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Attenuating Effect of Bifemelane on an Impairment of Mealtime-Associated Activity Rhythm in Aged and MK-801-Treated Rats

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SHIBATA, S., M. ONO, Y. MINAMOTO AND S. WATANABE. *Attenuating effect of bifemelane on an impairment of mealtime-associated activity rhythm in aged and MK-801-treated rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 207–210, 1995.—In the present experiment, we examined the attenuating effect of bifemelane hydrochloride (BF), 4-(*o*-benzyl phenoxy)-*N*-methylbutylamine hydrochloride, on the impairment of time perception caused by daily scheduled feeding using aged and MK-801-treated rats. When feeding was restricted to a single meal at a fixed time of day (1300–1700 h) for six successive days, young rats exhibited intense locomotor activity 1–3 h before feeding time. Intense locomotor activity was observed for 1200–1700 h even on the fasting day (day 7; mealtime-associated activity). Mealtime-associated activity was impaired in 24-mo-old rats and also in *N*-methyl-D-aspartate receptor antagonist, MK-801-treated rats. Daily injections of bifemelane at 1700 h for six successive days significantly attenuated the impairment of mealtime-associated activity on the seventh day in a dose-dependent manner in aged rats. In addition, cotreatment of MK-801 with bifemelane blocked the MK-801-induced impairment of mealtime-associated activity. The present study suggests that bifemelane has an enhancing effect on learning and memory performance, such as spatial and temporal perception.

Food anticipation Aging Activity Circadian rhythm Learning

BIFEMELANE HYDROCHLORIDE (BF), 4-(*o*-benzyl phenoxy)-*N*-methylbutylamine hydrochloride, is effective in the treatment of cerebrovascular disease as well as senile dementia in clinical trials (5,10). Treatment with BF exhibited antianoxic and memory retrieval effects in laboratory research animals (15). In addition, BF has been shown to prevent decreases in acetylcholine (ACh) levels as well as muscarinic receptor bindings by transient ischemia (1,9). Biochemical studies have revealed that a loss of cholinergic function occurs during aging (3,6,7,16). BF also has been shown to enhance the affinity of muscarinic receptors as well as choline acetyltransferase activity in cortical and subcortical regions of aged rats (2). Thus, BF may have useful applications in the treatment of CNS cholinergic dysfunction.

Treatment of rodents with the *N*-methyl-D-Aspartate (NMDA) receptor antagonist, MK-801, causes an impairment of learning and memory in several learning tasks (14,17). Recently, the depletion of NMDA receptors has been revealed in the gerbil hippocampus after transient ischemia (4). Furthermore, postischemic BF treatment almost completely prevented ischemia-induced decreases in the NMDA receptors (1).

When feeding is restricted to single meal scheduled at a fixed time for several days, rats develop intense locomotor activity 1–2 h before and during fixed mealtimes; this intense activity is observed even on the fasting day (mealtime-associated activity) (12,13). An age-related decline as well as MK-801-treated impairment was found in this mealtime-associated activity rhythm, suggesting the impairment of time perception in aging and MK-801-treated animals (12).

In the present study, we investigated whether BF could improve the impairment of mealtime-associated activity rhythms in old and MK-801-treated rats.

METHODS

Animals

Seven-week-old male Wistar rats were purchased from Kyudo Animal (Fukuoka, Japan) and maintained in tempera-

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ture-controlled animal quarters with food and water ad lib. We used 11-week-old rats for young animals and 24-mo-old rats for elderly ones. The animals were housed in groups with a 12-h light–dark cycle (lights on at 7000 h) until the experiment.

Procedure

Each animal was individually housed in a plastic cage (30 × 40 × 20 cm). Motor activity was measured using an area sensor (F5B; Omron, Japan), and the activity count (number of movements) was recorded and printed out at 1-h intervals (Intelligence Printer, Muromachi Kikai, Japan). After fasting for 24 h, restricted feeding was carried out for six successive days. During this period, animals were allowed access to food for 4 h from 1300 h (6 h after lights on). On day 7, food was withheld again. The increase in activity from 1200–1700 h on the fasting day was defined as mealtime-associated activity. Water was freely available throughout the experiment. The daily food intake was measured during the feeding period.

Drugs

The drugs used in this study were BF (Eizai, Japan) and (+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5,10-imine hydrogen maleate (MK-801, Funakoshi, Japan). These drugs were dissolved in distilled water. MK-801 and BF were administered by IP injection or oral gavage (p.o.), respectively. Control animals received saline for IP injection and water for oral gavage. BF was administered in three groups of rats. Because food intake or spontaneous locomotor activity during the feeding time might be affected by these drugs, all drugs were administered at the end of the feeding time for six successive days. Drugs were not administered on the fasting day (day 7). Apparently, IP administration of MK-801 induced strong locomotor activity in young controls fed ad lib. This may be due to an undefined effect of the IP injection, such as deviating pH.

