PLATELET ALPHA₂ ADRENORECEPTORS ARE DECREASED IN NUMBER AFTER ANTIDEPRESSANT THERAPY

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Abstract


1. Specific binding of ³H-clonidine to alpha₂ adrenoreceptors upon human blood platelet membranes is increased in patients with major depressive disorder (endogenous depression).
2. Specific binding of ³H-yohimbine to the platelet adrenoreceptor is not altered in endogenously depressed patients.
3. Other psychiatric disorders are not associated with alterations in the specific binding of either ³H-clonidine or ³H-yohimbine. In patients with severe congestive heart failure or with symptomatic coronary artery disease the number of platelet alpha₂ adrenoreceptors is actually decreased.
4. Treatment of endogenously depressed patients with tricyclic antidepressants, lithium salts or electroconvulsive therapy results in a decrease in the number of alpha₂ adrenoreceptors on blood platelet membranes.
5. These studies suggest that a supersensitivity of the alpha₂ adrenoreceptor might exist in patients with endogenous depression and that effective forms of therapy lead to a decrease in the number of neural alpha₂ adrenoreceptors which is reflected by a decrease in the number of these receptors upon blood platelet membranes.

Key words: alpha₂ adrenoreceptors, electroconvulsive therapy, human blood platelets, lithium salts, major depressive disorder, tricyclic antidepressants

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1. Introduction

The amine theory of depression postulates that the biological basis of endogenous depression (Major Depressive Disorder) is a lack of adequate neuronal release of amine neurotransmitters such as norepinephrine and serotonin (Bunney and Davis, 1965; Schildkraut, 1965). In recent years presynaptic receptors have been shown to play an important role in the regulation of neurotransmitter release (Langer, 1977, 1980; Starke, 1977, 1981; Westfall, 1977). Feedback inhibition of norepinephrine release by norepinephrine located in the synaptic cleft seems to be the primary means by which the release of this neurotransmitter is regulated. Stimulation of the presynaptic alpha2 adrenoreceptor mediates this inhibition of neurotransmitter release. It has been postulated that a supersensitivity of this receptor exists in the endogenous type of depression and that one result of treatment of this disease is a decrease in the sensitivity of this receptor (Garcia-Sevilla et al., 1981b; Smith et al., 1981; Smith and Garcia-Sevilla, 1982).

2. Alpha2 Adrenoreceptors in Experimental Animals

Several studies have shown that forms of therapy which are efficacious in the treatment of endogenous depression result in either a functional subsensitivity of the alpha2 adrenoreceptor (Crews and Smith, 1978) or a decrease in the number of these receptors located upon neural membranes isolated from the brains of experimental animals. Early functional studies in our laboratory showed that the long-term administration of tricyclic antidepressant drugs caused decreased sensitivity of alpha2 adrenoreceptors located upon noradrenergic neurons in the rat heart. Drugs which after chronic administration led to increased neuronal release of norepinephrine in the rat heart included both typical (clomipramine, desipramine and nortriptyline) and atypical (iprindole) tricyclic antidepressants (Crews and Smith, 1980). Later, long-term administration of amitriptyline to rats was found to decrease the number of alpha2 adrenoreceptors present in certain areas of the rat brain (Smith et al., 1981). Long-term administration of clorgylline, a monoamine oxidase inhibitor, also has been shown to decrease the number of alpha2 adrenoreceptors located upon neural membranes isolated from rat cerebral cortex (Cohen et al., 1982). Recently, the repeated administration of electroconvulsive shock to rats was found to decrease the number of alpha2 adrenoreceptors located upon neural membranes isolated from cerebral cortex, hippocampus and hypothalamus (Stanford and Nutt, 1982). Thus, a variety of different types of treatment for depressive illness all result in decreases in the number of alpha2 adrenoreceptors upon neurons in the rat brain.

