Novel Benzodioxan Derivative, 5-{3-[(2S)-1,4-Benzodioxan-2-ylmethyl]amino}propoxy]-1,3-benzodioxole HCl (MKC-242), with a Highly Potent and Selective Agonist Activity at Rat Central Serotonin_{1A} Receptors

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ABSTRACT—The present study characterizes the neurochemical profile of the newly synthesized compound 5-{3-[(2S)-1,4-benzodioxan-2-ylmethyl]amino}propoxy]-1,3-benzodioxole HCl (MKC-242). In in vitro experiments, MKC-242 had high affinity for serotonin_{1A} (5-HT_{1A}) receptors (K_i: 0.35 nM) and moderate affinity for \( \alpha_1 \)-adrenoceptors (K_i: 21 nM), whereas it had no appreciable affinity for any other neurotransmitter recognition sites studied and 5-HT transporter. MKC-242 (0.3–3.0 mg/kg, s.c.; 1–10 mg/kg, p.o.) caused presynaptic 5-HT_{1A}-receptor-mediated responses (decreases in 5-HT turnover and 5-HT release) and postsynaptic 5-HT_{1A}-receptor-mediated responses (hypothermia, an increase in serum corticosterone level and 5-HT_{1A} behavioral syndrome). The effects of MKC-242 on decarboxylase inhibitor-induced 5-hydroxytryptophan accumulation and rectal temperature were blocked by the 5-HT_{1A}-receptor antagonist N-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropanamide. The comparative studies on the in vivo responses induced by MKC-242 and the 5-HT_{1A}-receptor full agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) showed that MKC-242 and 8-OH-DPAT had similar efficacy at presynaptic 5-HT_{1A} receptors, whereas the former had less efficacy than the latter at postsynaptic 5-HT_{1A} receptors. Furthermore, MKC-242 partially inhibited forskolin-stimulated adenylate cyclase activity in hippocampal membranes. These findings suggest that MKC-242 acts as a full and partial agonist at pre- and postsynaptic 5-HT_{1A} receptors, respectively, in the central nervous system.

Keywords: Serotonin (5-HT), 5-HT_{1A} receptor, MKC-242, Agonist

Serotonin (5-HT) receptors are heterogeneous, being classified into the distinct main classes from 5-HT_1 to 5-HT_7 receptor families, and the 5-HT_1 family consists of several receptor subtypes (1–3). 5-HT_{1A} receptors of these receptor subtypes have been studied most intensively because of the availability of the 5-HT_{1A}-receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (4, 5) and the proposed clinical utilities of the 5-HT_{1A}-receptor agonists azapirones (buspirone, gepirone, ipsapirone and tandospirone) for treatment of anxiety and depression (6, 7). However, these 5-HT_{1A}-receptor agonists act not only at 5-HT_{1A} receptors but also at other sites; for example, buspirone, gepirone and ipsapirone markedly increase the turnover rate of dopamine (DA) in rat brain in a 5-HT_{1A}-receptor-independent mechanism (8). In addition, a metabolite of azapirone, 1-(2-pyrimidyl)piperazine, exhibits potent \( \alpha_2 \)-adrenoceptor antagonistic properties (9, 10) and exerts opposite effects to those associated with the stimulation of 5-HT_{1A} receptors (11). These findings argue against the hypothesis that 5-HT_{1A} receptors may be a molecular target for the non-benzodiazepine anxiolytics.

