British Journal of Pharmacology (2000) 129, 1028–1034

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Postsynaptic 5-hydroxytryptamine$_{1A}$ receptor activation increases *in vivo* dopamine release in rat prefrontal cortex

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1 5-Hydroxytryptamine (5-HT) plays a role in the regulation of 3,4-dihydroxyphenylethylamine (dopamine) neurons in the brain, but the precise mechanism of regulation by 5-HT$_{1A}$ receptors of dopamine release has not been defined. The present study describes the effect of 5-{3-[[(2S)-1,4-benzodioxan-2ylmethyl]amino]propoxy}-1,3-benzodioxole HCl (MKC-242), a highly potent and selective 5-HT$_{1A}$ receptor agonist, on dopamine release in the prefrontal cortex using microdialysis in the freely moving rat.

2 Subcutaneous injection of MKC-242 (0.3–1.0 mg kg^{-1}) increased extracellular levels of dopamine in the prefrontal cortex.

3 The effect of MKC-242 in the prefrontal cortex was antagonized by pretreatment with the selective 5-HT$_{1A}$ receptor antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)-cyclohexanecarboxamide (WAY100635; 1 mg kg^{-1}, i.p.).

4 Local application of WAY100635 (10 μM) *via* a microdialysis probe antagonized the effect of systemic MKC-242 in an increasing dopamine release, and locally infused 8-hydroxy-2-(di-n-propylamino)tetralin (10 μM) increased dopamine release in the prefrontal cortex.

5 MKC-242 increased cortical dopamine release in the rats pretreated with 5,7-dihydroxytryptamine (150 μg, i.c.v.) that caused an almost complete reduction in cortical 5-HT content.

6 The effect of MKC-242 to increase dopamine release was also observed in the hippocampus, but not in the striatum or nucleus accumbens.

7 Fluoxetine, a selective serotonin reuptake inhibitor, increased dopamine release in the prefrontal cortex, but not in the nucleus accumbens, while buspirone, a 5-HT$_{1A}$ receptor agonist, increased dopamine release in both brain regions.

8 The present results indicate that activation of postsynaptic 5-HT$_{1A}$ receptors increases dopamine release in a brain region-specific manner.

British Journal of Pharmacology (2000) **129,** 1028–1034

Keywords: 5-Hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors; MKC-242; fluoxetine; dopamine release; antidepressant; microdialysis

Abbreviations: CMC, carboxymethylcellulose; 5,7-DHT, 5,7-dihydroxytryptamine; MKC-242, 5-{3-[((2S)-1,4-benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; WAY100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide

Introduction

The 5-hydroxytryptamine (5-HT)$_{1A}$-receptor subtype has been implicated in anxiolytic and antidepressant responses (Taylor, 1990; Lucki *et al*., 1994). 5-HT$_{1A}$ receptors are localized not only on the serotoninergic cell bodies of the raphe nuclei, but also on non-serotoninergic neurons in various brain regions including the hippocampus and prefrontal cortex (Pazos & Palacios, 1985). Agonist activation of the somatodendritic 5-HT$_{1A}$ autoreceptors reduces 5-HT cell firing, synthesis and release, while activation of the postsynaptic 5-HT$_{1A}$ receptors affects non-serotoninergic neurons (Fletcher *et al*., 1993). We showed that 5-{3-[((2S)-1,4-benzodioxan-2-ylmethyl)amino]-propoxy}-1,3-benzodioxole (MKC-242), a potent and selective 5-HT$_{1A}$ receptor agonist with anxiolytic-like and antidepressant-like effects (Matsuda *et al*., 1995a,b; Abe *et al*., 1996; 1998a,b) facilitated *in vivo* release of noradrenaline (Suzuki *et al*., 1995) and acetylcholine (Somboothum *et al*., 1997) through activation of the postsynaptic and presynaptic 5-HT$_{1A}$ receptors, respectively. This effect on noradrenergic neurons (Chen & Reith, 1995; Hajos-Korcsok & Sharp, 1996; Hajos-Korcsok *et al*., 1999) may contribute to the antidepressant effect of 5-HT$_{1A}$ receptor agonists.

