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# Differential Effects of Subchronic Treatments with Atypical Antipsychotic Drugs on Dopamine $D_2$ and Serotonin 5-$HT_{2A}$ Receptors in the Rat Brain

Ichiro Kusumi, Yoshito Takahashi, Katsuji Suzuki,
Kensuke Kameda and Tsukasa Koyama

Department of Psychiatry, Hokkaido University School of Medicine,
Sapporo, Japan

Running headline: Effect of atypical antipsychotics on $D_2$ and 5-$HT_{2A}$ receptors

Correspondence:
Ichiro Kusumi, M.D., Ph.D.
Department of Psychiatry
Hokkaido University School of Medicine
North 15, West 7, Sapporo 060-8638, Japan
TEL: +81-11-716-1161 ext.5973
FAX: +81-11-736-0956
e-mail: ikusumi@med.hokudai.ac.jp

**Abstract**

The effects of 3-week treatment with a typical antipsychotic drug chlorpromazine and three atypical antipsychotic drugs (risperidone, olanzapine and perospirone) on the binding to dopamine $D_2$ and serotonin 5-$HT_{2A}$ receptors were examined in the rat striatum and frontal cortex, respectively. Subchronic treatment with chlorpromazine (10 mg/kg) and perospirone (1 mg/kg) significantly increased $D_2$ receptors, while no increase was observed with lower dose of chlorpromazine (5 mg/kg), perospirone (0.1 mg/kg), risperidone (0.25, 0.5 mg/kg) or olanzapine (1, 2 mg/kg). On the other hand, 3-week administration of chlorpromazine (5, 10 mg/kg) and olanzapine (1, 2 mg/kg) significantly decreased 5-$HT_{2A}$ receptors, but risperidone (0.25, 0.5 mg/kg) or perospirone (0.1, 1 mg/kg) had no effect. The measurement of *in vivo* drug occupation for $D_2$ and 5-$HT_{2A}$ receptors using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) suggested that high occupation of 5-$HT_{2A}$ receptors with lower $D_2$ receptor occupancy might be involved in the absence of up-regulation of $D_2$ receptors after subchronic treatment with some atypical antipsychotic drugs.

Key Words: Up-regulation, dopamine $D_2$ receptor, atypical antipsychotic drug, serotonin 5-$HT_{2A}$ receptor, in vivo occupation.

## Introduction

Serotonin 5-HT$_{2A}$ receptors are considered to be important in the therapeutical action of clozapine and other putative atypical antipsychotic drugs. A certain group of atypical antipsychotic drugs were characterized by high affinity for 5-HT$_{2A}$ receptors with lower or minimal affinity for dopamine D$_2$ receptors *in vitro* and *in vivo* (Meltzer et al., 1989; Matsubara et al., 1993; Takahashi et al., 1998a; 1998b). These characteristics may be relevant to their weak liability to induce extrapyramidal side effects or tardive dyskinesia, and possible efficacy for the treatment of the negative symptoms of schizophrenia (Kapur and Remington, 1996). It has been indicated that subchronic administration of typical antipsychotics such as haloperidol induces the up-regulation of striatal D$_2$ receptors, whereas that of atypical antipsychotics such as clozapine does not have such an effect (Rupniak et al., 1985; Wilmot and Szczepanik, 1989; O'Dell et al., 1990). We have previously shown that co-administration of 5-HT$_{2A}$ receptor antagonist attenuated the up-regulation of D$_2$ receptors induced by subchronic treatment with haloperidol so long as the *in vivo* occupation of 5-HT$_{2A}$ receptors is higher than that of D$_2$ receptors (Ishikane et al., 1997).

In this study, the effects of 3-week treatment with various 5-HT$_2$/D$_2$ receptor antagonists including both typical and atypical antipsychotics on the binding to D$_2$ and 5-HT$_{2A}$ receptors were examined in the rat striatum and frontal cortex, respectively. We also measured *in vivo* occupation of D$_2$ and 5-HT$_{2A}$ receptors after the administration of these drugs using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ).

