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The Influences of Reserpine and Imipramine on the 5-HT2 Receptor Binding Site and Its Coupled Second Messenger in Rat Cerebral Cortex

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Abstract

An investigation on the molecular mechanism of depression state, less attention was focused on changes at the intracellular messenger level. In this study the effects of reserpine, a monoamine depletor, and imipramine, an antidepressant, on serotonin-2 (5-HT2) receptor binding and its second messenger system of rat cerebral cortex were studied. The level of inositol 4-monophosphate (IP1) accumulation elicited by 100 μM 5-HT *via* activation of the 5-HT2 receptor on cerebral cortical slices at twelve hours after a single dose of reserpine (2 mg/kg, i.p.) was significantly higher in treated rats, when compared to that of saline-treated rats ; this significant level lasted for at least four days. The level of IP1 accumulation in rat cerebral cortical slices elicited by 100 μM serotonin was higher in the group pretreated with reserpine (0.25 mg/kg/day) sub-chronically for seven days than the group pretreated with normal saline. In the receptor binding study, the maximum binding (B_{max}) of 5-HT2 receptor binding was increased, when compared to the corresponding controls; whereas, the dissociation equilibrium constant (K_d) value of the 5-HT2 receptor was found unchanged in the reserpine treated group. Increases in the sensitivity of phosphoinositol (PI) turnover coupled with the 5-HT2 receptor were also found in the long-term (21 days) low dose (0.1 mg/kg/day) administration of reserpine. However, a long-term administration of imipramine (10 mg/kg/day) reduced the function of the PI turnover coupled with the 5-HT2 receptor. Results obtained from the combined use of reserpine and imipramine demonstrated that this combination was able to antagonize the super-sensitivity of the second messenger responses in 5-HT2 receptor induced by long-term treatment with reserpine.

Long term treatment with reserpine but not imipramine also caused an increase in the B_{max} of the 5-HT2 receptor. This up-regulation of the 5-HT2 receptor by reserpine could be antagonized by imipramine, if a combined treatment was employed. However, this combination of imipramine with an additional phospholipid liposome did not enhance or decrease the imipramine's effect on the 5-HT2 receptor, or on its coupled second messenger level. In summary, reserpine induced up-regulation of the postsynaptic monoamine receptor and its coupled second messenger responses (such as IP1 formation). Imipramine was capable of antagonizing these same events in a depression animal model with reserpine. This study demonstrated the dynamic changes and adaptability of the receptor system, followed by changes in PI turnover. The results provide an explanation at the molecular level for the bases of depression and the role of antidepressant drugs effects on those pathological linking elements.

Key Words: 5-HT2 receptor, antidepressant, imipramine, rat cerebral cortex, reserpine

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Introduction

In the early 1950s, researchers noticed that drugs such as reserpine which decreased monoamine precipitated depression (5), and drugs such as imipramine, which increased monoamine relieved depression (15). The Biogenic Amine Hypothesis has been proposed in the treatment of depression illnesses, which states that depression is caused by the deficiency of monoamines, particularly noradrenaline and serotonin (14). According to this hypothesis, depression can be alleviated by drugs that increase the availability of noradrenaline and serotonin. One method of increasing monoamine levels centers around the action of monoamine oxidase (MAO). The function of MAO is to degrade the neurotransmitters of adrenaline or serotonin. Another method is to increase monoamine involves blocking the process of reuptake. Drugs were developed in the 1959 that blocked reuptake and are still widely used today. These drugs are called tricyclic antidepressants (TCAs), an example of which is imipramine (14, 19).

The Biogenic Amine Hypothesis has been the cornerstone of research on depression for more than half a decade. However, an important fact cannot be explained by the Biogenic Amine Hypothesis. Laboratory tests indicate that antidepressants such as TCAs and MAO inhibitors increase available neurotransmitters quite rapidly, within a matter of hours. Yet, typically, clinical relief takes much longer as a person suffering from depression may not experience significant relief for as long as six to eight weeks (23, 27).

The Biogenic Amine Hypothesis alone cannot explain the delay in the time of onset of the clinical relief of depression of up to six to eight weeks (24). Recall how the body compensates for a deficit or excess of neurotransmitter. Super-sensitivity is a compensatory response of the postsynaptic neuron when too little stimulation is received. The neuron attempts to compensate for the lack of stimulation by increasing receptor responsiveness. Over time, the postsynaptic neuron may also compensate for the lack of stimulation by synthesizing additional receptor sites. This process is known as up-regulation (23).

By increasing the amount of neurotransmitter in the cleft, the responsiveness can be normalized in a process known as desensitization by decreasing receptor sensitivity. Therefore the hypothesis proposes that depression is the result of a pathological alteration (supersensitivity and up-regulation) in receptor sites, which results from too little stimulation by monoamines, *i.e.* a deficiency of noradrenaline and serotonin in the cleft (19).

Early in the 1980's, drugs were introduced to selectively block serotonin reuptake, resulting in more serotonin available in the cleft. These drugs were known as selective serotonin reuptake inhibitors (SSRIs). Unlike the TCAs, which are non-selective, the SSRIs may have fewer serious side effects and are therefore easier for patients to tolerate. The development of SSRIs has led to the Serotonin-only Hypothesis which emphasizes the role of serotonin in depression and downplays noradrenaline (9).

