Effects of Acute and Chronic Administration of MCI-225, a New Selective Noradrenaline Reuptake Inhibitor With 5-HT_3 Receptor Blocking Action, on Extracellular Noradrenaline Levels in the Hypothalamus of Stressed Rats

Ying-Liang Wu¹,², Masami Yoshida¹, Hiroyuki Emoto¹, Hideo Ishii¹, Kiminori Koga¹ and Masatoshi Tanaka¹—*

¹Department of Pharmacology, Kurume University School of Medicine, Asahi-Machi 67, Kurume 830-0011, Japan
²Department of Physiology, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang, Liaoning 110015, China

Received August 27, 1999   Accepted February 4, 2000

ABSTRACT—In the present study, we investigated the effects of acute and chronic systemic administration of MCI-225 (4-(2-fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidine monohydrate hydrochloride), a newly-developed selective noradrenaline (NA) reuptake inhibitor with 5-HT_3-receptor-blocking action, on extracellular NA levels in the hypothalamus of stressed and non-stressed rats by utilizing intracerebral microdialysis. Acute administration of MCI-225 (3 and 10 mg/kg, p.o.) significantly and dose-dependently increased extracellular NA levels in the hypothalamus in non-stressed rats. Footshock for 20 min also significantly increased NA levels in the hypothalamus of both groups of rats pretreated with vehicle and MCI-225. Although chronic administration of MCI-225 (3 or 10 mg/kg, p.o. for 14 days) did not alter the basal extracellular NA levels in the hypothalamus, the stress-induced increases in extracellular NA levels were significantly lower in rats chronically treated with MCI-225 (10 mg/kg) than those of rats pretreated with vehicle for the same period. The increase in extracellular NA levels induced by MCI-225 challenge (3 or 10 mg/kg, p.o.) was not different between rats chronically treated with MCI-225 or vehicle. These results suggest that MCI-225 enhances extracellular NA levels in the hypothalamus in both non-stressed and stressed rats by inhibiting NA uptake and that chronic systemic administration of MCI-225 did not alter basal extracellular NA levels, but reduced the increase in NA release caused by footshock stress. These data suggest the possibility that MCI-225 might possess anxiolytic and/or antidepressant properties.

Keywords: MCI-225, Footshock stress, Noradrenaline, Hypothalamus, Intracerebral microdialysis

MCI-225 (4-(2-fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidine monohydrate hydrochloride, see Fig. 1) is a novel psychoactive compound that has been reported to have antidepressant-like activity with primary activity in the central nervous system (1–5). Moreover, it improves amnesia in scopolamine-treated rats and basal forebrain-lesioned rats (1, 3), reduces resistance to extinction of the food-rewarded runway response in dorsal noradrenergic bundle-lesioned rats, and improved the reduction in the number of approaches to a novel object in DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine)-treated Mongolian gerbils (2).

MCI-225 exhibited a potent noradrenaline (NA) uptake inhibition property as shown by the fact that it inhibits selectively NA uptake not only in vitro but also in vivo. It is further reported that MCI-225 inhibited synaptosomal NA uptake, reduced reserpine-induced hypothermia, potentiated yohimbine-induced lethality in mice (4), inhibited α-methyl-p-tyrosine-induced NA depletion, in-

* To whom correspondence should be addressed.
creased extracellular NA level and decreased DOPEG (3,4-
dihydroxy-phenylethylene glycol) level in the hypothalamus of rats (6). MCI-225 is not only a selective NA uptake in-
hibitor but also an antagonist of 5-HT3 receptors and may have antidepressant activity as potent as tricyclic anti-
depressants (TCAs) in animal models (4). An antagonistic action of MCI-225 against 5-HT3 receptors might also con-
tribute to its antidepressant action and reduce its anti-
cholinergic action. Since 5-HT3 antagonists are reported to reduce immobility in the forced swimming test (7), it may be possible that 5-HT3 receptor antagonist action itself contributes to the reduction immobility by MCI-225. In addition, 5-HT3 enhanced acetylcholine release induced by potassium in rat ventrolateral cortex (8) and ameliorated amnesia induced by scopolamine or basal forebrain lesion (9, 10), and MCI-225 reduced amnesia in both scopol-
amine-treated rats (3) and basal forebrain-lesioned rats (1). From these findings, MCI-225 appears to be a promising candidate for a new type of antidepressant (4).

