Interaction of Orally Administered 5-{3-[[((2S)-1,4-Benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole (MKC-242) with 5-HT_{1A} Receptors in Rat Brain

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ABSTRACT—The present study was carried out to clarify whether orally administered 5-{3-[[((2S)-1,4-benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole (MKC-242), a serotonin_{1A} (5-HT_{1A})-receptor agonist having potent anxiolytic-like and antidepressant-like effects in animal models, binds to 5-HT_{1A} receptors in rat brain. Quantitative autoradiography showed that orally administered MKC-242 (0.1-0.5 mg/kg) caused a significant decrease in [3H]8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) binding in the hippocampus and dorsal raphe nucleus sections. The decrease in the binding by MKC-242 was observed up to 4 hr after administration, and the effective doses were similar to those in its anxiolytic-like effect in the animal models. Repeated treatment of MKC-242 (0.5 mg/kg/day, p.o.) or buspirone (30 mg/kg/day, p.o.) for 2 weeks did not affect [3H]8-OH-DPAT binding in both sections. These results suggest that orally administered MKC-242 at the low doses that do not show 5-HT_{1A}-receptor-mediated in vivo responses such as the hypothermic effect, adrenocortical effect and the decrease in 5-HT turnover passes the blood-brain barrier and subsequently binds to 5-HT_{1A} receptors in rat brain. In addition, they indicate that repeated stimulation of the receptors by the agonists does not affect the number of the binding sites.

Keywords: 5-{3-[[((2S)-1,4-Benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole (MKC-242), 5-HT_{1A}-receptor agonist, Autoradiography, Anxiolytic
graphy of 5-HT_{1A} receptors with [H]8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in order to provide evidence that the drug indeed binds to the receptors in rat brain. We also demonstrate that repeated stimulation of 5-HT_{1A} receptors does not alter the number of the binding sites in the brain.

MATERIALS AND METHODS

Animals

Male Wistar rats (Shimizu Lab. Supplies Co., Ltd., Kyoto) were maintained under controlled environmental conditions (22±1°C; 12-12 hr light-dark cycle, lights on at 08 hr 00 min; 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.

Materials

[3H]8-OH-DPAT (4,440 GBq/mmol) was obtained from New England Nuclear (Boston, MA, USA). Autoradiographic [H]micro-scales were obtained from Amersham Japan (Tokyo). MKC-242 and tandospirone citrate were synthesized at Mitsubishi Chemical Co. (Yokohama). Buspirone HCl and 5,7-dihydroxytryptamine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). For in vitro addition, MKC-242 was dissolved in 0.02 M acetic acid to make a 5 mM solution, and subsequent dilutions were made in assay buffer. For oral administration (1.0 ml/kg), MKC-242 was suspended in 0.5% carboxymethylcellulose. 5,7-Dihydroxytryptamine was dissolved in 0.9% NaCl containing 0.2% ascorbic acid.

Receptor autoradiography

Rats (200–300 g) were decapitated, and the brains were rapidly removed, frozen in dry ice powder and stored at −80°C. Coronal sections (20-μm-thick) were cut at −20°C, thaw mounted onto glass slides coated with a solution of 0.5% gelatin containing 0.05% chromic potassium sulfate and stored at −20°C until use for usually less than 2 weeks. The labeling experiment was carried out according to the procedure reported by Chalmers and Watson (13). Briefly, slice sections were preincubated at room temperature for 30 min in 0.17 M Tris-HCl, pH 7.6 containing 4 mM CaCl2 and 0.1% ascorbic acid. Subsequently, the sections were incubated with 2 nM [H]8-OH-DPAT in the fresh buffer for 60 min at room temperature. After incubation, the sections were washed with the fresh buffer (2×5 min) at 4°C and dried in a stream of cold air. Non-specific binding was determined in the presence of 2 μM 5-HT. In the Scatchard analysis, the concentration of [H]8-OH-DPAT varies from 0.25 to 5 nM. The sections and autoradiographic standard (Amersham Japan) were exposed to 3H-sensitive Hyperfilm at room temperature for 14 to 21 days. The exposure period depended on the intensity of the radioactivity on the slices. Quantitative analysis of the autoradiogram was carried out by an image analysis system developed by NIH (Image 1.59). The specific activity was estimated by the Amersham protocol using a 3H microscale and shown as fmol/mg tissue.