## Methods

Male Wistar rats (120-160g, 6 week-old) supplied from Japan SLC Inc. (Shizuoka, Japan) were used for all experiments. For subchronic administration experiments, the test compound (chlorpromazine 5, 10

mg/kg, perospirone 0.1, 1 mg/kg, risperidone 0.25, 0.5 mg/kg and olanzapine 1, 2 mg/kg) or vehicle (0.15% tartaric acid) was administrated intraperitoneally to the rats once a day for 3 weeks. After a complete wash-out period for one week, the rats were sacrificed by decapitation. The doses of test compounds were chosen as corresponding to the clinical dosage. For *in vivo* receptor occupancy measurements, the rats received the test compound or vehicle intraperitoneally 1 hour before EEDQ (8 mg/kg; dissolved in water/ethanol, 1:1, v/v; 4 mg/ml) injection. Twenty-four hours after EEDQ injection, the rats were sacrificed by decapitation. The dissected cerebral cortices and striata were frozen on dry ice and stored at -70$^{o}$C until they were used.

The tissue membranes were prepared, and saturation and *in vivo* occupancy experiments were performed according to the method of Matsubara et al. (1993) and Ishikane et al. (1997). The $D_2$ and 5-$HT_{2A}$ receptors were labeled with [$^3$H]spiperone (19.1 Ci/mmol) and [$^3$H]ketanserin (60.0 Ci/mmol), respectively. Nonspecific binding was determined in the presence of 1 μM (+)butaclamol (for $D_2$) or 2 μM methysergide (for 5-$HT_{2A}$ receptor binding). Six concentrations of [$^3$H]spiperone (0.01-0.32 nM) and [$^3$H]ketanserin (0.1-3.2 nM) were used for saturation experiments. The dissociation constant ($K_d$) and the total number of binding sites ($B_{max}$) were estimated using the non-linear regression program EBDA/LIGAND.

Receptor occupation was estimated by measuring the radioactivity that had escaped EEDQ-induced reduction, and was calculated by the following equation: percent receptor occupation (%) = {(drug/EEDQ) - (EEDQ)}/{(drug)-(EEDQ)} x 100 (Matsubara et al., 1993). Statistical comparisons were made by analysis of variance followed by a Duncan new multiple range test.

## Results

Three-week treatment with chlorpromazine (10 mg/kg) and perospirone (1 mg/kg) significantly increased the number of $D_2$ receptors in the striatum compared to vehicle, while no increase was observed by that with lower dose of chlorpromazine (5 mg/kg), perospirone (0.1 mg/kg), risperidone (0.25, 0.5 mg/kg) or olanzapine (1, 2 mg/kg) (Table 1). On the other hand, subchronic administration of chlorpromazine (5, 10 mg/kg) and olanzapine (1, 2 mg/kg) significantly decreased 5-$HT_{2A}$ receptors in the frontal cortex, but risperidone (0.25, 0.5 mg/kg) or perospirone (0.1, 1 mg/kg) had no effect on the 5-$HT_{2A}$ receptors (Table 2). No significant difference was found in $K_d$ value for both [$^3$H]spiperone and [$^3$H]ketanserin binding among treatment groups (Table 1 and 2).

The *in vivo* drug occupation for $D_2$ and 5-$HT_{2A}$ receptors after acute administration were shown in Table 3. Treatments with low dose of perospirone (0.1 mg/kg), risperidone (0.25, 0.5 mg/kg) and olanzapine (1, 2 mg/kg) showed higher occupation of 5-$HT_{2A}$ receptors than that of $D_2$ receptors. On the other hand, administration of chlorpromazine (5, 10 mg/kg) and higher dose of perospirone (1 mg/kg) exhibited almost same occupancy of 5-$HT_{2A}$ and $D_2$ receptors.

