LETTERS TO THE EDITOR

Neurochemical Correlates of Sleep Disorder of Rats Administered with Methylmercury Chloride

Keywords: Methylmercury chloride—Sleep disorder—Norepinephrine—5-Hydroxytryptamine—5-Hydroxyindole acetic acid—Tryptophan—Slow wave sleep—Paradoxical sleep

In the preceding study, it was found that methylmercury chloride (MMC)-dosed rats show a suppression of paradoxical sleep (PS) during light periods and an increase in the amounts of both slow wave sleep (SWS) and PS during dark periods. Since Jouvet's monoamine theory of sleep was presented, many studies have been undertaken to investigate the role of brain monoamines in sleep mechanisms. At present, it is assumed that catechol- and indole-amines are possibly involved in the regulation of sleep. The present study was designed to investigate the role of brain monoamines in the sleep disorder of MMC-dosed rats and attempts to elucidate the MMC-induced sleep disorder from the standpoint of changes in the concentrations of brain monoamines.

MMC was administered orally to 12 Sprague-Dawley male rats 8 weeks age at a dose of 15 mg MMC/kg body weight/day for two consecutive days. Twelve rats served as a control group and received vehicle administration of sesame seed oil. On the 17th day after the first MMC dose, 12 rats (6 each from the experimental and the control groups) were decapitated in the morning (10:00–11:00) and the other 12 at night (23:00–24:00). The brain of each rat was removed quickly and dissected into four regions, cerebral hemispheres, diencephalon and midbrain, pons and medulla, and cerebellum. The brain samples were stored at $-70^\circ$C until analysis. The dissected brains were weighed, homogenized with 10 volumes of 0.1 N HClO$_4$ containing 5 nmoles EDTA/ml and centrifuged at 4$^\circ$C. Concentrations of norepinephrine (NE), 5-hydroxytryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA) and tryptophan in the supernatant fluid were determined by high performance liquid chromatography with continuous fluorometric monitoring. For the NE measurement, the sample was injected into a Zipax SCX column, eluted with 0.025 M Na$_2$HPO$_4$ and converted to the fluorescent derivative by the THI method. The concentrations of the three indoles were determined by elution in a Yanapak ODS-T column with 12% MeOH-0.1 M Na$_2$HPO$_4$ (pH 3.1) and the formation of OPA derivatives.

Fig. 1 shows means and standard errors of NE, 5-HT, 5-HIAA and tryptophan levels of the three brain regions of both MMC-dosed and control rats in the morning and at night. When the concentrations of the monoamines of the MMC-
dosed rats were compared with those of the control rats, the following facts were observed. In the morning, NE levels were significantly higher in the pons and medulla and in the diencephalon and midbrain and lower in the cerebral hemispheres of MMC-dosed rats than in the controls. In the dark period, NE levels of the pons and medulla and of the diencephalon and midbrain tended to be

Fig. 1. Comparison of NE, 5-HT, 5-HIAA and tryptophan levels of some brain regions in MMC-dosed and control rats.
A = pons + medulla,  B = diencephalon + midbrain,  C = cerebral hemispheres.
Open and hatched bars indicate the values for MMC-dosed and control rats, respectively. Vertical lines indicate standard error of the mean.
* p<0.05,  ** p<0.01 (t test).
lower in the treated rats. 5-HT and 5-HIAA levels of the diencephalon and midbrain of the MMC-dosed rats tended to be higher than in the controls in the light period and to be lower in the dark period. Tryptophan levels of the three regions were significantly lower in the treated rats at night.

According to the literature\textsuperscript{6-12), PS appears to be suppressed by enhanced activity of NE-containing neurons in the locus coeruleus. Lesions of the mesencephalic tegmentum destroying the group A of catecholaminergic neurons and the isthmus destroying the NE bundle both result in a decreased brain NE level and produce a selective increase in SWS and an increase in both SWS and PS in the cat, respectively\textsuperscript{6,7). Alpha-methylparatyrosine, a tyrosine hydroxylase inhibitor, increases both SWS and PS and decreases the brain NE level in the cat\textsuperscript{8). Injection of 6-hydroxydopamine\textsuperscript{9), a toxin of catecholamine neurons, and alpha-methylparatyrosine\textsuperscript{10 produce an increased PS and a lowered brain NE level in the rat. Single unit activities of the locus coeruleus neurons containing NE are most active during wakefulness, much less active during SWS and nearly silent during PS\textsuperscript{11,12). The present study shows that in the morning the NE level of the pons and medulla is higher for the MMC-dosed rats than for the control rats. The previous study\textsuperscript{1}) also showed that occurrence of PS is suppressed during the first few hours of the light period. Therefore, considering the above reports in the literature\textsuperscript{6-12), we infer from our experiment that the MMC-induced suppression of PS during the early light period is due to the elevated NE level in the pons and medulla.

Studying the effect of maternal exposure to MMC on the brain monoamines of the male offspring of the rat, Sobotka et al.\textsuperscript{13) found a reduced NE level in the midbrain-diencephalon and a depressed 5-HT turnover in the pups at 28 days of age. Taylor and Distefano\textsuperscript{14) reported that rat pups showed decreased levels of 5-HT, 5-HIAA and NE in the whole brain during the first few days after MMC administration though the increased 5-HT turnover was recognized afterwards. In our study, the decreased rate of 5-HT turnover in the hind brain during the dark period was associated with increased amounts of both SWS and PS during the dark period. A central serotonergic system consisting mainly of the raphe nuclei is presumably related to induction and maintenance of SWS. SWS is reduced by pharmacological and surgical manipulation of the serotonergic system and SWS is also associated with enhanced turnover of 5-HT\textsuperscript{3). Our finding of the decrease in the 5-HT turnover during the dark period associated with an apparent increase in the amounts of SWS and PS during the same period cannot be explained by the serotonergic theory of SWS induction. Since MMC exerts an inhibitory effect on central cholinergic function as discussed in the preceding paper\textsuperscript{1), it will be necessary to study the neurochemical effects of MMC not only on the central monoaminergic but also on the cholinergic function which is also responsible for sleep mechanisms.
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REFERENCES


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