Previous studies also suggest a role for mesocortical dopaminergic pathways in mood regulation and antidepressant drug action (Willner, 1983; 1997; Knable & Weiberger, 1997) and their possible regulation by 5-HT$_{1A}$ receptors needs to be clarified. Arborelius *et al*. (1993), Rasmusson *et al*. (1994), Tanda *et al*. (1994) and Kuroki *et al*. (1996) reported that systemic administration of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a typical 5-HT$_{1A}$ receptor agonist, increased 3,4-dihydroxyphenylethylamine (dopamine) release in rat prefrontal cortex. In those studies, however, the possibility was not excluded that an α_2-adrenoceptor antagonistic action of 8-OH-DPAT may have contributed to the effect on dopamine release (Crist & Surprenant, 1987; Gobert *et al*., 1995). Furthermore, it is not known whether the 5-HT$_{1A}$ receptors modulating cortical dopamine release are localized presynaptically or postsynaptically. In this study, we examined the effect of the selective 5-HT$_{1A}$ receptor agonist, MKC-242,

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on dopamine release in rat prefrontal cortex using an *in vivo* microdialysis technique.

Methods

Animals and drugs

Male Wistar rats were maintained under controlled environmental conditions (22±1°C; 12 h–12 h light-dark cycle, lights on at 08:00, food and water *ad libitum*) for at least 1 week before being used for the experiments during which time they were accustomed to being handled. Procedures involving animals and their care were conducted according to Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

MKC-242 and N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide (WAY100635) were gifts from Mitsubishi Chemical Co. (Yokohama, Japan). Fluoxetine was a gift from Eli Lilly and Co. (Indianapolis, U.S.A.). All other chemicals used were of the highest commercially available purity. All drugs were freshly prepared. MKC-242 for s.c. injection (1 ml kg^{-1}) was suspended in 0.5% w v^{-1} carboxymethylcellulose (CMC). 5,7-Dihydroxytryptamine (5,7-DHT) was dissolved in 0.9% w v^{-1} NaCl containing 0.2% ascorbic acid. Other drugs were dissolved in 0.9% w v^{-1} NaCl. For direct administration into the prefrontal cortex *via* the dialysis probe, drugs were dissolved in a salt solution (composition, mM: NaCl 147, KCl 4, $CaCl_2$ 2.3).

Microdialysis procedure

Rats (250–350 g) were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.) and stereotaxically implanted with guide cannulae (one site per animal) for the dialysis probes (Eicom, Japan) at the prefrontal cortex (A +3.2, L −1.2, V 5.2, from bregma and dura), hippocampus (A −5.3, L −4.4, V 6.5), striatum (A +0.7, L −2.6, V 6.5) and nucleus accumbens (A +1.7, L −1.2, V 7.6) (Paxinos & Watson, 1986) as reported previously (Suzuki *et al*., 1995; Somboothum *et al*., 1997). The active probe membrane for the prefrontal cortex, hippocampus and striatum was 3 mm in length and that for the nucleus accumbens was 2 mm. The day after surgery the probes were perfused at a constant flow rate of 2 μl min^{-1} with Ringer's solution. A 4 h stabilization period was allowed before 20 min microdialysis samples (40 μl) were taken and immediately injected onto a h.p.l.c. column for subsequent assay of dopamine. The probe recovery for dopamine was 7.9±0.2% (n=6).

Analysis of dialysates

Dialysates were assayed by h.p.l.c. with electrochemical detection: Eicompak CA-5ODS column (2.1 mm i.d.×150 mm: Eicom, Kyoto, Japan) and graphite electrode (Eicom) set at +450 mV against an Ag/AgCl reference electrode were used as described previously (Suzuki *et al*., 1995). The mobile phase contained 80 mM sodium phosphate buffer (pH 6.0), 500 mg L^{-1} sodium octanesulphonic acid, 150 μM EDTA and 20% v v^{-1} methanol.

Lesions of serotoninergic neurons with 5,7-DHT

Rats (220–250 g) were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.). Lesions of 5-HT neurons were carried out by injection of 5,7-DHT (150 μg as free base, 20 μl per rat, over a period of 2 min) into the lateral ventricles (A −0.8, L −1.5 and V 4.5 from skull surface) as reported previously (Suzuki *et al*., 1995; Somboothum *et al*., 1997). Desipramine at 25 mg kg^{-1}, i.p. was injected 30 min before 5,7-DHT to protect noradrenergic neurons. The animals were used 2 weeks after the i.c.v. injection.

Determination of brain amines and their metabolites

The rats were killed by decapitation, and the samples for HPLC were prepared from the frontal cortex as previously reported (Matsuda *et al*., 1989). Eicompak CA-5ODS (4.6 mm i.d.×150 mm) (Eicom) was used for 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and noradrenaline assays. The mobile phase contained 40 mM sodium acetate-citrate buffer (pH 3.5), 170 mg L^{-1} sodium octanesulphonic acid, 15 μM EDTA and 13% v v^{-1} methanol. Other conditions were reported previously (Matsuda *et al*., 1995b).