5-HT_{1A} receptors are localized presynaptically on serotonergic perikarya in raphe nuclei and postsynaptically on non-serotonergic neurons in several brain regions (12, 13). The activation of the former receptors causes...
decreases in 5-HT synthesis and release, while that of the latter receptors causes induction of corticosterone secretion, hypothemia and behavioral effects (14, 15). The inhibition by 5-HT1A-receptor ligands of forskolin-stimulated adenylate cyclase activity is also considered to be mediated by postsynaptic 5-HT1A receptors (16). These observations suggest that pre- and postsynaptic 5-HT1A receptors differ in their functions. In this line, previous studies show that postsynaptic 5-HT1A receptors may be involved in the anticonflict/antidepressant effects of azapirones (17–20), although there are contradictory observations (21, 22). In order to elucidate the mechanism for the pharmacology of 5-HT1A-receptor agonists, it appears to be important not only to discover potent and selective 5-HT1A receptor agonists but also to study how the ligands act on pre- and postsynaptic 5-HT1A receptors. In this paper, we examined the neurochemical profile of the new compound MKC-242 (Fig. 1), which was developed as a 5-HT1A-receptor-related anxiolytic (23). The present study demonstrates that MKC-242 acts as a full agonist at presynaptic 5-HT1A receptors and as a partial agonist at postsynaptic 5-HT1A receptors. Certain of these results were communicated to the Japanese Neurochemical Society (24).

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 8:00; food and water ad libitum) for at least 1 week before being used for the experiments.

In vitro radioligand binding studies

In general, the rats were killed by decapitation, and their hippocampi, striata and cerebral corticies were immediately dissected according to Glowinsky and Iversen (25). The tissues were homogenized, and the membrane fractions were prepared for binding assays. The following assays were used (the description of each assay given in the following order: recognition site, [3H]ligand, the concentration, tissue, non-specific binding): 5-HT1A, 8-OH-DPAT, 0.2 nM, hippocampus, 10 μM 5-HT (26); 5-HT1A, 2 nM, hippocampus, 1 μM spiperone (26); 5-HT1B, 5-HT, 2 nM, striatum, 10 μM 5-HT (27); 5-HT2C, 5-HT, 2 nM, striatum, 10 μM 5-HT (27); 5-HT2A, spiperone, 0.25 nM, cortex, 1 μM 5-HT (26); 5-HT3, GR65630, 0.2 nM, cortex, 1 μM 5-HT (28); 5-HT transporter, paroxetine, 0.05 nM, cortex, 100 μM 5-HT (29); α1-adrenergic, prazosin, 0.15 nM, cortex, 10 μM phentolamine (30); α2-adrenergic, clonidine, 0.4 nM, whole brain, 1 μM clonidine (26); β-adrenergic, CGP12177, 0.25 nM, cortex, 10 μM propranolol (30); DA D1, SCH23390, 0.5 nM, cortex, 100 μM (+)SKF38393 (31); DA D2, raclopride, 1 nM, striatum, 1 μM butaclamol (32); benzodiazepine, diazepam, 2 nM, cortex, 1 μM diazepam (26); GABA, SR95531, 6.5 nM, whole brain, 100 μM GABA (33).

Adenylate cyclase assay

Rat hippocampi were homogenized in 20 volumes of 0.32 M sucrose, 1 mM EGTA, 5 mM EDTA, 5 mM dithiothreitol and 20 mM Tris-HCl (pH 7.4) and then centrifuged at 39,000 x g for 10 min. The pellet was suspended in the homogenizing solution and used for the enzyme assay. Adenylate cyclase activity was determined according to the method of De Vivo and Maayani (16), except that the amount of cAMP formed was measured by a radioimmunoassay with an assay kit (34).

Determination of amines and their metabolites

The rats were killed by decapitation, and the samples for HPLC were prepared from their brain regions as previously reported (26). Eicompak MA-5ODS (4.6 × 150 mm) (Eicom, Kyoto) and Eicompak CA-5ODS (4.6 × 150 mm) (Eicom) were used for 5-hydroxytryptophan (5-HTP), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dihydroxyphenylalanine (DOPA), DA and 3,4-dihydroxyphenylacetic acid (DOPAC) assays and for norepinephrine (NE) and 3-methoxy-4-hydroxyphenylglycol (MHPG) assays, respectively. The mobile phases were as follows: 15 mM sodium acetate-citrate buffer (pH 2.7) containing 150 mg/l sodium 1-octanesulfonate, 15 μM EDTA and 12(v/v)% methanol for 5-HTP; 40 mM sodium acetate-citrate buffer (pH 3.5) containing 220 mg/l sodium 1-octanesulfonate, 15 μM EDTA and 13–17% methanol for 5-HT, 5-HIAA, DA and DOPAC; 95 mM sodium phosphate buffer (pH 6.0) containing 1.85 mM sodium 1-octanesulfonate, 134 μM EDTA and 5% methanol for NE and MHPG. Other conditions were reported previously (26).

