Down-Regulation of Central Serotonin S2 Receptors after Repeated Treatment with Quinupramine in Rats

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Abstract—The present studies were undertaken to determine whether the repeated administration of quinupramine caused down- or up-regulation of β-, α2-adrenergic, serotonin S2, imipramine and muscarinic cholinergic receptors, as had been demonstrated for tricyclic and atypical antidepressant drugs. Quinupramine administered at 10 mg/kg (p.o.) twice daily for 10 days caused a down-regulation of serotonin S2 receptors in the frontal cortex of the rat as determined by [3H]-ketanserin binding. However, quinupramine did not alter the binding populations of β-adrenergic, muscarinic cholinergic and α2-adrenergic receptors in the rat brain as determined by the Scatchard analysis of the [3H]ligand binding data. Differing from quinupramine, imipramine caused down-regulation of β-adrenergic and serotonin S2 receptor bindings, and it caused slight but significant up-regulation of muscarinic cholinergic receptor bindings. These results show that the antidepressant activity of quinupramine is associated with the central serotonin system, but not with the β-adrenergic system. Accordingly, quinupramine, chemically one of the typical tricyclic antidepressant drugs, seems to be pharmacologically one of the atypical antidepressant drugs, and it was suggested that the central serotonin system plays an important role in the antidepressant activity of quinupramine.

The catecholamine and serotonin hypotheses on affective disorders were initiated and fortified from experimental and clinical observations. Though many antidepressant drugs, e.g., tricyclics and monoamine oxidase inhibitors, potentiate the action of biogenic amines in the CNS by blocking one of the two major pathways for monoamine inactivation, i.e., metabolism and reuptake, there are a number of antidepressant drugs which do not potentiate the central effects of biogenic amines (1). Moreover, many drugs that have no antidepressant activities inhibit monoamine uptake systems (1). If the blockade of monoamine uptake is a crucial step in relieving the symptoms of depression, it cannot be understood why antidepressant drugs which are monoamine uptake inhibitors must be given for 2 to 3 weeks before their antidepressant effects become evident.

There are additional interactions between antidepressant drugs and monoamine function that have been emphasized for their therapeutic action: (i) an apparent antagonism by many antidepressant drugs on various pre- and post-synaptic receptors and (ii) a down- or up-regulation of various pre- and post-synaptic receptors (1, 2).

We (3) reported that quinupramine possessed high affinity to the central muscarinic cholinergic and histamine H1 receptors. In the present study, in order to obtain additional information concerning the mechanism of the antidepressant activity of quinupramine, an attempt was made to
determine whether quinupramine could cause the down- or up-regulation of the postsynaptic transmitter receptors after repeated administration, as has been suggested for imipramine, which was used for comparison in this study.

Materials and Methods

Animal maintenance and drug treatment: Male Sprague-Dawley rats (220–270 g) from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) were used throughout the experiments. The rats were housed under standard laboratory conditions with water and food ad libitum and light-dark cycles of 12 hr. They received twice daily oral administrations of vehicle, quinupramine (5 or 10 mg/kg) or imipramine (10 mg/kg) for the number of days stated in the Results.

Preparation of tissue samples and radioligand binding studies: After decapitation, brains were rapidly removed and chilled in ice-cold saline. According to the method of Glowinski and Iversen (4), the specific regions were dissected over ice and were homogenized in appropriate volumes of ice-cold 50 mM Tris-HCl buffer with an Ultraturrax® (Janke & Kunkel KG, Staufen, West Germany) at 0°C for 15 sec.

The [3H]dihydroalprenolol binding assay was performed essentially according to the method of Bylund and Snyder (5). Cerebral cortices were homogenized in 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 8.0) and centrifuged at 49,000xg for 15 min at 4°C. The pellet was rehomogenized in the same buffer, centrifuged as above, and the resulting pellet was resuspended in 40 vol. of buffer per gram of original wet weight of tissue. Incubations were done in duplicate and were conducted at 23°C for 20 min. Specific binding of [3H]dihydroalprenolol was defined as the difference between the total binding and the binding observed in the presence of 5 μM propranolol.