In 1983, the first positive results began to appear: fluoxetine was as effective an antidepressant as the classical tricyclic drugs and, moreover, showed far fewer adverse effects (20). Feighner (9) began talking of a "New Generation of Antidepressants". Between 1984 and 1987 clinical trials with fluoxetine multiplied, and finally, in December 1987, the FDA definitively approved its clinical use, under the trade name of Prozac. Numerous clinical trials subsequently confirmed that the anti-depressive efficacy of fluoxetine and was also confirmed in patients with major depression and melancholia (12).

It is well known that in the depression state of animals or human being, there is a decrease in monoamine levels in certain regions of the brain. Clinical results revealed that the serotoninergic system is more involved than the adrenergic system in this situation, based on the effective of serotonergic uptake inhibitor, fluoxetine. In this study, the possibility of a relationship between the serotonergic receptor of 5-HT2 and its secondary messenger inositol phosphates were investigated at the state of depression.

In addition, a report by Drago *et al.* (7) indicates that the combination of phospholipid liposomes can enhance the effectiveness of imipramine's pharmacological action. It was endeavored to discover whether this enhanced effectiveness of imipramine would be present in the receptor level of 5-HT2 and its second messenger level. In the studies, the effect of reserpine, imipramine, phospholipids on 5-HT2 receptor level, a selective [^{3}H] ligand on ketanserin was used (16). For investigation on the effect of these agents on the second messenger level that coupled to 5-HT2 receptor, a [^{3}H] ligand of inositol was used in the PI turnover (4, 29).

Materials and Methods

Myo-[2-^{3}H] inositol (16.5 Ci/mmole) and [Ethylene-^{3}H]-Ketanserin hydrochloride (60 Ci/mmole) were purchased from New England Nuclear Co. (Boston, MA, U.S.A.). Serotonin creatine sulfate (nonradioactive), bovine serum albumin, tris-(hydroxymethyl) aminomethane, reserpine, imipramine, bovine brain extract (for phospholipids), and lithium chloride were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ketanserin tartrate was acquired from Research Biochemical

Incorporated (Natick, MA, U.S.A). Fetal bovine serum (FBS), Ham's F-10 cultured medium and modified Eagle's medium (MEM) were purchased from Gibco Company (Carlsbad, CA, U.S.A.) Cultured T-75 flaskes and 60 mm petri dishes were obtained from Nunc. Co. (Roskilde, Denmark). Dowex-1 × 8 (100-200 mesh in the formate form) was obtained from BioRad Co. (New York, NY, U.S.A). GF/B filter paper was purchased from Whatman Co. (Sanford, ME, U.S.A.). Coommassie protein assay reagent was obtained from Pierce Co. (New Brighton, MN, U.S.A.). Other chemicals of the highest purity were acquired from commercial sources.

Rats Treated with Normal Saline, Reserpine, and/or Imipramine

Experiments were performed on male Sprague-Dawley rats (150-250 g, obtained from the Experimental Animal Center, National Yang-Ming University, Taipei, Taiwan). Animals were housed in groups of four per cage, with a 12 h light – dark cycle (first light 07:00 h). Food and tap water were available *ad libitum*. After the animals were obtained from animal center, and kept in cages, a one day's adaptation period was given prior to drug treatment.

In a single dose treatment of reserpine, rats were injected with 2 mg/kg by intra-peritoneal (i.p.) route. Treated animals were sacrificed at 6, 12, 24, 48, 96 h and seven days after drug treatment. In rats treated with 0.25 mg/kg/day reserpine for seven days, animals were sacrificed 24-h after the last treatment for further studies.

For the subchronic reserpine treatment study, a group of animals were treated with reserpine 0.1 mg/kg/day by i.p. route for five days. In addition, a second group of animals were treated with 0.1 mg/kg/day, i.p. of reserpine for 21 days, and a third group of animals were treated with 10 mg/kg/day, i.p. of imipramine for 21 days. In a fourth group, the animals were treated with reserpine plus imipramine simultaneously as before.

All experimental procedures in rats described were reviewed and approved by the Institutional Animal Ethics Committee.

Rat Cerebral Cortical Slices for Phosphoinositol (PI) Turnover Study

To study the serotonin stimulated PI turnover in the cerebral cortex region, rats were killed by decapitation and their brains were removed at room temperature. The brain was placed in ice-cold Krebs-Hensleit buffer (KHB; in mM: NaCl 119; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25), and the specific brain region was dissected. The KHB buffer (pH 7.4) was gassed under an atmosphere of O_2/CO_2 (95/5%) for at least 30 min before use in the experiment. Brain slices (350 × 350 μm) were prepared using a tissue chopper. Slices were suspended in KHB and incubated for at least 30 min at 37°C in a shaking water bath. During the periods of incubation, the slices were washed three times with warm (37°C) KHB. Serotonin induced changes in phosphoinositide metabolism were measured by the method of Berridge *et al*. (4) with minor modification.

