Repeated Treatment with Levoprotiline, a Novel Antidepressant, Up-Regulates Histamine H₁ Receptors and Phosphoinositide Hydrolysis Response In Vivo

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ABSTRACT—The effects of repeated administration of levoprotiline, a novel type of tetracyclic antidepressant on histamine H₁, muscarinic acetylcholine and α₁-adrenergic receptors and the response of phosphoinositide hydrolysis (PI) stimulated by histamine in the cortex of the rat brain were investigated. Histamine H₁ receptors were up-regulated to 120% and PI response stimulated by histamine was enhanced to 160%-200% after repeated treatment with levoprotiline (20 mg/kg, i.p., once a day for 28 days) when compared to that of the saline-treated group. No significant alterations of muscarinic acetylcholine and α₁-adrenergic receptors were observed. This demonstrates that the repeated treatment with levoprotiline has prominent action on the regulation of histamine H₁ receptors and PI response coupling to histamine H₁ receptors in vivo.

Keywords: Levoprotiline, Histamine H₁ receptor, PI response, Cerebral cortex (rat), Antidepressant

A characteristic feature of antidepressant therapy is a delay in the onset of clinical efficacy, about 10–14 days being needed for standard antidepressants to bring about a clinical response (1). To explain this delay, it is postulated that receptor regulation changes during this lag phase, the onset of clinical efficacy of antidepressants only appearing after receptor modulation. There are many reports on the mechanism of receptor regulation including a down regulation of β-adrenergic (2), serotonin 5-HT₂ (3) and dopamine DA₁ (4) receptors which appears after repeated treatment with antidepressants. Such alterations in receptor regulations lead to a change in relevant post-receptor signal transduction systems such as in protein kinase C via the phosphoinositide hydrolysis (PI) response and in protein kinase A via the adenylate cyclase system. Conn and Sanders-Bush (5) showed that repeated treatment with mianserin decreases the number of 5-HT₂ receptors and suppresses PI response stimulated by 5-HT.

Levoprotiline (R-1-(2-hydroxy-3-methylaminopropyl)-dibenzo[b,e]bicyclo[2,2,2]octadiene hydrochloride) (Fig. 1), is an R(−)-enantiomer of oxaprotiline, a hydroxylated derivative of the tetracyclic antidepressant compound maprotiline, and it has been shown to be an active antidepressant in clinical studies (6). Biochemical studies on levoprotiline do not show any remarkable action on the central monoaminergic systems (7). In this present study, the effect of repeated administration of levoprotiline on the regulation of histamine H₁, muscarinic acetylcholine (mACh) and α₁-adrenergic receptors and the post-receptor signal transduction system were investigated.

Fig. 1. Chemical structure of levoprotiline (R-1-(2-hydroxy-3-methylaminopropyl)dibenzo[b,e]bicyclo[2,2,2]octadiene).
MATERIALS AND METHODS

Animals
Male Wistar rats (SLC, Shizuoka, Japan) weighing 100–150 g at the beginning of the treatment were used, and they were kept under standard laboratory temperatures (20–26°C) and humidity (40–60%) with free access to food and water.

Drugs
Levoprotiline was dissolved in distilled water and diluted to the appropriate concentrations for use and administered p.o. at a dose of 20 mg/kg, once a day for 28 days. Physiological saline was administered as a control.

