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Effect of acute treatment with YM992 on extracellular serotonin levels in the rat frontal cortex

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Abstract

(*S*)-2-[[(7-fluoroindan-4-yl)oxy]methyl]morpholine monohydrochloride (YM992) is a novel putative antidepressant exhibiting both selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibition and 5-HT_{2A} receptor antagonism. In vivo microdialysis revealed that a single treatment with YM992 (3, 10, 30 mg/kg i.p.) dose-dependently increased extracellular 5-HT levels in the rat frontal cortex. Fluoxetine, citalopram and venlafaxine also produced significant increases in 5-HT levels at doses of 10–30 mg/kg. However, the increase in 5-HT levels induced by YM992 was significantly larger than increases elicited by these three compounds at 30 mg/kg. The combined administration of *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidine-methanol (MDL100,907) (a selective 5-HT_{2A} receptor antagonist) and citalopram produced no additional increase in 5-HT levels compared with citalopram treatment alone. YM992 moderately enhanced [^{3}H]5-HT release from rat cerebral cortex synaptosomes using different mechanisms than *p*-chloroamphetamine. In comparison, 10-μM fluoxetine markedly induced 5-HT release in vitro, while citalopram and venlafaxine had no noticeable effect on release. YM992 produces a more robust increase of 5-HT levels acutely than other antidepressants in vivo and the effect may be due to 5-HT releasing properties of the drug.

Keywords: Microdialysis; Serotonin (5-hydroxytryptamine, 5-HT); Selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor; Venlafaxine; YM992; Antidepressant

1. Introduction

Depression is a common affective disorder characterized by a progressive, distinct loss of functional activity (Stassen and Angst, 1998). Evidence from several sources indicates that disruption of serotonin(5-hydroxytryptamine, 5-HT)-mediated neurotransmission in the brain significantly contributes to the pathophysiology of depression (Åsberg et al., 1976; Leonard, 1995), and that enhancement of 5-HT neurotransmission underlies the therapeutic effects of many antidepressant treatments (Blier and De Montigny, 1994). Indeed, selective serotonin reuptake inhibitors in current use such as fluoxetine (Prozac®) are potent antidepressive drugs (Feighner and Boyer, 1991). These drugs block the neuronal reuptake of 5-HT, resulting in increased neurotransmitter levels in the synaptic cleft. Evidence concerning these 5-HT levels was gathered using in vivo microdialysis, a technique widely used in neurochemical analysis to determine extracellular neurotransmitter levels in situ (Saito et al., 1996). Studies using this technique have reported that selective serotonin reuptake inhibitors increase in extracellular 5-HT levels in several discrete regions of rat brain (Fuller, 1994; Saito et al., 1996). Selective serotonin reuptake inhibitors also elevate 5-HT concentrations in the raphe nuclei of the midbrain. These nuclei contain inhibitory somatodendritic 5-HT_{1A} autoreceptors, which act to limit synaptic 5-HT levels (Bel and Artigas, 1992; Invernizzi et al., 1992). However, after several weeks of treatment with selective serotonin reuptake inhibitors, these 5-HT_{1A} autoreceptors become desensitized, resulting in increased 5-HT release in receiving projections from these nuclei. This change might explain, at least in part, the delayed onset of action of these drugs in treating major depression (Blier and De Montigny, 1994).

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(*S*)-2-[[(7-fluoroindan-4-yl)oxy]methyl]morpholine monohydrochloride (YM992) is a novel compound that both inhibits 5-HT reuptake and antagonizes 5-HT_{2A} receptors (Hatanaka et al., 1996; Takeuchi et al., 1997). It shows potent antidepressant-like activities in several animal models of depression (Takeuchi et al., 1997). In the present study, the effects of acute YM992 treatment on extracellular 5-HT levels in the rat frontal cortex were investigated using in vivo microdialysis. These results were compared with those of two selective serotonin reuptake inhibitors in current clinical use (fluoxetine and citalopram) as well as venlafaxine, a serotonin and norepinephrine reuptake inhibitor. In addition, the effects of YM992 on [^{3}H]5-HT release from rat cerebral cortex synaptosomes were examined to clarify the relative contribution of stored 5-HT to the total amount of 5-HT increase.