Measurement of Inositol Phosphate (IP1) Production

5-HT-stimulated PI hydrolysis was determined by monitoring the accumulation of the total [^{3}H]-inositol phosphate ([^{3}H]IP1) in the rat cerebral cortex slices in the presence of 5 mM LiCl, essentially as described by Brown *et al*. (6). Cross-chopped slices (350 × 350 μm) were incubated at 37°C for 60 min in a Krebs Henseleit buffer (KHB) gassed with O_2/CO_2 (95/5%). Fifty microliter aliquots of the slices were then incubated with [^{3}H]myo-inositol (0.3 μCi) for 30 min. After that, serotonin in defined concentrations with 5 mM LiCl was followed and incubated at 37°C for 45 min, the incubations were terminated by the addition of chloroform/methanol (1:2 v/v). After adding more chloroform and water (Brown *et al*., 1984), [^{3}H]-IP1 was separated from the aqueous phase by ion-exchange chromatography using the Dowex-50 resin in the Cl^- form. [^{3}H]-Phospholipids were separated from the chloroform phase by evaporation overnight at room temperature (26, 28). PI hydrolysis was expressed as the ratio [^{3}H]IP1 (dpm) and [^{3}H]-PI (dpm) × 100.

Rat Cerebral Cortex Homogenate Membranes for 5-HT2 Receptor Binding Study

Male rats were decapitated, their brains removed and the cerebral cortices were dissected for membrane preparation. Cerebral cortices were immersed in an ice-cold sucrose solution (320 mM) containing 2 mM EDTA and 5 mM $MgCl_2$ and homogenized using a Potter-Elvenjem glass/Teflon homogenizer. The homogenate was centrifuged at 1,000 × *g* for five min, and the pellet discarded. The supernatant was diluted four-fold with ice-cold Tris buffer (50 mM, pH 7) and centrifuged at 48,000 × *g* for 10 min. The pellet was resuspended in 50 mM Tris/2 mM EDTA/5 mM $MgCl_2$ buffer using a Polytron disrupter, and incubated at 37°C for 10 min. The pellet was resuspended in the same buffer, incubated again (30°C for 40 min) and centrifuged at 48,000 × *g* for 10 min. The final washed pellet was resuspended in assay buffer (50 mM Tris 950/2 mM EDTA/5 mM $MgCl_2$, pH 7.4) to a protein content of about 4 mg/ml. The

homogenate was stored at -30°C prior to use.

Measurement of [^{3}H]Ketanserin Binding to Rat Brain Synaptic Membranes of 5-HT2 Receptor Sites

5-HT2 receptors were labeled with [^{3}H]ketanserin essentially as described by Kendall and Nahorsi (13). Briefly, rat cerebral cortical membranes (about 0.6 mg protein) were incubated for 15 min at 37°C in 1ml of assay buffer (50 mM Tris, pH 7.7 plus 5.7 mM ascorbic acid, 10 µM pargyline and 4 mM $CaCl_2$ containing 1 nM [^{3}H]ketanserin, competing drugs and, when appropriate, serotonin. Incubations were terminated by rapid filtration through glass fibre filters followed by washing with ice cold buffer (50 mM Tris, pH 7). Non-specific binding was determined in the presence of 2 µM methysergide, and specific binding represented about 80% of total binding (*i.e.* total binding substract non-specific binding).

Statistical Analysis

Data were analysed by using one-way ANOVA followed by Dunett's test. Results were expressed as means ± SEM. Differences with $P < 0.05$ were considered significant.

Results

Effect of 5-HT-Induced Phosphoinositol (PI) Turnover in Cerebral Cortex Slices from Rats by a Single High Dose of Reserpine Treatment

Rats were injected singly with reserpine 2 mg/kg, i.p., and the brain removed either at 6, 12, 24, 48, 96 hrs or seven days after injection for further investigation. In preparation for the PI turnover study, 100 µM serotonin was added into the cerebral cortex slices of reserpine or vehicle (saline) treated animals. The 5-HT-induced PI turnover rate was found to be significantly increased in the 12 hrs reserpine treated group, and reached a maximal of 181 ± 12% in the 48 hrs reserpine treated group. The 5-HT-induced PI turnover was 140 ± 12.5% of the basal level at the 48 hrs time point in the saline-treated group. No increase was found in the seven days treated group when compared to that of the saline treated group (Fig. 1).

Effect of 5-HT-Induced PI Turnover in Cerebral Cortex Slices from Rats by Sub-Chronic Reserpine Treatment

Rats were treated with reserpine 0.25 mg/kg/day for 7 days, their brains removed, and the cerebral cortex slices were incubated with different concentrations of serotonin for 60 min. The PI turnover rate was found to be significantly increased in the 10 and 100 µM of serotonin treated group when compared to that of the saline treated rats. The 5-HT-induced PI rate at 100 µM of serotonin was 193 ± 15% in reserpine treated group, whereas, the PI rate was 157 ± 9% in the saline treated group (Fig. 2).