The [3H]ketanserin binding was performed in the frontal cortex according to the method of Leysen et al. (6). The homogenates were centrifuged (1,000xg for 10 min and twice 50,000xg for 10 min at 4°C), and the resulting pellet was resuspended in 20 vol. of 50 mM Tris-HCl buffer (pH 7.6). Incubations were conducted at 37°C for 30 min. Specific binding was defined as the difference in radioactivity bound in the absence and presence of 10 μM cinanserin.

The [3H]quinuclidinyl benzilate binding was performed in the whole brain without cerebellum according to the method of Innis et al. (7). The homogenates were centrifuged (twice 50,000xg for 10 min at 4°C), and the resulting pellet was resuspended in 100 vol. of 50 mM Tris-HCl buffer (pH 7.7). Incubations were conducted at 23°C for 60 min. Specific binding was defined as the difference in radioactivity bound in the absence and presence of 1 μM atropine.

The binding assay for [3H]clonidine, [3H]imipramine and [3H]spiperone was performed according to the method of U'Prichard et al. (8), Raisman et al. (9) and Peroutka and Snyder (10), respectively. Specific binding was defined as the difference in radioactivity bound in the absence and presence of 10 μM clonidine for [3H]clonidine, 10 μM desipramine for [3H]imipramine and 10 μM cyprohaptadine for [3H]spiperone binding.

After incubation, labelled membranes were collected and washed (3 times with 5 ml of ice-cold 50 mM Tris-HCl buffer) by rapid filtration under the vacuum through a glass fiber filter (GC-50, 0.5 μm; Toyo Roshi Co., Ltd., Tokyo, Japan) using a filtration manifold. The radioactivity remaining on the filter was counted using a liquid scintillation spectrometer (Mark III, Searle Analytic, Des Plaines IL, U.S.A.) in 8 ml of Biofluor® (New England Nuclear, Boston, MA, U.S.A.). The kinetic parameters (Kd: equilibrium dissociation constant, Bmax: maximal number of binding sites) of the specific binding of the various ligands were analyzed according to Scatchard (11).

Protein amounts: Protein contents of brain membrane preparations were determined by the modified Lowry method (12), using bovine serum albumin as the standard protein.

Statistics: Student's t-test was used for comparing significant differences between the mean values of vehicle- and drug-treated rats.

Drugs and chemicals: [Ethylene-3H]-ketanserin hydrochloride (95 Ci/mmol), [benzene ring-3H]clonidine hydrochloride
J41.5 Ci/mmol) and [N-methyl-3H]imipramine hydrochloride (76.7 Ci/mmol) were purchased from New England Nuclear. 1-[4,6-Propyl-3H]dihydroalprenolol (78 Ci/mmol), 1-quinuclidinyl[phenyl-4-3H]benzilate (35.5 Ci/mmol) and [phenyl-4-3H]spiperone (17 Ci/mmol) were from Amersham International (Buckinghamshire, England). [3H]Ligands were stored at -20°C and diluted in standard assay buffer for each ligand immediately before use.

Chemicals were obtained from the following sources: quinupramine (LM-208, Groupe Pharmuka, Gennevilliers, France and Nippon Shoji Kaisha, Ltd., Osaka, Japan), imipramine hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.), cinanserin hydrochloride (E.R. Squibb & Sons, Princeton, NJ, U.S.A.), propranolol hydrochloride (Nakarai Chemicals, Ltd., Kyoto, Japan), clonidine hydrochloride (Nippon Boehringer Ingelheim, Co. Ltd., Kawanishi, Japan), atropine sulfate (E. Merck, Darmstadt, West Germany), cyproheptadine hydrochloride (Merck Sharp & Dohme, Rahway, NJ, U.S.A.) and desipramine hydrochloride (Ciba-Geigy Japan Ltd., Takarazuka, Japan). Other chemicals of reagent grade were obtained commercially.

