six tubes for total binding and six tubes for non-specific binding (i.e., for α_2 -adrenoceptors the total binding had no additions and nonspecific binding had 10 μM NE added). According to a previous characterization study (Piletz et al., 1993), 8 nM p-[^{125}I]clonidine should be 8x greater than the K_D concentration for platelet α_2 -adrenoceptors, and therefore this should approximate the B_{max} . Similarly, the binding assay of IR-1 sites was performed at 8 nM p-[^{125}I]clonidine. In this case there were the same 6 total binding tubes containing 10 μM NE (revealing total non-adrenergic binding) and 6 non-specific binding tubes containing additional 10 μM moxonidine (an imidazoline compound to displace IR-1 binding), which is the NE-masking technique (Ernsberger et al, 1995b) According to our previous characterization study (Piletz et al., 1993), the specific binding of 8 nM p-[^{125}I]clonidine in the presence of 10 μM NE mask should approximate the K_D level for the platelet IR-1 site; and therefore this should be half the B_{max} .

2.4 Depressed Patients Were Positive Controls

Because the present study utilized only one radioligand concentration (8 nM), it was necessary to ensure that the assay was still capable of detecting actual down-regulation. To ensure this, six pre/post-treatment pairs of platelet samples were retrieved from which Bmax values were already known *from a previous study* (Piletz et al., 1996b). These six samples – herein called “depression controls” – were taken from the frozen (−80°C) supply of a previous study (Piletz et al., 1996b) which had shown platelet IR-1 down-regulation after desipramine treatment (Piletz et al., 1996b). The six patients had met Research Diagnostic Criteria for Major Depressive Disorder, unipolar subtype (Piletz et al., 1996b), and by six weeks of desipramine treatment they had all responded with a significant reduction in HAM-D scores (Piletz et al., 1996b). These samples had remained frozen for 5–6 years. No attempt was made to match a “depression control” sample with a current control sample in any specific regard (i.e., age or sex). The six “depression control” samples were analyzed along with the present samples according to the one-point binding assay.

2.5 Statistical Analyses

For comparisons over time, repeated measures analysis of variance (R-ANOVA) was performed followed by Dunnett’s multiple comparison test (comparing to time 0). In some cases, a two-tailed paired t test was also performed to compare between two groups. Values represent mean $\pm$ standard errors (SEM) unless specified.

3. Results

3.1 Desipramine Concentrations

The average dose of desipramine was 145.6 mg/day (range = 50–200 mg daily). Over weeks 2–6 of the study, this dose corresponded to an average plasma concentration of 186 ng/ml (range 88–424 ng/ml). One subject failed to reach a plasma desipramine concentration of 130 ng/ml during the 6-week portion of the study. During the second week on desipramine, this subject developed postural tachycardia that persisted and prevented a dose increase beyond 75 mg/day (Table 1). Another subject may have been a slow metabolizer, since plasma concentrations of 329–424 ng/ml were observed at weeks 2 and 4 on a desipramine dose of 100 mg/day; the maintenance dosage for this slow metabolizer was therefore adjusted to 75 mg/day. This subject reported adverse events such as dry mouth, constipation and blurred vision. These side effects were not unusual, since the same types of side effects were reported by most subjects (Table 2). No differences were observed between males and females in plasma desipramine concentrations.

3.2 Blood Pressure and Heart Rate

Table 1 summarizes the blood pressure and heart rate values during treatment. Desipramine did not significantly change systolic or diastolic blood pressure. This was true whether considering supine or standing blood pressures (Table 1). An attempt was also made to statistically combine the mean blood pressure values of week-2 and week-6 (giving a “total-effect mean” post-desipramine cardiovascular measure) for comparison to baseline, but there was still no significant change in blood pressure after treatment. On the other hand, heart rate increased on desipramine ($p = 0.01$ for supine position and $p = 0.0001$ for standing position; Table 1). When orthostatic changes were compared to baseline, the effect of desipramine was also significant in systolic blood pressure ($p = 0.04$) and heart rate ($p = 0.03$) (Table 1). Although the extent of these changes was small, it indicated that desipramine had produced physiological effects.