Data analysis

The average of three fractions before drug administration was defined as 100% (control), and the subsequent perfusate levels were expressed as a percentage of the control. Statistical analyses were conducted by two-way ANOVA followed by Tukey's test or Student's *t*-test. P values of 5% or less were considered statistically significant.

Results

Basal level of dopamine, corrected with probe recovery, in the prefrontal cortex was 44±6 pg per fraction (mean±s.e.mean of eight determinations). Subcutaneous injection of MKC-242 at 0.3 and 1.0 mg kg^{-1} increased extracellular dopamine levels by about 50% in the prefrontal cortex (Figure 1). The maximal effect of MKC-242 to increase dopamine release was observed at 60 min after injection, and duration of the effect seemed to be longer in the higher dose than in the lower dose. MKC-242 at 0.1 mg kg^{-1} was ineffective (data not shown). Pretreatment with the 5-HT$_{1A}$ receptor antagonist, WAY100635, blocked the effect of MKC-242 on dopamine release in the prefrontal cortex, although the antagonist alone did not affect the basal release of dopamine (Figure 2). Two-way ANOVA analysis showed that the effect of WAY100635 on MKC-242-induced dopamine release was significant (saline/MKC-242 vs WAY100635/MKC-242: $F_{(11.127)}=2.252$, $P=0.015$). Local perfusion of WAY100635 *via* a dialysis probe during the experiment also attenuated the effect of MKC-242 in increasing cortical dopamine release (Figure 3). Two-way ANOVA analysis showed that the effect of WAY100635 on MKC-242-induced dopamine release was significant (saline/MKC-242 vs WAY100635/MKC-242: $F_{(11.132)}=2.344$, $P=0.011$). The effect of 8-OH-DPAT administered locally *via* a dialysis probe depended on the period of perfusion with the agonist: 8-OH-DPAT at 10 μM for 60 min increased dopamine release, but perfusion for 20 min did not (Figure 4). For local application of a 5-HT$_{1A}$ agonist, we used 8-OH-DPAT instead of MKC-242 because of its hydrophobicity.

Lesions of serotoninergic neurons with 5,7-DHT caused a marked reduction in 5-HT (by 97%) and 5-HIAA (by 96%) levels, while it did not affect significantly noradrenaline or dopamine levels in the prefrontal cortex (Table 1). There was no difference in basal dopamine release between control and 5,7-DHT-treated groups: the dopamine levels (mean±s.e.-

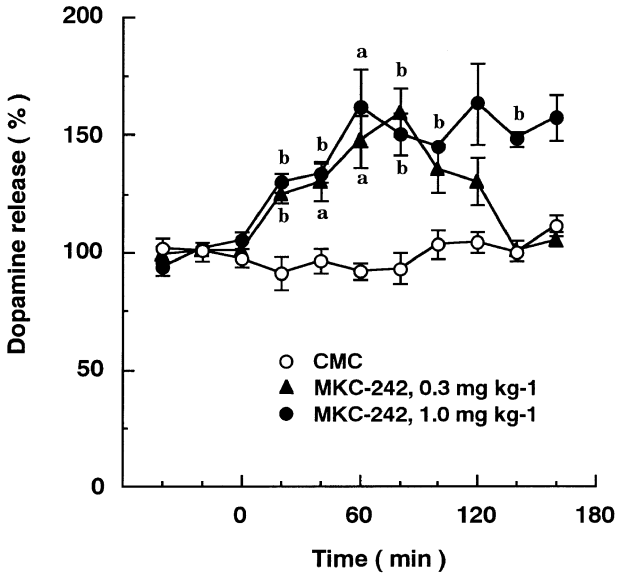

Figure 1 Effect of s.c. injection of MKC-242 on extracellular dopamine levels in the prefrontal cortex. Rats were treated with CMC and MKC-242 at zero time. Points are means ± s.e.mean of 3–5 rats. $^{a}P<0.05$, $^{b}P<0.01$, compared with the corresponding value of vehicle (two-way ANOVA followed by Tukey's test).