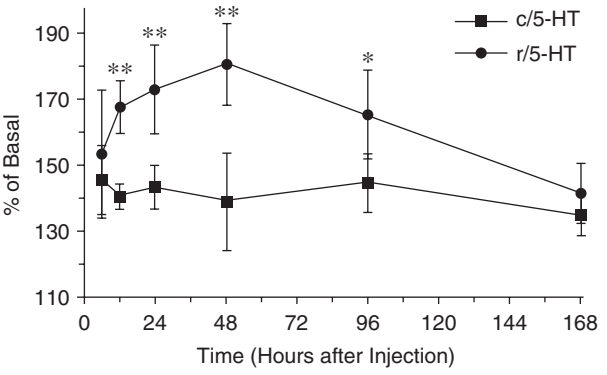

Fig. 1. Effect of a single dose of reserpine (2 mg/kg i.p.) treatment in rats on the level of serotonin (100 µM) stimulated IP1 accumulation/[^{3}H]myo-inositol incorporation in cerebral cortical slices. All values were calculated as a percentage of the basal level (in the absence of serotonin); means ± SEM, N = 4. c/5-HT: Cerebral cortical slices obtained from saline-treated rats and stimulated by serotonin. r/5-HT: Cerebral cortical slices obtained from reserpine-treated rats and stimulated by serotonin. *Significantly different from the saline-treated group ($P < 0.05$). **Significantly different from the saline-treated group ($P < 0.01$).

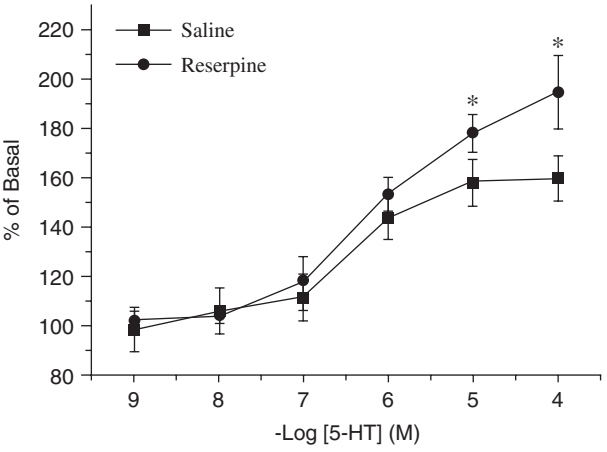

Fig. 2. Dose-response curves of 5-HT induced increase in the levels of IP1 accumulation/[^{3}H]myo-inositol incorporation in cerebral cortical slices from control and reserpine pretreated (0.25 mg/kg/day, for seven days) rats. Results are expressed as means ± SEM of the basal level (in the absence of 5-HT). Experiments were performed four times with triplicates. *Significantly different from that of the saline-treated group ($P < 0.05$).

Table 1. Effect of repeated reserpine treatment (seven days) on the dissociation equilibrium constant (K_d) and the maximal binding site (B_{max}) of specific [^{3}H]ketanserin at homogenate membranes of the rat cerebral cortex

Mode of Treatment	B_{max} (fmol/mg protein)	K_d (nM)
Saline	210.1 ± 21.8	16.95 ± 4.2
Reserpine	309.3 ± 17.9*	15.63 ± 6.3

Values are the means ± SEM of four saline- and four reserpine-treated rats. Specific [^{3}H]ketanserin binding was measured over a concentration range of 0.05-5.0 nM. *$P < 0.01$ compared with saline-treated group.

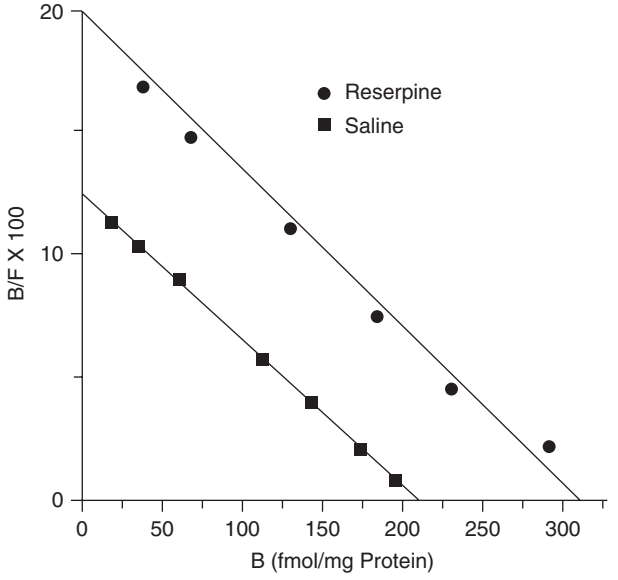

Fig. 3. Scatchard analysis of specific [^{3}H]ketanserin binding in crude synaptic membranes from the cerebral cortex of saline- and reserpine-treated (0.25 mg/kg/day, for seven days) rats. The concentration of [^{3}H]ketanserin used varied from 0.05 to 5.0 nM. Dissociation equilibrium constant (K_d) and maximal number of binding sites (B_{max}) calculated from this figure are given in Table 1.

Effect on [^{3}H]Ketanserin Binding to the Crude Synaptic Membrane of the Cerebral Cortex of Rats by Sub-Chronic Reserpine Treatment

Rats were treated with reserpine 0.25 mg/kg/day for seven days, their brains removed, and the crude synaptic membranes from the cerebral cortex of the rats were prepared for [^{3}H]ketanserin binding study. The binding was measured over a serotonin concentration range from 0.05 to 5.0 nM. The maximal binding B_{max} values of the reserpine treated group was 309.3 ± 17.9 fmole/mg protein, and that of the saline treated group was 210.1 ± 21.8 fmole/mg protein. The K_d value of the reserpine treated group was 15.63 ± 6.3 nM and was 16.95 ± 4.2 nM for the saline treated group (Table 1 and Fig. 3). Therefore, it was found that the B_{max} was significantly increased in the reserpine treated group; whereas there was no significant difference between the K_d values of reserpine and saline treated control groups.