3.3 Adverse Effects

Table 2 summarizes adverse events in response to a semi-structured questionnaire. None of the subjects requested to be discontinued from the study. However, all subjects complained of dry mouth, which persisted throughout treatment for 9 of the 12 subjects. Eleven subjects also complained of dizziness, usually within two weeks of drug initiation. Dizziness abated within subsequent weeks for 10 of the 11 subjects. Complaints of poor appetite did not correspond with measured weight loss. Ten of the 12 subjects had weight decline during the study. Most weight loss occurred by week-4 (mean = 3.42 ± 1.12 kg; range from -10.0 to $+2.0$ kg). By week-8 the average loss in weight from baseline was 3.95 ± 1.50 kg (range: -10.0 to $+3.5$ kg). Five of the 12 subjects had a sustained decrease of more than 3% of baseline body weight. These events required little treatment, except two laxative prescriptions for constipation, and several subjects used analgesics (self-administered on occasion over the counter) for headaches. These effects were anticipated based on the known muscarinic and/or $\alpha 1$ adrenergic blocking properties of desipramine. No differences in these somatic effects were observed between males and females.

3.4 Psychiatric Rating Scales

On the self-rating Beck Depression Inventory, the total score was zero for all subjects at baseline and all subsequent ratings. COVI anxiety scale scores were also zero at baseline and endpoint. However, the physician-rated Hamilton Depression Scale worsened from an average score of $1.0 (\pm 1.28 \text{ SD})$ at baseline to $2.8 (\pm 2.0 \text{ SD})$ after six weeks on desipramine ($p = 0.02$, two tailed t test). The increase in HAM-D score reflected mild to moderate sleep disturbances, decreased appetite, transient physical discomforts, and dysphoric mood changes (Table 2). The maximal HAM-D scores were 4.0 at baseline and 7.0 after six weeks on desipramine. None of the subjects developed a disturbance meeting criteria for clinical disorder of mood. There was no difference in HAM-D scores between genders.

3.5 Western Blots

The 33 kD band was analyzed on western blots from platelet total membranes of healthy subjects at pretreatment, after 1, 2, 4, and 6 weeks on desipramine, and after a two-week discontinuation period (until week-8). As shown in Fig. 1, the immunodensity of the 33 kD band gradually increased to a maximum, by week-6, of 26% higher than baseline ($p < 0.01$ by R-ANOVA and Dunnet's multiple comparison test). Over weeks 2–4, this rise was marginally significant ($p = 0.06$ at week-2 and $p = 0.03$ at week-4 by two-tailed t test) compared to baseline at week-0. Two weeks after treatment discontinuation, the 33 kD band's immunodensity had returned to baseline (Fig. 1). Over weeks 2–6, no statistical significance emerged when attempting to correlate the 33 kD band (or its percent rise scores) with somatic or mood changes. For instance, no correlation was noted with reduction in body-mass index ($r^2 = 0.14$, $p = 0.24$) or the slight rise in HAM-D scores ($r^2 = 0.001$, $p = 0.91$) after desipramine. There were no apparent differences between the 6 males and 6 females.

3.6 Radioligand Binding

Results of p-[^{125}I] clonidine binding to platelet IR-1 and α_2 -adrenoceptors are shown in Fig. 2. The "depression controls" were 6 patients who in our previous study had shown significant differences in pre- versus post-treatment Bmax values for p-[^{125}I]clonidine binding to platelet IR-1 and α_2 -adrenoceptors (Piletz et al., 1996b). After re-analysis, the levels of 8nM p-[^{125}I] clonidine binding to platelet IR-1 and α_2 -adrenoceptors were virtually identical to those previously found (Piletz et al., 1996b) - and hence no loss of binding due to 5–6 years of freezer storage (-80°C) was noted. This finding from our earlier "depression controls" thus demonstrated that the single-point (8 nM) radioligand binding assay is capable of detecting down-regulation of IR-1 sites (Fig. 2A). That is, the "depression controls" showed down-

regulation of platelet IR-1 binding sites between 0 – 6 weeks on desipramine ($p=0.007$ by two-tailed t test; Fig. 2A). An up-regulation of α_2 -adrenoceptors was also detected comparing post-desipramine to pre-desipramine in the depressed patients (Fig. 2C) (Piletz et al., 1996b). The extent of up-regulation of platelet α_2 -adrenoceptors between 0 – 6 weeks on desipramine was borderline significant ($p = 0.05$; Fig. 2C).