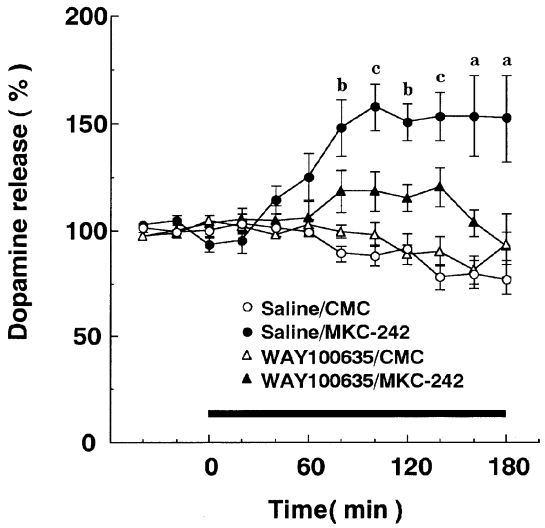

Figure 3 Effect of local application of WAY100635 on MKC-242-induced increase in extracellular dopamine levels in the prefrontal cortex. MKC-242 at 1.0 mg kg^{-1} was injected s.c. at 60 min. WAY100635 at 10 μM was perfused into the cortex *via* the dialysis probe for the time indicated by the horizontal bars. Points are means ± s.e.mean of 4–7 rats. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, compared with the corresponding value of saline/CMC treatment (two-way ANOVA followed by Tukey's test).

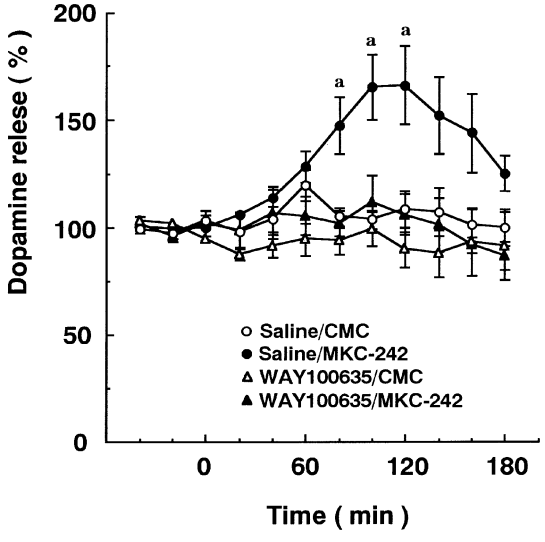

Figure 2 Effect of WAY100635 on MKC-242-induced increase in extracellular dopamine levels in the prefrontal cortex. MKC-242 at 1.0 mg kg^{-1} was injected s.c. at 40 min. WAY100635 at 1.0 mg kg^{-1} was administered s.c. 30 min before the agonist. Points are means ± s.e.mean of 5–7 rats. $^{a}P<0.05$, compared with the corresponding value of saline/CMC treatment (two-way ANOVA followed by Tukey's test).

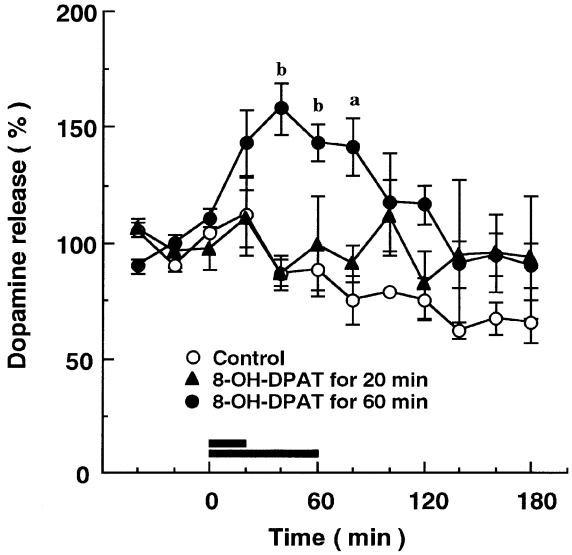

Figure 4 Effect of local application of 8-OH-DPAT on extracellular dopamine levels in the frontal cortex. 8-OH-DPAT at 10 μM was perfused into the cortex *via* the dialysis probe for the time indicated by the horizontal bars. Points are means ± s.e.mean of 3–5 rats. $^{a}P<0.05$, $^{b}P<0.01$, compared with the corresponding value of control (two-way ANOVA followed by Tukey's test).

Table 1 Effect of serotoninergic neuronal lesions with 5,7-DHT on noradrenaline (NA), dopamine (DA), 5-HT and their metabolite levels (ng g^{-1} tissue) in rat frontal cortex

	NA	*DA*	*DOPAC*	*HVA*	*5-HT*	*5-HIAA*
Vehicle	338 ± 30	148 ± 27	11 ± 1	24 ± 0	265 ± 28	113 ± 7
5,7-DHT	249 ± 20	127 ± 52	15 ± 4	15 ± 2	8 ± 1***	5 ± 0***

Rats were injected with vehicle and 5,7-DHT 14 days before the experiments. Results are means ± s.e.mean from 4–6 rats. ***$P<0.001$, compared with vehicle (Student's *t*-test).