Receptor binding assay
Rats were decapitated 24 hr after the last drug administration, and their cerebral cortices were dissected under dry ice and stored at −80°C until assay. Rat cortex membrane samples were prepared by homogenization for 20 sec (set 9) with a Polytron homogenizer and were subjected to \[^{3}H\]mepyramine binding (histamine H\textsubscript{1} receptor) (8), \[^{3}H\]quinuclidinyl benzilate (QNB) binding (mACh) (8) and \[^{3}H\]prazosin binding (α\textsubscript{1}-adrenergic receptor) (9). Briefly, the membrane preparations were added to the test tube containing various concentrations of \[^{3}H\]mepyramine, \[^{3}H\]QNB or \[^{3}H\]prazosin and drugs in the Tris buffer, with a final volume of 0.6 ml. The assay tubes were incubated for 30 min at 25°C for \[^{3}H\]mepyramine binding, 60 min at 25°C for \[^{3}H\]QNB binding and 40 min at 25°C for \[^{3}H\]prazosin binding. The reaction was terminated by rapid vacuum filtration through Whatman GF/B filters, which were rinsed immediately with 20 ml of ice cold Tris buffer. Radioactivity retained on the filters was measured with a scintillation counter (Beckman LC2000). Nonspecific bindings were determined in the presence of 1 μM triprolidine for \[^{3}H\]mepyramine binding, 0.3 μM atropine for \[^{3}H\]QNB binding and 1 μM phentolamine for \[^{3}H\]prazosin binding. Protein was assayed by the method of Lowry et al. (10).

PI response
Accumulation of \[^{3}H\]inositol 1-phosphate in slices from the cerebral cortex were measured essentially as described by Berridge et al. (11). In brief, cross chopped tissue slices (350 × 350 μm), prepared with a McIlwain tissue chopper, were washed and preincubated at 37°C for 30 min in Krebs-Ringer solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO\textsubscript{4}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4}, 25 mM NaHCO\textsubscript{3}, 1.3 mM CaCl\textsubscript{2}, and 11.7 mM d-glucose) and gently aerated with O\textsubscript{2}/CO\textsubscript{2} (95:5 vol./vol.). Cortical slices were washed twice and transferred to micro test tubes and allowed to settle under gravity. Portions (50 μl) of these slices were added to 230 μl Krebs-Riger solution, containing 0.3 μM myo-[2-\[^{3}H\]inositol (1 μCi per incubation) and 10 mM LiCl, in the micro test tube. The mixture was incubated at 37°C for 60 min by agitation in a water bath before the addition of 10 μl of histamine (final concentration, 1 mM). After incubation for a further 60 min, the reaction was terminated by the addition of 600 μl perchloric acid (4%; vol./vol.) and cooled under ice for 20 min. Slices were separated by centrifugation at 15,000 rpm for 5 min, dissolved in 1 N NaOH and stored for protein assay. A portion (500 μl) of the supernatant was neutralized with 5 N KOH and subjected to a further centrifugation at 15,000 rpm for 5 min. The resulting supernatant (500 μl) was diluted to 2.0 ml with distilled water (water soluble extract).

The water soluble extract was applied to columns containing 1 ml of Dowex AG 1 × 8 (formate form; Bio Rad Laboratories, U.S.A.) and eluted with 1) distilled water, 2) 5 mM-univalent tetraborate/60 mM-sodium formate, 3) 0.1 M-formic acid/0.2 M ammonium formate, 4) 0.1 M-formic acid/0.4 M ammonium formate, 5) 0.1 M-formic acid/1.0 M ammonium formate. \[^{3}H\]Inositol 1-phosphate was eluted in the fraction of 3 (11). The tritium content of 2 ml of fraction 3) was determined by scintillation counting.

Compounds
Radioactive ligands (specific activity between brackets) were purchased from Du Pont/NEN Research Products (U.S.A.): \[^{3}H\]mepyramine (23.9 Ci/mmol), \[^{3}H\]QNB (43.6 Ci/mmol), \[^{3}H\]prazosin (82.0 Ci/mmol). D-myo-[\[^{3}H\]inositol (81.5 Ci/mmol) was purchased from Amersham, Japan, Ltd. The non-radioactive reagents, atropine hydrochloride, lithium chloride, histamine dihydrochloride, were purchased from Nacalai Tesque Co., Ltd., (Japan); and mepyramine maleate and triprolidine hydrochloride were purchased from Sigma Chemical Co. (U.S.A.).

Statistical analysis
The results were statistically analysed by Student’s t-test and Dunnett’s multiple comparison test.