2. Materials and methods

2.1. Materials and administration

YM992 and *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidine-methanol (MDL100,907) were synthesized by the Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd. (Ibaraki, Japan). Fluoxetine (Eli Lilly and Co., Indianapolis, IN), citalopram (H. Lundbeck, Copenhagen, Denmark) and venlafaxine (Wyeth-Ayerst, Princeton, NJ) were obtained from commercially available preparations by extraction, refining and purity-checking at the Institute. *p*-Chloroamphetamine was purchased from Sigma (St. Louis, MO). [^{3}H]5-HT (529 GBq/mmol) was purchased from Amersham (Buckinghamshire, UK). All other chemicals were obtained from standard commercial sources. For the in vivo microdialysis study, YM992, venlafaxine and citalopram were dissolved in saline; MDL100,907 was dissolved in saline to which a few drops of Tween 80 was added (Kehne et al., 1996); and fluoxetine was dissolved in distilled water. Acute systemic administration of each drug was carried out intraperitoneally (1 or 2 ml/kg).

2.2. Animals and surgery

All experiments conformed with the regulations of the Animal Experimentation Ethics Committee of Yamanouchi Pharmaceutical Co., Ltd., Male Wistar rats (SLC, Shizuoka, Japan), weighing 260–380 g, were maintained on a 12-h light/dark cycle, with food and water available ad libitum. Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The skull was exposed and a small hole was drilled to allow implantation of a guide cannula (Eicom, Kyoto, Japan) in the frontal cortex (A: 3.7 mm, L: 2.0 mm, V: 1.5 mm, coordinates relative to bregma, Paxinos and Watson, 1986). The guide cannula was fixed in the skull with dental cement.

2.3. In vivo microdialysis and chromatographic analysis

At least 5 days after surgery, microdialysis probes (0.22 mm outer diameter, 3 mm exposed membrane; Eicom) were implanted into the frontal cortex and perfused with Ringer's solution (mM: NaCl, 147; KCl, 2.7; $CaCl_2$, 1.2; $MgCl_2$, 0.8) at a flow rate of 2 μl/min. Dialysates collected from the frontal cortex were directly injected into the high-performance liquid chromatography system every 20 min by an autoinjector (AS-10; Eicom). 5-HT in the dialysate was separated on a reverse-phase column (Eicompak CA-5ODS; Eicom) and detected with an electrochemical detector (ECD-100 or ECD-300; Eicom). The mobile phase consisted of 0.1 M phosphate buffer (pH 6.0), containing 400 mg/l of sodium-1-octanesulfonate, 50 mg/l of Na_2EDTA, and 20% methanol. The flow rate of the mobile phase was 1 ml/min. After basal 5-HT levels stabilized, each drug was administered to freely moving rats at Time 0. Values are expressed as a percentage of the mean of the last three measurements taken before drug administration in each animal.

2.4. [^{3}H]5-HT release from rat cerebral cortex synaptosomes

Male Wistar rats (SLC), weighing 160–200 g, were used. The animals were sacrificed by decapitation, and the cerebral cortices were dissected from two rats per experiment. Synaptosomes were prepared according to previously described methods (Harada and Maeno, 1979). Synaptosomes from about 50 mg of wet tissue were suspended in 1 ml of Krebs buffer (aerated with 95% O_2 and 5% CO_2) containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 11 mM glucose, 1 mM L-(+)-ascorbic acid, 0.067 mM EDTA and 0.1 mM pargyline to inhibit metabolism of [^{3}H]5-HT. The suspension was then added to an equal volume of the same buffer containing [^{3}H]5-HT (final concentration 50 nM). After 10 min incubation at 37°C, the solution was centrifuged at 12,000 × *g* for 5 min. The pellet was washed with cold Krebs buffer two times and resuspended. The [^{3}H]5-HT-loaded synaptosomes (0.1 ml) were added to 0.9 ml of pre-warmed buffer in the presence or absence of a drug and incubated at 37°C for 3 min. To measure the effect of drugs on [^{3}H]5-HT release stimulated by 1 μM *p*-chloroamphetamine or 1 μM YM992, each drug (1 μM) was mixed with either *p*-chloroamphetamine- or YM992-containing buffer before addition of the [^{3}H]5-HT-loaded synaptosomes. The reaction was terminated by cooling in an ice bath, and centrifuged at 12,000 × *g* for 1 min at 4°C. Radioactivity in the supernatant was counted with a liquid scintillation counter (Tri-Carb®-2500TR;

Packard Instrumental, Meriden, CT). To determine nonspecific release, incubation was performed in an ice bath. Data are expressed as a percentage of basal release (defined as 100%) in the absence of drugs.

