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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 (six males and six females) met stringent inclusion and exclusion criteria for physical and mental health. They received desipramine for 6 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 6 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-[^{125}I]clonidine to IR-1 and α_2-adrenoceptors in healthy subjects did not change during desipramine treatment. The immunodensity of the 33 kDa 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 kDa 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 et al., 2005; Hamilton, 1992), and intraocular pressure (Campbell and 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 et al., 1996c). Several endogenous compounds have been proposed as putative neurotransmitters for IR: agmatine, harmane, and imidazole-ribotide (Halaris and Piletz, 2003; Musgrave and Badoer, 2000; Prell et al., 2004). None of the classical monoamines have substantial affinity for IR

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(Bousquet, 1997). Imidazoline receptors have been linked to neuroplasticity (Piletz et al., 2003b), have been postulated to be a possible state marker for depression (Halaris and Piletz, 2003), and may be a target for anxiolytic drug action (Zhu et al., 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 et al., 1996a; Piletz et al., 1991; Piletz et al., 1996b; Piletz et al., 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 et al., 1997). In two western blotting studies, the immunoreactivity of IRAS in 33 kDa and 45 kDa bands (peptides of IRAS) (Zhu et al., 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-[^{125}I]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 6 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 6 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 6 weeks of desipramine treatment, and after 2 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 and 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 h 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 h. No other medications, such as over-the-counter analgesics, were allowed from two days prior to the blood drawings and a record of such over-the-counter medication 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