Acute administration (10 mg/kg, p.o.) and repeated administration (1 or 10 mg/kg per day, p.o. for 5 days) of MCI-225 significantly inhibited the rise in pain threshold induced by footshock stress (J. Eguchi, personal communication). The analgesia induced by footshock stress is believed to be due to opioids in the brain, since it is attenuated by opioid antagonists such as naloxone (11, 12).

Our results have demonstrated that there is a close relationship between the opioid peptide system and the noradrenergic system in the hypothalamus (13, 14). Moreover, it has been well known that hypothalamus-pituitary-adrenal axis might be involved in pathological mechanisms of depression. Thus, in the present study, we investigated the effect of acute and chronic systemic administration of MCI-225 on extracellular NA levels in the hypothalamus of stressed and non-stressed rats using footshock for 20 min by utilizing intracerebral microdialysis.

MATERIALS AND METHODS

Subjects

Male Wistar rats (260 – 320 g, purchased from Kyusyu Animal Corp., Kumamoto) were used as subjects. They were housed in a temperature-controlled room (24 ± 1°C) under a 12-h light-dark cycle (light on 07:00 and off 19:00) provided food and water ad libitum. In order to reduce the influence of handling on drug administration, the rats were handled 5 min daily for 7 days. All animal procedures were performed in accordance with the Guiding Principles for The Care and Use of Laboratory Animals, approved by the Japanese Pharmacological Society and approved by Committee of Animal Experimentation, Kurume University School of Medicine.

Drugs and administration

MCI-225 (lot No. P-PM-56605L, provided by Mitsubishi-Tokyo Pharmaceuticals, Inc., Yokohama) was emul-
ified with 0.5% Tween 80, which was also used for the control and p.o. in a volume of 0.1 ml/100 g body wt. Rats were randomly divided into the control group, the MCI-225 3 mg/kg group and the MCI-225 10 mg/kg group. In the chronic experiment, rats in the control and MCI groups were treated with 0.5% Tween 80 or MCI-225 (3 or 10 mg/kg) p.o. for 14 days, respectively. On the fifteenth day, the dialysis experiment was performed. In the challenge experiment, after stabilized basal output of NA was obtained, MCI-225 (3 or 10 mg/kg, p.o.) was administered. In the footshock stress experiment, after basal output of NA was stabilized, rats were exposed to foot-
shock stress for 20 min.

Probe insertion

As previously described (15), one day before the experiment, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and mounted on a Kopf stereotaxic frame with the tooth bar set 3.3 mm below the interaural line. A U-shape dialysis probe made by ourselves, which consisted of a cellulose hollow fiber tubing (0.25-mm diameter; mol-
ecular weight cutoff, 50,000; semipermeable membranes of 5-mm thickness, covering a dorsoventral distance of 2.5 mm, were exposed to the brain tissue), was implanted into the anterior hypothalamus. The coordinate for placement of the tip of the probes were: AP = −1.8 mm, ML 0.7 mm, DV 9.0 mm from the bregma and the dural surface, ac-
cording to the atlas of Paxinos and Watson (16). Rats were given 24 h to recover from surgery.