2.5. Statistics

Basal 5-HT levels in rat frontal cortex were evaluated by one-way analysis of variance (ANOVA) by treatment group. For in vivo microdialysis studies, the effects of drugs were analyzed by two-way repeated-measures ANOVA. When significant differences were found, post-hoc comparisons were made with Dunnett's test. The maximum increase of 5-HT levels of drugs at a dose of 30 mg/kg and the effects of drugs on basal [^{3}H]5-HT release from rat cerebral cortex synaptosomes were compared by Tukey's test. The effects of drugs on [^{3}H]5-HT release by *p*-chloroamphetamine or YM992 were analyzed by Student's *t*-test.

3. Results

3.1. Effects of YM992, fluoxetine, citalopram and venlafaxine on in vivo extracellular 5-HT levels in the rat frontal cortex

Basal 5-HT levels in rat frontal cortex were 98.7 ± 6.7 fmol/40 μl dialysate ($n = 61$). These basal values did not differ significantly (one-way ANOVA) among treatment groups (data not shown). Administration of YM992 produced a dose-dependent increase in extracellular 5-HT levels (two-way repeated-measures ANOVA; treatment: $F(3,17) = 6.72$; $P < 0.01$, time: $F(9,153) = 30.5$; $P < 0.001$, interaction: $F(27,153) = 3.76$; $P < 0.001$, Fig. 1). The 5-HT levels rose 4.5 times above basal levels at a YM992 dose of 3 mg/kg, about 5.5 times at a dose of 10 mg/kg and about 7.5 times at a dose of 30 mg/kg (Fig. 1). Fluoxetine (two-way repeated-measures ANOVA; treatment: $F(2,13) = 10.14$; $P < 0.01$, time: $F(9,117) = 6.2$; $P < 0.001$, interaction: $F(18,117) = 3.77$; $P < 0.001$),