Lesions of 5-HT neurons with 5,7-dihydroxytryptamine

Rats (140–150 g) were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). Lesions of 5-HT neurons were induced by injection of 5,7-dihydroxytryptamine (150 μg as free base, 20 μl par rat, over a period of 2 min) into lateral ventricles (A −0.8, L −1.5 and V 4.5 from skull surface) (14) as described previously (15). Desipramine at 25 mg/kg, i.p. was injected 30 min before 5,7-dihydroxytryptamine to protect noradrenergic neurons. The animals were used 2 weeks after the i.c.v. injection. The determination of brain amine contents by an HPLC method (16) showed that the treatment with 5,7-dihydroxytryptamine caused a marked reduction in 5-HT (by 96%) and its metabolite 5-hydroxyindoleacetic acid (by 97%) as previously reported (14).

Statistics

Statistical analyses were conducted by two-way or one-way ANOVA followed by either Fisher's least significant difference (LSD) test, the Duncan test or Student's t-test with statistical data analysis software (SPSS, Chicago, IL, USA). P values of 5% or less were considered statistically significant.

RESULTS

In vitro incubation of the dorsal raphe nucleus and hippocampus sections with [H]8-OH-DPAT shows the specific binding. In vitro addition of the 5-HT_{1A}-receptor agonists MKC-242 and buspirone to the medium decreased the specific binding of [H]8-OH-DPAT in a concentration-dependent manner: IC_{50} values of MKC-242 and buspirone were 0.43 and 76.5 nM, respectively. Their K_{i} values were calculated to be 0.13 and 27.1 nM, respectively (Fig. 1). In some experiments, the sections were treated with the drugs, subsequently washed with the incubation buffer and then incubated with [H]8-OH-DPAT. Under such conditions, the inhibitory effect of buspirone on the binding disappeared, while that of MKC-242 was still observed (Fig. 1).

Figure 2 shows a typical autoradiography of the
Fig. 1. Effect of in vitro addition of 5-HT\textsubscript{1A}-receptor agonists on $[^3\text{H}]$8-OH-DPAT binding in rat hippocampus. Slice sections pre-incubated in the Tris buffer were incubated with $[^3\text{H}]$8-OH-DPAT for 60 min in the presence of MKC-242 (open circle) and buspirone (closed circle) at the indicated concentrations. In the other experiment, the slices were pretreated with MKC-242 (open square) and buspirone (closed square) for 60 min and then washed with fresh buffer for 30 min and incubated with $[^3\text{H}]$8-OH-DPAT for 60 min. Points are means±S.E.M. of 3–6 experiments.

Fig. 3. Effect of oral administration of MKC-242 on the specific binding of $[^3\text{H}]$8-OH-DPAT in the hippocampus and dorsal raphe nucleus. Rats were sacrificed at 60 min after administration of MKC-242 at the indicated doses, and receptor autoradiography was carried out in the hippocampus (A) and dorsal raphe nucleus (B) sections. Points are means±S.E.M. of 6–16 experiments. *$P<0.05$, ***$P<0.001$, vs control rats (Duncan test).

Fig. 2. Typical receptor autoradiographs of $[^3\text{H}]$8-OH-DPAT binding to brain coronal sections of rats treated with MKC-242. The rats were orally administered with the vehicle (A, B) and MKC-242 (C, D), and the brain sections were incubated with $[^3\text{H}]$8-OH-DPAT. Non-specific binding (in the presence of 2 $\mu$M 5-HT) was less than 10% of the total binding (not shown). The sections correspond to the stereotaxic atlas of Paxinos and Watson (14) as follows: A and C, plate 31; B and D, plate 49.
specific binding of \([^{3}H]8\text{-OH-DPAT}\) in the hippocampus and dorsal raphe nucleus from rats treated with MKC-242. Acute oral administration of MKC-242 (0.1–0.5 mg/kg) resulted in a dose-dependent decrease of \([^{3}H]8\text{-OH-DPAT}\) binding in both brain regions (Fig. 3). Scatchard analysis showed that MKC-242 decreased the \(B_{\text{max}}\) value in both regions and increased the \(K_d\) value only in the hippocampus (Table 1). Typical plots are shown in Fig. 4. The effect of MKC-242 was maximal at 1 hr after administration and was observed up to 4 hr (Fig. 5).

Lesions of 5-HT neurons with 5,7-dihydroxytryptamine resulted in a slight increase in \([^{3}H]8\text{-OH-DPAT}\) binding in the hippocampus (Fig. 6A) and a marked decrease in the binding in the dorsal raphe nucleus (Fig. 6B). MKC-242 (0.5 mg/kg, p.o.) significantly decreased \([^{3}H]8\text{-OH-DPAT}\) binding in both regions from rats treated with 5,7-dihydroxytryptamine (Fig. 6).