Microdialysis procedure

Rats (250–350 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic ap-
paratus. A hole was drilled in the skull for implantation of a guide cannula (Eicom) according to Paxinos and Watson (35). The guide was cemented to the skull along with an anchoring skull screw. The probe tip was positioned in the hypothalamus (AP -1.8, ML 0.7, DV -9.5 relative to the bregma). After the rats recovered from surgery, the probe in which the active probe membrane was 2 mm was perfused at a constant flow rate of 2 μl/min with artificial cerebrospinal fluid (140 mM NaCl, 3.35 mM KCl, 1.15 mM MgCl₂, 1.26 mM CaCl₂, 1.20 mM Na₂HPO₄ and 0.30 mM NaH₂PO₄, pH 7.4) containing 1 μM citalopram, a 5-HT-uptake inhibitor. Every 20 min, perfusates were collected and immediately injected onto HPLC column with electrochemical detection for the 5-HT assay.

**Determination of rectal temperature**

Rats were habituated to a rectal probe (Baily Instrument, Clifton, NJ, USA) three times before the experiment was started. Body temperature was recorded with the rectal probe (inserted 2.5 cm).

**Determination of serum corticosterone level**

The serum corticosterone concentration was determined spectrofluorometrically by the method of Solem and Brinck-Johnsen (36).

**Behavioral observations**

The rats were pretreated with reserpine (1 mg/kg, s.c.) 18 hr before administration of 8-OH-DPAT and MKC-242. Observation sessions of 45 sec duration began 5 min later, and they were repeated every 3 min over a period of 12 min. The rats were observed by the procedure of Tricklebank et al. (37) and scored for individual components of the 5-HT behavioral syndrome (forepaw treading, lateral head weaving, hindlimb abduction and flat body posture) on a 4-point ranked intensity scale (0=absent, 1=equivocal, 2=present and 3=intense). These values were summed for each rat over the 12-min observation period to give a maximum possible score of 15.

The palpebral aperture was evaluated by an experienced observer “blind” as to the treatment as reported by Millan et al. (38). The action of MKC-242 was compared with that of prazosin.

**Drugs**

(±)MKC-242 and its enantiomers were synthesized at Pharmaceutical Laboratory I, Yokohama Research Center, Mitsubishi Chemical Co. (Yokohama). 5-HT creatine sulfate, 5-HTP, diazepam, L-DOPA, MHPG hemipiperazine salt, NE HCl, (−)propranolol HCl and spiperone were from Sigma Chemical Co. (St. Louis, MO, USA). 8-OH-DPAT HBr, (+)-butaclamol HCl, mianserine HCl and (±)SKF38393 were from Research Biochemicals, Inc. (Natick, MA, USA). 3-Hydroxybenzylhydrazine 2HCl (NSD 1015), 5-HIAA, DOPAC, phenolamine HCl and pargyline HCl were from Nacalai Tesque (Kyoto). DA HCl was from Wako Chemicals (Osaka). Clonidine HCl was from Boehringer Mannheim (Mannheim, FRG). All radioligands were obtained from NEN (Boston, MA, USA). Citalopram HBr and N-tert-butyl-3-(4-(2-methoxyphenyl) piperazin -1 - yl) - 2 - phenylpropanamide (WAY-100135) were gifts from H. Lundbeck A/S (Copenhagen, Denmark) and Wyeth Research Ltd. (Maidenhead, UK), respectively.

For the in vitro studies, MKC-242, its enantiomer, and racemate were dissolved in 0.02 M acetic acid to make a 5-mM solution, and subsequent dilutions were made in distilled water. For s.c. administration, they were suspended in 0.5% carboxymethylcellulose.