3. Alpha2 Adrenoreceptors on Human Blood Platelets

Our studies with experimental animals led us to explore the possibility that endogenous depression might be associated with alpha2 adrenoreceptor supersensitivity and that treatment would result in decreases in the number of these receptors located in the central nervous system. The alpha2 adrenoreceptor has been found in a variety of nonneural tissues such as rat renal cortex (Woodcock et al., 1980), hamster adipocytes (Garcia-Sainz et al., 1980), human fat cells (Berlan and LaFontan, 1980; Tharp et al., 1981) and human blood platelets (Motulsky et al., 1980; Garcia-Sevilla et al., 1981a; Shattil et al., 1981). The human blood platelet has been used often as a model to evaluate changes in nerve cell function in patients with psychiatric disorders (Stahl, 1977). The blood platelet possesses alpha adrenoreceptors which when stimulated are responsible for the initiation of platelet aggregation by catecholamines (Ardlie et al., 1966). Numerous recent studies have produced clear evidence that this adrenoreceptor is of the alpha2 subtype. The present paper reviews studies done in our laboratory upon platelet alpha2 adrenoreceptors in a variety of patients with psychiatric and nonpsychiatric disorders. Changes in the platelet alpha2 adrenoreceptor after treatment of endogenously depressed patients with various forms of therapy will be discussed.

4. Receptor Binding Studies in Endogenously Depressed Patients

4.1 Subjects Studied

Receptor binding techniques provide a powerful means of evaluating changes in the number
Platelet adrenoreceptors and antidepressant therapy

A partial agonist and an antagonist which have high affinity for the alpha_2 adrenoreceptor (Greenberg et al., 1976; Prichard et al., 1977). During the past three years the binding of either H-clonidine or H-yohimbine, or both ligands, to isolated platelet membranes has been determined in a sizeable population of patients with a variety of clinical conditions. Among these were patients with the following diagnoses: major depressive disorder, other depressive disorders, panic disorder, severe congestive heart failure and symptomatic coronary artery disease. Psychiatric patients were evaluated and treated in the Department of Psychiatry and patients with cardiovascular disorders in the Cardiology Unit, University of Michigan Medical Center, Ann Arbor. The procedures for diagnosing major depressive disorder have been described previously (Garcia-Sevilla et al., 1981b). Of a total of 35 drug-free psychiatric patients, the diagnosis of probable or definite major depressive disorder at the time of the initial study was subsequently confirmed by consensus diagnosis in 29 patients. Most of these patients met the criteria for probable or definite endogenous subtype according to Research Diagnostic Criteria. All patients with the exception of those in congestive heart failure were totally drug free for a period of two weeks prior to the initial platelet receptor binding studies. Patients in congestive heart failure were treated at the time of the initial studies with a combination of diuretics and cardiac glycosides, but did not receive either adrenergic agonists or antagonists. Specific binding of either H-clonidine or H-yohimbine, or both, to platelet membranes was determined prior to the onset of treatment in a blind manner with respect to the patient's diagnosis. Whenever possible, for the psychiatric patients the determinations were repeated subsequent to treatment with tricyclic antidepressants, lithium carbonate, or after electroconvulsive therapy.

4.2 Platelet Receptor Binding Techniques

All determinations were performed upon fresh platelet samples. Preliminary studies were carried out in which the isolated platelet membranes were frozen and stored at -70°C for a period of 7 days. Freezing caused significant changes in the number of specific binding sites for both H-clonidine and H-yohimbine. Consequently, only freshly prepared platelet membranes are used in our studies. Approximately 50 ml of blood is collected by venipuncture in plastic centrifuge tubes which contain (8:1 v/v) acid-citrate-dextrose (ACD) solution as anticoagulant (National Institutes of Health Formula A: 0.8% citric acid, 2.2% trisodium citrate and 2.45% dextrose). The blood is centrifuged at 160 x g for 10 min (25°C) and the resulting platelet-rich plasma is titrated to pH 6.5 with ACD solution and recentrifuged at 5100 x g for 15 min (25°C) to sediment the platelets. The platelet pellet is washed twice with 5 ml of Tyrode buffer (NaCl 137 mM; KCl, 2.7 mM; NaHCO_3 12.0 mM; dextrose 0.56 mM; pH 6.8) and recentrifuged at 5100 x g for 15 min (4°C). The washed pellet is lysed by homogenization in 2 ml of ice-cold hypotonic buffer (Tris-EDTA, 5 mM; pH 7.5). After centrifugation at 39000 x g for 10 min (4°C), the platelet membranes are resuspended in the Tris incubation buffer (Tris-Cl, 50 mM; MgCl_2 10 mM; pH 7.5) used in the binding assay. Total H-clonidine and H-yohimbine binding is measured with one ml aliquots of the fresh platelet membranes which are incubated in duplicate with shaking at 25°C. Nonspecific binding is determined by adding unlabelled clonidine or yohimbine, 10^(-4) M, in addition to the labelled ligand, to a second pair of incubates. Specific binding is defined as the difference between total and nonspecific binding. Further details of the experimental procedures have been previously described (Garcia-Sevilla et al., 1981a,b).

