α2-Adrenoreceptors in rat brain are decreased after long-term tricyclic antidepressant drug treatment

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(Accepted November 13th, 1980)

Key words: α2-adrenoreceptor — chronic amitriptyline — tricyclic antidepressant

After two weeks of twice-daily administration of amitriptyline to rats, the binding of [3H]clonidine to presynaptic α2-adrenoreceptors was decreased in membranes isolated from 5 areas of the rat brain. After one day of treatment, binding did not differ from saline treated controls. In vitro, a high concentration of amitriptyline caused a competitive inhibition of [3H]clonidine binding but did not alter the number of binding sites. The decrease in the number of α2-adrenoreceptor binding sites after two weeks of amitriptyline treatment would explain the subsensitivity of these receptors which occurs after prolonged administration of antidepressant drugs.

An important mechanism by which the neuronal release of norepinephrine is regulated is by stimulation of a presynaptic autoreceptor, the α2-inhibitory adrenoreceptor. When this receptor is stimulated, the further release of noradrenaline is inhibited which thus provides a means for autoregulation of the amount of norepinephrine within the synaptic cleft. Recently, we reported that the administration of various antidepressant drugs for 2–3 weeks leads to an increased norepinephrine release from adrenergic neurons in the isolated rat left atrium which is secondary to the development of subsensitivity of the presynaptic α2-adrenoreceptor. This subsensitivity of the presynaptic α2-adrenoreceptor might be an important mechanism by which antidepressant drugs could enhance the neuronal release of norepinephrine. The purpose of the present study was to determine whether the long-term administration of amitriptyline, a widely used tricyclic antidepressant, causes changes in presynaptic α2-adrenoreceptors in various areas of the rat brain which could result in increased norepinephrine release. Receptor binding techniques provide a powerful means of evaluating changes in the number and/or affinities of receptors. [3H]Clonidine is an agent which has high affinity for the presynaptic α2-adrenoreceptor. In order to evaluate changes in this receptor, we studied the specific binding of [3H]clonidine to receptors upon neural membranes prepared from homogenates of various areas of the rat brain.

Homogenates were made from the following brain areas isolated from male, Sprague–Dawley rats (220–240 g): amygdala, hippocampus, anterior caudate nucleus,
hypothalamus and locus coeruleus. The various areas of the rat brain were identified histologically. The area which is referred to as the locus coeruleus consisted of a section of brain stem 1 mm thick which was medial and inferior to the superior cerebellar peduncle, superior to the nucleus of the Vth cranial nerve and which contained the mesencephalic sensory nucleus of the trigeminal nerve as well as the locus coeruleus. Rats were treated with amitriptyline every 12 h either for one day or for two weeks and then were killed 12 h after the last injection. Brain areas isolated from 6 rats were pooled for each experiment. The brain areas were homogenized in 5 ml of ice-cold Tris-sucrose buffer which consisted of 5 mM Tris (hydroxymethyl) aminomethane, 0.25 M sucrose and 1 mM MgCl₂.6H₂O adjusted to pH 7.4 with 1 N HCl. The homogenates were centrifuged at 1000 × g for 10 min and the supernatants were saved and recentrifuged at 40,000 × g. The crude membrane pellet was washed twice with ice-cold Tris-incubation buffer, and recentrifuged at 40,000 × g. The incubation buffer was composed of 50 mM Tris (hydroxymethyl)aminomethane and 10 mM MgCl₂.6H₂O adjusted to pH 7.5 with 6 N HCl. The final pellet was resuspended in Tris-incubation buffer. To measure total binding, 1 ml aliquots of the neural membranes were incubated in duplicate for 30 min at 25 °C with concentrations of [³H]clonidine (sp. act. 22.2 Ci/mmol, N.E.N., Boston, Mass.) which ranged from 10⁻⁹ M to 6.4 × 10⁻⁸ M. Non-specific binding was determined by adding unlabeled clonidine, 10⁻⁵ M, as well as [³H]clonidine to a second pair of incubates. Incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters and by washing with two 10 ml aliquots of Tris incubation buffer (25 °C). Results are expressed as femtomoles of [³H]clonidine specifically bound per mg of protein.