**Data analyses**

Tests of significance were made by one-way ANOVA followed by the Duncan and LSD multiple tests, with P values smaller than 0.05 being considered significant.

**RESULTS**

**Affinity of MKC-242 for neurotransmitter recognition sites in rat brain**

Table 1 summarizes the in vitro receptor binding profile of MKC-242. MKC-242 showed high affinity for the 5-HT₁A-recognition site. Moreover, it was about 200-fold, 500-fold and more than 1,000-fold more active at the 5-HT₁A site than at the DA D₂ site; at the 5-HT₂A site; and

<table>
<thead>
<tr>
<th>Binding sites</th>
<th>IC₅₀ (nM)</th>
<th>Kᵢ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT₁A</td>
<td>0.39 ± 0.09</td>
<td>0.35 ± 0.08</td>
</tr>
<tr>
<td>5-HT₁,non-A</td>
<td>&gt;1,000</td>
<td></td>
</tr>
<tr>
<td>5-HT₁B</td>
<td>829 ± 337</td>
<td></td>
</tr>
<tr>
<td>5-HT₂A</td>
<td>229 ± 17</td>
<td>172 ± 13</td>
</tr>
<tr>
<td>5-HT₂C</td>
<td>&gt;1,000</td>
<td></td>
</tr>
<tr>
<td>5-HT₃</td>
<td>&gt;1,000</td>
<td></td>
</tr>
<tr>
<td>5-HT transporter</td>
<td>430 ± 24</td>
<td>360 ± 20</td>
</tr>
<tr>
<td>α₁</td>
<td>25 ± 2</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>α₂</td>
<td>995 ± 64</td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>&gt;1,000</td>
<td></td>
</tr>
<tr>
<td>D₁</td>
<td>&gt;1,000</td>
<td></td>
</tr>
<tr>
<td>D₂</td>
<td>117 ± 6</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>&gt;1,000</td>
<td></td>
</tr>
<tr>
<td>GABA_a</td>
<td>&gt;1,000</td>
<td></td>
</tr>
</tbody>
</table>

Results are means ± S.E. of four to six experiments.
at the 5-HT<sub>B</sub>, 5-HT<sub>C</sub>, 5-HT<sub>D</sub>, 5-HT transporter, α<sub>2</sub>-adrenergic, DA D<sub>1</sub>, benzodiazepine and GABA<sub>A</sub> sites, respectively. At the α<sub>1</sub>-adrenergic site, its affinity was about 60-fold lower than that at the 5-HT<sub>A</sub> site. The interaction with the 5-HT<sub>A</sub>-recognition site was stereospecific in that MKC-242 ((S)-enantiomer) was 15 times more active than the (R)-enantiomer: the K<sub>i</sub> values of the (R)-enantiomer and the racemate in [<sup>3</sup>H]8-OH-DPAT binding were 5.65 ± 1.23 and 1.56 ± 0.62 nM (means ± S.E. of four experiments), respectively. With regard to the affinity for 5-HT<sub>A</sub> receptors and selectivity for 5-HT<sub>A</sub> receptors versus DA D<sub>2</sub> receptors, MKC-242 was more potent and more selective than the reference compounds, the azapirones: K<sub>i</sub> values (means ± S.E. of three to four experiments) of buprione for 5-HT<sub>A</sub> and DA D<sub>2</sub>-receptor sites were 24 ± 4 and 142 ± 7 nM, respectively, and those of tandospirone were 45 ± 8 and 673 ± 29 nM, respectively.

**Brain 5-HT and catecholamine turnover**

Table 2 shows the effect of MKC-242 on the ratios of 5-HIAA/5-HT, MHPG/NE and DOPAC/DA, simple indicators of 5-HT, NE and DA turnover, respectively, in various brain regions. MKC-242 at 0.3–1.0 mg/kg (s.c.)