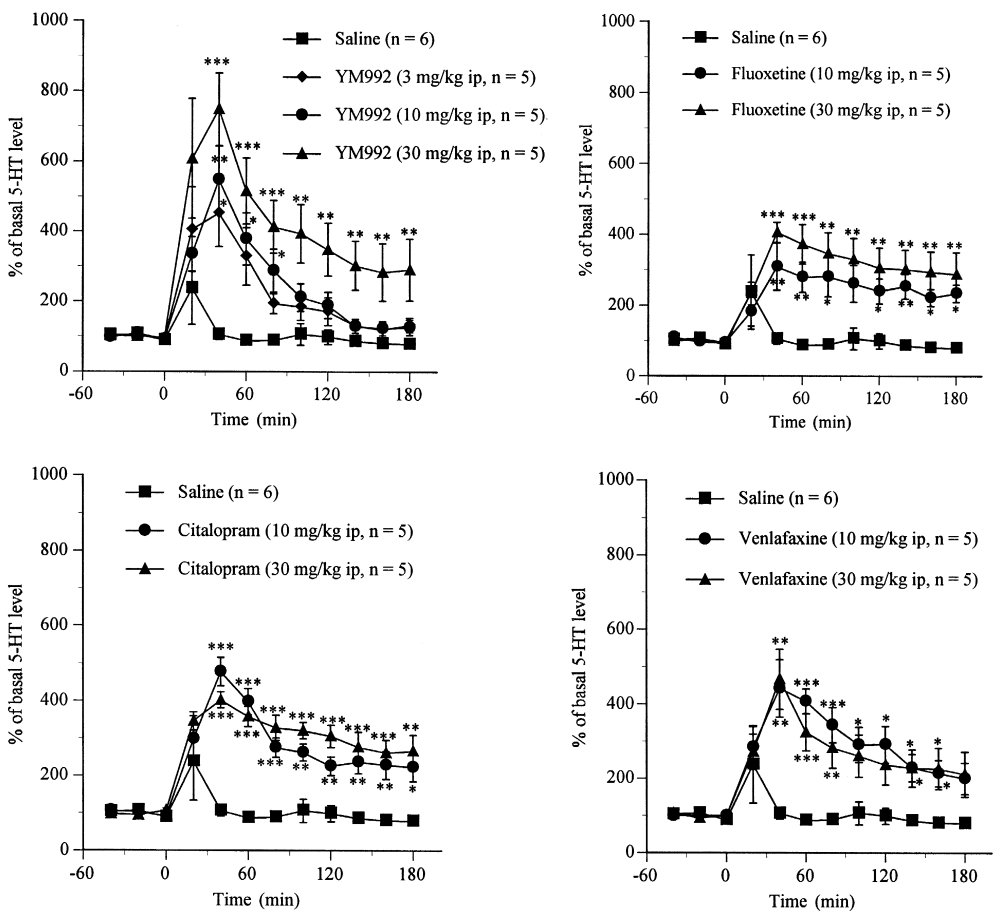

Fig. 1. Effects of acute treatment with YM992, fluoxetine, citalopram or venlafaxine on in vivo extracellular 5-HT levels in the frontal cortex of freely moving rats. Saline, YM992 (3, 10, 30 mg/kg i.p.), fluoxetine (10, 30 mg/kg i.p.), citalopram (10, 30 mg/kg i.p.) and venlafaxine (10, 30 mg/kg i.p.) were injected at Time 0. Results are expressed as a percentage of the mean 5-HT levels of the last three measurements taken before drug administration. Each value represents the mean ± S.E. of five or six rats. Significant differences from corresponding saline-treated rats were assessed by two-way repeated-measure ANOVA followed by Dunnett's test ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

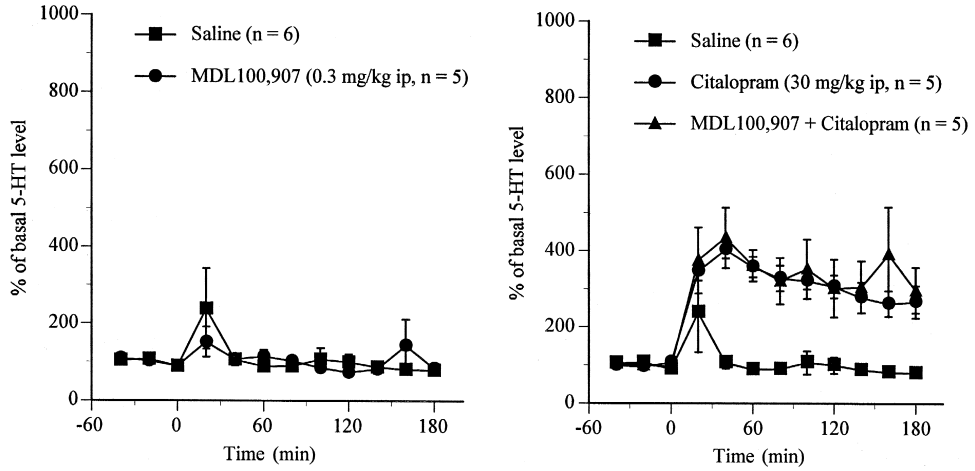

Fig. 2. Effects on in vivo extracellular 5-HT levels due to acute treatment with MDL100,907 alone and in combination with citalopram. Saline, MDL100,907 (0.3 mg/kg i.p.), citalopram (30 mg/kg i.p.) or MDL100,907 (0.3 mg/kg i.p.) plus citalopram (30 mg/kg i.p.) were injected at Time 0. Results are expressed as a percentage of the mean 5-HT levels of the last three measurements taken before drug administration. Each value represents the mean ± S.E. of five or six rats. There was no significant difference in 5-HT levels between MDL100,907 treatment and saline treatment ($F(1,9) = 0.03$; $P > 0.1$), or between citalopram-only treatment and MDL100,907 plus citalopram treatment ($F(1,8) = 0.19$; $P > 0.1$), according to two-way repeated-measures ANOVA.

citalopram (two-way repeated-measures ANOVA; treatment: $F(2,13) = 25.62$; $P < 0.001$, time: $F(9,117) = 14.43$; $P < 0.001$, interaction: $F(18,117) = 4.57$; $P < 0.001$) and venlafaxine (two-way repeated-measures ANOVA; treatment: $F(2,13) = 7.85$; $P < 0.01$, time: $F(9,117) = 13.31$; $P < 0.001$, interaction: $F(18,117) = 4.41$; $P < 0.001$) also significantly increased 5-HT levels (Fig. 1). However, YM992 produced a significantly larger increase in 5-HT levels than fluoxetine, citalopram or venlafaxine at 40 min after injection when all agents were administered at a dose of 30 mg/kg. (Tukey's test; $P < 0.05$).

