Effect of a New Psychoactive Compound, MCI-225, on Brain Monoamine Metabolism in Rats

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ABSTRACT—Effect of MCI-225 on brain monoamine metabolism was examined in rats. MCI-225 (30 mg/kg, p.o.) had no influence on noradrenaline (NA) levels, but significantly inhibited the NA turnover in the hippocampus and hypothalamus. This compound also significantly increased the 5-hydroxyindoleacetic acid (5-HIAA)/5-hydroxytryptamine ratio in the cerebral cortex, hippocampus and striatum; and it enhanced the probenecid-induced 5-HIAA accumulation in the striatum. In the microdialysis study, MCI-225 markedly increased the NA output, but decreased the 3,4-dihydroxyphenylethylenglycol output from the hypothalamus of urethane-anesthetized rats. These results suggest that MCI-225 enhances both noradrenergic and serotonergic function by inhibiting NA uptake and accelerating 5-HT turnover, respectively.

Keywords: MCI-225, Monoamine metabolism, Extracellular noradrenaline concentration

MCI-225, 4-(2-fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidine, is a newly synthesized compound that improves the impairment of spatial learning induced by scopolamine in mice (1). However, the action mechanisms of this compound are not clear. MCI-225 also reduces the resistance to extinction of a food-rewarded runway response induced by lesion of the dorsal noradrenergic bundle in rats (1). Therefore, in the present study, we examined the effect of MCI-225 on monoamine metabolism in the rat brain.

First, male Wistar rats (250–280 g) were treated with MCI-225 (10, 30 and 100 mg free base/kg, p.o.) and then killed 1, 2 and 4 hr later. The cerebral cortex, hippocampus, striatum and hypothalamus were immediately dissected and stored at −80°C until assay. Noradrenaline (NA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were simultaneously determined by direct injection of the supernatant of tissue homogenates into a high-performance liquid chromatograph (HPLC) with an electrochemical detector, as previously described (2).

Secondly, monoamine turnover was estimated from the depletion of NA and DA induced by α-methyl-p-tyrosine (α-MT; 250 mg/kg, i.p.) (3) or the accumulation of 5-HIAA induced by probenecid (200 mg/kg, i.p.) (4). MCI-225 (30 mg/kg, p.o.) was administered 1 hr before α-MT or probenecid treatment.

In the third experiment, we examined the effect of MCI-225 on the extracellular concentrations of NA and 3,4-dihydroxyphenylethylenglycol (DOPEG) in the rat hypothalamus by the in vivo microdialysis technique, as described previously (5). In brief, rats were anesthetized with urethane (1.1 g/kg, i.p.) and placed on a stereotaxic apparatus. A U-shaped probe with a 4-mm dialysis membrane (BDP-UI-12-02; Eicom, Kyoto) was inserted into the medial part of the hypothalamus. The coordinates were set to P 2.8 mm from bregma, L 0.7 mm, 10.5 mm below the skull according to the atlas of König and Klippel (6). Ringer’s solution was perfused at 2 μl/min, and the perfusates were collected every 30 min. The NA and DOPEG levels in the perfusates were determined by HPLC with electrochemical detection after purification by alumina adsorption. MCI-225 (30 mg/kg, p.o.) was administered 3 hr after the start of perfusion.

MCI-225 hydrochloride (a gift from Mitsubishi Kasei Corp., Yokohama) was emulsified with 0.5% Tween 80.
α-MT hydrochloride methyl ester (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in saline. Probencid (Sigma) was first dissolved in 0.15 N NaOH, and the pH was reduced to 8.5. Results were statistically evaluated by analysis of variance; and Dunnett’s test and Student’s t-test were subsequently performed in the first and second experiments, respectively.

Although the NA or DA levels in the brain regions examined did not change 2 hr after MCI-225 treatment at any doses, the cortical and striatal 5-HIAA levels were significantly increased by about 15% at doses of 30 and/or 100 mg/kg of this compound (Table 1). The ratio of 5-HIAA/5-HT was also significantly increased by MCI-225 in the cerebral cortex, hippocampus and striatum (data not shown). When examined 1 and 4 hr after MCI-225 treatment, no significant changes in monoamine levels were observed, except in the cerebral cortex where the 5-HT levels decreased by 11% 4 hr after 30 and 100 mg/kg of this compound (data not shown).