<table>
<thead>
<tr>
<th>Regions</th>
<th>Dose (mg/kg)</th>
<th>5-HIAA/5-HT</th>
<th>MHPG/NE</th>
<th>DOPAC/DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>s.c.</td>
<td>0</td>
<td>0.481±0.011</td>
<td>0.123±0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3</td>
<td>0.294±0.017**</td>
<td>0.113±0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.313±0.032**</td>
<td>0.097±0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>0.279±0.011**</td>
<td>0.181±0.021**</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>0</td>
<td>0.375±0.045</td>
<td>0.150±0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.299±0.021</td>
<td>0.177±0.033</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>0.264±0.019</td>
<td>0.161±0.023</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>0.240±0.011**</td>
<td>0.255±0.011*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>s.c.</td>
<td>0</td>
<td>0.521±0.014</td>
<td>0.092±0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3</td>
<td>0.435±0.021**</td>
<td>0.091±0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.426±0.031**</td>
<td>0.081±0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>0.364±0.019**</td>
<td>0.092±0.009</td>
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<td></td>
<td>p.o.</td>
<td>0</td>
<td>0.459±0.030</td>
<td>0.102±0.019</td>
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<td></td>
<td></td>
<td>1.0</td>
<td>0.398±0.020</td>
<td>0.108±0.015</td>
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<td>3.0</td>
<td>0.394±0.030</td>
<td>0.135±0.028</td>
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<td>10.0</td>
<td>0.340±0.017**</td>
<td>0.166±0.019</td>
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<td>Striatum</td>
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<td>0</td>
<td>0.853±0.021</td>
<td>NT</td>
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<td></td>
<td>0.3</td>
<td>0.699±0.030**</td>
<td>NT</td>
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<td></td>
<td></td>
<td>1.0</td>
<td>0.672±0.031**</td>
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<td></td>
<td>3.0</td>
<td>0.675±0.015**</td>
<td>NT</td>
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<tr>
<td></td>
<td>p.o.</td>
<td>0</td>
<td>NT</td>
<td>0.080±0.003</td>
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<tr>
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<td>1.0</td>
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<td>0.081±0.003</td>
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<td>3.0</td>
<td>NT</td>
<td>0.092±0.003*</td>
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<td></td>
<td></td>
<td>10.0</td>
<td>NT</td>
<td>0.106±0.003*</td>
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<tr>
<td>Hypothalamus</td>
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<td>0.411±0.010</td>
<td>0.014±0.001</td>
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<tr>
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<td>p.o.</td>
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<td>0.021±0.004</td>
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<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.278±0.020*</td>
<td>0.021±0.003</td>
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<tr>
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<td></td>
<td>3.0</td>
<td>0.240±0.019**</td>
<td>0.022±0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>0.196±0.005**</td>
<td>0.042±0.004**</td>
</tr>
</tbody>
</table>

Rats were decapitated 1 hr after administration of MKC-242 at the indicated doses. Results are means ± S.E. of four to six rats. NT, not tested. *P < 0.05, **P < 0.01, compared with the vehicle (Duncan's multiple test).
MKC-242, a Potent 5-HT₁A Agonist

Fig. 2. Effects of MKC-242 (0) and 8-OH-DPAT (●) on decarboxylase inhibitor-induced 5-HTP accumulation. MKC-242 (0.1-1.0 mg/kg) and 8-OH-DPAT (0.05-1.0 mg/kg) were subcutaneously injected 1 hr before the rats were decapitated. NSD-1015 at 100 mg/kg was intraperitoneally administered 30 min after MKC-242 or 8-OH-DPAT. 5-HTP was determined in the hypothalamus (A), hippocampus (B) and cortex (C) of rats. Brain 5-HTP content was not detected in rats untreated with NSD-1015. Points are means±S.E. of 4-6 rats. **P<0.01, compared with the vehicle (Duncan's multiple test).

Fig. 3. Antagonism by WAY100135 of MKC-242-induced decrease in 5-HTP level after decarboxylase inhibition in rat brain regions. WAY100135 (10 mg/kg, s.c.) was administered 30 min before MKC-242 (0.5 mg/kg), which was given 1 hr before rats were decapitated. NSD-1015 at 100 mg/kg was intraperitoneally administered 30 min after MKC-242 or 8-OH-DPAT. 5-HTP was determined in the hypothalamus (A), hippocampus (B) and cortex (C) of rats. Brain 5-HTP content was not detected in rats untreated with NSD-1015. Points are means±S.E. of 5 rats. **P<0.01, compared with the vehicle (Duncan's multiple test).

