Chronic Effects of Imipramine and Lithium on Postsynaptic 5-HT_{1A} and 5-HT_{1B} Sites and on Presynaptic 5-HT_{3} Sites in Rat Brain

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Abstract—The effects of chronic treatment with imipramine, a tricyclic antidepressant, or lithium, an antimanic-depressive illness drug, on postsynaptic serotonin-1A (5-HT_{1A}) and 5-HT_{1B} sites and on presynaptic 5-HT_{3} sites in the frontal cortex and hippocampus from rat brains were studied. Chronic i.p. administration (21 days) of imipramine reduced the maximum number of binding sites (B_{max}) for postsynaptic 5-HT_{1A} as monitored by the radioligands 3H-5-HT or 3H-8-hydroxy-2-(di-n-propylamino)tetralin (3H-8-OH-DPAT), but did not change the B_{max} for postsynaptic 5-HT_{1B} and presynaptic 5-HT_{3} in either the frontal cortex or the hippocampus. Chronic i.p. administration (21 days) of lithium reduced the B_{max} for postsynaptic 5-HT_{1A} sites in the hippocampus, but not in the frontal cortex. There was a specific difference between imipramine and lithium regarding the inhibitory effect on postsynaptic 5-HT_{1A} sites in the frontal cortex. In addition, lithium decreased the affinity of presynaptic 5-HT_{3} sites in the hippocampus. These findings may be also consistent with the inhibitory effect of lithium on presynaptic autoreceptors, which results in an increase of 5-HT release. It is concluded that enhanced 5-HT neurotransmission which develops during chronic treatment with imipramine or lithium seems to be related to the down-regulation of postsynaptic 5-HT_{1A} receptors in addition to postsynaptic 5-HT_{2} receptors, which may also have an important role in the antidepressant effects of these drugs.

Chronic but not acute treatment with tricyclic antidepressants reduced the density of serotonin (5-HT) receptors in rat brain tissues (1, 2). Most tricyclic antidepressants reduced the 5-HT_{2} binding sites in cerebral cortical areas, though they had no effect on 5-HT_{1} binding sites except for imipramine (3). This suggests that the down-regulation of 5-HT_{2} receptors may be more relevant to antidepressant activity.

Long-term administration of lithium, antimanic-depressive illness drug, also induced a significant reduction of 5-HT_{1} and 5-HT_{2} sites in the hippocampus (4), but not in the cerebral cortex (5). Recently, using 3H-5-HT or 3H-8-OH-DPAT (3H-8-hydroxy-2-(di-n-propylamino)tetralin) as the ligand, the postsynaptic 5-HT_{1A} and 5-HT_{1B} sites could be easily distinguished in the cerebral cortex and hippocampus. In addition, presynaptic 5-HT_{3} sites selectively labeled by 3H-8-OH-DPAT were found in the cerebral cortex and striatum (6).

In the present study, we have extended our previous findings with imipramine and lithium and assessed the effects of chronic administration of both drugs on postsynaptic 5-HT_{1A}, 5-HT_{1B} and presynaptic 5-HT_{3} sites in the cerebral cortex and hippocampus.

Materials and Methods

1. Drugs

Drugs were obtained from the following sources: 5-hydroxy[G-3H] tryptamine creatinine sulphate (3H-5-HT, 20 Ci/mmol) and 3H-8-hydroxy-2-(di-n-propylamino)tetralin
(\(^3\)H-8-OH-DPAT, 223 Ci/mmol) from Amer- 
sham; 5-HT, imipramine hydrochloride and 
LiCl from Sigma; and 8-OH-DPAT from 
Research Biochemicals. Other compounds 
were of the highest analytical grade.

2. Animals and drug treatments

Male Wistar rats (230–270 g, Japan Lab. 
Animals) were kept for at least 7 days in a 
controlled environment (22±2°C, 55±5% 
humidity, lights on 7 a.m.—7 p.m., food and 
water ad libitum) before being used. Imi-
pramine (20 mg/kg, i.p.) or LiCl (2 mEq/kg, 
i.p.) were administered once daily at 10 a.m. 
for 21 consecutive days. Control animals 
received an equivalent volume of saline. The 
animals were used 24 hr after the last injection.

