

*Short Communication***Lack of Enhanced Effect of Antipsychotics Combined With Fluvoxamine on Acetylcholine Release in Rat Prefrontal Cortex**Yukio Ago¹, Maiko Sato¹, Shigeo Nakamura¹, Akemichi Baba², and Toshio Matsuda^{1,3,*}¹Laboratory of Medicinal Pharmacology and ²Laboratory of Molecular Neuropharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan³Department of Experimental Disease Model, The Osaka-Hamamatsu Joint Research Center For Child Mental Development, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan

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Abstract. We have shown that coadministration of sulpiride and fluvoxamine preferentially increases the release of dopamine in the prefrontal cortex. To study the possible role of the cortical cholinergic system in this effect, we combined several other antipsychotic drugs with fluvoxamine and examined the effects on acetylcholine release in rat prefrontal cortex. Risperidone and clozapine significantly increased the release of acetylcholine but sulpiride did not, and fluvoxamine did not enhance the effects of the antipsychotics. These results further support the previous suggestion that the cortical dopamine system plays an important role in the effects of antipsychotic drugs administered in combination with fluvoxamine.

Keywords: antipsychotic, fluvoxamine, acetylcholine release

Selective serotonin reuptake inhibitors (SSRIs) are widely used for the treatment for depression, but about 30% – 50% of patients – those with so-called treatment-resistant depression – do not initially respond to SSRIs (1, 2). Furthermore, up to 40% of patients with schizophrenia respond inadequately to conventional antipsychotics; because their negative symptoms and cognitive impairments are often refractory and persistent, such patients are difficult to treat with antipsychotic drugs (3). Clinical studies show that the combination of an SSRI and a typical or atypical antipsychotic agent is effective in treating monotherapy-resistant depression, refractory negative symptoms, and cognitive dysfunction in schizophrenia (4 – 6). In this regard, neurochemical studies show that the combination of an antipsychotic with an SSRI causes extracellular dopamine, noradrenaline, and serotonin levels to increase in rat prefrontal cortex (7 – 9). We have also shown that the coadministration of a dopamine D₂-receptor antagonist, such as sulpiride or haloperidol, and the SSRI fluvoxamine increases the release of dopamine but not the release of serotonin or noradrenaline in rat prefrontal cortex (10).

In this regard, we further reported that sulpiride augments the antidepressant effect of fluvoxamine (11). These findings suggest that the prefrontal dopaminergic system plays a key role in the effects of combination therapy. It should be noted that the prefrontal cholinergic system, like the dopaminergic system, may play a key role in the ability of atypical antipsychotic drugs in improving faulty cognition and other negative symptoms of schizophrenia (12). However, it is not known whether the cholinergic system is involved in the effect of antipsychotic drugs and SSRIs used in combination. The present study examined the effects of coadministration of antipsychotics and fluvoxamine on *in vivo* acetylcholine (ACh) release in rat prefrontal cortex.

Male Wistar rats weighing 250 to 350 g at the beginning of the experiments were used. The animals were maintained under controlled environmental conditions (22 ± 1°C; 12:12-h light-dark cycle, lighting on at 08:00 h; food and water *ad libitum*) for at least 1 week before use in the experiments. The procedures to handle the animals and their care were conducted according to Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. The following drugs were used: fluvoxamine (Solvay Seiyaku KK, Kawagoe), sulpiride (Fujisawa Pharmaceutical Co., Ltd., Osaka), and risperidone and

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clozapine (Sigma, St. Louis, MO, USA). All other chemicals used were of the highest commercially available purity. Sulpiride and clozapine were dissolved in 0.1 M HCl adjusted to pH 6 to 7 with 0.1 M NaOH. Risperidone was dissolved in saline (0.9% NaCl solution) containing <0.1% v/v acetic acid. Fluvoxamine was dissolved in saline. The drugs were systemically injected at 1 mL/kg.