**Results**

1. Effect of duration of repeated quinupramine treatment on the β-adrenergic and serotonin S2 receptor binding: In the initial studies, rats received quinupramine at a dose of 5 mg/kg, twice daily by oral administration and were sacrificed at various time intervals. In all cases, rats were sacrificed 24 hr after the final administration, and brain membrane fractions from the frontal cortices were assayed for 0.84 nM [3H]dihydroalprenolol and 0.49 nM [3H]spiperone bindings. We observed no effect of single quinupramine treatment on the specific binding of [3H]dihydroalprenolol and [3H]spiperone. We observed no effect of single quinupramine treatment on the specific binding of [3H]dihydroalprenolol and [3H]spiperone in the rat frontal cortex. Twice daily treatment with quinupramine reduced serotonin S2 receptors labelled by [3H]spiperone in the rat frontal cortex, whereas no effect was observed in β-adrenergic receptors labelled by [3H]dihydroalprenolol (Fig. 1). Statistically significant (P<0.05) decrease in serotonin S2 receptors labelled by [3H]spiperone was apparent as early as 1 week after initiation of treatment. Maximal reduction of about 35% in serotonin S2 receptors labelled by [3H]spiperone was observed after 2 weeks of treatment.

From these results and pharmacokinetic data, (i) the biological half-life of the brain concentrations of quinupramine was about 10 hr, and (ii) steady-state concentrations in the brain were observed 7 days after single daily administration of quinupramine (N. Yokoyama et al., unpublished data); the rats treated twice daily for 10 days (total of 19 administrations) with vehicle or antidepressant drugs (10 mg/kg, p.o.) were used for subsequent experiments.

Since quinupramine and/or imipramine were found to possess substantial affinity for serotonin S2, muscarinic cholinergic receptors and imipramine binding sites (3), rats were...
killed 48 hr after the final administration to prevent the influence of residual drugs in the brain. In other receptor binding studies, all rats were killed 24 hr after the final administration.

$[^{3}H]$Spiperone is a very popular ligand for serotonin S$_2$ receptors, but $[^{3}H]$ketanserin is a far more selective ligand for serotonin S$_2$ receptors than $[^{3}H]$spiperone, which labels dopamine D$_2$ receptors with higher affinity than serotonin S$_2$ receptors (6). Recently, we found that quinupramine possessed higher affinity for $[^{3}H]$ketanserin binding sites than $[^{3}H]$spiperone binding sites, with K$_{1}$-values of 2.0 and 56 nM, respectively (H. Sakamoto et al., unpublished data). Consequently, we used $[^{3}H]$ketanserin as the ligand for serotonin S$_2$ receptors in subsequent studies.

2. Effect of repeated quinupramine treatment on the $\beta$-adrenergic receptor binding in rat cerebral cortex: Figure 2 shows the Scatchard analysis of the specific $[^{3}H]$-dihydroalprenolol binding measured in brain membrane fractions from the cerebral cortices of rats 24 hr after the final administration with vehicle or antidepressant drugs (10 mg/kg, p.o., b.i.d.). Quinupramine, when administered twice daily for 10 days had a slight but not significant effect on $[^{3}H]$-dihydroalprenolol binding. In contrast, imipramine induced a significant decrease in the B$_{\text{max}}$ value without apparent change of the K$_{D}$ value (Table 1).

3. Effect of repeated quinupramine treatment on the serotonin S$_2$ receptor binding in rat frontal cortex: Figure 3 shows the Scatchard analysis of $[^{3}H]$ketanserin specific binding measured in brain membrane fractions prepared from the frontal cortices of rats treated twice daily for 10 days with vehicle or antidepressant drugs (10 mg/kg, p.o.). In such a treatment schedule, quinupramine and imipramine (48 hr after the final administration) induced a significant decrease in the maximum number of binding sites (B$_{\text{max}}$ values) without apparent changes of dissociation constant, K$_{D}$ values (Table 1).