3. Tissue preparation for binding assays

Animals were killed by decapitation and 
their brains rapidly removed at 4°C. The hippo-
campus and frontal cortex were dissected 
out according to the method of Glowinski and 
Iversen (7). Crude synaptic membrane frac-
tions and crude mitochondrial P2 fractions 
were prepared according to the method 
described previously (1, 8). The P2 fractions 
were dispersed in 10 volumes (vol./wt.) of 
ice-cold 50 mM Tris-HCl (pH 7.4 at 23°C) 
by using a Polytron® disrupter (PCU-2) for 
10 sec and then incubated at 37 °C for 10 min 
to remove endogenous 5-HT (9). The pellets 
were then collected by centrifugation at 
48,000 g for 10 min and resuspended in final 
assay buffer (50 mM Tris-HCl, pH 7.4–8.2) 
containing 10 μM nialamide to yield a final 
concentration of 300–500 μg protein/ml 
using a Polytron® disrupter for 10 sec. The 
membranes were stored in liquid nitrogen or 
kept deep frozen at -80°C until used.

4. Binding assays

All binding assays were performed by the 
microassay method of Gozlan et al. (6).

4.1. \(^3\)H-5-HT: The measurement of \(^3\)H-5-HT binding to 5-HT\(_{1A}\) sites was performed 
as follows: a 0.8 ml aliquot of membrane 
suspension in 50 mM Tris-HCl (pH 7.4 at 23°C) 
by using a Polytron® disrupter (PCU-2) for 
10 sec and then incubated at 37°C for 10 min 
to remove endogenous 5-HT (9). The pellets 
were then collected by centrifugation at 
48,000 g for 10 min and resuspended in final 
assay buffer (50 mM Tris-HCl, pH 7.4–8.2) 
containing 10 μM nialamide to yield a final 
concentration of 300–500 μg protein/ml 
using a Polytron® disrupter for 10 sec. The 
membranes were stored in liquid nitrogen or 
kept deep frozen at -80°C until used.

4.2. \(^3\)H-8-OH-DPAT: The measurement of 
\(^3\)H-8-OH-DPAT binding to postsynaptic 5-
HT\(_{1A}\) sites was performed as follows: a 0.8 ml 
aliquot of membrane suspension was in-
cubated for 10 min at 37°C in 50 mM Tris-
HCl plus 0.1% ascorbate, pH 7.4, containing 
0.1 ml of \(^3\)H-8-OH-DPAT solution (0.1–50 
μM). The reaction was stopped by the ad-
dition of 5 ml of ice-cold Tris-HCl buffer. 
They were then rapidly vacuum-filtered 
through Whatman GF/B filters and rinsed 2 
times with 5 ml of the same buffer. The filters 
were placed in vials with 5 ml Univer-gel® 
(Nakarai) scintillation cocktail. The radio-
activity in the filter was determined by a liquid 
scintillation spectrometer at an efficiency of 
40–50%. Non-specific binding was defined 
by adding 10 μM 5-HT. For the measure-
ment of \(^3\)H-8-OH-DPAT binding to pre-
synaptic 5-HT\(_3\) sites, membranes were sus-
pended in 0.8 ml of 50 mM Tris-HCl plus 0.1% 
ascorbate, pH 7.4, containing 1 mM N-ethyl-
maleimide (NEM) to inactivate postsynaptic 
sites, and 0.1 ml of \(^3\)H-8-OH-DPAT (1–50 
μM) (6). Samples were incubated for 10 min 
at 37°C. The subsequent procedures were 
carried out as described for \(^3\)H-8-OH-DPAT. 
Every determination of binding was performed 
in triplicate. Protein was assayed by the Lowry 
method (10).