Decreased 5-HT turnover without changes in NE and DA turnover, while at 3.0 mg/kg, it decreased 5-HT turnover with concomitant increases in NE and DA turnover. An MKC-242-induced decrease in 5-HIAA/5-HT ratio was observed at 0.5-2 hr after injection, and it disappeared by 4 hr (data not shown). Oral administration of MKC-242 showed a similar effect. MKC-242 decreased the 5-HIAA/5-HT ratio in all brain regions, although the degree of the decrease was smaller in the hippocampus and striatum than in the hypothalamus and cortex (data not shown). The effects of MKC-242 and 8-OH-DPAT on 5-HT turnover were compared by determining the 5-HTP accumulation induced by the decarboxylase inhibitor NSD-1015, another useful index of 5-HT turnover (Fig. 2). Both compounds dose-dependently decreased 5-HTP accumulation in the hypothalamus, hippocampus and cortex; MKC-242 was less potent than 8-OH-DPAT, but there was no difference in the maximum effect (efficacy) between these compounds. The effect of MKC-242 on 5-HTP accumulation was attenuated by WAY100135, a
5-HT$_{1A}$-receptor antagonist (Fig. 3).

5-HT release

Figure 4 shows the effect of MKC-242 on 5-HT release in the hypothalamus of a freely-moving rat. Administration of MKC-242 decreased the 5-HT release in a dose-dependent manner.

Body temperature

Both MKC-242 and 8-OH-DPAT caused a dose-dependent decrease in rectal temperature, although the maximum decrease by MKC-242 was smaller than that by 8-OH-DPAT (Fig. 5A). The hypothermic effect of MKC-242 at 0.5 mg/kg reached the maximum at 30–60 min after injection and disappeared by 2 hr (data not shown). The effect of MKC-242 on body temperature was attenuated by pretreatment with WAY100135 (Fig. 6).

Corticosterone secretion

Injection of MKC-242 increased the serum corticosterone level, although the maximum effect by MKC-242 was smaller than that by 8-OH-DPAT (Fig. 5B). The effect of MKC-242 at 0.5 mg/kg reached the maximum at 60 min after injection and disappeared by 2 hr (data not shown).

Behavioral studies

Administration of MKC-242 and 8-OH-DPAT to reserpine-treated rats elicited typical 5-HT-syndrome behaviors such as flat body posture, forepaw treading, hindlimb abduction and lateral headweaving (Fig. 7). The maximum effect by MKC-242, which was observed at 2–5 mg/kg, was smaller than that of 8-OH-DPAT. Subcutaneous injection of MKC-242 at 0.1–2 mg/kg did not elicit ptosis (data not shown).

Forskolin-stimulated adenylate cyclase

MKC-242 and 8-OH-DPAT inhibited the activity of forskolin-stimulated adenylate cyclase in rat hippocampal membranes (Fig. 8). The effects of these compounds were statistically significant, although the degree of inhibition was small (about 20%). The EC$_{50}$ values for MKC-242 and 8-OH-DPAT were estimated to be approximately 3.5 nM and 15 nM, respectively.
Fig. 6. Antagonism by WAY100135 of MKC-242-induced hypothermia in rats. WAY100135 was administered 30 min before MKC-242 (0.5 mg/kg). Rectal temperature was measured before and 30 min after MKC-242. Results are means±S.E. of 5–13 rats. ***P<0.001, compared with no drug. mP<0.001, compared with MKC-242 (LSD multiple test).

Fig. 7. Effects of MKC-242 (○) and 8-OH-DPAT (●) on behavior in reserpine-treated rats. Behavior was evaluated between 5 and 20 min after s.c. injection of MKC-242 and 8-OH-DPAT at the indicated doses. Results of 8-OH-DPAT and MKC-242 are means of 2–3 rats and means ±S.E. of 4–5 rats, respectively.