![Fig. 2. Scatchard analysis of specific $[^{3}H]$-dihydroalprenolol ($[^{3}H]$DHA) binding in rat cerebral cortex. Rats were treated for 10 consecutive days with quinupramine (10 mg/kg, p.o., b.i.d.), imipramine (10 mg/kg, p.o., b.i.d.) or vehicle and were killed 24 hr after the final treatment. Each point represents the mean±S.E.M. of 3 animals. Concentrations of $[^{3}H]$dihydroalprenolol between 0.02 and 4.4 nM were used. Receptor density (B$_{\text{max}}$) and dissociation constant (K$_{D}$) were calculated and summarized in Table 1.](image1)

![Fig. 3. Scatchard analysis of specific $[^{3}H]$-ketanserin binding in rat frontal cortex. Rats were treated for 10 consecutive days with quinupramine (10 mg/kg, p.o., b.i.d.), imipramine (10 mg/kg, p.o., b.i.d.) or vehicle and were killed 48 hr after the final treatment. Each point represents the mean±S.E.M. of 3 animals. Concentrations of $[^{3}H]$ketanserin between 0.03 and 3.6 nM were used. Receptor density (B$_{\text{max}}$) and dissociation constant (K$_{D}$) were calculated and summarized in Table 1.](image2)
Table 1. Effects of repeated antidepressant drug treatment on various neurotransmitter receptor bindings in rat brain

| Receptor                | $B_{\text{max}}$ (fmol/mg protein) | $K_D$ (nM) |
|-------------------------|-----------------------------------|------------|
|                         | Control  | Quinupramine | Imipramine | Control  | Quinupramine | Imipramine |
| $\beta$-Adrenergic      | 56.0±0.8 | 50.0±4.2     | 33.6±1.8** | 0.406±0.007 | 0.397±0.041 | 0.336±0.027 |
| Serotonin $S_2$         | 243±3    | 194±16*      | 178±7*     | 0.581±0.035 | 0.591±0.092 | 0.503±0.060 |
| Muscarinic cholinergic  | 981±33   | 1087±44      | 1152±44*   | 0.104±0.003 | 0.101±0.014 | 0.179±0.005*** |
| $\alpha_2$-Adrenergic   | 104±3    | 101±8        | 113±6      | 2.87±0.13   | 3.42±0.39  | 3.42±0.18   |
| Imipramine              | 672±43   | 695±23       | 689±19     | 7.84±0.72   | 7.26±0.54  | 8.07±0.51   |

Rats were administered orally with quinupramine (10 mg/kg, p.o., b.i.d.), imipramine (10 mg/kg, p.o., b.i.d.) or vehicle for 10 consecutive days (total of 19 administrations), and were killed 24 ($\alpha_2$- and $\beta$-adrenergic) or 48 (muscarinic cholinergic, serotonin $S_2$ and imipramine) hr after the final administration. Rat brain membrane fractions were incubated with $[^3H]$dihydroalprenolol in a range from 0.02 to 4.4 nM; $[^3H]$ketanserin, from 0.03 to 3.6 nM; $[^3H]$-quinuclidinyl benzilate, from 0.015 to 2.7 nM; $[^3H]$clonidine, from 0.1 to 27 nM; $[^3H]$imipramine, from 0.08 to 14 nM. Each value represents the mean ±S.E.M. of 3 animals, each performed in duplicate. *$P<0.05$, **$P<0.01$, ***$P<0.005$, when compared to the values obtained from the vehicle control.
ment on the muscarinic cholinergic receptor binding in rat whole brain without cerebellum. Figure 4 shows the Scatchard analysis of \([^{3}H] \text{quinuclidinyl benzilate}\) specific binding measured in brain membrane fractions from the rat whole brain without cerebellum 48 hr after the final administration. Quinupramine, when administered twice daily for 10 days, had a slight but not significant effect on \([^{3}H]\) quinuclidinyl benzilate binding. In contrast, after such a treatment schedule, imipramine induced a significant change in the \(K_D\) value with a slight but significant \((P<0.05)\) change of \(B_{\text{max}}\) value (Table 1).

