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Sulpiride in Combination with Fluvoxamine Increases *in vivo* Dopamine Release Selectively in Rat Prefrontal Cortex

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Coadministration of atypical antipsychotics and selective serotonin reuptake inhibitors (SSRIs) enhances the release of monoamines such as dopamine (DA), norepinephrine (NE), and serotonin (5-HT) in the prefrontal cortex. To clarify the role of DA-$D_{2/3}$ receptors in the combination effect, we examined the effects of coadministration of the selective DA-$D_{2/3}$ antagonist sulpiride and the SSRI fluvoxamine on amine neurotransmitter release in rat prefrontal cortex. Sulpiride (10 mg/kg, i.p.) and fluvoxamine (10 mg/kg, i.p.) alone did not affect extracellular DA levels, while their coadministration caused a significant increase in DA levels. Sulpiride alone did not affect extracellular levels of 5-HT and NE in the prefrontal cortex, while fluvoxamine alone caused a marked increase in 5-HT levels and a slight increase in NE levels. Sulpiride did not affect the fluvoxamine-induced increases in extracellular levels of 5-HT and NE. The DA-$D_{2/3}$ antagonist haloperidol (0.1 mg/kg) in combination with fluvoxamine also caused a selective increase in extracellular DA levels in the cortex. Coadministration of sulpiride and fluvoxamine did not affect extracellular DA levels in the striatum. Combination of systemic sulpiride and local fluvoxamine did not increase the DA levels, but that of systemic fluvoxamine with local sulpiride increased. The combination effect in increasing prefrontal DA levels was antagonized systemically, but not locally, by the 5-HT_{1A} antagonist WAY100635 at a low dose. These findings suggest that the combination of prefrontal DA-$D_{2/3}$ receptor blockade and 5-HT_{1A} receptor activation in regions other than the cortex plays an important role in sulpiride and fluvoxamine-induced increase in prefrontal DA release.
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Keywords: sulpiride; fluvoxamine; combination; dopamine (DA) release; prefrontal cortex

Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are widely used for the treatment for depression, but about 30–50% of patients do not initially respond to SSRIs (Ferrier, 1999; Nelson, 1999; Barbui and Hotopf, 2001). Clinical studies show that atypical antipsychotics such as risperidone and olanzapine are effective when added to an SSRI in the case of depression in which treatment with an SSRI alone is not effective (called treatment-resistant depression) (O'Connor and Silver, 1998; Ostroff and Nelson, 1999; Shelton *et al*, 2001). On the other hand, Zhang *et al* (2000) using *in vivo* microdialysis techniques demonstrated that combination of olanzapine with fluoxetine produced robust, sustained increases in extracellular dopamine (DA), norepinephrine (NE), and 5-HT levels in rat prefrontal cortex. These effects may contribute to the clinical effect of combination therapy of atypical antipsychotics with SSRI. However, the neurochemical mechanism for the combination effect appears to be complicated, since olanzapine has wide-ranging effects on numerous neurotransmitter systems (Bymaster *et al*, 1996; Schotte *et al*, 1996; Zhang and Bymaster, 1999).

SSRIs increase extracellular 5-HT levels and the increased 5-HT may interact with specific subtypes of 5-HT receptors. Previous studies show that 5-HT receptor subtypes modulate DA-$D_{2/3}$ receptor blockade-induced DA release. Lucas and Spampinato (2000) reported that the DA-$D_{2/3}$ receptor antagonist haloperidol-induced increase was reduced by the 5-HT_{2A} receptor antagonist SR 46349B and potentiated by the 5-HT_{2A} receptor agonist 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane in the striatum. In contrast, Liégeois *et al* (2002) reported that haloperidol-induced DA release was potentiated by the 5-HT_{2A} receptor antagonist M100907 in the prefrontal cortex. These results suggest that 5-HT_{2A} receptors regulate haloperidol-induced DA release in a brain region-specific manner. With respect to 5-HT_{1A} receptors, Ichikawa and Meltzer (1999) reported that the 5-HT_{1A} receptor agonist 8-OH-DPAT potentiated the DA-$D_{2/3}$ receptor antagonist sulpiride-induced DA release in the prefrontal cortex and the potentiation was abolished by the 5-HT_{1A} receptor antagonist WAY100635.

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These results suggest that 5-HT_{1A} receptor agonist potentiates prefrontal DA release in combination with DA-$D_{2/3}$ receptor antagonism. However, it remains to be determined whether combination of typical antipsychotics and SSRIs affects the *in vivo* release of amine neurotransmitter including DA in the frontal cortex. The present study demonstrates that coadministration of sulpiride and fluvoxamine, an SSRI, selectively activates dopaminergic neurons in the prefrontal cortex, but not in the striatum. We also examined the mechanism for the coadministration-induced increase in prefrontal DA release.

MATERIALS AND METHODS

Animals and Drugs

Male Wistar rats weighing 250–350 g at the beginning of the experiments were used. The animals were maintained under controlled environmental conditions ($22 \pm 1^{\circ}$C; 12–12 h light–dark cycle, lights on at 0800 h; food and water *ad libitum*) for at least 1 week before being used in the experiment. The procedures involving 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, Saitama, Japan); sulpiride (Fujisawa Pharmaceutical Co., Osaka, Japan); *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY100635) (Mitsubishi Pharma Co., Yokohama, Japan); haloperidol (Sigma, St Louis, MO, USA). All other chemicals used were of the highest commercially available purity. Fluvoxamine and WAY100635 were dissolved in saline (0.9% NaCl solution). Sulpiride was dissolved in 0.1 M HCl and was adjusted to pH 6–7 with 0.1 M NaOH. Haloperidol was dissolved in saline containing less than 0.1% v/v acetic acid. The drugs were i.p. injected at 1 ml/kg.

Microdialysis

Rats were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.) and stereotaxically implanted with guide cannula (one site per animal) for the dialysis probe (Eicom, Kyoto, Japan) at the prefrontal cortex ($A + 3.2$ mm, $L-0.6$ mm, $V-5.2$ mm, from the bregma and skull) or caudate putamen ($A + 0.2$ mm, $L-2.6$ mm, $V-7.0$ mm) (Paxinos and Watson, 1986). The active probe membrane was 3 mm in length. On the day following surgery, the probe was perfused at a constant flow rate of 2 μl/min with Ringer's solution (147.2 mM NaCl, 4.0 mM KCl, and 2.2 mM $CaCl_2$, Fuso Pharmaceutical Industries, Ltd., Osaka, Japan). A stabilization period of 3 h was allowed before the experiment. Two separate experiments were carried out for the determination of 5-HT, DA, and NE release as previously reported (Ago *et al*, 2002, 2003). In one experiment, 10 min microdialysis samples (20 μl) were taken and then immediately injected onto an HPLC column for a simultaneous assay of 5-HT and DA. In the other experiment, 30 min microdialysis samples (60 μl) were taken and then immediately injected onto an HPLC column for an NE assay.