Microdialysis

Prior to drug challenge or the stress treatment, rats were individually placed into the footshock box for 3 h for habitation to the novel environment and the microdialysis probe was continuously perfused at a constant flow rate of 2 μl/min with an artificial cerebrospinal fluid (aCSF) with the following composition: 140 mM NaCl, 3.35 mM KCl, 2.5 mM CaCl2, 1.35 mM Na2HPO4 and 0.30 mM NaH2PO4, pH 7.4. In the acute administration experiment, after habitation, MCI-225 was administered and left for 1 h. Then 20 min footshock was delivered through the floor grid at an intensity of 0.3 mA for 5-s duration at 30-s intervals by a fixed impedance AC stimulator. In the chronic administration experiment, after stable basal output of NA was obtained, rats were either challenged by MCI-225 at 3 mg/kg (or 10 mg/kg, p.o.) or exposed to footshock stress for 20 min. The experiments were carried out between 12:00 and 18:00. After the experiment, the position of the dialysis probe was verified by histological examination.
**Assay of NA**

The microdialysis samples (40 µl/20 min; starting 60 min before, then during 20 min of stress and for 180 min after) were collected and directly injected into the HPLC-ECD system (Eicom Co., Kyoto) for analysis. The column (Eicom Pak CA-5, ODS: 4.6 × 150 mm; Eicom Co.) and a mobile phase composed of 0.1 M NaH₂PO₄ buffer (pH 6.0), 5% methanol, 400 mg/l l-octanesulfonate and 0.13 mM EDTA were used for NA assay. The column temperature was kept at 25°C and applied potential was set at +400 mV (versus an Ag/AgCl reference electrode). In the experiments using a Ca²⁺-free aCSF, CaCl₂ was removed and MgCl₂ was added to retain isotonicity. In the K⁺-evoked experiments, the concentration of KCl in the aCSF was increased to 100 mM and the concentration of NaCl was reduced to 40 mM to maintain isotonicity. All reagents used were of analytical grade.

Identification of the NA peak in the chromatogram was established by comparing its retention time with the retention time of NA obtained from a standard. The addition of the NA standard (10 pg/100 µl) into the aCSF caused a clear sharp peak. Absence of Ca²⁺ in the perfusing medium almost completely blocked the NA peak and high-K⁺ aCSF perfused for 5 min increased the peak by more 10 times the baseline level (data not shown).

**Data analysis**

Data were converted to a percent of three baseline samples and are presented as the mean ± S.E.M. values. Data analysis was performed using StatView software (1998 SAS Institute, Inc., Berkeley, CA, USA). In order to analyze the effect of the compound on extracellular NA levels, NA levels (for the 80–180 min samples) after drug administration or stress exposure were analyzed by two-way analysis of variance for repeated measures and Dunnett’s test.

In order to analyze the change in NA level induced by drug challenge or stress exposure, a comparison between NA levels after the experimental administration and their baseline was made by means of Student’s paired t-test; a P-value less than 0.05 was taken as the criterion for significance.

**RESULTS**

**NA basal extracellular levels in the hypothalamus**

Basal extracellular NA levels of the six groups of naïve rats were not significantly different (Table 1; F₄,32 = 0.115, P = 0.9881). Similarly, basal NA levels did not differ significantly between chronic drug administration groups and their respective control groups (Table 2; F₄,30 = 0.398, P = 0.5325, and F₁,24 = 1.415, P = 0.2458, respectively). Therefore, for the purposes of graphical presentation, the levels of NA in the post-treatment samples are expressed as a percent of the mean baseline value.

**Effects of acute systemic administration of MCI-225 on extracellular NA levels in the hypothalamus of rats under non-stress condition**

Two-way ANOVA for repeated measures showed a significant difference in NA levels after drug administration in three groups tested (Fig. 2; for the 80–180-min samples, F₂,1₄ = 4.360, P = 0.0337). MCI-225 at 10 mg/kg caused significant increases in extracellular NA levels in the hypothalamus after the drug administration compared with the control group (for the 80–180-min samples, F₁,9 = 6.899, P = 0.0275). Although MCI-225 at 3 mg/kg showed no significant increase in extracellular NA levels in the hypothalamus after the drug administration compared with the control group (for the 80–180-min samples, F₁,1₀ = 1.707, P = 0.2206), NA levels at the 120-min sample were significantly increased compared with baseline values (128.8%, P = 0.0475). MCI-225 at 10 mg/kg increased NA concentration to 176.1%, 176.3%, 157.0% and 153.4% at the 120-, 140-, 160- and 180-min sample, respectively (P = 0.0701, 0.0333, 0.0005 and 0.0213, respectively).

