Receptor Alterations Associated With Serotonergic Agents: An Autoradiographic Analysis

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Controversy exists concerning whether receptor down regulation is involved in the efficacy of antidepressants. Many investigators believe that norepinephrine (NE) receptor down-regulation is more important than serotonin (5-HT) receptor down-regulation. The ability to accurately determine which receptor types or subtypes have been down-regulated has been impaired by the lack of sufficiently specific ligands for labeling these receptor subtypes. Studies that have attempted to examine 5-HT, receptor down-regulation have used [3H]ketanserin as the ligand of choice to label 5 HT2 receptors, but this ligand also labels a nonselective site. The binding of [3H]ketanserin to sites other than 5 HT2 receptors can be examined and controlled for by autoradiographic techniques. The authors briefly review potential problems involved in analyzing receptor binding after antidepressant treatment and present new findings of receptor alterations in rat brain as examined by autoradiographic techniques following chronic exposure to fluoxetine (a selective 5-HT uptake blocker that has been shown to be an effective antidepressant). Laboratory animals injected with fluoxetine showed receptor down-regulation (reduced density) in the serotonergic system. A provocative and potentially important finding of this study is that this selective 5-HT uptake blocker also down-regulates β-adrenergic receptors in the CNS.


Monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and third generation antidepressants (which are unrelated to the tricyclic moiety) have been shown to be efficacious in relieving depressive symptomatology.

MODE OF ACTION OF ANTIDEPRESSANTS

Virtually all of these antidepressant agents are thought to act by increasing the concentration of certain neurotransmitter substances within the synaptic cleft. Two of the biogenic amines thought to be involved in depression are norepinephrine (NE) and serotonin (5-HT). Whereas MAOIs act by reducing the degradation of 5-HT or NE by interfering with monoamine oxidase, TCAs and most third generation antidepressants act by limiting the reuptake of the neurotransmitter after it is released into the synaptic cleft. These drugs are specific for either NE or 5-HT or may affect both. The chronic presence of the neurotransmitter in the synaptic cleft, regardless of the cause, induces a down-regulation of receptors on the postsynaptic membranes. The down-regulation of postsynaptic receptors closely follows the time course of the clinical efficacy of these compounds in relieving depression. This observation led many investigators to suggest that receptor down regulation may be associated with the ability of antidepressants to alleviate depression. The biogenic amine hypothesis of depression indicates that insufficient neurotransmitter substance is available and proposes that antidepressants act by prolonging the presence of the neurotransmitter substance at the terminal membranes. The latter effect, however, is immediate and thus does not correlate well with the time course of clinical efficacy of these compounds.

AUTORADIOGRAPHIC LOCALIZATION OF "RECEPTORS" FOR ANTIDEPRESSANTS

Autoradiographic techniques have been used to show the presence of receptors for TCAs on presynaptic membranes. By microscopically defining the location of these uptake inhibiting receptors, autoradiography allows us to correlate a drug receptor with regions showing nerve terminals containing the associated neurotransmitter. Imipramine, the prototype of the TCAs, is available in tritiated form and can be used to localize imipramine binding sites in the central nervous system. This substance is subsequently degraded in vivo to desmethylimipramine, which is also available in tritiated form and can be used to localize its receptors. Several studies have shown that imipramine is specific for 5 HT terminals and does not overlap onto NE terminals. In contrast, its demethylated metabolite, desmethylimipramine, affects only NE uptake and does not overlap onto 5-HT-containing neurons. Thus, these two compounds can be used to identify which 5-HT or NE-containing nerve terminals are associated with their antidepressant action. In areas where imipramine receptors were localized, the presence of 5-HT-containing nerve terminals have also been established. The reverse, however, was not true; many areas were found in which 5-HT nerve terminals were present in the absence of localizable imipramine binding sites.

The receptors for antidepressants such as imipramine...
may also undergo changes associated with chronic amphetamine exposure. Autoradiographic techniques have demonstrated that imipramine binding sites are dynamic structures. These sites are synthesized in the nerve cell bodies of 5-HT-containing neurons, are transported across place to place along the neuritic membrane, and undergo receptor turnover. Thus, the effectiveness of chronic treatment with tricyclic antidepressants may be limited by the way the cell recognizes the presence of and responds to the therapy.

**DEMONSTRATION OF RECEPTOR ALTERATIONS INDUCED BY ANTIDEPRESSANTS**

Determination of 5-HT receptors in response to the chronic presence of a 5-HT uptake blocker has been done extensively. The newer tricyclic antidepressants that are able to limit the uptake of 5-HT, however, have not been shown in all cases to affect the primary subtypes of 5-HT receptors that have been described. These studies were performed by assay of 5-HT type 1 (5-HT1) and 5-HT type 2 (5-HT2) receptors in brain membrane preparations from chronically treated animals. 15-HT receptors were identified using low concentrations of 5-HT-11 and 5-HT-12, and 5-HT receptors were analyzed using a tritiated form of the drug. 5-HT1A receptors are believed to be involved in the allostasis of the brain, while 5-HT2 receptors are believed to be involved in the allostasis of the brain. The importance of this work is in the demonstration that 5-HT receptors are dynamic structures that can be altered by chronic treatment with tricyclic antidepressants.