## Discussion

The present results suggest that high occupation of 5-$HT_{2A}$ receptors *in vivo* with lower occupation of $D_2$ receptors may be involved in the absense of up-regulation of $D_2$ receptors after subchronic treatment with some atypical antipsychotic drugs. This finding is in accordance with our earlier report of combined treatment with a $D_2$ receptor antagonist haloperidol and a 5-$HT_{2A/2C}$ receptor antagonist ritanserin (Ishikane et al., 1997). When both $D_2$ and 5-$HT_{2A}$ receptors were highly occupied (i.e. more than 60%) as treatment with 10 mg/kg of chlorpromazine or 1 mg/kg of perospirone,

$D_2$ receptors were up-regulated after subchronic treatment. However, the administration of 5 mg/kg chlorpromazine, which occupied about 40% of both $D_2$ and 5-$HT_{2A}$ receptors, did not induce $D_2$ receptor up-regulation. It is possible that striatal $D_2$ receptors may be up-regulated only when $D_2$ receptor occupancy is high (i.e. more than 60%) independent of cortical 5-$HT_{2A}$ receptor occupancy. But this possibility is incompatible with our previous result that the occupations of $D_2$ receptors are 44.3% and 68.0% by 0.1 mg/kg and 0.5 mg/kg of haloperidol, respectively, both of which induce $D_2$ receptor up-regulation. We have previously shown that subchronic administration of clozapine (10 mg/kg) or ORG 5222 (0.25 mg/kg) had no effect on striatal $D_2$ receptors. *In vivo* occupancy of $D_2$ and 5-$HT_{2A}$ receptors were 10.9% and 50.5% for 10 mg/kg of clozapine, and 43.8% and 83.7% for 0.25 mg/kg of ORG 5222, respectively (Ishikane et al., 1997). Therefore, these findings suggest that higher *in vivo* occupation of 5-$HT_{2A}$ receptors than that of $D_2$ receptors may contribute to their unique properties of some atypical antipsychotics. Moreover, in this study, we examined the effect of dose of various drugs on $D_2$ receptor up-regulation. The dose of each antipsychotic agent indeed influenced the occupancy of both $D_2$ and 5-$HT_{2A}$ receptors as shown in Table 3. Thus, whether subchronic treatment with the antipsychotic agent induces $D_2$ receptor up-regulation may depend on not only what the drug is, but the dose of the drug. These results suggest that the setting of clinical dose of a 5-$HT_2$/$D_2$ receptor antagonist may be very important for atypical antipsychotic actions.

The precise mechanism by which 5-$HT_{2A}$ receptor antagonism may attenuate the $D_2$ receptor up-regulation is uncertain from the present data. One possible examination may be that 5-$HT_{2A}$ receptor blockade affects raphe neurons which then influence neural dopamine function. It was reported that lesion of the dorsal raphe nucleus resulted in increased

dopamine metabolites in the substantia nigra, suggesting a tonically active inhibition of dopamine neurons from dorsal raphe (Dray et al., 1976; Nicolaou et al., 1979). Similarly, acute 5-HT$_{2A}$ antagonist treatment enhanced the activity of nigrostriatal dopamine-containing neurons (Ugedo et al., 1989), presumably resulting in enhanced dopamine release and receptor stimulation (Saller et al., 1990). Dopamine agonist administration was reported to attenuate haloperidol-induced D$_2$ receptor supersensitivity (List and Seeman, 1979).