Fig. 8. Effects of MKC-242 (○) and 8-OH-DPAT (●) on the activity of forskolin-stimulated adenylate cyclase in rat hippocampal membranes. Adenylate cyclase activity was determined in the presence of 10 μM forskolin. The points are means±S.E. of 8–10 determinations. *P<0.05, **P<0.01, compared with the value without MKC-242 and 8-OH-DPAT (Duncan’s multiple test).

DISCUSSION

In vitro radioligand binding showed that MKC-242 had an extremely high affinity for 5-HT1A receptors (Table 1). The interaction between MKC-242 and 5-HT1A receptors was stereospecific: MKC-242 ((S)-enantiomer) had 15-times higher affinity for the receptors than the (R)-enantiomer. The difference in the potency between these enantiomers was also observed in their hypothermic effect (data not shown). In tests of selectivity, MKC-242 showed low affinities for other 5-HT-receptor subtypes, 5-HT transporter and other neurotransmitter receptors except for α1-adrenoceptors. It is reported that some 5-HT1A-receptor ligands such as NAN-190, BMY 7378 and SDZ 216-525 (38–41) act as α1-adrenoceptor antagonists. In the separate experiments, we have observed that MKC-242 at 100 μM blocked the NE-induced increase in intracellular Ca2+ concentration in cultured rat astrocytes (data not shown). This in vitro result suggests that MKC-242 may be a weak α1-adrenoceptor antagonist. In contrast, the compound (0.1–2 mg/kg, s.c.) did not elicit ptosis (data not shown), an in vivo response to peripheral
agonists have been recently reported (K, value in [3H]8-
(38, 44), and the following highly potent 5-HT1A receptor
 tors have been developed as summarized by Millan et al.
partial agonist at postsynaptic 5-HT1A receptors. That of
8-OH-DPAT, suggesting that MKC-242 acts as a
modulate NE release in the hypothalamus and hippocam-
the anti-immobility effect of 5-HT1A-receptor agonists in
be a useful research tool for exploring the functional roles
MKC-242 contrasts with azapirones in the following respects:
MKC-242 can not give rise to 1-(2-pyrimidyl) piper-
MKC-242 at 0.3 – 1.0 mg/kg does not affect DA turnover
(Table 2), whereas buspirone is reported to act as a DA
agonist at postsynaptic 5-HT1A receptors. It is
considered that postsynaptic 5-HT1A receptors may be
the azapirones (7), appear to be full agonists at
postsynaptic 5-HT1A receptors, although they have not
been fully characterized. MKC-242 is similar to FG5893
(48) and MDL 73005EF (38) with respect to their interac-
tion with postsynaptic 5-HT1A receptors: they act as par-
tial agonists at the postsynaptic sites. However, FG5893 is
also a potent 5-HT2A receptor antagonist (48) and MDL
73005EF has lower affinity for 5-HT1A receptors than
MKC-242 (51). Furthermore, it is noteworthy that MKC-
242 acts as a full agonist at postsynaptic 5-HT1A
receprons. The present study was performed to deter-
mine how MKC-242 affects these 5-HT1A receptors.
It is well-known that the decrease in 5-HT turnover is
mediated by presynaptic 5-HT1A receptors (15). Adminis-
tration of MKC-242 decreased not only 5-HT turnover
determined by decreases in the 5-HIAA/5-HT ratio
(Table 2) and the accumulation of 5-HTP (Fig. 2), but
also 5-HT release (Fig. 4). The maximum effect of
MKC-242 on 5-HT turnover was almost similar to that of
8-OH-DPAT, a full agonist of the receptors: there was no
difference in the efficacies of these drugs. Furthermore,
the effect of MKC-242 on the accumulation of 5-HP was
blocked by WAY100135 (Fig. 3). These findings suggest
that MKC-242 acts as a full agonist at presynaptic
5-HT1A receptors. The effect of MKC-242 on postsynaptic
5-HT1A receptors was examined in both in vitro and in
vivo systems. MKC-242 partially inhibited forskolin-