### Table 1. The NA basal concentrations in dialyses from the anterior hypothalamus of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MCI-225 (mg/kg, p.o.)</th>
<th>n</th>
<th>NA baseline (pg/40 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-stress</td>
<td>Vehicle 0 6</td>
<td>2.08 ± 0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCI-225 (low dose) 3 6</td>
<td>2.13 ± 0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCI-225 (high dose) 10 5</td>
<td>1.92 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Stress</td>
<td>Vehicle 0 7</td>
<td>1.86 ± 0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCI-225 (low dose) 3 7</td>
<td>1.99 ± 0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCI-225 (high dose) 10 7</td>
<td>2.11 ± 0.28</td>
<td></td>
</tr>
</tbody>
</table>

n = number of animals. Baseline was defined as the mean of the three baseline samples prior to the onset of treatment. Data are shown as the mean ± S.E.M.

### Table 2. The NA basal concentrations in dialyses from hypothalamus of rats after the chronic systemic administration of MCI-225 for 14 consecutive days

<table>
<thead>
<tr>
<th>Groups</th>
<th>MCI-225 (mg/kg × days)</th>
<th>n</th>
<th>NA baseline (pg/40 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0 x 14</td>
<td>19</td>
<td>2.14 ± 0.17</td>
</tr>
<tr>
<td>MCI-225 (low dose)</td>
<td>3 x 14</td>
<td>19</td>
<td>1.99 ± 0.14</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0 x 14</td>
<td>13</td>
<td>3.52 ± 0.44</td>
</tr>
<tr>
<td>MCI-225 (high dose)</td>
<td>10 x 14</td>
<td>13</td>
<td>4.23 ± 0.41</td>
</tr>
</tbody>
</table>

n = number of animals. Baseline was defined as the mean of the three baseline samples prior to the onset of treatment. Data are shown as the mean ± S.E.M.
Effects of acute systemic administration of MCI-225 on extracellular NA levels in the hypothalamus of rats under 20 min footshock stress condition

As for the stress experiment, NA levels were significantly different in three groups tested (Fig. 3; for the 80–180-min samples, $F_{2.10}=4.752$, $P=0.0220$). Footshock stress for 20 min significantly increased extracellular NA levels in the hypothalamus in the control group (0 mg/kg group) at the 100- and 120-min samples after stress (209.6% and 141.1%, $P=0.0211$ and 0.0189, respectively). MCI-225 at 10 mg/kg significantly enhanced increases in NA levels in the hypothalamus after footshock stress exposure compared with the control group ($F_{1,12}=7.963$, $P=0.0154$). However, MCI-225 at 3 mg/kg seemed to enhance the stress-induced increase in extracellular NA level, but no significant difference was obtained ($F_{1,12}=2.637$, $P=0.1306$). Compared with baseline value, NA levels were not significantly different at every sample after vehicle administration in the control group before stress exposure. However, the NA levels in the MCI-225 10 mg/kg group were significantly increased from 20- to 60-min samples after MCI-225 administration.