For the assay of 5-HT and DA, an Eicompak PP-ODS column (4.6 mm i.d. × 30 mm: Eicom) was used, and the potential of the graphite electrode (Eicom) was set to + 400 mV against an Ag/AgCl reference electrode. The mobile phase for the 5-HT and DA assay contained 100 mM sodium phosphate buffer (pH 6.0), 500 mg/l decanesulfonic acid, 134 μM EDTA, 1% (vol/vol) methanol. For the NE assay, an Eicompak CA-5ODS column (2.1 mm i.d. × 150 mm: Eicom) was used, and the potential of the graphite electrode (Eicom) was set to + 500 mV against an Ag/AgCl reference electrode. The mobile phase for NE assay contained 100 mM sodium phosphate buffer (pH 6.0), 400 mg/l octanesulphonic acid, 134 μM EDTA, 5% (v/v) methanol.

After each experiment, microinjection (0.5 μl) of 1% Evans Blue dye was made through the cannula. The rats were decapitated, and the brains were removed and frozen in cold isopentane. Serial frozen sections were sliced, and the probe position was histologically verified.

Statistics

All microdialysis data were calculated as percent change from dialysate basal concentrations with 100% defined as the average of three fractions before drug administration. The statistical analyses were performed using one-way or two-way analysis of variance (ANOVA) for treatment as between-subjects factor and repeated measures with time as within-subject factor. The statistical analyses were performed using a software package (Stat View 5.0) for an Apple Macintosh computer. The *P*-values of 5% or less were considered statistically significant. The effects of vehicle treatments were not discussed, since they did not produce significant changes in the *in vivo* release of 5-HT, DA, and NE in rat prefrontal cortex and striatum.

RESULTS

In the brain microdialysis, basal levels of 5-HT, DA, and NE in dialysate (incorrected for *in vitro* probe recovery) were expressed as pg/fraction. The basal extracellular 5-HT, DA, and NE levels (means ± SEM) in the prefrontal cortex were 0.77 ± 0.06 pg/20 μl, 0.41 ± 0.03 pg/20 μl, and 3.67 ± 0.27 pg/60 μl, respectively ($n = 80$ for 5-HT and DA, $n = 39$ for NE) and striatum were 0.29 ± 0.02 pg/20 μl, 4.62 ± 0.70 pg/20 μl, and 0.91 ± 0.09 pg/60 μl, respectively ($n = 22$ for 5-HT and DA, $n = 21$ for NE).

Figure 1 shows the effects of fluvoxamine and sulpiride alone and in combination on the *in vivo* release of 5-HT, DA, and NE in rat prefrontal cortex. Fluvoxamine at 10 and 20 mg/kg alone caused a sustained increase in 5-HT release ($F(28, 224) = 8.690$; $P < 0.0001$) (Figure 1a), and at 10 mg/kg caused a slight increase in NE release ($F(7, 71) = 8.842$; $P < 0.0001$). However, it did not affect DA release ($F(28, 224) = 0.869$; NS). Sulpiride (10 mg/kg) alone did not affect the release of 5-HT ($F(14, 119) = 1.141$; NS), DA ($F(14, 119) = 1.218$; NS), and NE ($F(7, 63) = 1.147$; NS). Coadministration of sulpiride at 3 and 10 mg/kg and fluvoxamine (10 mg/kg) significantly increased prefrontal DA release ($F(28, 209) = 3.196$; $P < 0.0001$), while sulpiride did not affect the fluvoxamine-induced increase in 5-HT ($F(28, 209) = 1.013$; NS) or NE release ($F(7, 63) = 1.693$; NS). The combination of sulpiride at 10 mg/kg and fluvoxamine

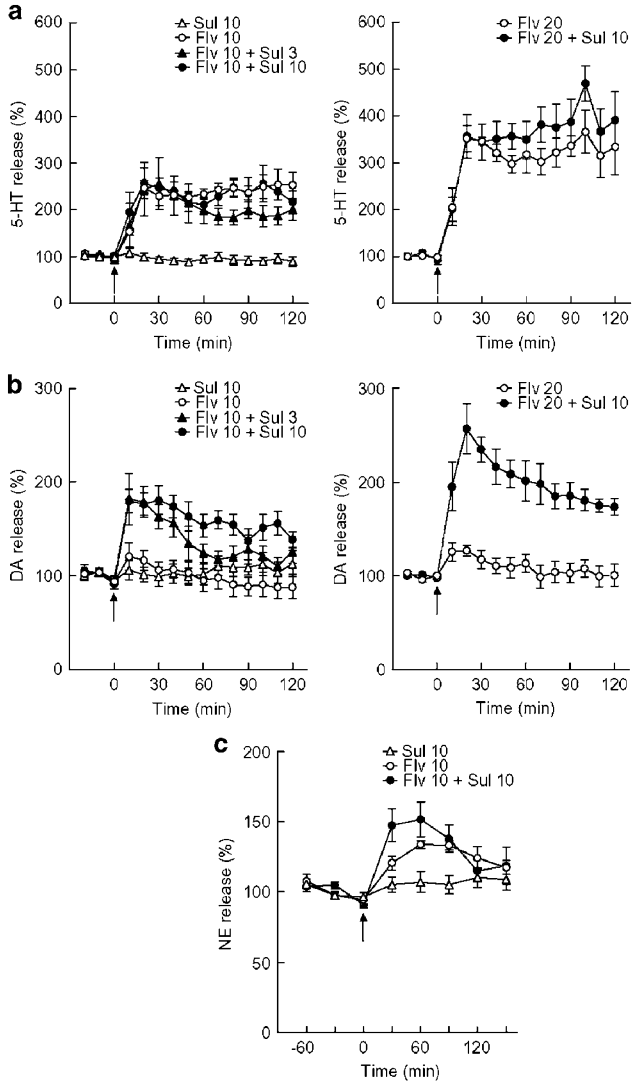

Figure 1 Effects of sulpiride and fluvoxamine alone and in combination on the *in vivo* release of 5-HT (a), DA (b), and NE (c) in rat prefrontal cortex. Sulpiride at 3 mg/kg (Sul 3) and 10 mg/kg (Sul 10) and fluvoxamine at 10 mg/kg (Flv 10) and 20 mg/kg (Flv 20) were i.p. administered at 0 time (arrow). Vehicle alone did not affect the extracellular levels of monoamines (not shown). Results are means ± SEM of four to five rats.