In contrast to depressed patients, the binding of 8 nM p-[125 I] clonidine to IR-1 and α_2 -adrenoceptors in healthy subjects was not different after desipramine treatment (Fig 2B and Fig. 2D). There was a trend for increased α_2 -adrenoceptor binding after 6 weeks of desipramine (Fig. 2D), similar to that seen with the “depression controls” (Fig. 2C), but it did not reach statistical significance (Fig. 2D, $p = 0.22$ by two-tailed t test). A decrease in platelet α_2 -adrenoceptor binding was also observed two weeks after discontinuation of desipramine (Fig. 2D: $p=0.04$ by two-tailed t test), while the discontinuation of desipramine had no effect on platelet IR-1 binding sites (Fig. 2B, $p > 0.8$ by two-tailed t test). Thus, in healthy subjects, platelet α_2 -adrenoceptors and IR-1 were not regulated by desipramine in the same manner as seen in depressed patients (compare Fig. 2 A,C with Fig. 2 C,D). The results were similar in males and females.

4. Discussion

Human platelets have been of special interest to psychiatric research because they exhibit biochemical properties similar to those of monoaminergic, notably serotonergic, nerve endings. These enucleated cells possess serotonin reuptake sites – a major target for antidepressant drugs – and the release of serotonin from platelets can be inhibited by α_2 -adrenoceptor agonists (Shams et al., 1991). A number of early studies had shown increased platelet α_2 -adrenoceptors in depression (Garcia-Sevilla, 1989; Garcia-Sevilla et al. 1986). Yet, an equal number of negative studies now exist of platelet α_2 -adrenoceptors in depression, and it has been documented that most of the earlier positive studies had utilized clonidine as radioligand (Fu et al., 2001; Garcia-Sevilla et al., 1999; Halaris et al., 2003; Mokrani et al. 2000; Piletz, Halaris, & Ernsberger, 1994; Schittcatté et al., 2002). Other α_2 -adrenergic radioligands and molecular probes had been utilized (Garcia-Sevilla et al., 1999), but most findings for up-regulated α_2 -adrenoceptors have used clonidine (Halaris et al., 2003). Hence the question: What makes clonidine unique? The answer remains uncertain. One hypothesis has emphasized supersensitive receptors, which connotes a state of the α_2 -adrenoceptor that is stabilized by agonists, like clonidine (Garcia-Sevilla et al., 1999). This state has been conceptualized as the agonist-induced high-affinity state of α_2 -adrenoceptors that exists in ternary complex with G-proteins (Garcia-Sevilla et al., 1999). A different and not mutually exclusive hypothesis postulates that clonidine binds to a non-adrenergic IR-1 site which is up-regulated in depression (Piletz et al., 1994).

The IR-1 protein has been associated with IRAS (Li et al., 2006; Zhang & Abdel-Rahman, 2006), a membrane-linked protein without similarity to G-protein coupled receptors (Piletz et al., 2000). IRAS interacts with a number of membranous receptors, such as the mu-opioid receptor, of relevance to mood disorders (Piletz et al., 2003b; Piletz et al., 2000a; Wu et al., 2005). Exactly how IR-1 transduces signal is an area of active research, but one suggestion is that IR-1 modulates the shape of cells through interaction with integrins (Halaris et al., 2003). Furthermore, IR-1 inhibit neurotransmitter release in some neurons, similar to the manner of α_2 -adrenoceptors. For instance, IR-1 inhibit NE release in peripheral cells (Molderings et al., 2002) and reduce GABAergic transmission in striatal medium spiny neurons (Tanabe et al., 2006).

The main finding of the present study is that there is no change in p-[125 I]clonidine binding at either α_2 -adrenoceptor or IR-1 sites in healthy subjects treated with desipramine for six weeks.

This “lack of a finding” on IR-1 in healthy subjects is in sharp contrast with nearly all other previous studies that have shown robust antidepressant drug effects on platelet IR-1 sites (Garcia-Sevilla et al., 1996; Piletz et al., 1991; Piletz et al., 1996b; Zhu et al., 1999; Zhu et al., 1997). Down-regulation of IR-1 sites in treated depressed patients has been reported irrespective of type of antidepressant used (Garcia-Sevilla et al., 1996; Piletz et al., 1991; Piletz et al., 1996b; Zhu et al., 1999; Zhu et al., 1997). The antidepressants studied to date with platelet IR-1 in depressed patients are: desipramine (n=40), bupropion (n=17), fluoxetine (n=16), citalopram (n=7), clomipramine (n=1), and imipramine (n=1) (Garcia-Sevilla et al., 1996; Piletz et al., 1991; Piletz et al., 1996b; Zhu et al., 1999; Zhu et al., 1997). In the present study we again confirmed that desipramine induced down-regulation of platelet IR-1 sites in six re-analyzed depressed patients (Fig. 2). Thus, the finding of no down-regulation in platelet IR-1 in healthy subjects treated with desipramine is in sharp contrast with a substantial body of findings with depressed patients showing significant down-regulation (Halaris et al., 2003).