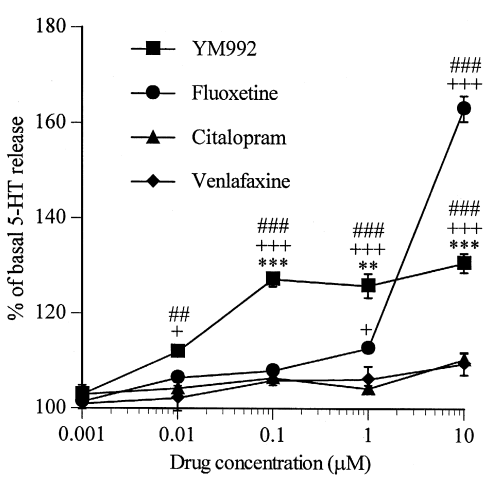

Fig. 3. Effects of YM992, fluoxetine, citalopram and venlafaxine on in vitro basal [^{3}H]5-HT release from rat cerebral cortex synaptosomes. The data are expressed as a percentage of basal 5-HT release (defined as 100%) in the absence of drugs. Each value represents the mean ± S.E. ($n = 4$). Significant differences from fluoxetine ($^{**}P < 0.01$, $^{***}P < 0.001$), citalopram ($^{+}P < 0.05$, $^{+++}P < 0.001$) or venlafaxine ($^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$) were assessed by Tukey's test.

3.2. Effects of MDL100,907 and combined treatment of MDL00,907 and citalopram on extracellular 5-HT levels in the rat frontal cortex

MDL100,907, a selective 5-HT$_{2A}$ receptor antagonist, induced no significant increase in extracellular 5-HT levels (two-way repeated-measures ANOVA; treatment: $F(1,9) = 0.03$; $P > 0.1$, Fig. 2). Combined administration of MDL100,907 and citalopram produced no additional increase in 5-HT levels compared with citalopram treatment

Table 1
Effects of YM992, fluoxetine, citalopram and venlafaxine on in vitro [^{3}H]5-HT release from rat cerebral cortex synaptosomes induced by *p*-chloroamphetamine
The data are expressed as a percentage of basal 5-HT release (defined as 100%) in the absence of drugs. Each value represents the mean ± S.E. ($n = 4$). Significant differences from treatment of *p*-chloroamphetamine alone were assessed by Student's *t*-test.

Drug (concentration)	Percentage of basal 5-HT release
p-Chloroamphetamine (1 μM)	186.5 ± 3.3
p-Chloroamphetamine (1 μM) + YM992 (1 μM)	129.7 ± 1.8***
p-Chloroamphetamine (1 μM) + fluoxetine (1 μM)	120.8 ± 1.5***
p-Chloroamphetamine (1 μM) + citalopram (1 μM)	111.1 ± 1.2***
p-Chloroamphetamine (1 μM) + venlafaxine (1 μM)	126.6 ± 2.9***

$^{***}P < 0.001$.

Table 2
Effects of fluoxetine, citalopram and venlafaxine on in vitro [^{3}H]5-HT release from rat cerebral cortex synaptosomes induced by YM992
The data are expressed as a percentage of basal 5-HT release (defined as 100%) in the absence of drugs. Each value represents the mean ± S.E. ($n = 4$). Significant differences from treatment of YM992 alone were assessed by Student's *t*-test.

Drug (concentration)	% of basal 5-HT release
YM992 (1 μM)	127.2 ± 2.6
YM992 (1 μM) + Fluoxetine (1 μM)	118.2 ± 2.3*
YM992 (1 μM) + Citalopram (1 μM)	110.9 ± 1.5**
YM992 (1 μM) + Venlafaxine (1 μM)	118.3 ± 0.9*

*$P < 0.05$.
**$P < 0.01$.

alone (two-way repeated-measures ANOVA; treatment: $F(1,8) = 0.19$; $P > 0.1$, Fig. 2).

3.3. Effects of YM992, fluoxetine, citalopram and venlafaxine on in vitro basal [^{3}H]5-HT release from rat cerebral cortex synaptosomes

YM992 concentration-dependently induced [^{3}H]5-HT release from rat cerebral cortex synaptosomes, reaching a maximum at 0.1 μM (Fig. 3). [^{3}H]5-HT release by YM992 was significantly higher than that by 0.01–10 μM citalopram and venlafaxine, and 0.1–1 μM fluoxetine (Fig. 3). 10 μM of fluoxetine also induced marked [^{3}H]5-HT release compared with YM992, citalopram and venlafaxine treatment (Fig. 3).