5. Effect of repeated quinupramine treatment on the \(\alpha\)-adrenergic receptor and imipramine binding in rat brain: Repeated treatment of quinupramine and imipramine did not alter either the \(B_{\text{max}}\) or \(K_D\) values of the binding of \([^{3}H]\) clonidine or \([^{3}H]\) imipramine binding measured in brain membrane fractions from the rat whole brain without cerebellum and cerebral cortex, respectively (Table 1).

Discussion

The results from the present work confirmed the finding of several other studies (2), in that the repeated administration of imipramine caused a down-regulation of \(\beta\)-adrenergic and serotonin \(S_2\) receptor binding in rat brain. In addition, the present study revealed the fact that the repeated administration of quinupramine led to down-regulation in serotonin \(S_2\) receptor binding but not in \(\beta\)-adrenergic receptor binding.

The down-regulation of \(\beta\)-adrenergic receptor binding density may be correlated with the sensitivity of the noradrenaline-coupled adenylate cyclase system (13). However, mianserin, which has little effect on the central monoamine uptake system, fails to alter the central \(\beta\)-adrenergic receptor binding in spite of the fact that this drug induces subsensitivity of the noradrenaline-coupled adenylate cyclase system (14). This suggests that both phenomena are not necessarily interdependent and that the antidepressant drug-induced down-regulation may be affected by an action other than one at the noradrenaline-coupling step (2). Information about the effects of repeated quinupramine treatment on the noradrenaline-coupled adenylate cyclase system are not yet available.

Some of the most commonly used antidepressant drugs, e.g., tricyclics and mianserin, have potent serotonin receptor-blocking properties (15). This lead to the hypothesis that antidepressant drugs may act by reducing serotonin neurotransmission in some areas of the brain by blocking the central serotonin receptor (16). Long-term treatment with antidepressant drugs have been shown to reduce the number of serotonin \(S_2\) receptors labelled by \([^{3}H]\) spiperone in rat brain (2). This change in \([^{3}H]\) spiperone binding in rat brain is, however, difficult to explain on the basis of serotonin \(S_2\) receptor-blocking activity, since a blocking action at a receptor should lead to an up-regulation of the number of transmitter receptors (17). In this paper, we demon-

Fig. 4. Scatchard analysis of specific \([^{3}H]\)-quinuclidinyl benzilate (\([^{3}H]\)QNB) binding in rat whole brain without cerebellum. Rats were treated for 10 consecutive days with quinupramine (10 mg/kg, p.o., b.i.d.), imipramine (10 mg/kg, p.o., b.i.d.) or vehicle and were killed 48 hr after the final treatment. Each point represents the mean±S.E.M. of 3 animals. Concentrations of \([^{3}H]\) quinuclidinyl benzilate between 0.015 and 2.7 nM were used. Receptor density (\(B_{\text{max}}\)) and dissociation constant (\(K_D\)) were calculated and summarized in Table 1.
strated that both imipramine and quinupramine caused a down-regulation of serotonin S2 receptors labelled by [3H]ketanserin. Quinupramine possesses high affinity for serotonin S2 receptor labelled by [3H]-ketanserin in rat frontal cortex, with a \( K_i \) value of 2.0 nM, and its potency for inhibition of serotonin uptake was only one eleventh that of imipramine (H. Sakamoto et al., unpublished data). Further work is required to clarify the mechanisms of reduction of serotonin S2 receptor binding by quinupramine. It is postulated that antidepressant drugs may activate especially presynaptic receptors for comodulator in the serotonin nerve terminals, which subsequentely leads to a change in comodulator release and in postsynaptic comodulator activity, followed by a reduction in the number of serotonin S2 receptors (16, 18).