On the other hand, it is not known from our data why risperidone and perospirone which are potent 5-HT$_{2A}$ receptor antagonists both *in vitro* and *in vivo*, do not induce 5-HT$_{2A}$ receptor down-regulation in the frontal cortex. Only *in vivo* occupancy of D$_2$ and 5-HT$_{2A}$ receptors cannot explain these mechanisms. Moreover, it does not appear that extracellular 5-HT level is directly related to the 5-HT$_{2A}$ receptor down-regulation. Ichikawa et al. (1998) have reported that risperidone (1 mg/kg) and clozapine (20 mg/kg) significantly increased extracellular 5-HT concentrations in the rat medial frontal cortex and nucleus accumbens but that olanzapine (1 and 10 mg/kg), MDL-100,907 (1 mg/kg) , a selective 5-HT$_{2A}$ receptor antagonist, or haloperidol (0.1 and 1 mg/kg) had no significant effect in either region. Hertel et al. (1997) also indicated that risperidone (0.2, 0.6 and 2 mg/kg) dose-dependently increased extracellular 5-HT levels in the rat frontal cortex, which may be related to its alpha$_2$-adrenoceptor antagonistic action. Matsubara et al. (1989) reported the down-regulation of 5-HT$_2$ receptors following a single injection of clozapine (20 mg/kg). However, this rapid down-regulation of 5-HT$_2$ receptor sites is characteristic of some but not all atypical antipsychotic drugs. They have concluded that dibenzo-epines such as clozapine, loxapine, amoxapine and chlothiapine consistantly down-regulated 5-HT$_2$ receptors in frontal cortex after acute treatment. Kuoppamaeki et al. (1995) reported no change of 5-HT$_{2A}$ receptors after 2-

week treatment with risperidone (0.3 mg/kg) and also pointed the possibility that administration of structurally different 5-HT2A antagonists could lead to various 5-HT2A receptor adaptation other than down-regulation.

In this study, 3-week intraperitoneal injection of perospirone (0.1 and 1 mg/kg) did not induce 5-HT2A receptor down-regulation in the rat frontal cortex. However, Ohno et al. (1995) indicated that 2-week oral administration of perospirone (10 mg/kg) significantly reduced the density of 5-HT2A receptors in the same region. In our study, the animals were decapitated one week after the last injection, while the withdrawal of oral treatment was 72 hr in the study of Ohno et al. It has been shown that the 5-HT2A receptor down-regulation is reversed faster after stopping drug treatment than D2 receptor up-regulation (Leysen et al., 1986). Thus, the possibility exists that differences in the treatment protocol or the pharmacokinetics of the test compounds may explain the different results. Moreover, ritanserin has been recently reported to be more effective in lowering 5-HT2A receptor numbers when co-administered with haloperidol than when administered alone (Szczepanik and Wilmot, 1997). This finding suggests a possibility of interaction between D2 and 5-HT2A receptor populations. The mechanisms of 5-HT2A receptor down-regulation are not known from the present study, thus further investigations are needed to elucidate the 5-HT2A receptor adaptation after subchronic administration of 5-HT2A antagonists.

In conclusion, our study indicates that high *in vivo* occupation of 5-HT2A receptors with lower occupation of D2 receptors may underlie the absence of striatal D2 receptor up-regulation after the subchronic treatment with some atypical neuroleptics. These characteristics may be relevant to the atypicality for at least a certain group of atypical antipsychotic drugs. Some atypical antipsychotics have high affinity not only for D2 and/or 5-HT2A

receptors, but also for $D_3$, $D_4$, $5HT_6$ and $5HT_7$ receptors (Sokoloff et al., 1990; Van Tol et al., 1991; Roth et al., 1994). Therefore, in future, we should also investigate the interactions between these receptors in order to develop new therapeutic approaches to the treatment of schizophrenia and drug-related side effects.