RESULTS

Receptor binding assay
The results of receptor binding assay on histamine H\textsubscript{1}, mACh and α\textsubscript{1}-adrenergic receptors are shown in Table 1. Specific \[^{3}H\]mepyramine binding to the membrane after repeated treatment with levoprotiline and
saline gave $B_{max}$ values of 98.0 ± 2.3 and 80.6 ± 2.0 fmol/mg protein, respectively (Table 1). The number of $[^{3}H]$mepyramine binding sites increased significantly after repeated administration of levoprotiline. Specific $[^{3}H]$QNB binding to the membrane after repeated treatment with levoprotiline and saline gave $B_{max}$ values of 469.7 ± 8.0 and 461.3 ± 17.8 fmol/mg protein, respectively (Table 1). No significant change was observed in the levoprotiline-treated group. Specific $[^{3}H]$prazosin binding to the membrane after repeated treatment with levoprotiline and saline gave $B_{max}$ values of 103.6 ± 2.8 and 102.0 ± 5.0 fmol/mg protein, respectively (Table 1). No significant change of $[^{3}H]$prazosin binding sites was observed after repeated administration of levoprotiline.

**Effect of levoprotiline on the accumulation of $[^{3}H]$inositol 1-phosphate stimulated by histamine**

It is well-established that Li⁺ amplifies the agonist-dependent PI response in the brain (12). Li⁺ exerts a profound alteration in inositol metabolism by inhibiting the conversion of myo-inositol 1-phosphate into myo-inositol by the enzyme myo-inositol 1-phosphatase (13).

$[^{3}H]$inositol 1-phosphate in the slice was accumulated by incubation with 10 mM LiCl. The basal level of $[^{3}H]$inositol 1-phosphate of saline- and levoprotiline-treated slices were increased to about 180 and 250% by the incubation with LiCl, respectively (Table 2). The effect of repeated treatment with levoprotiline on the accumulation of $[^{3}H]$inositol 1-phosphate stimulated by histamine is also given in Table 2. In the saline-treated group, the accumulation of $[^{3}H]$inositol 1-phosphate was enhanced by 0.1 mM and 1 mM histamine to 126 and 148%, respectively, when compared with the basal level in the presence of 10 mM LiCl. However, in the levoprotiline-treated group, the accumulation of $[^{3}H]$inositol 1-phosphate was enhanced by 0.1 mM and 1 mM histamine to 130 and 200%, respectively. When compared between these two groups, the accumulations of $[^{3}H]$inositol 1-phosphate enhanced by 0.1 mM and 1.0 mM histamine in the levoprotiline-treated group were greater than those in the saline-treated group by 160% (P < 0.05) and 200% (P < 0.01), respectively.

**DISCUSSION**

In the present study, the effects of repeated administration of levoprotiline on the binding sites of $[^{3}H]$mepyramine, $[^{3}H]$QNB and $[^{3}H]$prazosin in the cortex of the rat brain were examined. It is well-known that antidepressants have various pharmacological actions such as cholinergic receptor blocking or $\alpha_{1}$-receptor blocking action (14). Their anticholinergic action may be partly shown by unwanted side effects such as mouth dryness, difficulty of urination and pulsus fre-

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**Table 1. Alterations of specific $[^{3}H]$mepyramine, $[^{3}H]$QNB and $[^{3}H]$prazosin binding sites in rat cerebral cortex after repeated treatment with levoprotiline**

| Binding      | $B_{max}$ (fmol/mg protein) |
|--------------|-----------------------------|
| Treatment    | $[^{3}H]$mepyramine | $[^{3}H]$QNB | $[^{3}H]$prazosin |
| Saline       | 80.60 ± 2.0               | 461.3 ± 17.8 | 102.0 ± 5.0      |
| Levoprotiline| 98.00 ± 2.3*              | 469.7 ± 8.0  | 103.6 ± 2.8      |

Concentration of $[^{3}H]$mepyramine, $[^{3}H]$QNB and $[^{3}H]$prazosin were successively developed from 10 nM to 0.313 nM. Each result represents the mean ± S.E. of $B_{max}$ values of eight rats after Scatchard analysis. Statistically significant difference from the saline-treated group (*P < 0.01, using Student's t-test).