4.3 Binding of 3H-Clonidine and 3H-Yohimbine to Platelet Membranes from Endogenously Depressed Patients

The specific binding of 3H-clonidine to platelet alpha_2 adrenoreceptors in a selected group of drug-free patients with major depressive disorder is significantly higher than that obtained in a similar control population (Table 1). Although the specific binding of H-yohimbine was also increased in the population of patients with major depressive disorder, this increase was smaller than that seen with H-clonidine and was not significant statistically (Table 2). It is possible that the two ligands bind to different forms of the same receptor and that the form of this receptor to which H-clonidine binds is that which is abnormal in patients with major depressive disorder.
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>$B_{\text{max}}$ (fmol/mg protein)</th>
<th>$K_D$ (nM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>32 ±2</td>
<td>5.5 ±0.6</td>
<td>26</td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>** 46 ±2</td>
<td>5.6 ±0.6</td>
<td>29</td>
</tr>
<tr>
<td>Non-major depressive disorder</td>
<td>29 ±6</td>
<td>5.1 ±0.9</td>
<td>6</td>
</tr>
<tr>
<td>Panic disorder $\S$</td>
<td>36 ±4</td>
<td>8.5 ±1.4</td>
<td>9</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>** 24 ±2</td>
<td>3.4 ±0.6</td>
<td>5</td>
</tr>
<tr>
<td>Coronary artery disease $\S$</td>
<td>30 ±3</td>
<td>7.3 ±1.3</td>
<td>5</td>
</tr>
</tbody>
</table>

Each value represents the mean ± the standard error of the mean. $B_{\text{max}}$, the maximum number of specific binding sites; $K_D$, the dissociation constant. $\S$ Approximately one-half of the patients with panic disorder met the DSM III criteria for agoraphobia with panic disorder. $\S$ All of the patients in this category had symptoms of angina pectoris. n= number of subjects. * P<.05, ** P<.0005.

5. Other Psychiatric and Non-Psychiatric Disorders

In patients with panic disorder or in psychiatric patients who did not meet the criteria for major depressive disorder, specific binding of $^3$H-clonidine to platelet alpha$_2$ adrenoreceptors was the same as to membranes from normal subjects. Specific binding of tritiated yohimbine was actually decreased significantly in those patients with panic disorder. The decrease in $^3$H-yohimbine binding might reflect either transient or sustained increases in plasma catecholamine content. Patients with severe congestive heart failure and those with symptomatic coronary artery disease also had marked reductions in the specific binding of $^3$H-yohimbine. Specific binding of $^3$H-clonidine was also reduced in patients with severe congestive heart failure, and in these patients there was a good correlation between plasma catecholamine content and the number of platelet alpha$_2$ adrenoreceptors.