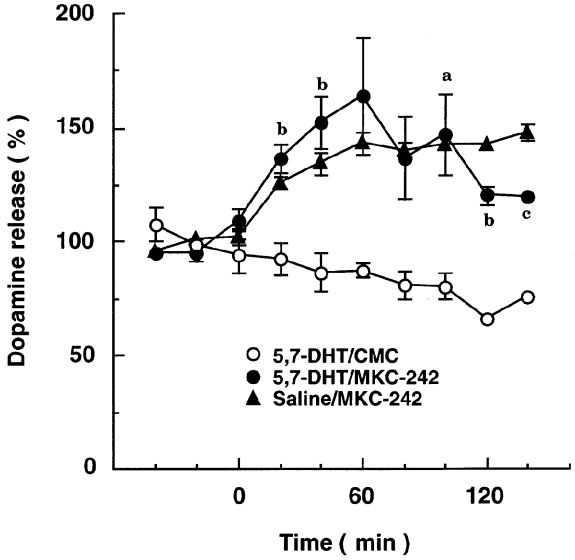

Figure 5 Effect of pretreatment with 5,7-DHT on MKC-242-induced increase in extracellular dopamine levels in the prefrontal cortex. MKC-242 at 1.0 mg kg^{-1} was injected s.c. 14 days after rats were injected i.c.v. with saline and 5,7-DHT at 150 μg. Points are means$\pm$s.e.mean of 4–8 rats. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, compared with 5,7-DHT/CMC (two-way ANOVA followed by Tukey's test).

mean, $n=9-11$) in the prefrontal cortex of vehicle- and 5,7-DHT-treated rats were 46 ± 10 and 39 ± 5 pg per fraction, respectively. MKC-242 increased extracellular dopamine levels in the prefrontal cortex of 5,7-DHT-treated rats: there was no difference in MKC-242-induced increase in dopamine release between saline and 5,7-DHT-pretreated rats (Figure 5).

The effect of MKC-242 on dopamine release was also studied in other brain regions. Systemic administration of MKC-242 at 1.0 mg kg^{-1} increased dopamine release in the hippocampus by about 100%, but not in the striatum or nucleus accumbens (Figure 6). The maximal effect of MKC-242 to increase dopamine release in the hippocampus was observed at 80 min and continued until 140 min.

Figure 7 shows the effects of buspirone and fluoxetine on dopamine release in the prefrontal cortex. Subcutaneous injection of these drugs at 3 and 10 mg kg^{-1} caused a dose-dependent increase in extracellular dopamine levels in the prefrontal cortex. Buspirone and fluoxetine at 10 mg kg^{-1} caused 3 fold and 2 fold increases in cortical dopamine release, respectively. The maximal effects by buspirone and fluoxetine were observed at 60–120 and 40 min after injection, respectively. Fluoxetine as well as MKC-242 did not affect dopamine release in the striatum or nucleus accumbens, while buspirone increased dopamine release by about 50% in the nucleus accumbens (Figure 8).

Discussion

Previous studies suggest that serotoninergic regulation of the meso-cortical dopaminergic pathway projecting from the ventral tegmental area to the prefrontal cortex plays a role in the effects of antidepressants (Willner, 1983; Tanda *et al*., 1994; Carlson *et al*., 1996) and atypical antipsychotic drugs (White & Wang, 1983; Meltzer, 1996). For example, activation of 5-HT$_{1B}$ (Iyer & Bradberry, 1996), 5-HT$_{2A}$ (Gobert & Millan, 1999) or 5-