Data Analysis

Results are expressed as the mean ± SEM. Activity levels were fairly consistent for each individual animal, although they differed between groups. The assessment of food anticipatory behavior was performed by comparison of the absolute activity levels of individual rats and by determination of the activity ratios before the introduction of food restriction and on the fasting day. Therefore, the percentage of mealtime-associated anticipation was defined as [(activity count during 1200–1700 h on the fasting day/total activity count before restriction) × 100/5]. The significance of differences between groups was determined with one-way analysis of variance followed by Duncan or Student *t*-test.

RESULTS

Figure 1 presents the activity patterns of young, aged, and young MK-801-treated rats. Activity levels of rats not presented showed a comparable pattern. Activity counts over 24 h were 1431 ± 142 (n = 5) in young rats, 642 ± 84 (n = 5) in aged rats ($p < 0.01$, Student t-test), and 2379 ± 293 (n = 5) in MK-801-treated young rats ($p < 0.05$). All rats exhibited a nocturnal activity rhythm. Activity during night was lower in aged rats compared with the young ones. In contrast, MK-801 increased the activity; this increase lasted for about 3 h.

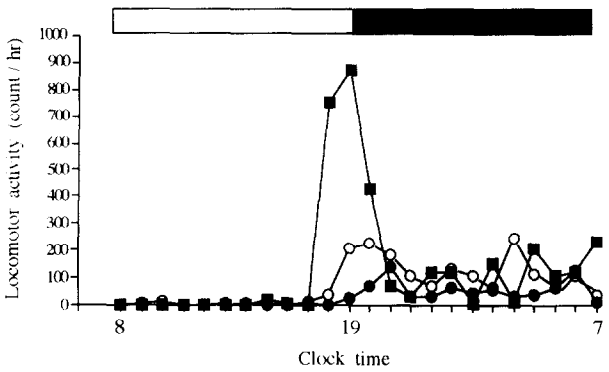

FIG. 1. Locomotor activity patterns of young, aged, and MK-801 treated rats for a 24-h period under a free-feeding schedule. The horizontal axis indicates the time of the day. The open column at the top of panel shows the light period; the solid column shows the dark period. The vertical axis indicates the locomotor activity (count/h). ○, young rat treated with saline; ●, aged rat treated with saline; ■, young rat treated with MK-801 (0.2 mg/kg, IP).

Aged Rats

The level of mealtime-associated activity for a representative young and aged rat on the fasting day is shown in Fig. 2 for a 24-h period. A young rat exhibited an apparent increase in daytime activity level that seems to be associated with meal expectation. In contrast to a young rat, an aged one exhibited no mealtime-associated activity on the fasting day, but this rat exhibited a normal increase in nocturnal activity (Fig. 2). We examined the effect of BF on the impairment of mealtime-associated activity in an aged rat. An old rat treated with BF (20 mg/kg per 6 days) increased the level of mealtime-associated activity on the fasting day (Fig. 2).

In contrast to the young rats, the aged ones failed to exhibit mealtime-associated activity on the fasting day (Fig. 3) ($p < 0.01$, Student t-test). Daily administration of BF significantly ($F(3, 19) = 9.4$, $p < 0.01$) attenuated the impairment of the

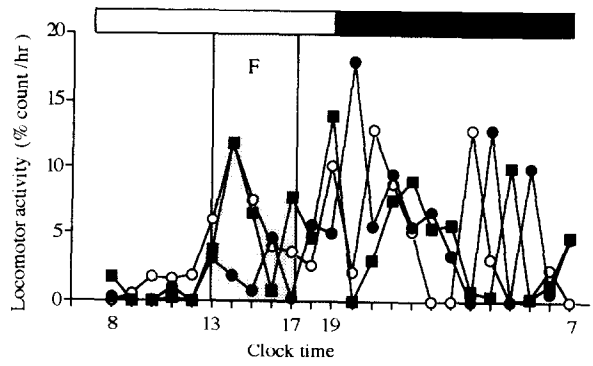

FIG. 2. Representative mealtime-associated activity patterns of young, control, and bifemelane-treated aged rats on the fasting day. The horizontal axis indicates the time of the day. The open column at the top of panel shows the light period; the solid column shows the dark period. The vertical axis indicates the percentage of activity per hour. ○, young rat treated with saline; ●, aged rat treated with saline; ■, aged rat treated with bifemelane (20 mg/kg, p.o.). Feeding restriction and drug injection at 1700 h were conducted for six successive days; treatments were withdrawn on the day 7. F = feeding time.