6. Effects of Antidepressant Therapy

Specific binding of either $^3$H-clonidine or $^3$H-yohimbine to platelet membranes has been measured in patients treated with tricyclic antidepressants, lithium carbonate or electroconvulsive therapy (Table 3). These patients were selected because they received no other type of drug therapy. Treatment with either imipramine hydrochloride or with amitriptyline hydrochloride produced significant decreases in the $B_{\text{max}}$ of high affinity binding sites for
Table 2
Specific Binding of $^3$H-yohimbine to Platelet Alpha$_2$
Adrenoreceptors in Normal Subjects and Patients
with Psychiatric and Non-Psychiatric Disorders

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>$B_{max}$ (fmoles/mg protein)</th>
<th>$K_D$ (nM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>165 ± 12</td>
<td>4.0 ± 0.5</td>
<td>16</td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>183 ± 9</td>
<td>5.2 ± 0.8</td>
<td>18</td>
</tr>
<tr>
<td>Panic disorder §</td>
<td>113** ± 19</td>
<td>4.1± 0.5</td>
<td>8</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>94** ± 9</td>
<td>4.4 ± 0.4</td>
<td>9</td>
</tr>
<tr>
<td>Coronary artery disease §</td>
<td>103** ± 7</td>
<td>4.4 ± 0.9</td>
<td>13</td>
</tr>
</tbody>
</table>

Each value represents the mean ± the standard error of the mean. $B_{max}$, the maximum number of specific binding sites; $K_D$, the dissociation constant. § Approximately one-half of the patients with panic disorder met the DSM III criteria for agoraphobia with panic disorder. * All of the patients in this category had symptoms of angina pectoris. n = number of subjects. * P< .05, ** P< .0005.

$^3$H-clonidine (31% ± 6%, P < .025, n=8). Treatment with lithium carbonate produced significant decreases in the maximum number of high affinity binding sites for $^3$H-yohimbine (31% ± 8%, P < .05, n=4). Electroconvulsive therapy decreased the specific binding of both $^3$H-clonidine (49% ± 4%, P < .0005, n=7) and $^3$H-yohimbine (41% ± 9%, P < .005, n=5).

7. Conclusions

The number of alpha$_2$ adrenoreceptors upon human blood platelet membranes to which $^3$H-clonidine binds specifically is increased in patients who are endogenously depressed. A similar increase in the specific binding of $^3$H-yohimbine is not observed. Other psychiatric and nonpsychiatric disorders are not associated with similar alterations in the number of alpha$_2$ adrenoreceptors. Treatment of endogenously depressed patients with tricyclic antidepressants, lithium carbonate or electroconvulsive therapy decreases the number of alpha$_2$ adrenoreceptors upon platelet membranes. It is suggested that there is a supersensitivity of alpha$_2$ adrenoreceptors upon neurons in the central nervous system of patients with major depressive disorder and that effective forms of therapy result in a decrease in the number of these receptors which is reflected by a similar change in the number of alpha$_2$ adrenoreceptors upon blood platelet membranes.
Table 3
High-affinity Binding of $^3$H-Clonidine and $^3$H-Yohimbine to Platelet Membranes Before and After Antidepressant Therapy

<table>
<thead>
<tr>
<th>Drug-free</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_D$ (nM)</td>
<td>$B_{max}$ (fmoles/mg protein)</td>
</tr>
<tr>
<td><strong>$^3$H-Clonidine Binding</strong></td>
<td></td>
</tr>
<tr>
<td>Tricyclics$^1$</td>
<td>6.5</td>
</tr>
<tr>
<td>± 1.0</td>
<td>± 5</td>
</tr>
<tr>
<td>ECT$^3$</td>
<td>5.3</td>
</tr>
<tr>
<td>± 1.5</td>
<td>± 6</td>
</tr>
<tr>
<td><strong>$^3$H-Yohimbine Binding</strong></td>
<td></td>
</tr>
<tr>
<td>Lithium$^2$</td>
<td>5.8</td>
</tr>
<tr>
<td>± 1.0</td>
<td>± 21</td>
</tr>
<tr>
<td>ECT</td>
<td>4.8</td>
</tr>
<tr>
<td>± 0.8</td>
<td>± 9</td>
</tr>
</tbody>
</table>

1. Patients were treated either with imipramine hydrochloride (125 to 250 mg/day) or with amitriptyline hydrochloride (125 to 150 mg/day) for a period of 2 to 5 weeks.
2. Patients were treated with lithium carbonate (900 to 1500 mg/day) for 3 weeks.
3. Electroconvulsive therapy was administered over a period of 2 to 3 weeks (7 to 10 treatments).

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References


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