5. Calculations

Data from the saturation experiments 
(Scatchard) were evaluated by non-linear 
computer-assisted curve fitting. Control 
and treated rats were compared using a two-
tailed Student’s t-test.
Results

The effects of imipramine and lithium on the various classes of 5-HT binding sites, postsynaptic 5-HT_{1A} and 5-HT_{1B} sites and presynaptic 5-HT_{3} sites, in the frontal cortex and hippocampus are shown in Table 1. Long-term administration of imipramine (20 mg/kg for 3 weeks) decreased significantly $B_{\text{max}}$ values for postsynaptic 5-HT_{1A+B} sites labeled with $^3$H-5-HT and postsynaptic 5-HT_{1A} sites labeled with $^3$H-5-HT or $^3$H-8-OH-DPAT in the frontal cortex and hippocampus, but there was no change in $K_d$ values, as compared to saline-treated rats (Fig. 1). Imipramine treatment affected neither the $B_{\text{max}}$ nor the $K_d$ values for postsynaptic 5-HT_{1B} sites labeled with $^3$H-5-HT and presynaptic 5-HT_{3} sites labeled with $^3$H-5-HT and $^3$H-8-OH-DPAT containing NEM.

On the other hand, long-term administration of lithium (2 mEq/kg for 3 weeks, 0.8±0.2 mEq/l serum) decreased significantly $B_{\text{max}}$ values for postsynaptic 5-HT_{1A+B} sites labeled with $^3$H-5-HT and postsynaptic 5-HT_{1A} sites labeled with $^3$H-5-HT and $^3$H-8-OH-DPAT, but did not alter $K_d$ values in the hippocampus. Lithium did not show any effect on postsynaptic 5-HT_{1B} sites, but increased $K_d$ values for presynaptic 5-HT_{3} in the hippocampus (Fig. 2). In the frontal cortex, there was no effect of lithium on postsynaptic 5-HT_{1A}, 5-HT_{1B} and presynaptic 5-HT_{3} sites.

Fig. 1. Scatchard plot of $^3$H-8-OH-DPAT binding to postsynaptic 5-HT_{1A} sites in the frontal cortex (A) and hippocampus (B) from rats treated with saline (C), imipramine (●) or lithium (▲) for 3 weeks. Each point represents the mean of 5 experiments, each assayed in triplicate.

Fig. 2. Scatchard plot of $^3$H-8-OH-DPAT binding to presynaptic 5-HT_{3} sites in the frontal cortex (A) and hippocampus (B) from rats treated with saline (C), imipramine (●) or lithium (▲) for 3 weeks. Each point represents the mean of 5 experiments, each assayed in triplicate.
Table 1. Effects of long-term administration of imipramine or lithium on the characteristics of 5-HT binding sites in the frontal cortex and hippocampus from rat brains

<table>
<thead>
<tr>
<th></th>
<th>Postsynaptic</th>
<th></th>
<th></th>
<th>Presynaptic</th>
<th></th>
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<tr>
<td></td>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt; (H-5-HT)</td>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt; (H-5-HT)</td>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt; (H-8-OH-DPAT)</td>
<td>5-HT&lt;sub&gt;1B&lt;/sub&gt; (H-5-HT)</td>
<td>5-HT&lt;sub&gt;6&lt;/sub&gt; (H-8-OH-DPAT)</td>
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<tr>
<td></td>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
<td>B&lt;sub&gt;max&lt;/sub&gt;</td>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
<td>B&lt;sub&gt;max&lt;/sub&gt;</td>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
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<tr>
<td>Frontal cortex</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>1.00 ± 0.12</td>
<td>556.2 ± 42.3</td>
<td>6.92 ± 0.91</td>
<td>233.1 ± 15.2</td>
<td>2.84 ± 0.33</td>
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<tr>
<td>Imipramine</td>
<td>1.28 ± 0.17</td>
<td>450.0 ± 40.2</td>
<td>6.73 ± 1.01</td>
<td>144.5 ± 19.3</td>
<td>2.81 ± 0.44</td>
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<td>Lithium</td>
<td>1.16 ± 0.16</td>
<td>526.1 ± 49.2</td>
<td>7.21 ± 0.96</td>
<td>221.3 ± 18.5</td>
<td>2.95 ± 0.33</td>
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<td>Hippocampus</td>
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<td>Saline</td>
<td>0.70 ± 0.11</td>
<td>760.3 ± 52.2</td>
<td>3.78 ± 0.51</td>
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<td>Imipramine</td>
<td>0.79 ± 0.12</td>
<td>610.2 ± 55.6</td>
<td>3.90 ± 0.42</td>
<td>245.2 ± 26.2</td>
<td>1.36 ± 0.12</td>
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<td>Lithium</td>
<td>0.86 ± 0.13</td>
<td>685.3 ± 58.9</td>
<td>3.62 ± 0.45</td>
<td>283.8 ± 23.1</td>
<td>1.49 ± 0.13</td>
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</table>