stimulated adenylate cyclase activity (Fig. 8). In contrast
to the in vivo experiments, the experiment on adenylate
cyclase inhibition did not show any significant difference
in efficacy between MKC-242 and 8-OH-DPAT. In this
assay system, it was difficult to make a distinction be-
tween a full agonist and a partial agonist, since the degree
of inhibition by the full agonist 8-OH-DPAT was small.
Administration of MKC-242 caused hypothermia (Fig.
5), an increase in serum corticosterone level (Fig. 7) and
5-HT1A behavioral syndrome (Fig. 8). The experiment
using WAY100135 suggests that the effect of MKC-242
on body temperature is mediated by 5-HT1A receptors. In
these responses, the efficacy of MKC-242 is lower than
that of 8-OH-DPAT, suggesting that MKC-242 acts as a
partial agonist at postsynaptic 5-HT1A receptors.
Several potent and selective ligands for 5-HT1A recep-
tors have been developed as summarized by Millan et al.
(38, 44), and the following highly potent 5-HT1A receptor
agonists have been recently reported (K, value in [3H]8-
OH-DPAT binding): LY293284 (0.07 nM) (45),
LY228729 (0.13 nM) (46), (+)-S 20499 (0.19 nM) (47),
FG5893 (0.7 nM) (48), (S)-LY-41 (0.7 nM) (49) and S
14506 (1.02) (50). The present study shows that MKC-242
is ranked among the most potent 5-HT1A-receptor
agonists. Most of these 5-HT1A-receptor agonists, unlike
the azapirones (7), appear to be full agonists at
postsynaptic 5-HT1A receptors.
Therefore, information on pre- and postsynaptic 5-HT1A
receptor mechanisms may contribute to a better under-
standing of the pharmacological effects of 5-HT1A-recep-
tors. Thus it is a promising candidate for
clinical evaluation as a treatment for anxiety and depres-
son, the effect does not appear to be related to the
direct action on DA D2 receptors in view of the high
selectivity of MKC-242 for 5-HT1A receptors versus DA
D2 receptors. Taking these facts together, it is considered
that MKC-242 is a novel compound with higher specificity
for 5-HT1A receptors and characteristics as a partial
agonist at postsynaptic 5-HT1A receptors.
In conclusion, MKC-242, a new compound, is a highly
potent and selective 5-HT1A-receptor agonist, and it acts
as a full agonist at presynaptic 5-HT1A receptors and a
partial agonist at postsynaptic 5-HT1A receptors. It is
considered that postsynaptic 5-HT1A receptors may be
responsible for the anxiolytic and antidepressant effects
of azapirones, partial 5-HT1A-receptor agonists (6, 7).
Therefore, the present study, together with the evidence
that MKC-242 has anticonflict and antidepressant-like
effects in animal models (23), suggests that MKC-242 may
be a useful research tool for exploring the functional roles
of 5-HT1A receptors. Thus it is a promising candidate for
clinical evaluation as a treatment for anxiety and depres-
sion. Using this compound, we have recently demon-
strated that postsynaptic 5-HT1A receptors play a role in
the anti-immobility effect of 5-HT1A-receptor agonists in
the forced swimming test (54), and the 5-HT1A receptors
modulate NE release in the hypothalamus and hippocam-
pus (55).

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REFERENCES


11 Martin P: 1-(2-Pyrimidinyl)piperazine may alter the effects of 5-HT1A agonists in the learned helplessness paradigm in rats. Psychopharmacology (Berlin) 104, 275 – 278 (1991)


21 Cervo L, Grignauchi G and Samarin R: 8-Hydroxy-2-(di-n-propylamino)tetralin, a selective serotonin1A receptor agonist, reduces the immobility of rats in the forced swimming test by acting on the nucleus raphe dorsalis. Eur J Pharmacol 158, 53 – 59 (1988)


31 Reader TA, Briere R, Gottberg E, Diop L and Grodin L: Specific [3H]SCH23390 binding to dopamine D1 receptors in