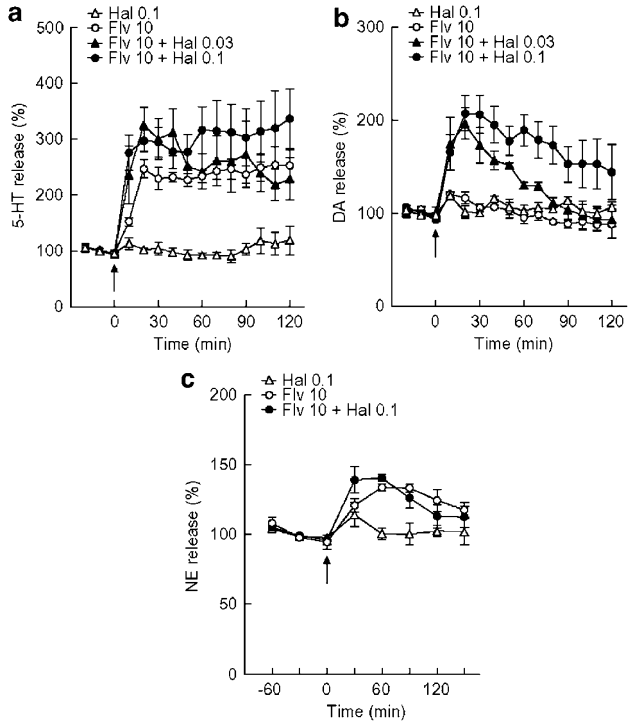

Figure 2 Effects of haloperidol and fluvoxamine alone and in combination on the *in vivo* release of 5-HT (a), DA (b), and NE (c) in rat prefrontal cortex. Haloperidol at 0.03 mg/kg (Hal 0.03) and 0.1 mg/kg (Hal 0.1) and fluvoxamine at 10 mg/kg (Flv 10) were i.p. administered at 0 time (arrow). Vehicle alone did not affect the extracellular levels of monoamines (not shown). Results are means ± SEM of four to five rats.

at 20 mg/kg also produced the robust increase in DA release ($F(14, 134) = 8.511$; $P < 0.0001$), while sulpiride did not affect the fluvoxamine-induced increase in 5-HT release ($F(14, 134) = 0.737$; NS). The increasing effect on DA release by sulpiride (10 mg/kg) was significantly greater in combination with 20 mg/kg of fluvoxamine than in that with 10 mg/kg of fluvoxamine ($F(14, 134) = 2.127$; $P < 0.05$).

The augmentation effect on DA release by coadministration of DA-$D_{2/3}$ antagonist and SSRI was also observed when haloperidol, another typical DA-$D_{2/3}$ antagonist, was used instead of sulpiride. Figure 2a shows the effects of haloperidol and fluvoxamine alone and in combination on the *in vivo* release of 5-HT in rat prefrontal cortex. Haloperidol at 0.1 mg/kg alone did not affect the release of 5-HT ($F(14, 119) = 0.556$; NS). The combination of haloperidol at 0.03 or 0.1 mg/kg and fluvoxamine at 10 mg/kg did not affect fluvoxamine-induced increase in 5-HT ($F(28, 194) = 1.278$; NS). Figure 2b shows the effects of haloperidol and fluvoxamine alone and in combination on the *in vivo* release of DA in rat prefrontal cortex. Haloperidol at 0.1 mg/kg alone did not affect the release of DA ($F(14, 119) = 1.379$; NS). The combination of haloperidol at 0.03 or 0.1 mg/kg and fluvoxamine at 10 mg/kg increased the DA release ($F(28, 194) = 3.144$; $P < 0.0001$). Figure 2c shows the effects of haloperidol and fluvoxamine alone and in combination on the *in vivo* release of NE in rat prefrontal cortex. Haloperidol at 0.1 mg/kg alone did not affect the release of NE ($F(7, 63) = 0.894$; NS). The combination of haloperidol at 0.1 mg/kg and fluvoxamine at 10 mg/kg did not affect the fluvoxamine-induced increase in NE release ($F(7, 71) = 1.700$; NS).

The effects of sulpiride and fluvoxamine alone and in combination on the *in vivo* release of 5-HT, DA, and NE were examined in rat striatum (Figure 3). Fluvoxamine at 10 mg/kg increased 5-HT release ($F(14, 149) = 17.230$; $P < 0.0001$) in the striatum, while sulpiride at 10 mg/kg decreased 5-HT release ($F(14, 119) = 4.266$; $P < 0.0001$). Sulpiride at 10 mg/kg did not affect the fluvoxamine (10 mg/kg)-induced increase in 5-HT release ($F(14, 134) = 0.466$; NS). Sulpiride at 10 mg/kg increased the DA release ($F(14, 119) = 20.440$; $P < 0.0001$) in the striatum, while fluvoxamine at 10 mg/kg did not affect the DA release ($F(14, 149) = 1.272$; NS). Fluvoxamine at 10 mg/kg did not affect the sulpiride (10 mg/kg)-induced increase in DA release ($F(14, 119) = 0.603$; NS). The NE release in the striatum was not affected by fluvoxamine at 10 mg/kg ($F(7, 63) = 0.804$; NS) and sulpiride at 10 mg/kg

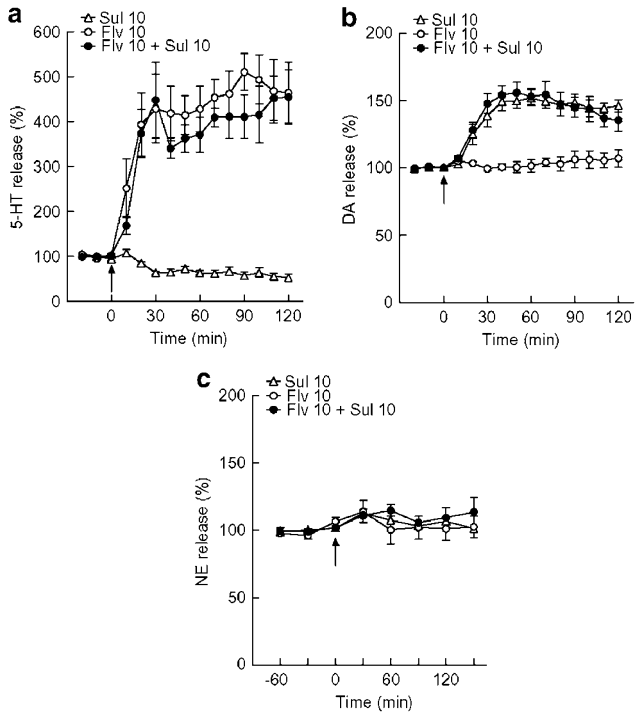

Figure 3 Effects of sulpiride and fluvoxamine alone and in combination on the *in vivo* release of 5-HT (a), DA (b), and NE (c) in rat striatum. Sulpiride at 10 mg/kg (Sul 10) and fluvoxamine at 10 mg/kg (Flv 10) were i.p. administered at 0 time (arrow). Vehicle alone did not affect the extracellular levels of monoamines (not shown). Results are means ± SEM of four to five rats.

