# Comorbidity of panic disorder and major depressive disorder: effects on platelet alpha$_2$ adrenergic receptors

Grunhaus LJ, Cameron O, Pande AC, Haskett RF, Hollingsworth PJ, Smith CB. Comorbidity of panic disorder and major depressive disorder: effects on platelet alpha$_2$ adrenergic receptors.
Acta Psychiatr Scand 1990: 81: 216–219.

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Adrenergic receptor dysregulation has been described as occurring in both major depressive disorder (MDD) and panic disorder. Measurements of platelet alpha$_2$ adrenergic receptors in these patients may be confounded by the coexistence or comorbidity of both diagnoses in the same patient. To explore this possibility, we measured platelet alpha$_2$ adrenergic receptors ($^3$H-clonidine and $^3$H-yohimbine binding) in 3 groups of patients (MDD only, panic disorder only, and those showing comorbidity of MDD and panic) and normal controls. Patients with comorbidity of MDD and panic disorder had significantly lower agonist binding ($^3$H-clonidine).

Key words: panic disorder; affective disorder; adrenergic receptor; comorbidity

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Accepted for publication August 19, 1989

Adrenergic receptor dysregulation has been proposed as occurring in both major depressive disorder (MDD) and panic disorder. Most of the evidence supporting these hypotheses has been obtained by measuring products of adrenergic receptor activity, i.e., plasma catecholamines, or metabolites of these secretions, i.e., plasma 3-methoxy-4-hydroxyphenylglycol (MHPG). More direct assessment of receptor function in humans is difficult because of the relative inaccessibility of brain tissue. Blood platelets seem to share properties with central neurons (the neuronal model concept of Pletscher (1)), and thus have been used as peripheral markers for receptor activity (2, 3). Many studies have measured alpha$_2$ adrenergic receptors in platelets of patients with either MDD or panic disorder; unfortunately, results have not been consistent and are difficult to interpret because of methodological differences between the studies (3–5). When alpha$_2$ receptor agonists ($^3$H-clonidine or $^3$H-p-aminoclonidine) are used as ligands on fresh platelet membranes, most studies (6–9), but not all (10) suggest that high-affinity binding sites are increased in patients with MDD; on the other hand, if frozen platelets are used as substrate (11), these differences disappear. When alpha$_2$ receptor antagonists ($^3$H-dihydroergocryptine, $^3$H-rauwolscine or $^3$H-yohimbine) are utilized as ligands, the findings are more heterogeneous, with authors reporting either higher (12–15), normal (8, 16–22), or lower (23) receptor numbers. In patients with panic disorder, agonist binding ($^3$H-clonidine) has been reported as normal (24) whereas antagonist binding ($^3$H-yohimbine or $^3$H-rauwolscine) has been observed as either lower (24, 25) than controls or not significantly different (26, 27).

A very relevant factor in assay variability is diagnostic heterogeneity (2). This may be particularly important if the diagnostic heterogeneity is a reflection of underlying pathophysiology. Recent studies suggest that when MDD and panic disorder occur together in the same patient (comorbidity), a more severe disorder may be present, possibly reflecting combined diathesis (28, 29). Some support for this hypothesis has emerged from studies of sleep electroencephalographic changes and urinary MHPG in patients with comorbidity of MDD and panic disorder compared with patients with MDD alone. Grunhaus et al. (30) and Dube et al. (31) reported that sleep electroencephalography recordings in patients with comorbidity of MDD and panic disorder were significantly different from those of MDD patients without comorbidity features, even though both groups meet the Research Diagnostic Criteria for MDD endogenous subtype. The comorbidity groups had less of an endogenous profile (higher REM latencies and less REM densities) in the sleep polysomnography recording. Garvey et al. (32) measured urinary MHPG in patients with MDD and subdivided them according to the presence of panic attacks, and found that those with

MDD and panic (comorbidity group) had significantly higher excretion of urinary MHPG.

To explore whether comorbidity of MDD and panic disorder is a factor in platelet alpha$_2$ adrenergic receptor variability, we compared 3 groups of patients (those with MDD alone, those with comorbidity of MDD and panic disorder and those with panic disorder alone) with normal controls. Our findings suggest that platelet alpha$_2$ adrenergic receptors are significantly lower in patients with comorbidity of MDD and panic disorder.