Long Term Treatment of Reserpine, Imipramine Alone or in Combination in Rats for Serotonin Induced PI Turnover Rate of Cerebral Cortex Slices

Rats were treated with saline, reserpine (0.1 mg/kg/day) or imipramine (10 mg/kg/day) alone or in a combination of reserpine (0.1 mg/kg/day and imipramine (10 mg/kg/day) for 21 days. The animals were then sacrificed and cerebral cortex slices were prepared for the serotonin induced PI turnover study. The PI turnover rate was 185.8 ± 11.0% in the reserpine treated group and was 166.4 ± 10.3% in the saline treated group. In a combination of reserpine and imipramine for 21 days, using 100 µM of serotonin resulted in a PI turnover rate of 155.5 ± 11.9% in the group treated with both reserpine and imipramine; whereas the PI turnover rate was 185.8 ± 11.0% in the reserpine treated only group. The combination of reserpine and imipramine resulted in a significant decrease, when compared with reserpine alone (Fig. 4).

Long Term Treatment of Reserpine, and Imipramine Alone or/and in Combination in Rats for [^{3}H]Ketanserin Binding to Synaptic Membranes of the Brains

The rats were treated long term with reserpine or imipramine alone or in combination for 21 days. The B_{max} was found to be significantly increased in the reserpine only treated group, when compared to that of the saline only treated group; whereas no significant differences were found in the K_d of both groups. The B_{max} was found significant decrease in imipramine, when compared to that of saline treated group; whereas no differences were found in the K_d of both groups. The B_{max} of the reserpine plus imipramine group had a significant decrease when compared to that of the reserpine treated group alone. The K_d values of all the studied groups were found to have no significant changes (Table 2).

Table 2. Specific [^{3}H]ketanserin binding at homogenate membranes of cerebral cortex of rats following long term treatment with reserpine, imipramine and its combination

Treatment	B_{max} (fmol/mg Protein)	K_d (nM)
Saline	206.13 ± 23.73	17.04 ± 4.15
Reserpine	314.34 ± 17.90[a]	17.62 ± 6.28
Imipramine	158.44 ± 6.12[b]	14.20 ± 2.62
Rsp., rsp. + Imi.	224.18 ± 17.90[c]	16.93 ± 1.70

Rats were divided into four groups and treated with saline, reserpine (0.1 mg/kg/day) and imipramine (10 mg/kg/day) alone or combination for 21 days, respectively. The rats of the last group were treated with reserpine for five days, then with both reserpine (rsp.) and imipramine (imi.) for 21 days. Results obtained from the Scatchard analysis of each binding experiment and the value are the means ± SEM of six experiments. Differences of B_{max} and K_d in the four groups were evaluated using one-way ANOVA followed by Dunett's test.
a, Significantly different from the saline-treated group ($P < 0.001$); b, Significantly different from the saline-treated group ($P < 0.01$); c, Significantly different from the reserpine-treated group ($P < 0.001$).

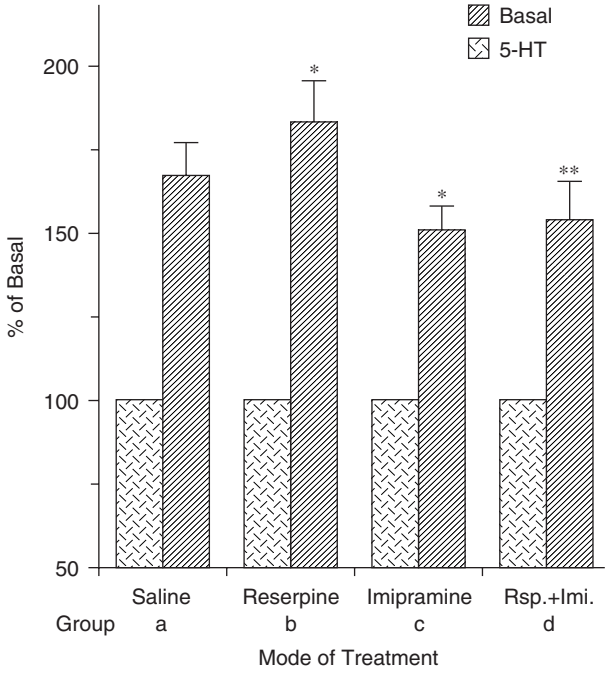

Fig. 4. Effect of serotonin (100 uM)-stimulated PI turnover (increase in the ratio of IP1 accumulation/[^{3}H]myo-inositol incorporation) of cerebral cortical slices from rats treated with normal saline (group a), reserpine (0.1 mg/kg/day, group b), and imipramine (10 mg/kg/day, group c) for 21 days and with reserpine for 5 days first, then, reserpine (rsp.) and imipramine (imi.) for 21 days (group d). Results are the means ± SEM of six independent experiments and are expressed as the percentage of the basal levels (in the absence of serotonin). Basal levels of the group a, b, c, and d are as follows (ratio of IP1/[^{3}H]myo-inositol incorporation): a, 0.195 ± 0.012; b, 0.191 ± 0.05; c, 0.196 ± 0.011; d, 0.209 ± 0.007. *Significantly different from the value of group a/5-HT ($P < 0.05$). **Significantly different from the value of group b/5-HT ($P < 0.01$).