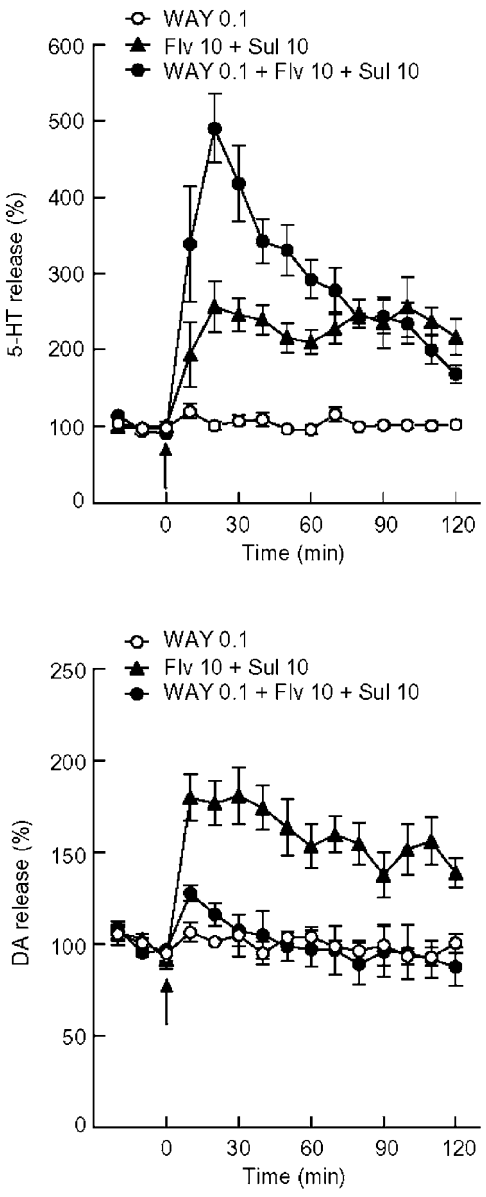

Figure 4 Effect of systemic WAY100635 on the changes in the *in vivo* release of 5-HT and DA induced by fluvoxamine plus sulpiride combination in rat prefrontal cortex. Fluvoxamine at 10 mg/kg and sulpiride at 10 mg/kg (Flv 10 + Sul 10) were i.p. administered at 0 time (arrow). WAY100635 at 0.1 mg/kg (WAY 0.1) was injected simultaneously with fluvoxamine and sulpiride. WAY100635 alone did not affect the release of 5-HT and DA. Results are means ± SEM of four to five rats.

($F(7, 71) = 0.257$; NS) alone, or in combination ($F(7, 31) = 1.297$; NS).

Figure 4 shows the effect of systemic WAY100635 on sulpiride plus fluvoxamine-induced changes in the *in vivo* release of 5-HT and DA in rat prefrontal cortex. WAY100635 at 0.1 mg/kg did not affect 5-HT and DA release ($F(14, 134) = 1.465$; NS for 5-HT, $F(14, 134) = 0.423$; NS for DA). WAY100635 at 0.1 mg/kg significantly blocked the increase in DA release ($F(14, 149) = 5.757$; $P < 0.0001$), while it potentiated the increase in 5-HT release ($F(14, 149) = 5.586$; $P < 0.0001$). Figure 5 shows the effect of local application of WAY100635 on sulpiride plus fluvoxamine-induced changes in the *in vivo* release of 5-HT and DA in the prefrontal cortex. Local application of WAY100635 at 10 μM via the microdialysis probe during the experiment did not affect the combination-induced increases in 5-HT and DA release ($F(14, 119) = 0.863$; NS for 5-HT, $F(14, 119) = 0.653$; NS for DA).

Figure 6 shows the combination effect of local application of fluvoxamine and systemic administration of sulpiride on the *in vivo* release of 5-HT and DA in rat prefrontal cortex. Local application of fluvoxamine at 50 nM via the microdialysis probe during the experiment increased 5-HT release ($F(14, 59) = 7.803$; $P < 0.0001$), while it did not affect DA release ($F(14, 59) = 1.321$; NS). These effects of local fluvoxamine were not affected by systemic administration of sulpiride at 10 mg/kg ($F(14, 119) = 0.599$; NS for 5-HT, $F(14, 119) = 0.603$; NS for DA). Figure 7 shows the combination effect of local application of sulpiride and systemic administration of fluvoxamine on the *in vivo* release of 5-HT and DA in rat prefrontal cortex. Local application of sulpiride at 10 μM via the microdialysis probe during the experiment did not affect the 5-HT and DA release ($F(14, 59) = 1.514$; NS for 5-HT, $F(14, 59) = 0.711$; NS for DA). Local sulpiride did not affect the fluvoxamine-induced increase in 5-HT release ($F(14, 134) = 1.094$; NS), while the combination of local, like systemic, sulpiride and fluvoxamine significantly increased the DA release ($F(14, 59) = 7.282$; $P < 0.0001$). The results of the present study are summarized in Table 1.