## Material and methods

Twenty-seven patients from the Depression and Anxiety Disorders Programs of the Psychiatry Department of the University of Michigan were recruited for this study. In order to be included, patients had to meet DSM-III diagnostic criteria for either MDD or panic disorder for the index episode. Patients with panic disorder were required to have had at least 1 panic attack per week during the preceding 4 weeks. Patients with MDD were required to have scored 15 or more points in the 17-item Hamilton Rating Scale for Depression (HRSD) (33). The evaluation process included diagnostic interviews with senior clinicians for consensus diagnosis and a comprehensive physical examination and thorough laboratory work-up to rule out possible illness. For 14 days prior to withdrawal of samples, patients and controls were kept free of all medications, including benzodiazepines, alcohol, street drugs, and any medication that could affect adrenergic receptors; drug-free status was verified with a urine drug screen that preceded blood sampling by 3 days. During the diagnostic interviews, patients with MDD were carefully questioned for the presence of panic attacks during the current episode of illness; those having panic attacks of sufficient severity and frequency to meet the criteria for panic disorder were later subcategorized as patients with comorbidity of MDD and panic disorder. Thus, participating patients were categorized into 3 groups: patients with MDD only (those never meeting criteria for anxiety diagnosis; $n = 14$); patients with panic disorder only (these never meeting criteria for a depression diagnosis; $n = 6$), and patients with comorbidity of MDD and panic disorder (those meeting criteria for both disorders; $n = 7$).

Fifteen volunteers with normal physical exam and laboratory studies were included as the control group. The psychiatric assessment of the normal volunteers was done using the Schedule for Affective Disorders and Schizophrenia (SADS) (34) and HRSD. Any volunteer with personal or first-degree family history of any psychiatric disorder or an HRSD > 5 was not accepted for the study. The rejection rate for normal volunteers was approximately 30%.

The research protocol was approved by the institutional review board in charge of supervising research in humans, and all subjects signed a written informed consent for participation in the protocol. All subjects fasted and remained supine after midnight preceding blood withdrawal. An antecubital catheter was inserted at 0730 and baseline samples were withdrawn 1 h later.

Controls were recruited with an attempt to stratify according to the age of the patients. Mean age was $34.4 \pm 11.6$ years for MDD patients, $31.1 \pm 6.9$ years for panic disorder patients and $34.3 \pm 6.9$ years for the controls ($F = 1.1$; NS). No sex differences were found between the groups.

The platelet alpha$_2$ adrenergic receptor binding assay has been described in detail elsewhere (6, 8). Briefly, immediately after withdrawal, blood was mixed with acid-citrate-dextrose anticoagulant buffer. Platelets were isolated, washed, and lysed, and platelet membranes were isolated. Specific binding of tritiated yohimbine or tritiated clonidine to platelet membranes was determined on fresh platelet membranes. Specific binding (total binding minus nonspecific binding) was approximately 85% for tritiated clonidine and 90% for tritiated yohimbine. A computerized program (35) that utilizes Scatchard (36) and Hill (37) transformations was used to develop preliminary estimates of the apparent dissociation contents (KD) and the maximum binding sites ($B_{max}$) for the 2 ligands. Final values for the 2 binding parameters were estimated by use of the nonlinear regression analysis program devised by Munson & Rodbard (38). Assays were performed by individuals blind to patient diagnosis.

Statistical comparisons included a one-way analysis of variance with post-hoc comparisons when significant differences occurred.

## Results

To explore our assumptions regarding the relevance of diagnostic heterogeneity, we first analyzed the results of the platelet receptor assay using the traditional DSM-III categories of MDD and panic disorder (Table 1A) and later separated from the MDD groups the patients with comorbidity (Table 1B).

Using the traditional DSM-III groups, no differences in receptor numbers ($B_{max}$) and dissociation constants ($K_d$) as measured with $^3$H-clonidine (agonist) and $^3$H-yohimbine (antagonist) binding between the groups were detected between patients and controls (see Table 1A for actual values). When patients were separated according to the comorbidity

Table 1. Platelet alpha$_2$ adrenergic receptor values in patients with MDD, panic disorder, comorbidity of MDD and panic disorder and normal controls ($B_{max}$ expressed as fmol/mg protein)