Decrease in the binding of [3H]ligand may be due to changes in the number of sites \( (B_{\max}) \) or in the dissociation constant \( (K_D) \). The Scatchard analysis of the binding of [3H]dihydroalprenolol or [3H]ketanserin indicated that the antidepressant drugs decrease the number of \( \beta \)-adrenergic or serotonin S2 receptor binding sites without any change in \( K_D \). On the other hand, the repeated treatment of imipramine but not of quinupramine causes a change in \( K_D \) with a change in \( B_{\max} \), and this observation does not agree with those of other investigators (5, 19). It was also reported that many of the anti-muscarinic cholinergic drugs developed tolerance accompanied by a up-regulation of muscarinic cholinergic receptor binding (20). Since quinupramine, one of the antidepressant drugs with a potent anti-muscarinic cholinergic activity, failed to alter the binding of rat brain muscarinic cholinergic receptors, it is unlikely that the change in \( K_D \)-value is due to influence of residual drugs in the brain.

Both quinupramine and imipramine failed to alter the binding populations of \( \alpha_2 \)-adrenergic receptors and imipramine binding sites in rat brain. Our results of the present study in \( \alpha_2 \)-adrenergic receptors confirmed the finding of several other investigators (21, 22). However, other groups reported that after the repeated treatment of anti-depressant drugs, an increase in \( \alpha_2 \)-adrenergic receptor density was observed in the rat cerebral cortex (23, 24). In contrast, according to Campbell and McKernan (25), the repeated administration of imipramine has been observed to cause a decrease in cortical \( \alpha_2 \)-adrenergic receptor binding. Similarly, Chuang et al. (26) reported that the repeated treatment of imipramine for 10 days reduced the binding of [3H]imipramine to the membranes in the hippocampus, but not in the cortex and cerebellum. These results obtained in many studies with long-term drug treatment are dependent upon a number of experimental factors. These include brain regions used, dosage, frequency and duration of administration, and the time interval between cessation of administration and commencement of the experiment. Furthermore, a limitation of all the experiments described above is the use of "normal" rats. Mann and Enna (27) have studied the effect of an atypical antidepressant drug, mianserin, using "muricidal" rats, an animal model commonly used in the screening for antidepressant drugs. They found that the cortical \( \beta \)-adrenergic receptor binding was decreased in muricidal rats in association with extinction of muricidal behavior, but not in the non-muricidal control after treatment for 6 days.

Quinupramine, unlike the other tricyclic antidepressant drugs, is a weak inhibitor of monoamine uptake systems (H. Sakamoto et al., unpublished data) and of [3H]imipramine binding sites (3). Further, quinupramine has been found to posses high affinity for muscaninic cholinergic receptors labelled by [3H]quinuclidinyl benzilate, histamine \( H_1 \) receptors labelled by [3H]pyrilamine (3) and serotonin S2 receptors labelled by [3H]ketanserin (H. Sakamoto et al., unpublished data), with \( K_i \)-values of 2.9, 9.1 and 2.0 nM, respectively, and to possess decreased central serotonin S2 receptor binding without altered \( \beta \)-adrenergic receptor binding after repeated treatment. From these observations, we can conclude that the pharmacological properties of quinupramine, chemically one of the typical tricyclic antidepressant drugs, are close to those of the atypical antidepressant drugs.
and that the central serotonin system plays an important role in the antidepressant activity of quinupramine.

Finally, about the central serotonin system, recent experiments have demonstrated (i) a molecular linkage between serotonin and noradrenaline systems at the level of β-adrenoceptors (28), (ii) an existence of imipramine like substance (endocoid) in rat brain (29) and (iii) a difference of [3H]-mianserin and [3H]ketanserin binding sites (18). It is expected that new approaches can lead to the finding of common mechanisms of action of antidepressant drugs.

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