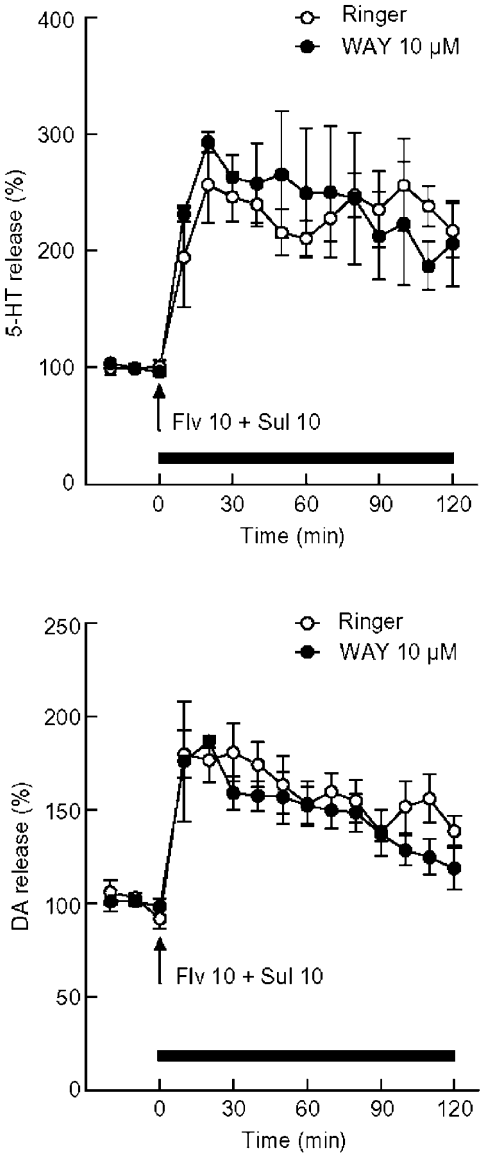

Figure 5 Effect of local application of WAY100635 on the changes in the *in vivo* release of 5-HT and DA induced by fluvoxamine plus sulpiride combination in rat prefrontal cortex. Rats were i.p. injected with fluvoxamine at 10 mg/kg plus sulpiride at 10 mg/kg (Flv 10 + Sul 10) at 0 time (arrow). Ringer's solution (open circles) or WAY100635 (WAY) at 10 μM (closed circles) was perfused into the cortex via a dialysis probe for the time indicated by the horizontal bar. Results are means ± SEM of three to five rats.

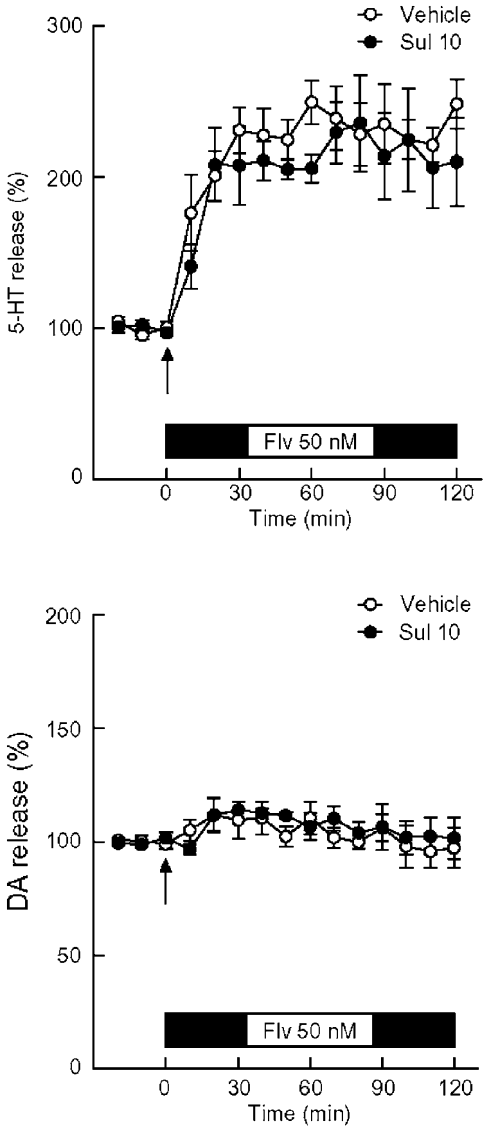

Figure 6 Effect of local application of fluvoxamine plus systemic sulpiride combination on the *in vivo* release of 5-HT and DA in rat prefrontal cortex. Rats were i.p. injected with vehicle (open circles) or sulpiride at 10 mg/kg (closed circles) (Sul 10) at 0 time (arrow). Fluvoxamine (Flv) at 50 nM was perfused into the cortex via the dialysis probe for the time indicated by the horizontal bar. Results are means ± SEM of four rats.

DISCUSSION

Zhang *et al* (2000) reported that the atypical antipsychotics olanzapine in combination with SSRI produced robust, sustained increases in extracellular levels of DA and NE. This effect may explain the clinical effectiveness of the combination therapy for treatment-resistant depression. The neurochemical mechanism for the increases in extracellular levels of monoamines, however, remains unknown. Olanzapine interacts with many receptors other than DA-D_2/D_3 receptors, while the typical antipsychotic sulpiride is a selective antagonist of DA-D_2/D_3 receptors (Sokoloff *et al*, 1990). The present study examined the effect of sulpiride instead of olanzapine on extracellular levels of monoamines to clarify the role of DA-D_2/D_3 receptor blockade in the synergistic effect of antipsychotic and SSRI. Sulpiride at 10 mg/kg alone did not affect the extracellular levels of these monoamines in the prefrontal cortex. Furthermore, fluvoxamine, an SSRI, at 10 mg/kg alone did not affect extracellular levels of DA in the prefrontal cortex, while it caused a marked increase in extracellular 5-HT levels and a slight increase in the NE levels. Under these conditions, the combination of sulpiride and fluvoxamine caused a marked increase in extracellular DA levels in the prefrontal cortex, while sulpiride did not affect the fluvoxamine-induced increases in the 5-HT and NE levels. The role of DA-D_2/D_3 receptor blockade in the combination effect is also supported by the observation that haloperidol, like sulpiride, in combination with fluvoxamine increases cortical DA