The effect of repeated treatment with MKC-242 (once a

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**Table 1.** Scatchard plot analysis of \([^{3}H]8\text{-OH-DPAT}\) binding in the slice sections of rats treated with MKC-242

| Regions          | Treatment     | \(B_{\text{max}}\) (fmol/mg tissue) | \(K_d\) (nM) |
|------------------|---------------|-----------------------------------|--------------|
| Hippocampus      | Control       | 99.6±10.8                        | 1.10±0.27    |
|                  | MKC-242       | 29.1±6.3**                       | 3.01±0.35**  |
| Dorsal raphe     | Control       | 66.9±8.0                         | 0.86±0.07    |
|                  | MKC-242       | 14.6±3.0***                      | 0.90±0.15    |

MKC-242 at 0.5 mg/kg and 0.5% carboxymethylcellulose (control) were orally administered 1 hr before sacrifice. Results are means±S.E.M. of 4–5 experiments. **P<0.01, ***P<0.001, vs control (Student's t-test).

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**Fig. 4.** Scatchard plots of \([^{3}H]8\text{-OH-DPAT}\) binding in the slice sections of rats treated with MKC-242. MKC-242 at 0.5 mg/kg (closed) and 0.5% carboxymethylcellulose (open) were orally administered 1 hr before sacrifice. Points are values from a representative experiment, which has been repeated.

**Fig. 5.** Time course of displacement of \([^{3}H]8\text{-OH-DPAT}\) binding caused by administration of MKC-242. Rats were sacrificed at the indicated time after administration of MKC-242 (0.5 mg/kg, p.o.). Points are means±S.E.M. of 3–4 experiments. *P<0.05, ***P<0.001, vs 0 time (LSD test).

**Fig. 6.** Effect of oral administration of MKC-242 on \([^{3}H]8\text{-OH-DPAT}\) binding in rats treated with 5,7-dihydroxytryptamine. Rats pretreated with vehicle (Sham) and 5,7-dihydroxytryptamine (5,7-DHT) were sacrificed at 60 min after vehicle (open column) and MKC-242 (0.5 mg/kg, p.o.) (hatched column) in the hippocampus (A) and dorsal raphe nucleus (B). Results are means±S.E.M. of 4–5 experiments. **P<0.001, vs control; †P<0.05, ††P<0.01, vs sham (two-way ANOVA followed by Student's t-test).
day for 2 weeks at 0.5 mg/kg, p.o.) and buspirone (once a day for 2 weeks at 30 mg/kg, p.o.) on [3H]8-OH-DPAT binding was examined. [3H]8-OH-DPAT binding in the dorsal raphe nucleus and hippocampus was not changed by the repeated treatment with MKC-242 or buspirone (Fig. 7: A and B). The significant decrease in [3H]8-OH-DPAT binding by MKC-242 (0.5 mg/kg, p.o., 1 hr) was observed even in rats treated chronically with MKC-242 (Fig. 7: C and D).

**DISCUSSION**

Previous studies show that peripherally injected 5-HT1A-receptor agonists have anxiolytic-like and anti-depressant-like effects in animal models. It is likely that these effects are mediated by an activation of central 5-HT1A receptors because the pharmacological effects are blocked by a 5-HT1A-receptor antagonist (17) and the compounds cause typical 5-HT1A-receptor-mediated in vivo effects (hypothermia, increased adrenocortical response and decreased 5-HT metabolism). However, we observed that the 5-HT1A-receptor agonist MKC-242 had an anxiolytic-like effect at the extremely low doses (12) that did not cause 5-HT1A-receptor-mediated in vivo responses such as hypothermia, increased adrenocortical response and decreased 5-HT turnover (11). This finding, unlike the experiment using a 5-HT1A-receptor antagonist (12), appears to be against the idea that the anxiolytic-like effect of MKC-242 is mediated by activation of central 5-HT1A receptors. Recently, Laporte et al. (18) have demonstrated by an in vivo labeling technique with the novel 5-HT1A-receptor antagonist radioligand [1H]WAY100635 that peripherally injected 5-HT1A-receptor agonists interacted with the receptors in mouse brain. In this study, we tried using a quantitative receptor autoradiography method to test whether orally administered MKC-242 at the low doses that do not show the 5-HT1A-receptor-mediated in vivo responses interacts with 5-HT1A receptors in rat brain.