Effect of footshock for 20 min on extracellular NA levels in the hypothalamus of rat chronically treated with MCI-225 or vehicle

Footshock stress for 20 min significantly increased extracellular NA levels in the hypothalamus at the 20- and 40-min samples in the rats chronically treated with vehicle (211.4% and 193.9%, $P=0.0082$ and 0.0265, respectively) and MCI-225 at 3 mg/kg (165.2% and 159.2%, $P=0.0001$ and 0.0007, respectively) compared with the respective
baseline values (Fig. 4), and they returned to the basal levels thereafter. The increases in extracellular NA levels in the hypothalamus from 20 to 180 min after the stress exposure, were not significantly different between the two groups ($F_{1,19} = 1.704, P = 0.2073$).

Stress exposure significantly increased extracellular NA levels in the hypothalamus at the 20-, 40- and 60-min samples (140.2%, 173.9% and 141.0%, $P = 0.0024, 0.0010$ and 0.0178, respectively) in the rats treated chronically with vehicle, and NA levels remained elevated until the end of experiment (Fig. 5). In contrast, in rats treated chronically with MCI-225 at 10 mg/kg, stress exposure significantly increased extracellular NA levels in the hypothalamus at the 20- and 40-min samples (118.3% and 119.6%, $P = 0.0153$ and 0.0848, respectively), and then NA levels returned to basal levels. Two-way ANOVA for repeated measures showed that the increases in extracellular NA levels in the hypothalamus from 20 to 180 min after the stress exposure were significantly different between the two groups ($F_{1,11} = 7.486, P = 0.0194$).

**Effect of MCI-225 challenge on extracellular NA levels in the hypothalamus of rats chronically treated with MCI-225**

Challenge with MCI-225 (3 mg/kg, p.o.) significantly increased extracellular NA levels in the hypothalamus from 20- to 80-min samples in rats chronically treated with MCI-225 at 3 mg/kg ($P < 0.05$), and then NA levels returned to basal levels (Fig. 6). Challenge with MCI-225 (3 mg/kg, p.o.) significantly increased extracellular NA levels in the hypothalamus at the 20-min sample and from 80- to 180-min samples ($P < 0.05$) in rats chronically treated with vehicle. Two-way ANOVA for repeated measures showed that the increase in extracellular NA levels in the hypothalamus from 80 to 180 min after the drug challenge were not significantly different between the two groups ($F_{1,15} = 0.881, P = 0.3628$).
Challenge with MCI-225 (10 mg/kg, p.o.) significantly increased extracellular NA levels in the hypothalamus at the 20-, 80–140- and 180-min samples (P<0.05) in the rats chronically treated with MCI-225 at 10 mg/kg (Fig. 7). Challenge with MCI-225 (10 mg/kg, p.o.) only significantly increased extracellular NA levels in the hypothalamus at the 180-min sample (P<0.05) in rats chronically treated with vehicle. Two-way ANOVA for repeated measures showed that the increase in extracellular NA levels in the hypothalamus from 80 to 180 min after the drug challenge were not significantly different between the two groups (F$_{1,10}$ = 1.718, P = 0.2192).

**DISCUSSION**

In the acute study, it was shown that MCI-225 increased extracellular NA levels in the hypothalamus of non-stressed rats in a dose-dependent manner and significant differences were observed between the MCI-225 10 mg/kg group and the vehicle group. As shown in Fig. 2, there seems to be two peaks of increases over time. A pharmacokinetic study showed that the T$_{max}$ of MCI-225 (10 mg/kg, p.o.) in rat blood serum was around 1.5 h after treatment (J. Eguchi, personal communication). Oishi et al. (6) reported that MCI-225 (30 mg/kg, p.o.) significantly increased NA output in the rat hypothalamus to a level 2.5 times higher than the basal value at 2.5–4 h after treatment. From these findings, the first peak (for the 20–60-min samples) on the time-course curve might be due to stimulation by the experimental manipulations used, and the second peak (for the 80–180-min samples) are considered to be due to the NA uptake-inhibiting property and 5-HT$_{3}$-receptor antagonist action of MCI-225. Matsumoto et al. (17) used microdialysis to show that
ondansetron, a 5-HT3-receptor antagonist, decreases the 5-HT- or fluoxetine-induced inhibition of NA release in the rat hippocampus; thus it seems to be possible that the 5-HT3-receptor antagonist action of MCI-225 decreased the endogenous 5-HT-induced inhibition of NA release.