**Table 2. Effect of repeated treatment with levoprotiline on the accumulation of $[^{3}H]$inositol 1-phosphate stimulated by histamine**

| Treatment      | Accumulation of $[^{3}H]$inositol 1-phosphate (DPM/mg protein) | % Saline-treated group |
|----------------|---------------------------------------------------------------|-----------------------|
|                | Saline       | Levoprotiline | Saline-treated group |
| LiCl 0 mM      | 1083.5 ± 34.4 | 1156.7 ± 53.4 | 110                    |
| + His 0.1 mM   | 1117.8 ± 31.1 | 1345.8 ± 28.9 | 120                    |
| + His 1.0 mM   | 1211.5 ± 51.5 | 1458.1 ± 43.9 | 120                    |
| LiCl 10 mM     | 1950.3 ± 82.7 | 2891.8 ± 91.2 | 148                    |
| + His 0.1 mM   | 2457.8 ± 36.5*| 3921.5 ± 82.6***| 160                   |
| + His 1.0 mM   | 2895.5 ± 78.2*| 5783.6 ± 133.4***| 200                   |

Values represent the mean ± S.E. of six determinations. *P < 0.05, **P < 0.01, compared with basal level values in the presence of 10 mM LiCl (Dunnett's multiple comparison test). ***P < 0.05, ****P < 0.01, compared with the values of the saline-treated group (Dunnett's multiple comparison test).
quens (15). The blocking of $\alpha_1$-adrenergic receptors may be a cause of orthostatic hypotension (16). Levoprotiline did not affect the number of $[^3H]$QNB and $[^3H]$prazosin binding sites, while levoprotiline caused a significant increase in the number of $[^3H]$mepyramine binding sites. These results suggest that levoprotiline does not affect the regulation of mACh and $\alpha_1$-adrenergic receptors but affects that of histamine $H_1$ receptors in the cerebral cortex.

Our previous in vitro studies have shown that levoprotiline has a potent affinity for histamine $H_1$ receptors (17) and a potent antihistaminergic action (18) in the central nervous system. The IC$_{50}$ value of levoprotiline in the $[^3H]$mepyramine binding assay was 5.5-fold smaller than that of mepyramine (17). Furthermore, a biochemical study has suggested that levoprotiline has no effect on the pre-synaptic biosynthesis of histamine in the cerebral cortex (19). Therefore, the up-regulation of histamine $H_1$ receptors after repeated treatment with levoprotiline may reflect the compensatory response to its potent histamine $H_1$ blocking action at the postsynapses of the central histaminergic neurons. There are some reports that refer to the relationship between the antihistaminergic action in the central nervous system and the mode of action of antidepressants. Kanof and Greengard (20) reported that the inhibitory action of histamine sensitive adenylate cyclase in cell-free preparations is a common feature of many antidepressants, and Kanba and Richelson (21) reported that many antidepressants are potent blockers of histamine $H_1$ receptors.

A receptor mediated hydrolysis of inositol phospholipids is well-established as a common mechanism for transducing various extracellular signals into the cell (22). For example, when histamine binds to histamine $H_1$ receptors, the phosphoinositide metabolism, the so-called “PI response”, is stimulated through an activation of guanosine triphosphate binding protein and phospholipase C (23). To evaluate the effect of levoprotiline on this post-receptor (second messenger) system, the effect of repeated treatment with levoprotiline on the accumulation of $[^3H]$inositol 1-phosphate stimulated by histamine was also examined. Repeated treatment with levoprotiline enhanced the accumulation of $[^3H]$inositol 1-phosphate stimulated by histamine. This suggests that the repeated treatment with levoprotiline also affected the second messenger system.

In conclusion, the repeated treatment with levoprotiline showed up-regulation of histamine $H_1$ receptors and enhanced PI response in vivo.

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