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Table 1. Effect of long-term treatment with various antipsychotics on dopamine D2 receptors in rat striatum

| | Dopamine D2 receptors | |
|---|---|---|
| | Bmax (fmol/mg tissue) | Kd (pM) |
| Vehicle | 18.0±1.5 | 42.9±3.6 |
| Chlorpromazine (5 mg/kg) | 20.1±1.1 | 43.6±2.4 |
| Chlorpromazine (10 mg/kg) | 22.8±3.2* | 46.0±6.0 |
| Perospirone (0.1 mg/kg) | 17.5±1.3 | 41.5±1.3 |
| Perospirone (1 mg/kg) | 22.6±3.2* | 44.0±5.4 |
| Risperidone (0.25 mg/kg) | 19.2±1.6 | 40.9±1.6 |
| Risperidone (0.5 mg/kg) | 21.2±2.3 | 43.8±5.0 |
| Olanzapine (1 mg/kg) | 20.4±3.5 | 42.8±5.2 |
| Olanzapine (2 mg/kg) | 19.8±1.1 | 43.5±2.8 |

Animals were treated once a day for 3 weeks with vehicle or test compounds, and were decapitated 1 week after the last injection. The total number of binding sites (Bmax) and the dissociation constant (Kd) for [3H]spiperone were estimated from saturation experiments using Scatchard analysis. Each value is the mean ± SEM. * $p<0.05$ vs Vehicle, N= 6

Table 2. Effect of long-term treatment with various antipsychotics on serotonin 5-HT2A receptors in rat cortex

| | Serotonin 5-HT2A receptors | |
|---|---|---|
| | Bmax (fmol/mg tissue) | Kd (pM) |
| Vehicle | 15.2±0.3 | 0.44±0.05 |
| Chlorpromazine (5 mg/kg) | 13.6±0.5* | 0.48±0.02 |
| Chlorpromazine (10 mg/kg) | 13.1±0.9** | 0.48±0.12 |
| Perospirone (0.1 mg/kg) | 15.7±1.5 | 0.46±0.01 |
| Perospirone (1 mg/kg) | 15.7±0.3 | 0.43±0.06 |
| Risperidone (0.25 mg/kg) | 15.7±1.2 | 0.44±0.01 |
| Risperidone (0.5 mg/kg) | 15.5±0.9 | 0.45±0.05 |
| Olanzapine (1 mg/kg) | 12.6±0.4** | 0.43±0.02 |
| Olanzapine (2 mg/kg) | 12.7±1.2** | 0.45±0.03 |

Animals were treated once a day for 3 weeks with vehicle or test compounds, and were decapitated 1 week after the last injection. The total number of binding sites (Bmax) and the dissociation constant (Kd) for [3H]ketanserin were estimated from saturation experiments using Scatchard analysis. Each value is the mean ± SEM.

* $p<0.05$ vs Vehicle, ** $p<0.01$ vs Vehicle, N= 6

Table 3. D2 & 5-HT2A receptor regulations and in vivo drug occupancy for D2 and 5-HT2A receptors

| Treatment | D2 Receptor regulatio | 5-HT2A | In vivo occupancy (%) D2 | 5-HT2A |
|---|---|---|---|---|
| Chlorpromazine (5 mg/kg) | → | ↓ | 37.6 | 42.7 |
| Chlorpromazine (10 mg/kg) | ↑ | ↓ | 61.4 | 63.4 |
| Perospirone (0.1 mg/kg) | → | → | 15.2 | 34.9 |
| Perospirone (1 mg/kg) | ↑ | → | 77.8 | 78.6 |
| Risperidone (0.25 mg/kg) | → | → | 12.4 | 64.6 |
| Risperidone (0.5 mg/kg) | → | → | 38.3 | 80.3 |
| Olanzapine (1 mg/kg) | → | ↓ | 29.4 | 69.7 |
| Olanzapine (2 mg/kg) | → | ↓ | 41.1 | 75.8 |

Animals received test compound or vehicle i.p. 1 hr before EEDQ (8 mg/kg) i.p. and were decapitated 24 hr after the EEDQ injection. Percent receptor occupancy was calculated by the following equation; receptor occupancy (%) = {(drug/EEDQ) - (EEDQ)} / {(drug) - (EEDQ)} x 100

↑: up-regulation, ↓: down-regulation, → : no change, N= 4-8