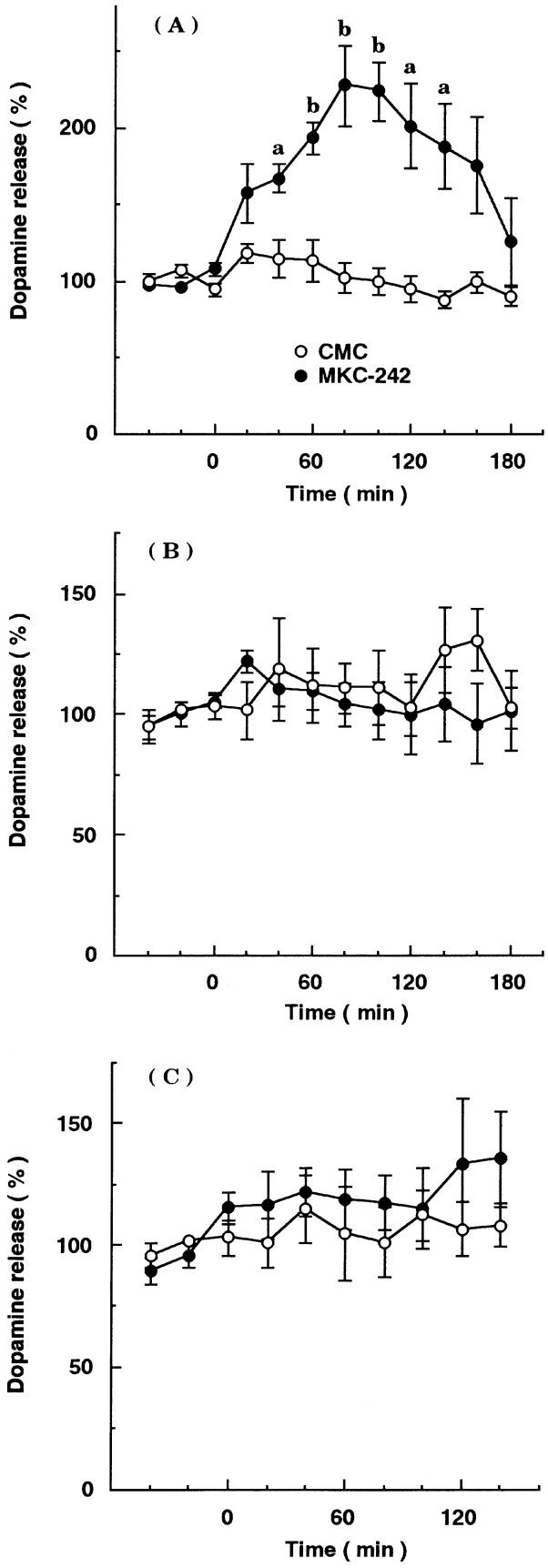

Figure 6 Effect of s.c. injection of MKC-242 on extracellular dopamine levels in the hippocampus (A), striatum (B) and nucleus accumbens (C). Rats were treated with saline and MKC-242 at 1.0 mg kg^{-1} at zero time. Points are means$\pm$s.e.mean of 4–6 rats. $^{a}P<0.05$, $^{b}P<0.01$, compared with the corresponding value of vehicle (two-way ANOVA followed by Tukey's test).

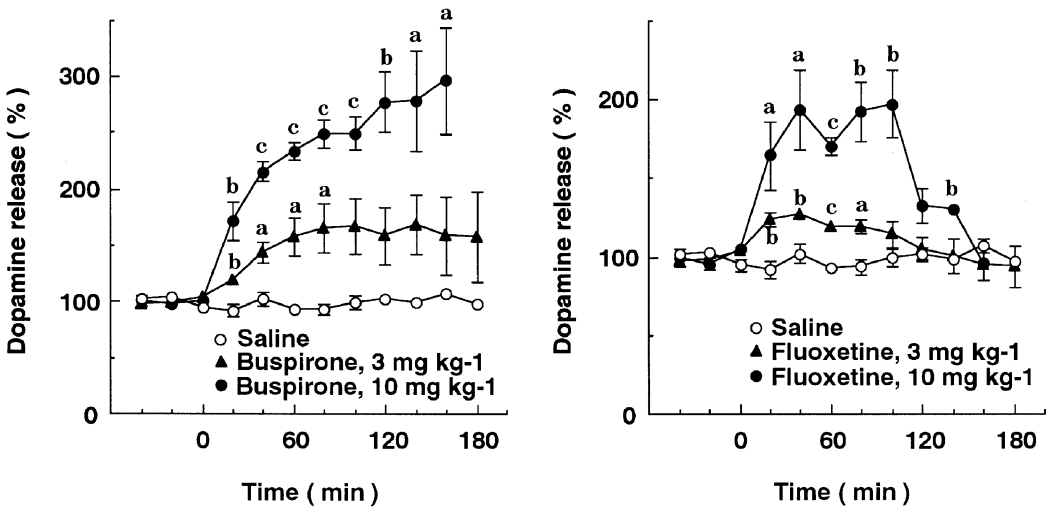

Figure 7 Effects of buspirone and fluoxetine on extracellular dopamine levels in the prefrontal cortex. Rats were treated with saline, buspirone and fluoxetine at zero time. Points are means±s.e.mean of 3–5 rats. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, compared with the corresponding value of saline treatment (two-way ANOVA followed by Tukey's test).