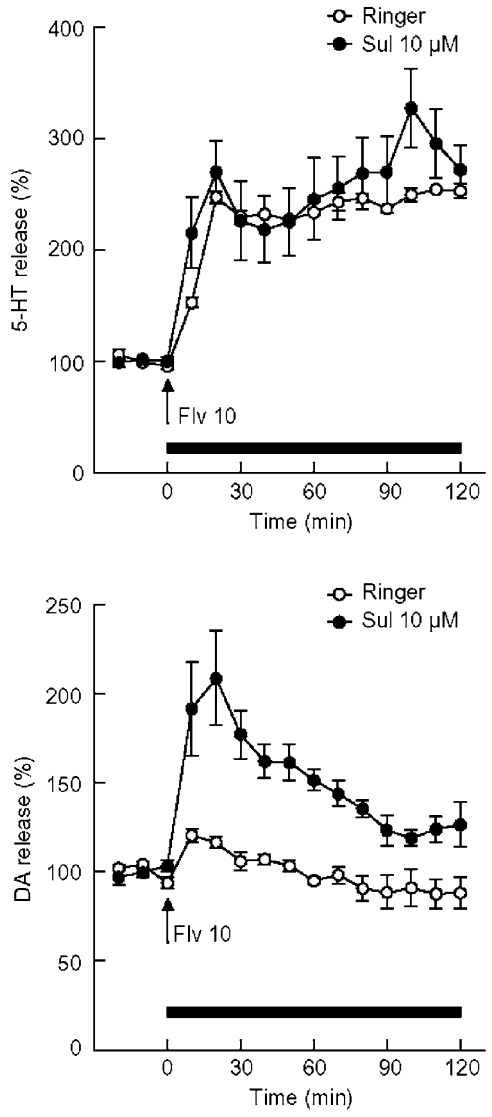

Figure 7 Effect of systemic fluvoxamine plus local application of sulpiride combination on the *in vivo* release of 5-HT and DA in rat prefrontal cortex. Rats were i.p. injected with fluvoxamine at 10 mg/kg (Flv 10) at 0 time (arrow). Ringer's solution (open circles) or sulpiride (Sul) at 10 μM (closed circles) was perfused into the cortex via a dialysis probe for the time indicated by the horizontal bar. Results are means ± SEM of four to five rats.

release. In contrast, Zhang *et al* (2000) reported that the combination of haloperidol with fluoxetine did not increase prefrontal DA levels more than fluoxetine alone. This may be due to a difference in the dose of haloperidol used between the previous (1.0 mg/kg haloperidol) and present (0.03 and 0.1 mg/kg) studies. In this line, Lucas *et al* (2000) reported that the SSRI citalopram enhanced striatal DA release induced by 0.01 mg/kg, but not 1.0 mg/kg, of haloperidol. These opposite effects of haloperidol may be explained by the effect on presynaptic or postsynaptic DA-D_2/D_3 receptors. Taken together, the present findings suggest that DA-D_2/D_3 receptor blockade in combination with 5-HT reuptake inhibition causes a selective increase in DA release in the prefrontal cortex.

Fluvoxamine increases extracellular 5-HT levels not only in the prefrontal cortex but also in other brain regions including the raphe nuclei where serotonergic cell bodies are localized. We first examined whether the increased 5-HT levels in the prefrontal cortex are involved in the effect of the combination of sulpiride and fluvoxamine. The fluvoxamine-induced increase in extracellular levels of 5-HT in the prefrontal cortex was not affected by sulpiride. This observation suggests that the increased levels of prefrontal 5-HT are not involved in the effect of the combination of sulpiride and fluvoxamine. In agreement with this suggestion, the combination of local application of fluvoxamine in the prefrontal cortex, which increased extracellular levels of 5-HT, and systemic sulpiride did not increase the extracellular levels of DA. This result suggests that the increased 5-HT in the prefrontal cortex does not play a role in the combination effect: the sites of action of systemic fluvoxamine which in combination with sulpiride increase DA release are regions other than the prefrontal cortex.

We secondly examined what kind of 5-HT receptor subtypes may be involved in the combination-induced increase in DA release in the prefrontal cortex. We have previously found that 5-HT_{1A} receptor activation causes DA release in the frontal cortex (Sakaue *et al*, 2000). Furthermore, Ichikawa *et al* (2001) reported that a combination of the 5-HT_{2A} antagonist M100907 and sulpiride increased DA release in rat prefrontal cortex and this effect was blocked by a low dose of WAY100635. In view of these observations, the present study examined the possible involvement of 5-HT_{1A} receptors in the combination-induced increase in DA release in the prefrontal cortex. We found that the combination effect of sulpiride and fluvoxamine was blocked by a low dose of WAY100635, a 5-HT_{1A} receptor antagonist. In this experiment, WAY100635 markedly enhanced the combination-induced increase in 5-HT release. This is in agreement with the previous observations that WAY100635-induced inhibition of presynaptic 5-HT_{1A} autoreceptors enhances SSRI-induced 5-HT release (Romero *et al*, 1996; Hjorth *et al*, 1997; Dawson and Nguyen, 1998). In view of the previous microdialysis study that local application of 5-HT facilitates DA release in the prefrontal cortex (Iyer and Bradberry, 1996), presynaptic 5-HT_{1A} receptor-mediated decrease in 5-HT release may lead to decrease in cortical DA release. It then appears that presynaptic 5-HT_{1A} receptor activation in the raphe is not involved in the combination-induced increase in cortical DA release. However, the present study showed that the combination effect was not affected by local application of WAY100635. This suggests that 5-HT_{1A} receptors responsible for combination-induced increase in cortical DA release are localized in regions other than the prefrontal cortex. This supports the point discussed above that the sites of action of systemic fluvoxamine, which in combination with sulpiride increase DA release, are regions other than the prefrontal cortex. Furthermore, the combination effect was blocked by a low dose of systemic WAY100635 which blocks preferentially presynaptic 5-HT_{1A} receptor-mediated responses (Hajós-Korcsok *et al*, 1999; Gobert *et al*, 1999; Ago *et al*, 2003). These findings suggest that fluvoxamine-induced activation of 5-HT_{1A} receptors, presumably presynaptic 5-HT_{1A} receptors, is involved in the combination effect of fluvoxamine and sulpiride in increasing DA release.

Table 1 Summary of the Findings of the Present Study

Regions	Drugs	5-HT release	DA release	NE release
Prefrontal cortex	Sul (i.p.)	—	—	—
	Flv (i.p.)	↑↑	—	↑
	Sul (i.p.)+Flv (i.p.)	↑↑	↑↑[a]	↑
	Hal (i.p.)	—	—	—
	Hal (i.p.)+Flv (i.p.)	↑↑	↑↑[a]	↑
	Sul (i.p.)+Flv (i.p.)+WAY (i.p.)	↑↑↑[b]	↑[b]	ND
	Sul (i.p.)+Flv (i.p.)+WAY (local)	↑↑	↑↑[a]	ND
	Sul (i.p.)+Flv (local)	↑↑	—	ND
	Sul (local)+Flv (i.p.)	↑↑	↑↑[a]	ND
Striatum	Sul (i.p.)	↓	↑	—
	Flv (i.p.)	↑↑	—	—
	Sul (i.p.)+Flv (i.p.)	↑↑	↑	—

Sul, sulpiride; Flv, fluvoxamine; Hal, haloperidol; WAY, WAY100635; —, no effect; ↑, increase; ↓, decrease.
[a]Significant change by coadministration.
[b]Significant change by WAY; ND, not determined.