Each rat was anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and stereotactically implanted with a guide cannula (one site per animal) to accommodate a dialysis probe (Eicom, Kyoto) in the prefrontal cortex (A +3.2 mm, L -0.6 mm, V -5.2 mm, from the bregma and skull), as reported previously (10, 13). The cannula was cemented in place with dental acrylic and the animal

kept warm and allowed to recover from anesthesia. Postoperative analgesia was provided by a single injection of buprenorphine (0.1 mg/kg, i.p.) (13). The active probe membrane was 3-mm-long. At surgery on the following day, the probe was perfused with Ringer's solution (147.2 mM NaCl, 4.0 mM KCl, and 2.2 mM CaCl_2 ; Fuso Pharmaceutical Industries, Ltd., Osaka) containing 10 nM neostigmine at a constant flow rate of 1 $\mu\text{L}/\text{min}$. A stabilization period of 3 h was established before the beginning of the experiments. The 20-min perfusates (20 μL) were taken, and isopropylhomocholine (250 fmol) was added to the samples as an internal standard; then the mixture was immediately injected onto an HPLC column for ACh assay. After the experiments, Evans Blue dye was microinjected through

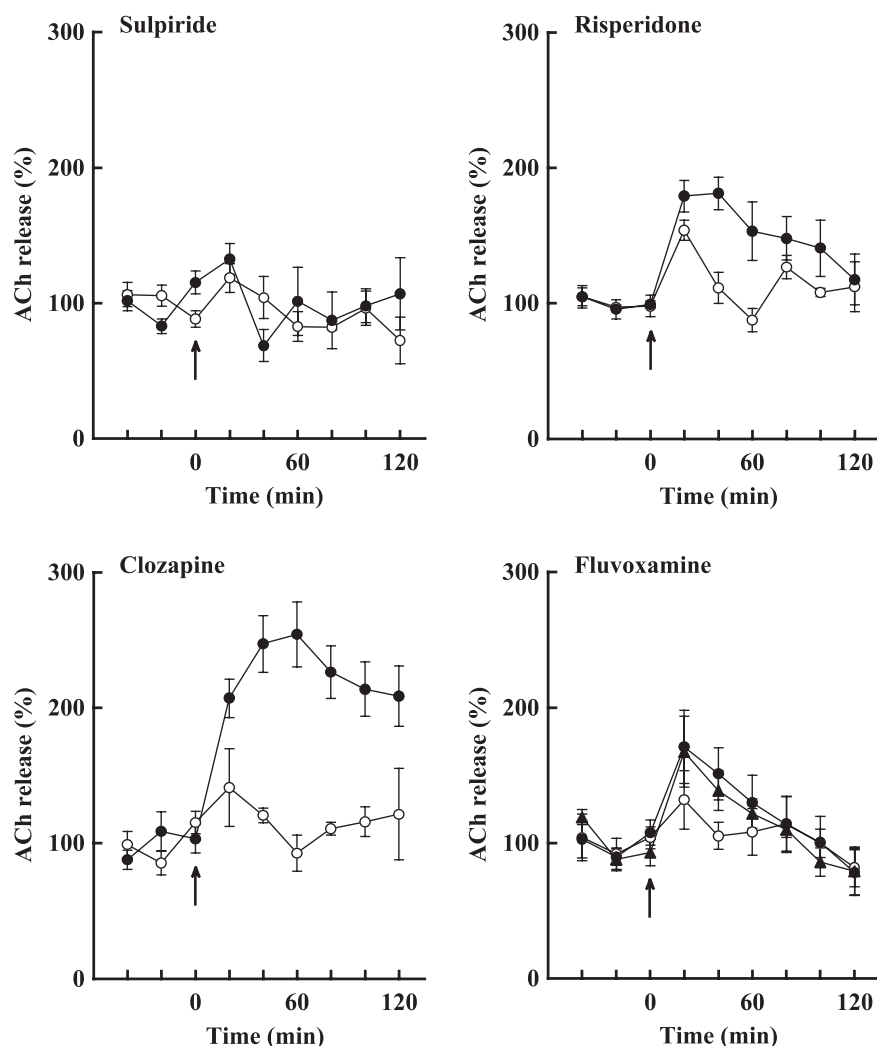


Fig. 1. Effects of sulpiride, risperidone, clozapine, and fluvoxamine on the in vivo release of ACh in rat prefrontal cortex. Sulpiride at doses of 0 (open circles) and 10 (closed circles) mg/kg, risperidone at doses of 0 (open circles) and 1 (closed circles) mg/kg, and fluvoxamine at doses of 0 (open circles), 10 (closed circles), and 20 (closed triangles) mg/kg were intraperitoneally administered at 0 min (arrow). Clozapine at doses of 0 (open circles) and 5 (closed circles) mg/kg were subcutaneously administered at 0 min (arrow). Values are expressed as the mean \pm S.E.M. of 3 to 6 rats.

the cannula to verify the probe position histologically. The ACh concentration in brain microdialysates was determined by HPLC with an electrochemical detector (HTEC-500, Eicom). Samples were injected into an analytical column (2.0 mm ID \times 150 mm, Eicompak AC-GEL; Eicom) in which ACh was separated before entering an enzyme column (3.0 mm ID, AC-Enzymapak; Eicom) containing