Effect of Phospholipids on the Long Term Treatment of Reserpine and/or Imipramine in Rats on [^{3}H]Ketanserin Binding to the Synaptic Membranes of the Brains

All rats received reserpine injections for five days. The rats were then divided into four groups and labeled with r, i, p and q. The rats of group r were treated with reserpine + vehicle, group i with reserpine + imipramine, group p with reserpine + phospholipids and group q with reserpine + imipramine + phospholipids.; there were two time periods before sacrificing the rats, *i.e.* seven days and 14 days. The dosages of the drugs were as follows: reserpine, 0.1 mg/kg/day; imipramine, 10 mg/kg/day; phospholipids, 15 mg/kg/day. Results were obtained from the Scatchard analysis of each binding experiment. The B_{max} of [^{3}H]ketanserin binding was found to be significantly decreased in the reserpine with imipramine treated animals when compared to the reserpine with vehicle group after 14 days of treatment but not after seven days of treatment. This difference was also found between the group with and without in the presence of phospholipids. There were no significant differences in the B_{max} or K_d values of the four different groups studied at seven days (Table 3).

Effect of Phospholipids on the Long Term Treatment of Reserpine and/or Imipramine in Rats on the Serotonin Induced PI Turnover Rate of the Cerebral Cortical Slices

The rats were treated with reserpine + vehicle (group r), reserpine + imipramine (group i), reserpine + phospholipids (group p) and reserpine + imipramine + phospholipids (group q) for seven days by intra-peritoneal injection. Before the combined

Table 3. Specific [^{3}H]ketansein binding at homogenate membranes of cerebral cortex of rats following long term treatment with reserpine + vehicle, reserpine + imipramine, reserpine + phospholipids, and reserpine + imipramine + phospholipids

A.	7 Days	
Mode of Treatment	B_{max} (fmol/mg Protein)	K_d (nM)
Reserpine + Vehicle (r)	287.6 ± 4.8	16.1 ± 1.6
Reserpine + Imipramine (i)	262.5 ± 17.7	18.7 ± 1.4
Reserpine + Phospholipids (p)	290.9 ± 15.9	19.7 ± 2.8
Reserpine + Imipramine + Phospholipids (q)	256.2 ± 20.0	20.4 ± 5.1
B.	14 Days	
Reserpine + vehicle (r)	302.4 ± 7.7	22.0 ± 1.9
Reserpine + imipramine (i)	242.7 ± 17.7*	23.1 ± 3.2
Reserpine + phospholipids (p)	289.5 ± 13.3	19.0 ± 3.6
Reserpine + imipramine + phospholipids (q)	246.9 ± 16.6*	16.7 ± 3.0

All rats received reserpine injection for five days. After that, they were divided into four groups and labeled with r, i, p, and q. The rats of group r were treated with reserpine + vehicle, group i with reserpine + imipramine, group p with reserpine + phospholipids, and group q with reserpine + imipramine + phospholipids. There were two time periods before scarificing the rats, *i.e.* seven days and 14 days. The dosages of the drugs were as follows: reserpine, 0.1 mg/kg/day; imipramine, 10 mg/kg/day; phospholipids, 15 mg/kg/day. Results obtained from the Scatchard analysis of each binding experiment and the value are the means ± SEM of three independent experiments. Differences in the B_{max} and K_d of the four groups were evaluated using one-way ANOVA followed by Dunnet's test. *Significantly different from the group r ($P < 0.01$).

treatment, all rats received reserpine injections for five days. The dosages of the drugs were as follows: reserpine, 0.1 mg/kg/day; imipramine, 10 mg/kg/day; phospholipids, 15 mg/kg/day. There were no changes in the serotonin-induced PI turnover amongst the four groups studied (Fig. 5).

Effect of Phospholipids on Long Term Treatment of Reserpine and/or Imipramine in Rats on the Serotonin Induced PI Turnover Rate of the Cerebral Cortical Slices

The rats were treated with reserpine + vehicle (group r), reserpine + imipramine (group i), reserpine + phospholipids (group p) and reserpine + imipramine + phospholipids (group q) for 14 days by i.p. injection. Before the combined treatment, all rats received reserpine injection for five days. The dosages of the drugs were as follows: reserpine, 0.1 mg/kg/day; imipramine, 10 mg/kg/day; phospholipids, 15 mg/kg/day. There were significant decreases between the reserpine with vehicle and reserpine with imipramine groups, and between the reserpine with phospholipids and reserpine, imipramine and phospholipids groups at 14 days treatment; these trends were not observed at seven days (Fig. 6). The addition of phospholipids, however, did not result in any significant changes in the observed difference between the combination of reserpine and imipramine under with or without the addition of phospholipids in this study (Fig. 6).