We also examined the site of action of sulpiride in the effect of the combination of sulpiride and fluvoxamine. Previous studies show that DA autoreceptors in the prefrontal cortex are coupled to regulation of the release of DA (Wolf and Roth, 1987; Bean *et al*, 1990), and prefrontal DA-D_2/D_3 receptors function as DA autoreceptors (Gobert *et al*, 1995; Koeltzow *et al*, 1998). The present study showed that a local application of sulpiride in the cortex, like systemic sulpiride, with systemic fluvoxamine increased extracellular levels of DA. The finding suggests that prefrontal DA-$D_{2/3}$ receptor blockade plays a role in the combination effect of sulpiride and fluvoxamine in increasing DA release. That is, fluvoxamine increases cortical DA release under the condition of prefrontal DA-$D_{2/3}$ receptor blockade. The effect of fluvoxamine may be explained by activation of 5-HT_{1A} receptors in regions other than the prefrontal cortex as discussed above. Fluvoxamine-induced increase in 5-HT activates 5-HT_{1A} autoreceptors which reduce serotonergic activity. This may disinhibit dopaminergic activity in the ventral tegmental area. This may lead to an increase in DA release in the prefrontal cortex, to which the ventral tegmental area projects dopaminergic neurons.

Koch *et al* (2004) have recently demonstrated that the combination effect of olanzapine and fluoxetine on monoamines is observed only in select brain regions such as the prefrontal cortex and hypothalamus. We also observed that the combination of sulpiride and fluvoxamine did not affect extracellular DA levels in the striatum. The region-specific effect of the combination appears to be of significance as discussed by Koch *et al* (2004). The prefrontal cortex plays an important role in mediating affect and cognition and has been considered to control behaviors with relevance to affect and reward (Miller and Cohen, 2001). The regional specificity for regulation of DA release is also reported in the effect of coadministration of 8-OH-DPAT and sulpiride on DA release (Ichikawa and Meltzer, 1999).

In general, drug metabolism plays a key role in changes in the pharmacological effects induced by the combined administration of two drugs. However, it is unlikely that the effect of the combination of sulpiride and fluvoxamine on DA release is due to pharmacokinetic interactions. In this study, we found that sulpiride did not affect fluvoxamine-induced increase in 5-HT and NE levels. The observation suggests that sulpiride does not affect metabolism of fluvoxamine. Furthermore, we showed that fluvoxamine did not increase the sulpiride-induced increase in the DA levels in the striatum. The result suggests that fluvoxamine does not affect the metabolism of sulpiride. In this line, Imondi *et al* (1978) reported that most of the sulpiride administered was the unchanged form in the urine and feces in human and monkeys.

Sulpiride has antidepressant activity at doses that are 4–5 times lower than usual neuroleptic values (Benkert and Holsboer, 1984; Maier and Benkert, 1994; Vergoni *et al*, 1995), and the effect is considered to be mediated by enhanced dopaminergic activity (Serra *et al*, 1990). The present study shows that coadministration of sulpiride and fluvoxamine causes a selective increase in prefrontal DA release. In view of the previous reports that various drugs with antidepressant potentials increase extracellular DA levels selectively in the rat prefrontal cortex (Tanda *et al*, 1994, 1996), the present finding suggests that the combination may have an antidepressant effect. In agreement with the suggestion, Renard *et al* (2001) reported that sulpiride significantly increased the anti-immobility effects of the SSRIs fluvoxamine and paroxetine in a mouse-forced swimming test. In addition, we have recently found in the separate experiment that the combination of sulpiride and fluvoxamine is more effective than either drug alone in reducing the immobility time in a mouse tail suspension test (unpublished). On the other hand, previous studies suggest that atypical antipsychotic drugs may improve negative symptoms and cognitive dysfunction in schizo-

phrenia partly via increasing cortical DA release (Moghaddam and Bunney, 1990; Kuroki *et al*, 1999; Meltzer and McGurk, 1999). Then, the present neurochemical study implies that the combination of sulpiride and fluvoxamine, like 5-HT_{1A} receptor agonist combined with sulpiride (Ichikawa and Meltzer, 1999), is effective not only for treatment of depression but also for improvement of cognitive dysfunction in schizophrenia.

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REFERENCES

Ago Y, Koyama Y, Baba A, Matsuda T (2003). Regulation by 5-HT_{1A} receptors of the *in vivo* release of 5-HT and DA in mouse frontal cortex. *Neuropharmacology* **45**: 1050–1056.

Ago Y, Sakaue M, Baba A, Matsuda T (2002). Selective reduction by isolation rearing of 5-HT_{1A} receptor-mediated dopamine release *in vivo* in the frontal cortex of mice. *J Neurochem* **83**: 353–359.

Barbui C, Hotopf M (2001). Amitriptyline v. the rest: still the leading antidepressant after 40 years of randomized controlled trials. *Br J Psychiatry* **178**: 129–144.

Bean AJ, During MJ, Roth RH (1990). Effects of dopamine autoreceptor stimulation on the release of colocalized transmitters: *in vivo* release of dopamine and neurotensin from rat prefrontal cortex. *Neurosci Lett* **108**: 143–148.

Benkert O, Holsboer F (1984). Effect of sulpiride in endogenous depression. *Acta Psychiatr Scand Suppl* **311**: 43–48.

Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, Moore NA, Tye NC *et al* (1996). Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology* **14**: 87–96.

Dawson LA, Nguyen HQ (1998). Effects of 5-HT_{1A} receptor antagonists on fluoxetine-induced changes in extracellular serotonin concentrations in rat frontal cortex. *Eur J Pharmacol* **345**: 41–46.

Ferrier IN (1999). Treatment of major depression: is improvement enough? *J Clin Psychiatry* **60**(Suppl 6): 10–14.

Gobert A, Rivet J-M, Audinot V, Cistarelli L, Spedding M, Vian J *et al* (1995). Functional correlates of dopamine D_3 receptor activation in the rat *in vivo* and their modulation by the selective antagonist, (+)-S 14297: II. Both D_2 and 'silent' D_3 autoreceptors control synthesis and release in mesolimbic, mesocortical and nigrostriatal pathways. *J Pharmacol Exp Ther* **275**: 899–913.

Gobert A, Rivet J-M, Cistarelli L, Melon C, Millan MJ (1999). Buspirone modulates basal and fluoxetine-stimulated dialysate levels of dopamine, noradrenaline and serotonin in the frontal cortex of freely moving rats: activation of serotonin$_{1A}$ receptors and blockade of α_2-adrenergic receptors underlie its actions. *Neuroscience* **93**: 1251–1262.

Hajós-Korcsok É, McQuade R, Sharp T (1999). Influence of 5-HT_{1A} receptors on central noradrenergic activity: microdialysis studies using (±)-MDL 73005EF and its enantiomers. *Neuropharmacology* **38**: 299–306.