Each value of K<sub>d</sub> (in nM) or B<sub>max</sub> (in fmol/mg protein) is the mean ± S.E.M. of 5 separate determinations in each group. *Significantly different from saline-treated rats at P < 0.05.
Discussion

We previously reported that the long-term administration of several tricyclic antidepressants produced a significant decrease in the $B_{max}$ value for 5-HT binding sites in rat whole brains (1). Chronic treatment with imipramine, one of the typical tricyclic antidepressants, was found to reduce the $B_{max}$ value for 5-HT binding sites in the frontal cortex, even though most other tricyclic antidepressants had no effect on 5-HT sites (3). The present study showed that imipramine decreased the $B_{max}$ value for postsynaptic 5-HT$_{1A}$ sites monitored by binding of the radioligand $^3$H-5-HT or $^3$H-8-OH-DPAT but did not change the $B_{max}$ for postsynaptic 5-HT$_{1B}$ sites in both the frontal cortex and hippocampus. The 5-HT$_{1A}$ sites selectively labeled by $^3$H-8-OH-DPAT in the hippocampus (11-14) are located in the postsynaptic membranes, and they are present in both the postsynaptic membranes and the pre- and presynaptic membranes in the cerebral cortex (15). Since no reduction of $^3$H-5-HT or $^3$H-8-OH-DPAT binding was detected following 5-HT selective degeneration induced by 5,7-dihydroxytryptamine in the cerebral cortex and hippocampus, it can be concluded that the 5-HT autoreceptors controlling the presynaptic 5-HT release correspond neither to 5-HT$_{1A}$ nor to 5-HT$_{1B}$ sites (16, 17). It was reported that the 5-HT$_{1A}$ sites link to adenylate cyclase in the hippocampus (18, 19). Therefore, the down-regulation of 5-HT$_{1A}$ sites induced by the long-term administration of imipramine may result in a decrease in 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. The relationship between the 5-HT$_{1A}$ sites and the GTP binding protein (Gs and/or Gi) coupled with adenylate cyclase deserves further investigation.

The effects of long-term lithium administration on pre- and postsynaptic processes involving 5-HT have been measured in rat hippocampus and cerebral cortex; results indicate that there is an increase of 5-HT release and a reduction of the $B_{max}$ value for 5-HT$_1$ and/or 5-HT$_2$ in the hippocampus, but not in the cerebral cortex (5, 20). Our data indicate that chronic administration of lithium to rats decreased the $B_{max}$ value for 5-HT$_{1A}$ sites labeled with $^3$H-5-HT or $^3$H-8-OH-DPAT in the hippocampus, but not in the frontal cortex. From the results that lithium caused the down-regulation of 5-HT$_{1A}$ sites, lithium as well as imipramine appears to take part in 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes. Since there has been evidence that chronic treatment with lithium enhances the 5-HT stimulated adenylate cyclase activity in the postsynaptic membranes.

Acknowledgment: We gratefully acknowledge the excellent technical assistance of Miss Chika Imai.
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