Discussion

Animals treated with reserpine have been used as a model for depression study in pathology and pharmacology (22). In this study, the data showed that when reserpine was administered alone by acute treatment with a single dose (Fig. 1), or chronic application for seven days or 21 days (Fig. 2), IP1 formation induced by serotonin was significantly increased, and 5 HT2 receptor sites were also increased significantly when compared with that of non-reserpine treated animal groups (Fig. 3 and Table 1). Our data revealed that when reserpine, a monoamine depletor, causes the monoamine level to decrease in the synaptic cleft (5), in order to compensate the decrease, there is an increase in the related receptor's numbers to balance its function. As a consequence of this event, the secondary messengers, such as inositol phosphates, production was found to increase. This observation is consistent with the Biogenic Amine Hypothesis and the Receptor Sensitivity Hypothesis which have been used for a long time to explain the cause of depression in animal model and human patients and for finding new drugs

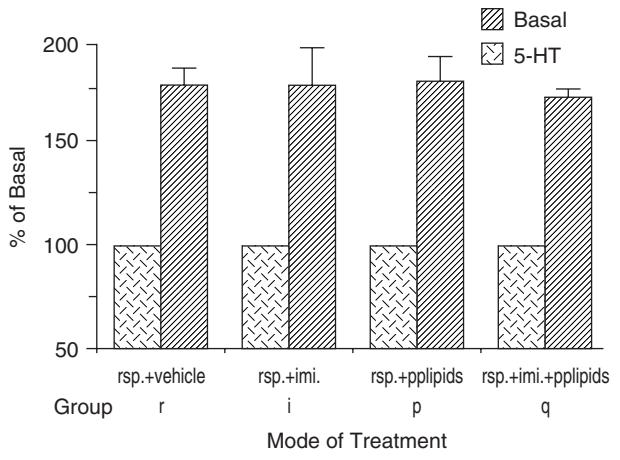

Fig. 5. Effects of serotonin (100 μM)-stimulated PI turnover (an increase in the ratio of IP1 accumulation/[^{3}H]myo-inositol incorporation) of cerebral cortical slices from the rats treated with reserpine + vehicle (group r), reserpine + imipramine (rsp. + imi., group i), reserpine + phospholipids (rsp. + pplipids., group p), and reserpine + imipramine + phospholipids (rep. + imi. + pplipids., group q) for seven days by i.p. injection. Before the combined treatment, all rats received reserpine injections for five days. The dosage of the drugs were as follows: reserpine, 0.1 mg/kg/day; imipramine, 10 mg/kg/day; phospholipids, 15 mg/kg/day. Results are the means ± SEM of three experiments with triplicate and are expressed as the percentage of the basal level (in the absence of serotonin). Basal levels of IP1 accumulation/[^{3}H]myo-inositol incorporation for the four groups are as follows: r, 0.199 ± 0.008; i. 0.195 ± 0.007; p, 0.194 ± 0.008; q, 0.205 ± 0.009.

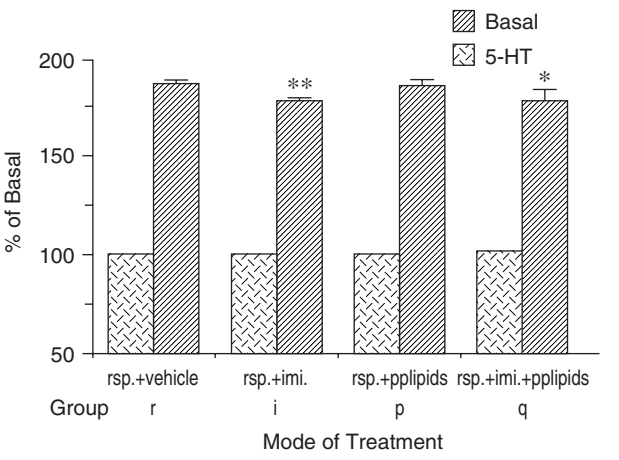

Fig. 6. Effects of serotonin (100 μM)-stimulated PI turnover (an increase in the ratio of IP1 accumulation/[^{3}H]myo-inositol incorporation) of cerebral cortical slices from the rats treated with reserpine + vehicle (group r), reserpine + imipramine (group i), reserpine + phohpholipids (group p), and reserpine + imipramine + phospholipids (group q) for 14 days by i.p. injection. Before the combined treatment, all rats received reserpine injections for five days. Drug doses were as follows: reserpine, 0.1 mg/kg/day; imipramine, 10 mg/kg/day; phospholipids, 15 mg/kg/day. Results are the means ± SEM of three experiments with triplicate and are expressed as the percentage of the basal level (in the absence of serotonin). Basal levels of IP1 accumulation/[^{3}H]myo-inositol incorporation for the four groups are as follows: r, 0.193 ± 0.007; i, 0.210 ± 0.009; p, 0.194 ± 0.002; q, 0.203 ± 0.004. *Significantly different from group r/5-HT ($P < 0.05$). **Significantly different from group r/5-HT ($P < 0.01$).

in the treatment of depression (19).