The goal of this study was to elucidate two divergent possibilities in interpreting the down-regulation of IR-1 associated with antidepressant drug use: one being a purely pharmacological effect of desipramine leading to IR-1 down-regulation, and the other being a mood state-dependent effect of desipramine leading to IR-1 down-regulation. Over six weeks of desipramine administration, none of our healthy subjects experienced mood elevation. On the contrary, a small but statistically significant rise in HAM-D scores was observed ($p = 0.02$) associated with dysphoric mood changes and signs of malaise attributable to the tricyclic antidepressant. In these subjects there was no down-regulation of either IR-1 or α_2 -adrenoceptors in platelets. Not only was there no down-regulation of IR-1, there was actually an up-regulation of the 33 kD band immunodensity during desipramine treatment of healthy subjects (Fig. 1 and further discussed below). Thus, the platelet IR-1-related immunoreactive band appeared to respond differentially to desipramine in healthy subjects compared to depressed patients. Since the distinguishing event (that did not occur in healthy subjects) was mood elevation/normalization seen in depressed subjects, it stands to reason that the down-regulation/normalization of platelet IR-1 seen exclusively in treated depressed patients (Fig. 2A) should be associated with mood improvement due to the antidepressant drug.

Previous studies have offered little clarification whether platelet IR-1 down-regulation is associated with mood state improvement or with a pharmacological effect independent of clinical response. In two previous studies, we found the degree of down-regulation of platelet IR-1 to be linearly associated with plasma antidepressant drug concentration (Halaris et al., 2003; Zhu et al., 1999). This finding left open the possibility that IR-1 down-regulation was driven by direct pharmacological effects rather than clinical improvement, and in those previous studies no correlation was found between the magnitude of platelet IR-1 down-regulation and overall HAM-D score improvement (Halaris et al., 2003). Yet, in another study a linear correlation was established between the extent of improvement in the psychomotor retardation subscore of the HAM-D scale and the extent to which the platelet 33 kD band down-regulated from before to after antidepressant treatment (Halaris et al., 2000). Another antidepressant treatment study showed down-regulation of platelet IR-1 in bupropion responders but not in bupropion non-responders (Zhu et al., 1999). Two other antidepressant studies, one of desipramine and one of fluoxetine, showed nearly identical degrees of down-regulations of platelet IR-1 in responders and non-responders to treatment (Piletz et al., 1996b). Our current study supports and extends the concept that platelet IR-1 density is a state-dependent marker of depression or depression-like mood states.

Not all our present data can be easily interpreted based on the assumption that IR-1 are a marker of mood state. In particular, the rise in platelet 33 kD band during desipramine treatment (Fig. 1) came as a surprise. By week-6 on desipramine, the immunodensity of the 33 kD band had risen nearly as much as α_2 -adrenoceptor binding sites in untreated depressed patients (Fig. 2).

It would be tempting to dismiss this observation as spurious (Fig. 2B), except that we had previously studied conditions where platelet IR-1 binding and 33 kD band immunodensity are discordant (Zhu et al., 2003a). This discordance was the very reason we employed two superficially redundant measures in this study. The discordance is thought to be due to the 33 kD band being an IRAS breakdown fragment (Zhu et al, 2003a). Furthermore, in transfected cells we previously observed a concordance between IRAS and α_2 -adrenoceptors (Chen, Zhu, & Piletz, 2003), similar to the possible concordance of 33 kD and α_2 -adrenoceptor down-regulation seen in this study during discontinuation (Figs. 1 and 2). Therefore the question remains: Why is the platelet 33 kD band elevated during desipramine treatment of healthy subjects which is opposite to that seen in antidepressant-treated patients (Zhu et al, 1999)? The answer will have to await further study.