**NEW FINDINGS REGARDING RECEPTOR DOWN-REGULATION INDUCED BY FLUOXETINE**

Using the highest resolution available with autoradiographic techniques, we have demonstrated that chronic treatment of fluoxetine up-regulates 5-HT1 and 5-HT2 receptors in rat brain, but only in specific cortical regions (unpublished). Furthermore, an interesting aspect of this study was the demonstration that chronic treatment of fluoxetine down-regulates 5-HT1 receptors in several discrete brain regions. The close association of the serotonergic and neuropeptide systems in depression is well known, and our efforts to support the concept that components of the serotonergic system may indirectly affect the other as well.

**METHOD**

**Tissue Preparation and Labeling**

In vitro autoradiography is performed by labeling tissue sections of brain tissue. The terms of human

| Table 1. Binding Conditions for Autoradiographic Autoradiography |
|--------------|---------------|---------------|----------------|
| Ligand       | Preincubation | Incubation    | Source         |
| 5-HT1 receptor | 5-HT1 receptor | 5-HT1 receptor | Reference       |
| [3H] Imipramine | [3H] Imipramine | [3H] Imipramine | Reference       |

**Autoradiographic Analysis of Receptor Alterations**

**RESULTS**

Localisation of Imipramine Binding Sites

Previous experiments with [3H]-imipramine demonstrated that imipramine binding sites were not associated with any known neurotransmitter receptor type. A highly specific [3H]-imipramine binding was not detectable in the presence of agents such as 5-HT. Autoradiographic localisation of these sites demonstrated that imipramine binding is concentrated in areas such as the hippocampus, anterior thalamic nuclei, CFA of the hypothalamus, lateral and baso medial nuclei of the amygdala, medial thalamic nuclei, subiculum, and dorsal raphe. Immunohistochemical localisation of 5-HT1A receptors within the central nervous system showed a marked overlap with the areas displaying the presence of imipramine binding. However, there was not a non-linear correlation of 5-HT1 receptors with imipramine binding sites. Lesions of the serotonergic system with the monoaminergic neurotoxin 5,7-DHT produced a marked reduction in the serotonin immunoreactivity in all regions included in the study. The results shown were obtained from the brains of prophylaxis-treated rats. In contrast, lesions produced by the monoaminergic agent, serotonin, in the serotonergic system did not result in the reduction of serotonin immunoreactivity. The serotonin neurons that project from the dorsal raphe to the nuclei in different areas was also reduced in the 5,7-DHT-treated rats. The results shown in the present study were obtained from the brains of prophylaxis-treated rats. In contrast, the results shown were obtained from the brains of rats treated with 5,7-DHT. The results shown were obtained from the brains of rats treated with 5,7-DHT.
Table 7. Receptor Allosteric Inhibitors by Chronic Fluoxetine Treatment in 10 animals during tissue ADPase activity

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Basal</th>
<th>Fluoxetine</th>
<th>% Change</th>
</tr>
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</table>
| Caudate | 1.5 ± 0.3 | 1.2 ± 0.2 | -20.0%
| Putamen | 2.0 ± 0.4 | 1.6 ± 0.3 | -20.0%


discussion

One data show that concentrations for uptake inhibiting compounds such as imipramine exert in various regions of the brain. The presence of these receptors can be correlated with the presence of the uptake inhibitor containing the serotonergic synapses affected by that particular compound. For instance, receptors for the 5-HT uptake inhibiting compound imipramine can be correlated with the presence of serotoninergic nerve terminals in many regions of the brain. These receptors are of specific transmitters such as 5-HT, 5-HT, and 5-HT, which are important for the regulation of the serotonergic system.

It was noted that there was a simple one-step correlation between the presence of the 5-HT uptake inhibiting compound trazodone and the presence of 5-HT terminals. In all regions where 5-HT uptake inhibiting sites were localized, the presence of 5-HT terminals could also be shown. In contrast, there were many regions where 5-HT terminals were present but did not exhibit significant 5-HT uptake activity associated with them. Thus, it appears that even the presence of a single 5-HT terminal is sufficient to influence the activity of this system. It is possible that the presence of 5-HT terminals could be correlated with the presence of the uptake inhibitor containing the serotonergic synapses affected by that particular compound.

Receptor transport has been identified for the receptors of several neurotransmitters. Presumably, receptor binding sites are present in the cell body, where the appearance of the transport system exists, and they are subsequently transported into the dendritic spines of the cell and, presumably, to the presynaptic receptors, along the axons as well. Many studies have documented that imipramine affects 5-HT uptake activity in several brain areas. The 5-HT uptake inhibiting compound imipramine can be correlated with the presence of the uptake inhibitor containing the serotonergic synapses affected by that particular compound.

In conclusion, it can be concluded that the presence of the 5-HT uptake inhibiting compound imipramine can be correlated with the presence of the uptake inhibitor containing the serotonergic synapses affected by that particular compound. For instance, receptors for the 5-HT uptake inhibiting compound imipramine can be correlated with the presence of serotoninergic nerve terminals in many regions of the brain. These receptors are of specific transmitters such as 5-HT, 5-HT, and 5-HT, which are important for the regulation of the serotonergic system.

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CONCLUSION

The data presented in this paper indicate the importance of studying the drug response of receptor allotropes associated with chronic antidepressant treatment. The study indicates areas of the brain that may be susceptible to clinical efficacy of antidepressant compounds and gives some insights into regions of the brain that may be responsible for depression. Hopefully, these insights will allow us to better understand mechanisms associated with depression and now drugs such as fluoxetine and further, experiments such as those performed to assess future research and developing compounds that may produce associations with depression and reduce side effects through specific drug therapy.

REFERENCES