3.4. Effects of YM992, fluoxetine, citalopram and venlafaxine on in vitro [^{3}H]5-HT release by p-chloroamphetamine

p-Chloroamphetamine, an agent which triggers 5-HT release, induced greater [^{3}H]5-HT release at 1 μM than YM992. This 5-HT releasing action was significantly inhibited by 1 μM concentrations of all tested drugs (Table 1).

3.5. Effects of fluoxetine, citalopram and venlafaxine on in vitro [^{3}H]5-HT release by YM992

YM992 significantly induced [^{3}H]5-HT release at 1 μM compared with fluoxetine, citalopram and venlafaxine (Fig. 3). Fluoxetine, citalopram and venlafaxine at 1 μM significantly blocked [^{3}H]5-HT release induced by YM992 (Table 2).

4. Discussion

In vivo microdialysis results showed that YM992 dose-dependently increases extracellular 5-HT levels in the rat frontal cortex. Fluoxetine, citalopram and venlafaxine also significantly increased 5-HT levels at doses of 10–30 mg/kg. However, the increase in 5-HT concentrations induced by YM992 was significantly larger than those induced by the other three drugs at a dose of 30 mg/kg. Monoamine uptake studies revealed that YM992 inhibits 5-HT uptake with a selectivity and potency similar to fluoxetine in vitro and in vivo (Hatanaka et al., 1996; Takeuchi et al., 1997). Thus, the larger increase in 5-HT levels by YM992 is probably not due to 5-HT uptake inhibition alone.

YM992 is a potent 5-HT$_{2A}$ receptor antagonist in addition to a serotonin reuptake inhibitor (Hatanaka et al., 1996; Takeuchi et al., 1997). Wright et al. (1990) reported that 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT$_{2A/2C}$ agonist, decreases 5-HT release in the rat frontal cortex. Also, 5-HT levels in the rat nucleus accumbens increased after acute treatment with the 5-HT$_{2A/2C}$ antagonist ritanserin (Devaud and Hollingsworth, 1991). Therefore, to clarify the contribution of 5-HT$_{2A}$ receptor antagonism to the total 5-HT increase, effects due to administration of either the selective 5-HT$_{2A}$ receptor antagonist MDL100,907 (Kehne et al., 1996) or the combined treatment of citalopram and MDL100,907 on in vivo extracellular 5-HT levels in the rat frontal cortex were investigated. MDL100,907 did not increase 5-HT levels, and the change in 5-HT levels due to combined administration of citalopram and MDL100,907 was not significantly different from that induced by citalopram alone. Thus, it is thought that the marked 5-HT increase induced by YM992 is not due to its 5-HT$_{2A}$ receptor antagonism.

Amphetamine-like compounds such as *p*-chloroamphetamine or fenfluramine are able to induce release of 5-HT in vitro and in vivo. This action is inhibited by serotonin uptake inhibitors (Gobbi et al., 1992; Sabol et al., 1992; Levi and Raiteri, 1993; Raiteri et al., 1995; Wichems et al., 1995). Thus, the effects of YM992 on [^{3}H]5-HT release from rat cerebral cortical synaptosomes was examined. YM992 significantly enhanced [^{3}H]5-HT release compared with 0.01–10 μM citalopram and venlafaxine, and 0.1–1 μM fluoxetine. Since all these drugs inhibit 5-HT uptake at concentrations less than 1 μM (Bolden-Watson and Richelson, 1993; Hatanaka et al., 1996; Noble and Benfield, 1997), the significant enhancement of [^{3}H]5-HT release by YM992 is thought to be due to its unique 5-HT releasing action, which these other three drugs do not possess.

p-Chloroamphetamine induced a larger [^{3}H]5-HT release than YM992. Furthermore, this release was almost fully inhibited by fluoxetine, citalopram and venlafaxine, as previously reported (Gobbi et al., 1992; Sabol et al., 1992; Levi and Raiteri, 1993; Wichems et al., 1995; Raiteri et al., 1995). YM992 (1 μM) also suppressed 5-HT release induced by *p*-chloroamphetamine in the same way, perhaps due to a blockade of the 5-HT transporter (Hatanaka et al., 1996). These results suggest that enhancement of 5-HT release from nerve terminals in the rat frontal cortex by YM992 contributes to its greater efficacy

than fluoxetine, citalopram and venlafaxine in increasing extracellular 5-HT levels, and uses a markedly different mechanism than *p*-chloroamphetamine.