MCI-225 (30 mg/kg, p.o.) significantly inhibited the α-MT-induced depletion of NA in the hippocampus and hypothalamus. However, neither the NA depletion in the cerebral cortex nor the DA depletion in the striatum was significantly inhibited (Table 2). On the other hand, the probencid-induced 5-HIAA accumulation was significantly enhanced in the striatum by MCI-225.

In microdialysis study, NA output from the hypothalamus was significantly increased by MCI-225 (30 mg/kg, p.o.) (F=6.41, P<0.01), and the levels were about 2.5 times higher than the basal value 2.5~4 hr after treatment (Fig. 1). In contrast, the DOPEG output was significantly decreased to about 50% of the basal level 1~4 hr after treatment (F=4.69, P<0.01).

In the present study, MCI-225 did not change the NA levels, but significantly inhibited the α-MT-induced NA depletion in the hippocampus and hypothalamus at a dose of 30 mg/kg (p.o.), where it significantly improves the behavioral impairment induced by lesion of dorsal noradrenergic bundle in rats (1). Changes in the monoamine turnover has been used as one of the methods for in vivo assessment of inhibition of monoamine uptake (7~10). Desipramine, an inhibitor of NA uptake, has been shown to decrease the α-MT-induced NA depletion (9) and the rate of NA synthesis (8, 11, 12). The reduction of NA turnover by MCI-225 may be due to its inhibitory action on NA uptake. This idea is further confirmed by the in vivo microdialysis study, which showed that MCI-225 increases the extracellular NA level, but decreases the

| Treatments (mg/kg, p.o.) | NA   | DA   | DOPAC | 5-HT | 5-HIAA |
|-------------------------|------|------|-------|------|--------|
| Cerebral cortex         |      |      |       |      |        |
| Tween 80                | 194±4|      |       | 259±16| 277±10 |
| MCI-225 (10)            | 189±11|      |       | 238±9 | 294±14 |
| (30)                    | 211±7 |      |       | 266±11| 320±7* |
| (100)                   | 200±14|      |       | 235±9 | 320±12*|
| Hippocampus             |      |      |       |      |        |
| Tween 80                | 213±5 |      |       | 286±15| 430±8  |
| MCI-225 (10)            | 197±14|      |       | 254±13| 436±12 |
| (30)                    | 249±11|      |       | 274±12| 449±14 |
| (100)                   | 212±12|      |       | 248±13| 468±13 |
| Hypothalamus            |      |      |       |      |        |
| Tween 80                | 1525±62| 408±31| 142±7 | 851±44| 766±11 |
| MCI-225 (10)            | 1455±71| 410±31| 145±10| 824±47| 789±23 |
| (30)                    | 1588±27| 394±13| 138±8 | 851±32| 787±12 |
| (100)                   | 1546±43| 406±34| 135±7 | 837±41| 803±23 |
| Striatum                |      |      |       |      |        |
| Tween 80                | 8013±222| 2750±111| 371±19 | 636±11 |
| MCI-225 (10)            | 7470±229| 2662±42 | 332±14 | 659±29 |
| (30)                    | 7669±286| 2694±41 | 364±15 | 732±18*|
| (100)                   | 7629±132| 2764±81 | 338±9 | 701±19 |

Each result represents the mean±S.E.M. of 5 animals. *P<0.05, as compared with the corresponding control (Tween 80) values.
DOPEG level in the hypothalamus. We previously showed that the NA output detected in this method is highly tetrodotoxin-sensitive and that monitoring both NA and DOPEG outputs is useful for obtaining detailed information about central NA metabolism in vivo (5).

When probenecid-induced 5-HIAA accumulation was examined, a significant enhancement by MCI-225 was observed only in the striatum. However, a significant increase in the 5-HIAA level was also observed in the cerebral cortex, and the 5-HIAA/5-HT ratio was markedly increased in the brain regions other than hypothalamus. Although it is difficult to explain the reason for regional differences in changes in each index, it seems that MCI-225 enhances the 5-HT turnover especially in the telencephalon.

The present study suggests that MCI-225 enhances both noradrenergic and serotonergic function in the brain by inhibition of NA uptake and acceleration of 5-HT turnover, respectively. The interaction of this compound with adrenergic (α1, α2, β1, and β2) or serotonergic (5-HT1 and 5-HT2) receptors is very weak in the binding studies (M. Egawa, personal communication). Although the detailed mechanisms for the pharmacological actions of MCI-225 (such as amelioration of cognition impairment) is unclear, changes in monoamine metabolism may be at least partly involved in these effects.

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