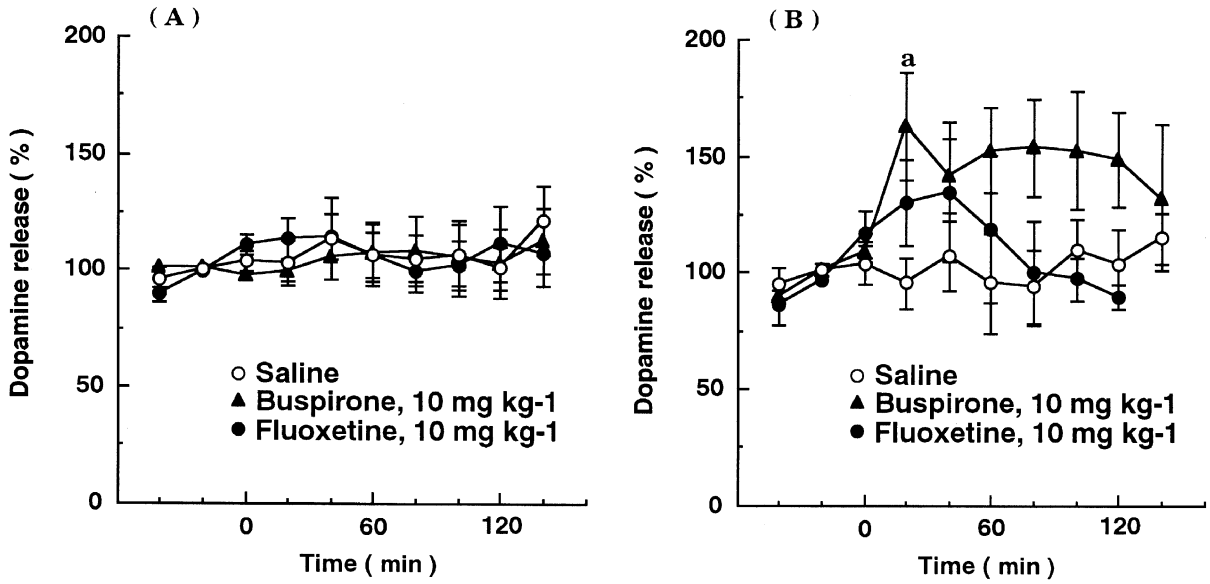

Figure 8 Effects of buspirone and fluoxetine on extracellular dopamine levels in the striatum (A) and nucleus accumbens (B). Rats were treated with saline, buspirone and fluoxetine at zero time. Points are means±s.e.mean of 5–7 rats. $^{a}P<0.05$, compared with the corresponding value of saline treatment (two-way ANOVA followed by Tukey's test).

HT_3 (Chen *et al*., 1992) receptors enhances directly or indirectly dopamine release in the prefrontal cortex, whereas activation of 5-HT$_{2C}$ receptors inhibits cortical dopamine release (Millan *et al*., 1998). The present study aimed to clarify the regulation by 5-HT$_{1A}$ receptors of cortical dopamine release using the selective 5-HT$_{1A}$ receptor agonist, MKC-242, and the selective 5-HT$_{1A}$ receptor antagonist, WAY100635. MKC-242 has high affinity for 5-HT$_{1A}$ receptors (K_i: 0.35 nM), and it is about 500 fold and more than 1000 fold more active at the 5-HT$_{1A}$ site than at the 5-HT$_{2A}$ site and at the 5-HT$_{1B}$, 5-HT$_{2C}$, 5-HT$_3$, α_2-adrenergic and dopamine D_1 sites, respectively (Matsuda *et al*., 1995b). The present study suggests that activation of the 5-HT$_{1A}$ receptors causes cortical dopamine release and the degree of the effect is similar to that of the 5-HT$_{2A}$ receptor agonist-induced cortical dopamine release (Gobert & Millan, 1999).

The present study demonstrates that treatment with 5,7-DHT that destroys presynaptic serotoninergic nerve fibres does not alter the effect of MKC-242 in increasing cortical dopamine release. This finding suggests that dopamine release is modulated in part by postsynaptic 5-HT$_{1A}$ receptors in the prefrontal cortex. Since exogenous 5-HT facilitated dopamine release in the prefrontal cortex (Iyer & Bradberry, 1996), it is possible that 5,7-DHT lesions could eliminate this action of endogenous 5-HT on dopamine release. However, the recent (Matsumoto *et al*., 1998) and present studies showed that there was no difference in basal dopamine release between vehicle- and 5,7-DHT-treated rats. This may be explained by 5,7-DHT-induced supersensitivity of 5-HT$_{1B}$ or 5-HT$_{2A}$ receptors (Van de Kar *et al*., 1989; Butler *et al*., 1990; Sijbesma *et al*., 1991), since these receptors can exert a stimulatory influence on dopamine release (Iyer & Bradberry, 1996; Gobert & Millan, 1999). The involvement of the postsynaptic 5-HT$_{1A}$ receptors in MKC-242-induced cortical dopamine release is further supported by the experiments of local application of a 5-HT$_{1A}$