Interestingly, [^{3}H]5-HT release by YM992 was also significantly inhibited by fluoxetine, citalopram and venlafaxine. It is thought that this significant suppression is not due to inhibition of [^{3}H]5-HT release through the 5-HT transporter, since that may be blocked by YM992 itself (Table 1). Instead, YM992 partly may be transported into the synaptosomes through the 5-HT uptake site. Once inside, it may enhance 5-HT release by an unknown mechanism. Although further studies are necessary to confirm this hypothesis, the inhibition of the entry of YM992 by the three compounds may result in this significant decrease of [^{3}H]5-HT release.

Fluoxetine (10 μM) also induced marked [^{3}H]5-HT release. Gobbi et al. (1995) have reported that 10 μM fluoxetine evokes a marked tritium outflow from rat cortex synaptosomes preloaded with [^{3}H]5-HT. Our results agree with their report. In contrast, citalopram and venlafaxine did not show such action. Therefore, high concentrations of fluoxetine exhibit characteristics unlike citalopram and venlafaxine in vitro. However, since there were no in vivo differences in 5-HT levels due to treatment with fluoxetine, citalopram or venlafaxine, the significance of the marked releasing effect of 10 μM fluoxetine is unclear.

In this microdialysis study, fluoxetine, citalopram and venlafaxine significantly increased extracellular 5-HT levels in the rat frontal cortex. This agrees with reports that fluoxetine (Jordan et al., 1994; Malagié et al., 1995), fluvoxamine (Bel and Artigas, 1992; Jordan et al., 1994), citalopram (Invernizzi et al., 1992), duloxetine (Kihara and Ikeda, 1995) and venlafaxine (Gur et al., 1999) significantly increase 5-HT levels in the rat frontal cortex, although the magnitude of the increases are slightly different. However, selective serotonin reuptake inhibitors have been reported to preferentially increase extracellular 5-HT levels in the raphe nuclei, which contain inhibitory somatodendritic 5-HT$_{1A}$ autoreceptors (Bel and Artigas, 1992; Invernizzi et al., 1992). These receptors suppress the firing activity of 5-HT neurons in the raphe nuclei (Blier and De Montigny, 1983; Chaput et al., 1986), which inhibits 5-HT release. Thus, increased extracellular 5-HT levels in the synaptic cleft reflect a balance between decreased 5-HT release and inhibition of 5-HT reuptake (Fuller, 1994; Malagié et al., 1995). YM992, like the selective serotonin reuptake inhibitors, inhibits the firing activity of the dorsal raphe 5-HT neuron of rats in vivo (Dong et al., 1999). Nevertheless, YM992 treatment yielded higher in vivo extracellular 5-HT levels in rat frontal cortex than treatment with two currently marketed selective serotonin reuptake inhibitors or venlafaxine, possibly due to its moderate 5-HT releasing action.

It has been suggested that enhancement of serotonergic neurotransmission, in particular that mediated by post-synaptic 5-HT$_{1A}$ receptors, can treat depressive disorders (Blier and De Montigny, 1994). Since post-synaptic 5-HT$_{2A}$ receptors are known to inhibit 5-HT$_{1A}$ receptor-mediated signal transduction, a concomitant blockade of the 5-HT$_{2A}$ receptor is likely to augment 5-HT$_{1A}$ receptor-mediated neurotransmission (Backus et al., 1990; Ashby et al., 1994; Borsini, 1994). Since YM992 produces robust elevation of extracellular 5-HT levels acutely and antagonizes 5-HT$_{2A}$ receptors, it may enhance neurotransmission by post-synaptic 5-HT$_{1A}$ receptors strongly and have a more potent antidepressant effect than selective serotonin reuptake inhibitors in current clinical use.

In summary, YM992 showed greater efficacy acutely than fluoxetine, citalopram and venlafaxine in increasing 5-HT levels in the rat frontal cortex, and was also shown to enhance [^{3}H]5-HT release from rat cerebral cortex synaptosomes using different mechanisms than *p*-chloroamphetamine. Although the precise nature of these mechanisms remains unknown, the action may contribute to a greater increase in extracellular 5-HT levels in the rat frontal cortex.

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