Hjorth S, Westlin D, Bengtsson HJ (1997). WAY100635-induced augmentation of the 5-HT-elevating action of citalopram: relative importance of the dose of the 5-HT_{1A} (auto)receptor blocker *versus* that of the 5-HT reuptake inhibitor. *Neuropharmacology* **36**: 461–465.

Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, Meltzer HY (2001). 5-HT_{2A} and D_2 receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J Neurochem* **76**: 1521–1531.

Ichikawa J, Meltzer HY (1999). *R*(+)-8-OH-DPAT, a serotonin$_{1A}$ receptor agonist, potentiated *S*(−)-sulpiride-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens but not striatum. *J Pharmacol Exp Ther* **291**: 1227–1232.

Imondi AR, Alam AS, Brennan JJ, Hagerman LM (1978). Metabolism of sulpiride in man and rhesus monkeys. *Arch Int Pharmacodyn* **232**: 79–91.

Iyer RN, Bradberry CW (1996). Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. *J Pharmacol Exp Ther* **277**: 40–47.

Koch S, Perry KW, Bymaster FP (2004). Brain region and dose effects of an olanzapine/fluoxetine combination on extracellular monoamine concentrations in the rat. *Neuropharmacology* **46**: 232–242.

Koeltzow TE, Xu M, Cooper DC, Hu X-T, Tonegawa S, Wolf ME *et al* (1998). Alterations in dopamine release but not dopamine autoreceptor function in dopamine D_3 receptor mutant mice. *J Neurosci* **18**: 2231–2238.

Kuroki T, Meltzer HY, Ichikawa J (1999). Effect of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *J Pharmacol Exp Ther* **288**: 774–781.

Liégeois J-P, Ichikawa J, Meltzer HY (2002). 5-HT_{2A} receptor antagonism potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and inhibits that in the nucleus accumbens in a dose-dependent manner. *Brain Res* **947**: 157–165.

Lucas G, De Deurwaerdère P, Caccia S, Spampinato U (2000). The effect of serotonergic agents on haloperidol-induced striatal dopamine release *in vivo*: opposite role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes and significance of the haloperidol dose used. *Neuropharmacology* **39**: 1053–1063.

Lucas G, Spampinato U (2000). Role of striatal serotonin$_{2A}$ and serotonin$_{2C}$ receptor subtypes in the control of *in vivo* dopamine outflow in the rat striatum. *J Neurochem* **74**: 693–701.

Maier W, Benkert O (1994). Treatment of chronic depression with sulpiride: evidence of efficacy in placebo-controlled single case studies. *Psychopharmacology* **115**: 495–501.

Meltzer HY, McGurk SR (1999). The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. *Schizophr Bull* **25**: 233–255.

Miller EK, Cohen JD (2001). An integrative theory of prefrontal cortex function. *Annu Rev Neurosci* **24**: 167–202.

Moghaddam B, Bunney BS (1990). Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an *in vivo* microdialysis study. *J Neurochem* **54**: 1755–1760.

Nelson JC (1999). A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. *Biol Psychiatry* **46**: 1301–1308.

O'Connor M, Silver H (1998). Adding risperidone to selective serotonin reuptake inhibitor improves chronic depression [letter]. *J Clin Psychopharmacol* **18**: 89–91.

Ostroff RB, Nelson JC (1999). Risperidone augmentation of selective serotonin reuptake inhibitors in major depression. *J Clin Psychiatry* **60**: 256–259.

Paxinos G, Watson C (1986). *The Rat Brain in Sterotaxic Coordinates*, 2nd edn Academic Press: San Diego.

Renard CE, Fiocco AJ, Clenet F, Hascoet M, Bourin M (2001). Is dopamine implicated in the antidepressant-like effects of

selective serotonin reuptake inhibitors in the mouse forced swimming test? *Psychopharmacology* **159**: 42–50.

Romero L, Hervás I, Artigas F (1996). The 5-HT_{1A} antagonist WAY-100635 selectively potentiates the presynaptic effects of serotonergic antidepressants in rat brain. *Neurosci Lett* **219**: 123–126.

Sakaue M, Somboonthum P, Nishihara B, Koyama Y, Hashimoto H, Baba A *et al* (2000). Postsynaptic 5-hydroxytryptamine$_{1A}$ receptor activation increases *in vivo* dopamine release in rat prefrontal cortex. *Br J Pharmacol* **129**: 1028–1034.

Schotte A, Janssen PF, Gommeren W, Luyten WH, Van Gompel P, Lesage AS *et al* (1996). Risperidone compared with new and reference antipsychotic drugs: *in vitro* and *in vivo* receptor binding. *Psychopharmacology* **124**: 57–73.

Serra G, Forgione A, D'Aquila PS, Collu M, Fratta W, Gessa GL (1990). Possible mechanism of antidepressant effect of L-sulpiride. *Clin Neuropharmacol* **13**(Suppl 1): S76–S83.

Shelton RC, Tollefson GD, Tohen M, Stahl S, Gannon KS, Jacobs TG *et al* (2001). A novel augmentation strategy for treating resistant major depression. *Am J Psychiatry* **158**: 131–134.

Sokoloff P, Giros B, Martres M-P, Bouthenet M-L, Schwartz J-C (1990). Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature* **347**: 146–151.

Tanda G, Bassareo V, Di Chiara G (1996). Mianserin markedly and selectively increases extracellular dopamine in the prefrontal cortex as compared to the nucleus accumbens of the rat. *Psychopharmacology* **123**: 127–130.

Tanda G, Carboni E, Frau R, Di Chiara G (1994). Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology* **115**: 285–288.

Vergoni AV, Forgione A, Bertolini A (1995). Chronic administration of *l*-sulpiride at non-neuroleptic doses reduces the duration of immobility in experimental models of 'depression-like' behavior. *Psychopharmacology* **121**: 279–281.

Wolf ME, Roth RH (1987). Dopamine neurons projecting to the medial prefrontal cortex possess release-modulating autoreceptors. *Neuropharmacology* **26**: 1053–1059.

Zhang W, Bymaster FP (1999). The *in vivo* effects of olanzapine and other antipsychotic agents on receptor occupancy and antagonism of dopamine D_1, D_2, D_3, $5HT_{2A}$ and muscarinic receptors. *Psychopharmacology* **141**: 267–278.

Zhang W, Perry KW, Wong DT, Potts BD, Bao J, Tollefson GD *et al* (2000). Synergistic effects of olanzapine and other antipsychotic agents in combination with fluoxetine on norepinephrine and dopamine release in rat prefrontal cortex. *Neuropsychopharmacology* **23**: 250–262.