TABLE I

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Control</th>
<th>Amitriptyline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kₐ (nM)</td>
<td>Bₘₐₓ (fmol/mg)</td>
</tr>
<tr>
<td>Amygdala</td>
<td>7.0 ± 0.9</td>
<td>367 ± 17</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>6.7 ± 1.3</td>
<td>155 ± 10</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>10.4 ± 0.2</td>
<td>127 ± 11</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>15.5 ± 0.3</td>
<td>289 ± 22</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>11.6 ± 2.2</td>
<td>196 ± 16</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01; *** P < 0.005 (Student’s t-test).
High affinity binding to the neural membranes was rapid, saturable and reversible by clonidine, $10^{-5}$ M. The regional distribution of specific binding in the rat brain was uneven (Table I). The highest degree of binding occurred in the amygdala and the lowest in the caudate nucleus. The other areas had intermediate amounts of specific binding. Non-specific binding represented only a small proportion of the total $^3$H-ligand bound and ranged from 6% in the amygdala to 18% in the caudate nucleus. None of the drug treatments altered non-specific binding to membranes isolated from any of the brain areas. Scatchard analyses suggested that only a single population of binding sites was present upon the isolated membranes.

The long-term administration of amitriptyline led to dose-dependent decreases in the binding of $[^3H]$clonidine in certain specific areas of the rat brain. In these experiments, the binding of $[^3H]$clonidine was evaluated at a concentration of $8 \times 10^{-9}$ M. Decreases in binding occurred in membrane preparations made from all areas of the brain and were caused by doses which ranged from 3 to 30 mg/kg. For example, a typical dose–response relationship is shown in Fig. 1A for the reduction in binding which occurred in the amygdala. The maximum decreases were $39 \pm 3\%$ ($P<0.001$) in the amygdala, $34 \pm 6\%$ ($P<0.01$) in the hippocampus, $29 \pm 6\%$ ($P<0.05$) in the locus coeruleus, $27 \pm 7\%$ ($P<0.05$) in the caudate nucleus and $48 \pm 9\%$ ($P<0.001$) in the hypothalamus. Scatchard plots were constructed for binding in the various brain areas isolated from saline-treated animals and from animals treated with amitriptyline, 10 mg/kg i.p., every 12 h for two weeks. Saturation curves and Scatchard plots for membranes isolated from the amygdala are shown in Fig. 1B. The maximum number of binding sites ($B_{\text{max}}$) were reduced significantly in the amygdala ($30 \pm 5\%$), hippocampus ($35 \pm 5\%$), caudate nucleus ($35 \pm 8\%$) and locus coeruleus ($22 \pm 6\%$), but not in the hypothalamus. The apparent dissociation constants ($K_d$) were not altered significantly in membranes isolated from any brain region except the hypothalamus where the $K_d$ was increased approximately two-fold (Table I). The differences between $[^3H]$clonidine binding in the hypothalamus and that in the other brain areas might be due to a higher proportion of postsynaptic $\alpha_2$-receptors, a lower proportion of noradrenergic nerve terminals, or the presence of a second binding site of lower affinity.

The changes in $[^3H]$clonidine binding observed after long-term administration of amitriptyline do not occur after the short term treatment of rats with this antidepressant. The binding of $[^3H]$clonidine, $8 \times 10^{-9}$ M, to neural membranes was studied after one day of amitriptyline administration (10 mg/kg i.p., administered twice at 12 h intervals). The amounts of $[^3H]$clonidine which were bound to membranes isolated from the brains of saline-treated rats were (in fmol/mg protein, n = 8): amygdala, 211 ± 12; hippocampus, 90 ± 7; locus coeruleus, 82 ± 8; hypothalamus, 100 ± 4; and caudate nucleus, 62 ± 7. The amounts of $[^3H]$clonidine which were bound in the same regions from the brains of rats treated acutely with amitriptyline were (n = 3): amygdala, 238 ± 7; hippocampus, 91 ± 5; locus coeruleus, 107 ± 13; hypothalamus, 99 ± 7; and caudate nucleus, 69 ± 9. Thus, the decreases in binding observed after two weeks of amitriptyline administration required prolonged exposure to the drug in
Fig. 1. Effects of long-term amitriptyline administration upon the specific binding of [3H]clonidine to rat brain membranes isolated from the amygdala. A: dose–response relationship for various doses of amitriptyline (solid bars) or for saline (open bar) administered i.p. every 12 for two weeks. Each bar represents the mean of 3–8 determinations. Each determination was performed on membranes pooled from 6 rat brains and incubated in the presence of [3H]clonidine, $8 \times 10^{-9}$ M. Vertical lines represent S.E.s of the mean. B: saturation and Scatchard plots for the binding of [3H]clonidine to rat brain membranes isolated from the amygdala. Large graph: saturation curves. Ordinate, amount of [3H]clonidine specifically bound; abscissa, concentration of [3H]clonidine in the incubate. Inset graph: Scatchard plots. Ordinate, [3H]clonidine specifically bound/[3H]clonidine free in incubate; abscissa, [3H]clonidine specifically bound. Solid circles, determinations done on amygdala membranes isolated from groups of 6 rats treated with saline twice daily for two weeks ($n = 4-8$); open circles, determinations done on amygdala membranes isolated from groups of 6 rats treated with amitriptyline, 10 mg/kg i.p. every 12 h for 2 weeks ($n = 3-6$). Specific binding refers to the difference between total binding and non-specific binding. Vertical lines represent standard errors of the mean. Regression lines are shown in the inset graph.
order to develop. This development of decreased binding was gradual and was maximal at two weeks of amitriptyline treatment.