immobilized AChesterase and choline oxidase, which converted the ACh to hydrogen peroxide. The hydrogen peroxide was detected by a platinum electrode (Eicom) set to +450 mV against an Ag/AgCl reference electrode. The mobile phase contained 50 mM potassium bicarbonate buffer (pH 8.3), 400 mg/L decanesulfonic acid, and 50 mg/L EDTA. All microdialysis data were calculated as percent change from dialysate basal concentrations, with 100% defined as the average of three fractions before administration. Analyses were made using two-way ANOVA for treatment as the intersubject factor and repeated measures with time as the intrasubject factor. Statistical analyses were made using the software package Statview 5.0J for Apple Macintosh computer (SAS Institute, Inc., Cary, NC, USA). A value of $P < 0.05$ was considered statistically significant.

The basal extracellular ACh level (mean \pm S.E.M. of 57 rats) in the prefrontal cortex was 232.1 ± 12.7 fmol/fraction in the presence of 10 nM neostigmine, an AChesterase inhibitor. Figure 1 shows the effects of sulpiride, risperidone, clozapine, and fluvoxamine on

the in vivo release of ACh in rat prefrontal cortex. Sulpiride, a typical antipsychotic (10 mg/kg), did not affect ACh release [$F(8, 48) = 1.271$, NS]. On the other hand, the atypical antipsychotics risperidone (1 mg/kg) and clozapine (5 mg/kg) increased ACh release in the prefrontal cortex [$F(8, 56) = 2.159$, $P = 0.0449$ for risperidone; $F(8, 40) = 8.092$, $P < 0.0001$ for clozapine]. Fluvoxamine at doses of 10 and 20 mg/kg did not affect ACh release [$F(8, 48) = 0.910$, NS for 10 mg/kg; $F(8, 48) = 1.042$, NS for 20 mg/kg]. Figure 2 shows the effects of fluvoxamine in combination with sulpiride, risperidone, and clozapine, respectively, on the in vivo release of ACh in rat prefrontal cortex. Coadministration of sulpiride (10 mg/kg) and fluvoxamine at doses of 10 and 20 mg/kg did not affect ACh release [$F(8, 64) = 0.978$, NS for 10 mg/kg; $F(8, 56) = 0.577$, NS for 20 mg/kg] (Fig. 2A). Fluvoxamine (10 mg/kg) did not affect risperidone (1 mg/kg)-induced [$F(8, 64) = 0.371$, NS] (Fig. 2B) and clozapine (5 mg/kg)-induced [$F(8, 32) = 0.648$, NS] (Fig. 2C) increases in ACh release.