Two brain regions (the dorsal raphe nucleus and hip-
pocampus) were used in this study to obtain the information on somadendritic and postsynaptic 5-HT_{1A} receptors. We observed that [^{3}H]8-OH-DPAT binding in the hippocampus was increased by 5,7-dihydroxytryptamine, whereas that in the dorsal raphe nucleus was decreased in agreement with the previous reports (19, 20). Scatchard analysis of receptor autoradiography showed that the K_d value of [^{3}H]8-OH-DPAT binding in tissue sections was similar to that in membrane receptor binding as reported previously (11, 21, 22). In addition, K_i values of MKC-242 and buspirone in this autoradiography experiment were similar to those reported in membrane receptor binding (11). Taken together, it is conceivable that [^{3}H]8-OH-DPAT binding in the dorsal raphe nucleus and hippocampus reported here reflects the densities of somadendritic and postsynaptic 5-HT_{1A} receptors, respectively. In receptor autoradiography experiments, the slice section mounted on a coverglass is usually incubated with buffer before the binding experiment to wash out endogenous ligands. Indeed, our pilot experiment showed that the binding was higher in washed slices than in unwashed slices. Under the conditions including a washing procedure, we could not detect any inhibitory effects of peripherally injected azapirones (30 mg/kg, p.o., 1 hr) such as buspirone and tandospirone on [^{3}H]8-OH-DPAT binding (data not shown). This may be due to a rapid dissociation of azapirones from the binding sites during in vitro washing. It appears that MKC-242 contrasts with buspirone and tandospirone with respect to the interaction with 5-HT_{1A} receptors. It should be noted that the slow dissociation of MKC-242 from 5-HT_{1A} receptors contributes to a quantitative autoradiographic study for the in vivo binding of the drug to the receptors. The most important finding in this study is that orally administered MKC-242 at the low doses that do not show the 5-HT_{1A}-receptor-mediated in vivo responses significantly decreases [^{3}H]8-OH-DPAT binding in the brain sections. This finding suggests that MKC-242 penetrates the blood-brain barrier and binds to 5-HT_{1A} receptors in the brain. We further observed that oral MKC-242 decreased [^{3}H]8-OH-DPAT binding in the hippocampus and dorsal raphe nucleus to the similar degree, and it decreased the binding even in rats treated with 5,7-dihydroxytryptamine. These results suggest that systemic MKC-242 interacts with not only somadendritic 5-HT_{1A} autoreceptors, but also postsynaptic 5-HT_{1A} receptors. It should be noted that these receptors differed in the response to MKC-242: the K_d value in the hippocampus, but not the dorsal raphe nucleus, was increased by MKC-242, although the B_{max} value was decreased in both regions. The observation supports the idea that presynaptic 5-HT_{1A} receptors are not same as postsynaptic 5-HT_{1A} receptors in the brain (23, 24). Although postsynaptic 5-HT_{1A} receptors are involved in the antidepressant-like effect of MKC-242 (16), it remains to be clarified whether the anxiolytic-like effect of the drug is mediated by presynaptic or postsynaptic 5-HT_{1A} receptors.

Electrophysiological studies suggest that desensitization of presynaptic 5-HT_{1A} receptors play an important role in the antidepressant-like effects of 5-HT uptake inhibitors and azapirones (25, 26), although neurochemical observations do not support this idea (10, 27). Welner et al. (28) reported using quantitative autoradiography that repeated treatment with gepirone (10 mg/kg/day, 3 weeks), a 5-HT_{1A}-receptor agonist, decreased [^{3}H]8-OH-DPAT binding in the raphe region, but not in the hippocampus. In contrast, the present study showed that repeated stimulation of 5-HT_{1A} receptors by MKC-242 or buspirone did not change [^{3}H]8-OH-DPAT binding in the dorsal raphe nucleus and hippocampus. The exact reason for the apparent discrepancy between the previous (28) and the present observations is not known, although it may be explained by a difference in the experimental conditions such as treatment period. Alternatively, the contrast implies the difference in pharmacological properties of the 5-HT_{1A}-receptor agonists gepirone, buspirone and MKC-242. The present finding suggests that desensitization of the receptor itself is not involved in the pharmacological effect of MKC-242, but it does not exclude the possible involvement of desensitization of the receptor functions. In this connection, Li et al. (29) have recently suggested that a reduction in G proteins might play a role in desensitization of 5-HT_{1A} receptors induced by fluoxetine.

In conclusion, the present study shows that orally administered MKC-242 at the low doses that do not show the 5-HT_{1A}-receptor-mediated in vivo responses penetrates the blood-brain barrier and binds to 5-HT_{1A} receptors in rat brain. The slow dissociation of MKC-242 from the receptors may contribute to the potent anxiolytic-like and antidepressant-like effects of this drug. The conclusion will be confirmed by future experiments using radiolabeled MKC-242 which is not commercially available. In addition, this study also shows that repeated stimulation of 5-HT_{1A} receptors by the agonists does not affect the number of the binding sites.

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