Our previous studies have documented that NA release from the anterior hypothalamus was significantly increased by psychological, footshock and immobilization stresses (15, 18) and that there is a close relationship between the opioid peptide system and the noradrenergic system in the hypothalamus (13, 14). Since MCI-225 could inhibit the footshock stress-induced rise in pain threshold (J. Eguchi, personal communication) and the analgesia induced by footshock stress is believed to be due to opioids in the brain (11, 12), we selected the footshock stress model to analyze the effect of MCI-225 on extracellular NA levels in the hypothalamus of stressed rats in the present study. Although footshock stress for 20 min significantly increased extracellular NA levels in the hypothalamus of control rats, pretreatment with MCI-225 at 3 mg/kg and 10 mg/kg further enhanced increases in the extracellular NA levels in this region induced by stress in a dose-dependent manner. These results might also be due to the NA uptake-inhibiting property and 5-HT3-receptor antagonist action of the drug (4). These findings indicate that acute administration of MCI-225 causes increases in the extracellular NA levels in the area in both stressed and non-stressed rats.

Repeated administration of MCI-225 (3 and 10 mg/kg × 14 day, p.o.) did not affect basal extracellular NA levels in the hypothalamus. However, increases in extracellular NA levels caused by footshock stress were dose-dependently reduced by repeated administration of the drug. This result suggests that chronic systemic administration of MCI-225 could attenuate increases in central noradrenergic activity caused by footshock stress. Because neuronal activation of the brain NA system has, in part, an important role in the provocation of anxiety and/or fear (14, 19–24), this effect of MCI-225 might be clinically relevant and contribute to the therapy of anxiety and depression.

After chronic administration of MCI-225, the increase of extracellular NA levels in the hypothalamus induced by challenge with the drug were not significantly different between rats chronically treated with vehicle or MCI-225. However, interestingly, we found that the increase in NA levels induced by MCI-225 challenge was less in the 10 mg/kg group and more in the 3 mg/kg group, relative to levels seen with acute administration of the drug to naive rats (see Fig. 2). As shown in Fig. 2, oral administration could be a stressor itself, since it increased significantly extracellular NA levels in the hypothalamus. The repeated oral administration for two weeks, which could be stressful for the rats, might change the responses to MCI-225 and induce the different results between acute administration of the drug to the naive rats and challenge with the drug after chronic treatment with vehicle. These results suggested that the mechanism of MCI-225 might be different and complicated depending on acute or chronic administration of the drug. Although detailed mechanisms for the pharmacological actions of MCI-225 are unclear, changes in monoamine metabolism and/or NA transport might be involved in the CNS effects of this novel compound.

Acknowledgments

We wish to thank Prof. Gary B. Glavin of the Department of Pharmacology and Therapeutics, University of Manitoba, Canada for his kind reviewing of an earlier version of this manuscript and helpful discussions. We are grateful to Mitsubishi-Tokyo Pharmaceuticals, Inc. for the generous supply of MCI-225.

REFERENCES

7 Thiebot MH and Martin P: Effects of benzodiazepines, 5-HT1A agonists and 5-HT1A antagonists in animal models sensitive to antidepressant drugs. In 5-HT1A Agonists, 5-HT1A Antagonists and Benzodiazepines. Their Comparative Behavioral Pharmacology, Edited by Rodgers RJ and Cooper SJ, pp 159–194, John Wiley & Sons, West Sussex (1991)
11 Akil H, Madden J, Patrick RL and Barchas JD: Stress-induced


16 Paxinos G and Watson C: The Rat Brain in Stereotaxic Coordinates, Plate 10 and Figure 10, Academic Press, London (1982)