Yamaguchi et al. (14) have reported that indeloxazine, an inhibitor of serotonin and noradrenaline reuptake, alone increases ACh release in the prefrontal cortex, suggesting that this effect might be mediated by endogenous serotonin. In contrast, the present study demonstrates that fluvoxamine alone does not affect ACh release in the prefrontal cortex. This may be due to differences in the experimental conditions such as the drugs used (indeloxazine vs fluvoxamine) and the

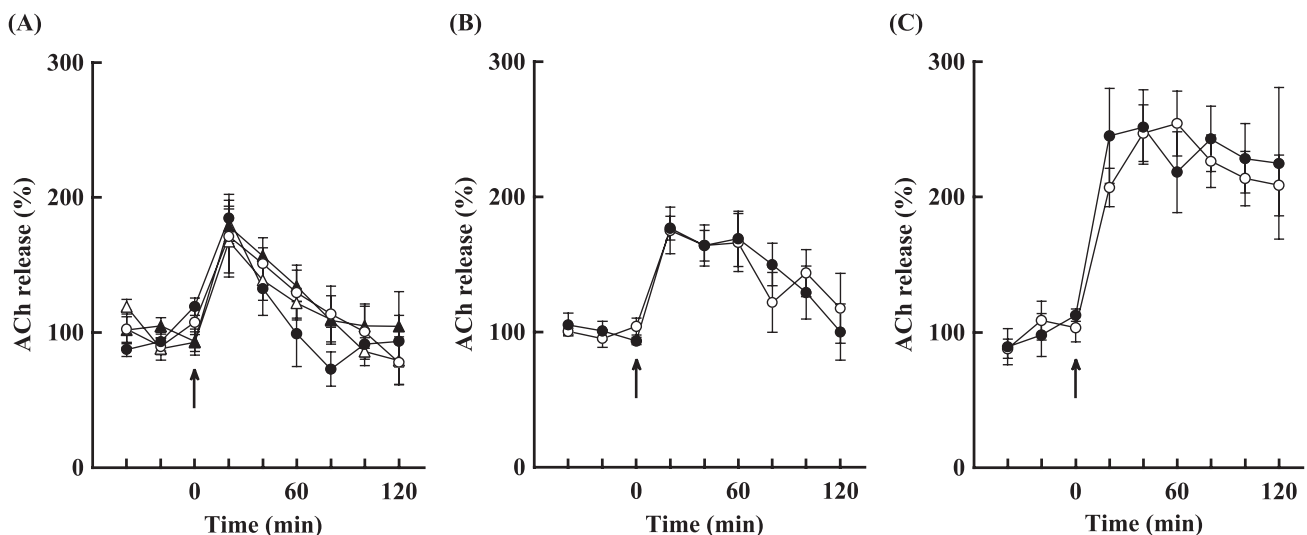


Fig. 2. Effects of fluvoxamine in combination with sulpiride, risperidone, and clozapine on the in vivo release of ACh in rat prefrontal cortex. A: Fluvoxamine at doses of 10 (circles) and 20 (triangles) mg/kg alone (open symbols) and in combination with sulpiride 10 mg/kg (closed symbols) were administered at 0 min (arrow). Values are expressed as the mean \pm S.E.M. of 4 to 6 rats. B: Risperidone 1 mg/kg alone (open circles) and in combination with fluvoxamine 10 mg/kg (closed circles) were administered at 0 min (arrow). Values are expressed as the mean \pm S.E.M. of 4 to 6 rats. C: Clozapine at 5 mg/kg alone (open circles) and in combination with fluvoxamine 10 mg/kg (closed circles) were administered at 0 min (arrow). Values are expressed as the mean \pm S.E.M. of 3 rats.

concentrations of AChE inhibitors included in the perfusate (1 μ M of physostigmine vs 10 nM of neostigmine). AChE inhibitors have generally been included in the microdialysis perfusate to protect the ACh from degradation; it should be noted, however, that in vivo ACh release is dependent on the concentration of the AChE inhibitor (15). In the presence of a low concentration of neostigmine, fluvoxamine affected neither risperidone- nor clozapine-induced increases in cortical ACh release. Furthermore, we observed that the combination of sulpiride and fluvoxamine did not cause an increase in extracellular ACh levels in the prefrontal cortex. These results suggest that the cholinergic system in the prefrontal cortex may have no major role in the effects resulting from the combined administration of antipsychotics and fluvoxamine. It is also unlikely that noradrenaline may be involved in the enhanced effects of antipsychotics combined with an SSRI since sulpiride or haloperidol does not enhance fluvoxamine-induced noradrenaline release in the prefrontal cortex (10).