To summarize, our findings are consistent with the hypothesis that the down-regulation of platelet IR-1 binding sites that has previously been seen during treatments of depressed patients with a variety of antidepressants is a marker of mood state improvement rather than of an antidepressant-induced pharmacological state.

Acknowledgements

This work was supported by NIMH grant MH42859.

The authors wish to express their sincere appreciation to Dr. He Zhu at the University of Mississippi Medical Center for his expert technical assistance.

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Figure 1.

Effects of desipramine treatment on immunodensities of the 33 kD IRAS band in platelet total membranes of healthy subjects. Western blotting was performed with IRBP antiserum and immunodensities were determined by densitometry of films. Values represent integrated optical density units (O.D.) normalized to a 5-point standard curve on each blot of 2.5 – 12.5 μ g total protein standard (the standard was a total membrane preparation of platelets from the Mississippi Blood Services, Jackson, MS). Bars represent mean $\pm$ SEM of values from 12 subjects.

* Compared with week-0, $p < 0.01$ (Repeated measures ANOVA with Dunnet's multiple comparison test).

Figure 2.

Binding of p-[¹²⁵I]clonidine binding to platelet imidazoline-1 receptors (IR-1) and α₂-adrenoceptors (α₂-AR) in desipramine-treated depressed patients (n= 6) and desipramine-treated healthy subjects (n= 12). The specific binding of 8 nM p-[¹²⁵I]clonidine to α₂-AR was defined by subtracting non-specific binding in the presence of 10 μM NE, and to IR-1 by subtracting additional non-specific binding in the presence of 10 μM moxonidine. Values are expressed as fmoles of radioligand specifically bound to each site per mg membrane protein. (A) IR-1 comparisons in depressed patients before desipramine (pre-DMI) and after 6 weeks of desipramine treatment. (B) IR-1 comparisons in healthy subjects before desipramine (pre-DMI), after 6 weeks of desipramine treatment, and after additional 2 weeks of desipramine discontinuation (ns: non-significance). (C) α₂-AR comparisons in depressed patients before desipramine (pre-DMI) and after 6 weeks of desipramine treatment. (D) α₂-AR comparisons in healthy subjects before desipramine (pre-DMI), after 6 weeks of desipramine treatment, and after additional 2 weeks of desipramine discontinuation.

TABLE 1

Vital Signs in Desipramine-treated Healthy Subjects

Postural Position	Measure*	0	Week of Study 2	6	Significance (p value*)
Supine:	Systolic BP (mm Hg)	111.7 ± 15.4	112.8 ± 14.9	118.5 ± 13.5	NS
	Diastolic BP (mm Hg)	73.3 ± 10.4	75.0 ± 9.3	76.4 ± 8.4	NS
	Heart Rate (bpm)	75.7 ± 9.9	88.0 ± 10.1	88.5 ± 14.2	0.01
Standing:	Systolic BP (mm Hg)	113.4 ± 15.3	103.3 ± 13.9	111.5 ± 14.1	NS
	Diastolic BP (mm Hg)	79.5 ± 10.9	76.5 ± 10.6	76.8 ± 8.9	NS
	Heart Rate (bpm)	84.8 ± 12.9	105.3 ± 9.3	105 ± 12.8	0.0001
Orthostatic:	Systolic BP (mm Hg)	+ 1.8 ± 7.0	- 9.5 ± 9.7	- 7.3 ± 15.2	0.04
	Diastolic BP (mm Hg)	+ 6.7 ± 8.0	+ 2.2 ± 6.4	+ 0.3 ± 6.1	NS
	Heart Rate (bpm)	+ 9.2 ± 8.7	+ 17.2 ± 9.9	+ 16.5 ± 7.6	0.03

* Based on Students t test (two-tailed) for mean blood pressure values of week-2 and week-6 (giving "total mean" post-desipramine blood pressure value) compared to week-0.

Values represent mean ± standard errors.

Abbreviations: BP, blood pressure; bpm, beats per minute

TABLE 2

Desipramine Side Effects in Healthy Subjects *

Side Effect	Percent Reporting
Dry mouth	100%
Dizziness	92
Headache	75
Constipation	58
Tiredness	50
Insomnia	50
Visual blurring	42
Poor appetite	33
Palpitations	25
Irritability	16

* Side effects reported by two or more subjects at least once during six weeks on desipramine.