Tricyclic antidepressants have been reported to compete in vitro for the binding of \(^{[3]}\text{H}\)WB4101 to \(\alpha\)-adrenergic receptors on rat brain membranes\(^{15}\). WB4101, \(2,6\)-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane, is an antagonist which binds selectively to the postsynaptic \(\alpha_1\)-adrenoceptor. The possibility that amitriptyline also might compete with \(^{[3]}\text{H}\)clonidine for the presynaptic \(\alpha_2\)-adrenoceptor was investigated. Only at high concentrations in vitro did amitriptyline, \(10^{-5}\) M, cause inhibition of the binding of \(^{[3]}\text{H}\)clonidine, \(8 \times 10^{-9}\) M. Binding to membranes prepared from all brain regions was decreased. The degree of inhibition of binding to membranes prepared from the various brain areas was \((n = 4)\): amygdala, \(48 \pm 5\% (P<0.001)\); hippocampus, \(35 \pm 6\% (P<0.001)\); caudate nucleus, \(30 \pm 9\% (P<0.05)\); hypothalamus, \(28 \pm 3\% (P<0.005)\); and locus coeruleus, \(35 \pm 4\% (P<0.01)\). In preliminary experiments Scatchard analyses of the binding of \(^{[3]}\text{H}\)clonidine to membranes isolated from the hippocampus indicate that in the presence of amitriptyline, \(10^{-5}\) M, the \(K_a\) for binding was increased 2.5-fold whereas the \(B_{max}\) was unchanged. Tang and Seeman\(^{14}\) recently reported that lower concentrations of amitriptyline in vitro did not affect the binding of \(^{[3]}\text{H}\)clonidine to membranes isolated from calf frontal cortex homogenates. Thus, the effects of amitriptyline in vitro differ from its effects after long-term administration in vivo in that in vitro the drug produces an inhibition which is competitive rather than one associated with a decrease in the number of binding sites.

Long-term administration of antidepressant drugs has been reported to decrease the number of binding sites for \(^{[3]}\text{H}\)dihydroalprenolol and \(^{125}\text{I}\)iodohydroxybenzyl-pindolol in rat brain membranes\(^{1,8}\). These ligands bind to \(\beta_1\)-receptors in the brain. A recent report suggested that changes in postsynaptic \(\alpha_2\) binding sites are secondary to changes in \(\beta_1\)-receptor sites\(^{8}\). However, several studies indicate that the decreased number of postsynaptic \(\beta_1\)-receptor binding sites seen after long-term antidepressant treatment are secondary to the decreases in the number of presynaptic \(\alpha_2\)-adrenoceptor sites which results in an increased release of noradrenaline. Administration of phenoxybenzamine, an antagonist of the \(\alpha_2\)-adrenoceptor, increases the rate at which desipramine, another tricyclic antidepressant, desensitizes \(\beta\)-receptors in rat cerebral cortex\(^9\). Destruction by 6-hydroxydopamine of the noradrenergic nerve terminals upon which many of the \(\alpha_2\)-adrenoceptors are located prevents the decrease in the number of beta receptors produced by long-term desipramine treatment\(^{11}\). Studies currently in progress in our laboratory indicate that the administration of 6-hydroxydopamine to neonatal rats also prevents the changes in the \(\alpha_2\)-adrenoceptor which are produced by long-term amitriptyline treatment. This observation indicates that the \(\alpha_2\)-adrenoceptors altered by antidepressant drug treatment are located upon adrenergic nerve terminals. Nevertheless, the precise relationship between changes in \(\beta\)-receptor sites and changes in \(\alpha_2\)-receptor sites in the brain remains to be determined.

The present study offers a molecular explanation for the increases in norepinephrine release from adrenergic neurons which occurs after long-term antidepressant
administration. The decrease in the number of presynaptic α2-adrenoreceptors also requires prolonged administration to develop fully. These findings are consistent with several recent studies which involve single cell recordings in the central nervous system and which suggest that a subsensitivity of the presynaptic α2-adrenoreceptor develops after the long-term administration of tricyclic antidepressant drugs. In addition to suggesting that the clinical mechanism of action of tricyclic antidepressant drugs is a loss of presynaptic α2-adrenoreceptors in specific areas of the central nervous system, this study leads one to speculate that endogenous depression might be the result of a supersensitivity (due perhaps to an increased number of presynaptic α2-adrenoreceptors) in the brain.