In conclusion, the present study shows the absence of effects due to the administration of antipsychotics together with fluvoxamine on ACh release in rat prefrontal cortex. Thus, the present and previous findings strongly imply that the activation of aminergic neurotransmission, especially by dopamine, in the prefrontal cortex plays an important role in the clinical effects of the combination.

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References

- 1 Ferrier IN. Treatment of major depression: is improvement enough? *J Clin Psychiatry*. 1999;60 Suppl 6:10–14.
- 2 Nelson JC. A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. *Biol Psychiatry*. 1999;46:1301–1308.
- 3 Meltzer HY, Burnett S, Bastani B, Ramirez LF. Effects of six months of clozapine treatment on the quality of life of chronic schizophrenic patients. *Hosp Community Psychiatry*. 1990;41:892–897.
- 4 O'Connor M, Silver H. Adding risperidone to selective serotonin reuptake inhibitor improves chronic depression. *J Clin Psychopharmacol*. 1998;18:89–91.
- 5 Lu ML, Lane HY, Chen KP, Jann MW, Su MH, Chang WH. Fluvoxamine reduces the clozapine dosage needed in refractory schizophrenic patients. *J Clin Psychiatry*. 2000;61:594–599.
- 6 Shelton RC, Tollefson GD, Tohen M, Stahl S, Gannon KS, Jacobs TG, et al. A novel augmentation strategy for treating resistant major depression. *Am J Psychiatry*. 2001;158:131–134.
- 7 Zhang W, Perry KW, Wong DT, Potts BD, Bao J, Tollefson GD, et al. Synergistic effects of olanzapine and other antipsychotic agents in combination with fluoxetine on norepinephrine and dopamine release in rat prefrontal cortex. *Neuropsychopharmacology*. 2000;23:250–262.
- 8 Denys D, Klompmaekers AA, Westenberg HG. Synergistic dopamine increase in the rat prefrontal cortex with the combination of quetiapine and fluvoxamine. *Psychopharmacology*. 2004;176:195–203.
- 9 Koch S, Perry KW, Bymaster FP. Brain region and dose effects of an olanzapine/fluoxetine combination on extracellular monoamine concentrations in the rat. *Neuropharmacology*. 2004;46:232–242.
- 10 Ago Y, Nakamura S, Baba A, Matsuda T. Sulpiride in combination with fluvoxamine increases in vivo dopamine release selectively in rat prefrontal cortex. *Neuropsychopharmacology*. 2005;30:43–51.
- 11 Ago Y, Harasawa T, Itoh S, Nakamura S, Baba A, Matsuda T. Antidepressant-like effect of coadministration of sulpiride and fluvoxamine in mice. *Eur J Pharmacol*. 2005;520:86–90.
- 12 Ichikawa J, Dai J, O'Laughlin IA, Fowler WL, Meltzer HY. Atypical, but not typical, antipsychotic drugs increase cortical acetylcholine release without an effect in the nucleus accumbens or striatum. *Neuropsychopharmacology*. 2002;26:325–339.
- 13 Nakamura S, Ago Y, Itoh S, Koyama Y, Baba A, Matsuda T. Effect of zotepine on dopamine, serotonin and noradrenaline release in the prefrontal cortex. *Eur J Pharmacol*. 2005;528:95–98.
- 14 Yamaguchi T, Suzuki M, Yamamoto M. Facilitation of acetylcholine release in rat frontal cortex by indeloxazine hydrochloride: involvement of endogenous serotonin and 5-HT₄ receptors. *Naunyn Schmiedeberg Arch Pharmacol*. 1997;356:712–720.
- 15 Moor E, Schirm E, Jacso J, Westerink BH. Effects of neostigmine and atropine on basal and handling-induced acetylcholine output from ventral hippocampus. *Neuroscience*. 1998;82:819–825.