receptor agonist and antagonist. The effect of MKC-242 in increasing cortical dopamine release was blocked by local application of WAY100635, and local application of 8-OH-DPAT increased dopamine release in the cortex. It should be noted that a long duration of WAY100635 or 8-OH-DPAT perfusion was required. This implies that the site of action of these drugs is away from the position of the probe: the postsynaptic 5-HT$_{1A}$ receptors modulating cortical dopamine release appear to be localized on sites other than dopaminergic nerve terminals.

Tanda *et al*. (1994) reported that 8-OH-DPAT increased dopamine release in the prefrontal cortex, but not in the nucleus accumbens. In line with this finding, MKC-242 increased dopamine release in the prefrontal cortex, but not in the striatum or nucleus accumbens. Furthermore, the 5-HT$_{1A}$ receptor agonist-induced increase in dopamine release was greater in the hippocampus than in the prefrontal cortex. The region-specific effect may be explained by the idea that dopamine release is modulated by postsynaptic 5-HT$_{1A}$ receptors. The 5-HT$_{1A}$ receptors are dense in the hippocampus, while they are sparse in the striatum and nucleus accumbens (Pazos & Palacios, 1985). In this regard, we have found that locally perfused 5-HT, but not 8-OH-DPAT, at 10 μM increased dopamine release in the striatum and nucleus accumbens in a WAY100635-insensitive manner (data not shown). This suggests that 5-HT receptor subtypes other than 5-HT$_{1A}$ receptors are involved in dopamine release in these brain regions. In contrast to MKC-242 and 8-OH-DPAT, systemic buspirone increased dopamine release in the nucleus accumbens. This observation is also reported by Tanda *et al*. (1994). Furthermore, the previous studies showed that systemic administration of buspirone and intrastriatal administration of ipsapirone enhanced dopamine release in the striatum (Algeri *et al*., 1988; Golemibiowska & Wedzony, 1993). These azapirone compounds act not only as 5-TH$_{1A}$ receptor agonists but also α_2 adrenoceptor and dopamine D$_2$ receptor antagonists. Since α_2 adrenoceptor and dopamine D$_2$ receptor antagonists increase dopamine release (Gobert *et al*., 1998; Matsumoto *et al*., 1998), it is likely that the effects of azapirones on these receptors contribute at least partly to the increase in dopamine release in the 5-HT$_{1A}$ receptor-poor regions.

The present study showed that MKC-242 as well as fluoxetine, a selective 5-HT reuptake inhibitor, preferentially increased dopamine release in the prefrontal cortex. The region-specific activation of the dopamine system by MKC-242 and fluoxetine may contribute to the antidepressant-like effect of these drugs. Furthermore, the present finding implies that the activation of this receptor may have a beneficial effect for treatment of schizophrenia, since activation of cortical dopaminergic system may be important for the ameliorating effect of atypical antipsychotic drugs on negative symptoms in schizophrenia (Meltzer, 1989; Andersson *et al*., 1995). In contrast, it is unlikely that the activation of cortical dopaminergic system contribute to the anxiolytic-like effect of 5-HT$_{1A}$ receptor agonists, since anxiogenic agents and stress cause dopamine release in the prefrontal cortex (Bradberry *et al*., 1991; Yoshioka *et al*., 1996; Matsumoto *et al*., 1998).

In conclusion, the present study demonstrates that the 5-HT$_{1A}$ receptor agonist MKC-242 preferentially increased extracellular dopamine level in the prefrontal cortex and hippocampus *via* activation of postsynaptic 5-HT$_{1A}$ receptors. The exact anatomical site of these receptors remains to be determined.

This research was supported by grants from the Ministry of Education, Science and Culture of Japan and from Mitsubishi Chemical Co., Yokohama, Japan. We acknowledge the donations of MKC-242 by Dr M. Egawa (Mitsubishi Chemical Co.), WAY100635 by Dr M. Egawa, and fluoxetine by Dr T. Okimura (Eli Lilly Japan K.K.).

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(Received September 9, 1999
Revised November 24, 1999
Accepted December 7, 1999)