Imipramine has been used to treat depressant patients for a long time (from 1950 up to now) (19). Imipramine treated animals using 10 mg/kg/day for 21 days caused a significant decrease of the B_{max} of the 5-HT2 receptors without changes to the K_d of the 5-HT2 receptor from the [^{3}H]ketanserin binding study compared to that of saline control and the reserpine treated group (Table 2). In the combination study of reserpine plus imipramine, the increase of the 5-HT2 receptor sites by reserpine alone, was found significantly decreased by the addition of imipramine (Table 2).

In the long term study, reserpine treated animals (0.1 mg/kg/day for 21 days), showed a significant increase in 5-HT induced IP1 formation; whereas in imipramine treated animals (10 mg/kg/day for 21 days), there was a significant decrease in 5-HT induced IP1 formation, when compared to saline treated group. In combination of reserpine plus imipramine treated animals, the increase in 5-HT induced IP1 formation by reserpine alone could be reduced significantly (Fig. 4).

In the long term study of reserpine plus imipramine application, the increases in the 5-HT2 receptor number by reserpine alone could be counterbalanced by the addition of imipramine in the 14 days treatment. However, there was no such change in the seven days treatment (Table 3). Besides, the IP1 production level induced by serotonin was also counter-balanced by imipramine in 14 days, but not in seven days (Fig. 5 and Fig. 6).

Many previous studies pointed out the importance of serotoninergic transmission, 5-HT2 receptor and their relationship with the depression state of patients or animals. For example, Fuxe *et al*. (10) reported that subchronic administration of imipramine-like drugs on depression animal caused a central serotoninergic transmission decrease including changes in serotonin synthesis and 5-HT2 receptor binding sites. Eison *et al*. (8) reported that in *ex vivo* studies, chronic oral administration of Nefazodone (a monoamine uptake inhibitor) produced a decrease in the density of brain cortical 5-HT2 receptor sites in reserpine induced depression rats. Gray and Roth (11) pointed out chronic antidepressant drug treatment

caused down-regulation of 5-HT2A receptors *via* receptor internalization mechanism.

In depression *in vivo* study and treatment, both reserpine (a vesicular monoamine transporter inhibitor) and imipramine (a monoamine uptake inhibitor) have a significant effect on both serotoninergic and adrenergic systems. Many reports showed changes in norepinephrine level and adrenergic β receptor level that might be important to the depressant state of animals and patients (1, 19, 21). In our study, changes in adrenergic β receptor activated cAMP formation in the brain were decreased by long-term administration of imipramine (10 mg/kg/day, i.p. for 21 days) in reserpine-induced depression rats (unpublished observation).

One of the main purposes of this study was to find out if the use of phospholipids could potentiate the effect of imipramine on the 5-HT2 receptor level and/or its secondary messenger IP products after the chronic application of imipramine and phospholipids. Unfortunately, the data obtained failed to show a significant effect on either parameter (Table 3 and Fig. 6). It could be concluded that while the observed potentiation of phospholipids on imipramine's effect was found in the animal model of constrained swimming test by Drago *et al*. (7), the potentiation of imipramine by phospholipids at the 5-HT2 receptor and molecular levels were not found. It is likely that through other molecular mechanisms, such as the adrenergic receptor pathway (21), or even certain testing models, the effectiveness of imipramine plus phospholipids may be observed.

The data collected are in agreement with the previous hypotheses proposed regarding the depression state of animal models and human patients. Monoamine depletion induced by reserpine is one of the methods used for the depressant animal model (22). Monoamine uptake inhibitors, such as imipramine, can increase amine level in the synaptic cleft and improve the depression state. However, the time course of the effectiveness did not match well with its amine level.

Receptor up-regulation by reserpine or down regulation by imipramine treatment is closer to the time course of the effectiveness in the treatment of the depression patients observed clinically. Unlike the changes in the amine level which occurs within hours or days, the time course of the treatment is usually more than two weeks. In this study, the focus was on the serotonin receptor system; however, the adrenergic receptor system, such as beta receptor system may play some role in the depression state.

Many reports reveal that the PI cycle is a second messenger system for numerous neurotransmitters including noradrenaline and serotonin (2). In this study, the secondary messenger of the PI level was found to change following the changes in the receptor and amine levels during the treatment of reserpine and/or imipramine.

Clinically, Barkai *et al*. (3) reported that depressed patients, both unipolar and bipolar, had markedly low levels of inositol in their cerebralspinal fluid (CSF). Levine *et al*. (17, 18) showed that the administration of 12 g/day of inositol for four weeks to depressed patients raised CSF inositol level by 70%. Tondao *et al*. (25) reported that lithium maintenance yielded a striking long-term reduction of depressive as well as manic morbidity in both bipolar disorder subtypes, with greater overall benefits in type II patients and with earlier treatment. Inositol phosphate levels in the neuronal cells have been linked to the mood of the patient as such, the use of lithium has been proposed to the treatment of manic depressive illnesses (30). The mode of action of lithium is proposed as an IP1 phosphatase inhibitor to interfere with PI turnover.

In the future, in addition to those drugs which work on the neurotransmitter and receptor levels, drugs effecting on the secondary messenger level that might have potential for the treatment of depression patients. Chemicals could be designed that have an activity similar to lithium in inhibiting IP1 phosphatase or phospholipase C (2). Such agents may have the therapeutic benefits of lithium, but with less toxicity, on the sodium ion competition effect.

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