A: according to DSM-ill diagnostic groups

| | | Clonidine | | Yohimbine | |
|---|---|---|---|---|---|
| | *n* | $B_{max}$ mean ± SEM | $K_d$ mean ± SEM | $B_{max}$ mean ± SEM | $K_d$ mean ± SEM |
| MDD | 21 | 32.6 ± 3.1 | 4.2 ± 5 | 112.0 ± 9.2 | 3.9 ± 0.3 |
| Panic | 6 | 26.6 ± 4 | 6.9 ± 2.8 | 106.8 ± 28.2 | 3.3 ± 0.6 |
| Controls | 15 | 36.8 ± 4.2 | 4.8 ± 0.7 | 115.7 ± 11.9 | 3.3 ± 0.4 |
| | | $F = 1.09$ NS | $F = 1.53$ NS | $F = 0.76$ NS | $F = 1.2$ NS |
| B: according to comorbidity groups | | | | | |
| MDD | 14 | 38.6 ± 3.5 | 4.1 ± 0.5 | 118.4 ± 11.5 | 3.6 ± 0.4 |
| MDD and panic | 7 | 20.7 ± 3.0 | 3.9 ± 0.2 | 107.5 ± 18.7 | 4.7 ± 0.7 |
| Panic | 6 | 26.6 ± 3.9 | 6.9 ± 2.7 | 106.8 ± 28.1 | 3.3 ± 0.6 |
| Controls | 15 | 36.8 ± 4.3 | 4.8 ± 0.7 | 115.7 ± 11.9 | 3.3 ± 0.4 |
| | | $F = 3.62$* | $F = 1.23$ NS | $F = 0.12$ NS | $F = 1.72$ NS |

* $P < 0.02$; MDD and panic vs controls: $P < 0.01$; MDD and panic vs MDD: $P < 0.01$.

group (Table 1B), significant differences appeared. $B_{max}$ for $^3$H-clonidine binding in patients with comorbidity of MDD and panic disorder is significantly lower than in controls and MDD patients (MDD: 38.6 ± 3.5, comorbidity group 20.7 ± 3.0, panic disorder group 26.6 ± 3.9, controls 36.8 ± 4.3 (fmol/mg protein); $F = 3.62$; $P = 0.02$). Interestingly, $B_{max}$ values for $^3$H-clonidine showed a trend toward being significantly lower in the panic disorder group than in the MDD group, suggesting that the lower agonist binding may be a feature of the panic groups. $^3$H-yohimbine binding appears to be lower in the panic groups (NS). Kd for agonist and antagonist binding were not significantly different between the groups.

## Discussion

Our results suggest that alpha$_2$ adrenergic receptors in platelets of patients who show comorbidity of MDD and panic disorder are significantly lower than those measured in normal controls or patients with MDD alone. These findings are of interest and support our initial assumption that patients with comorbidity of depression and panic are different from a pathophysiological perspective from patients with MDD alone. They also provide support to the suggestions that patients with comorbidity of MDD and panic disorder differ from patients with MDD alone on clinical and biological parameters. Patients with the combined diagnoses are likely to exhibit more severe psychopathology (28, 30, 39–41), greater number of affected relatives (28, 42), worse treatment outcome (39, 43), and greater lifelong impairment (41, 43).

These observations should be considered preliminary for several reasons:

- The number of patients studied is relatively small, and this may account for the differences observed between this study and previous reports from our group showing higher $^3$H-clonidine binding in MDD patients (7) and lower $^3$H-yohimbine binding in panic disorder patients (24).
- More direct measures of receptor function were not incorporated into the paradigm studied.
- The relationship between plasma catecholamines and receptor numbers ($B_{max}$) was not explored; it is possible that the lower $B_{max}$ observed in the population with panic attacks is associated with higher levels of circulating catecholamines.

A heightened adrenergic tone has been suggested to occur in patients with panic disorder (24, 44–56), in melancholic patients (47) and in patients with MDD with panic attacks (31). It is possible that adrenergic dysregulation is a feature common to patients with panic attacks regardless of the diagnosis. The findings we are reporting suggest that, in studies of alpha$_2$ adrenergic receptor function in MDD and panic disorder, the presence of comorbidity of these conditions may confound the results of biological studies.

The question of peripheral-central interactions must also be clarified. Charney et al. (44–46) have suggested that hyperactive presynaptic alpha$_2$ adrenergic receptors at the level of the locus coeruleus are a feature of panic disorder patients. This central upregulation of alpha$_2$ receptor could be conducive to peripheral downregulation, possibly through higher circulating catecholamine levels. Whether central upregulation and peripheral downregulation of alpha$_2$ receptors is a feature common to patients with high circulating catecholamines is an interesting possibility yet to be explored.

It is unclear whether the differences we describe represent true biological differences. Most certainly, replication and extension of these findings is necessary. If supported, it may suggest that lower platelet alpha$_2$ adrenergic receptors, as measured by agonist binding, are a feature more closely related to patients who show comorbidity of panic and MDD. Most certainly, combining studies of receptor function with actual receptor measurements is a necessary future step in clarifying this interesting question.

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