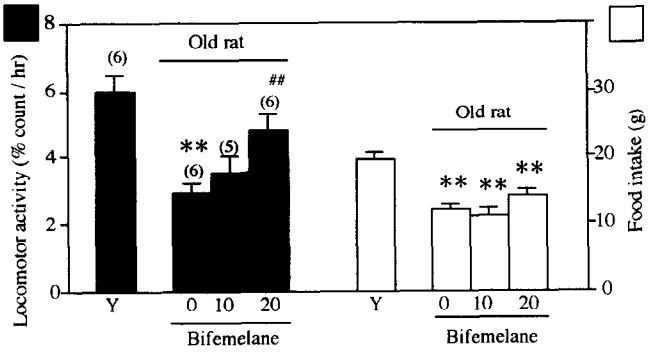

FIG. 3. Effect of bifemelane on the manifestation of mealtime-associated activity and food intake in aged rats. The left vertical axis indicates the mean percentage of the activity per hour from 1200–1700 h on the fasting day. The right vertical axis indicates the mean volume of food intake during 1300–1700 h on day 6 after restricted feeding. Feeding restriction and bifemelane (p.o. injection at 1700 h) were conducted for six successive days; treatments were withdrawn on day 7. Numbers in parentheses indicate the number of animals. $**p < 0.01$ vs. young rats; $\#\#p < 0.01$ vs. water-treated aged rats (Duncan's test).

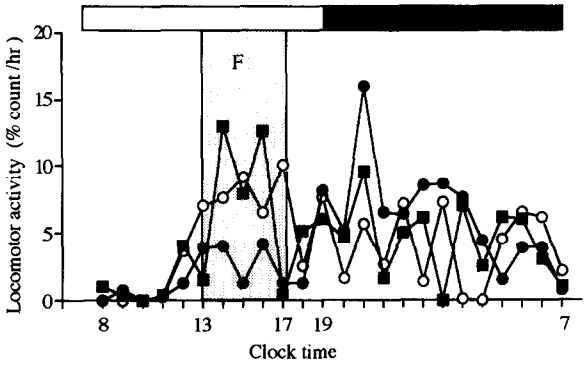

FIG. 4. Representative mealtime-associated activity patterns of saline-, MK-801-, and MK-801/bifemelane-treated young rats on the fasting day. The horizontal axis indicates the time of the day. The open column at the top of panel shows the light period; the solid column shows the dark period. The vertical axis indicates the percentage of activity per hour. ○, treatment with saline; ●, treatment with MK-801 (0.2 mg/kg, IP); ■, treatment with MK-801 (0.2 mg/kg, IP)/bifemelane (50 mg/kg, p.o.). Feeding restrictions and bifemelane injection at 1700 h and/or MK-801 injection at 1730 h were conducted for six successive days; treatments were withdrawn on day 7. F = feeding time.

development of the mealtime-associated activity in a dose-dependent manner (Fig. 3). The food intake of aged rats during food restriction was significantly lower than that of young rats ($p < 0.01$). BF administration to aged rats did not affect food intake during feeding restriction ($p > 0.05$) (Fig. 3). Under a free-feeding schedule, neither treatment with water nor BF (20 mg/kg) at 1300 h for six successive days led to an increase in activity from 1200–1700 h : 0.8 ± 0.4 ($n = 3$) % count/h in water-treated and 0.5 ± 0.2 ($n = 3$) in BF-treated aged rats.

MK-801-Treated Rats

In the next experiment, we examined the effect of BF on the MK-801-induced impairment of mealtime-associated activity. MK-801 increased activity during the 6 days of administration in young rats on scheduled feeding. Figure 4 shows the level of mealtime-associated activity of saline-, (0.2 mg/kg) MK-801-, and (0.2 mg/kg) BF/(50 mg/kg) MK-801-treated rats on the fasting day for a 24-h period. The appearance of mealtime-associated activity was impaired by daily treatment with MK-801 (0.2 mg/kg, IP) at 1730 h for six successive days (Figs. 4 and 5). Pretreatment with BF (50 mg/kg, p.o.) attenuated the MK-801-induced impairment of mealtime-associated activity (Fig. 4) in a dose-dependent manner [$F(4, 24) = 7.5$, $p < 0.01$] (Fig. 5). However, BF administration alone did not affect mealtime-associated activity (Fig. 5). Food intake during feeding restriction was not significantly [$F(4, 24) = 0.9$, $p > 0.05$] affected by either MK-801 (0.2 mg/kg) or MK-801 plus BF (20, 50 mg/kg) (Fig. 5).

DISCUSSION

In this experiment, mealtime-associated activity rhythm was impaired in 24-mo-old and MK-801-treated rats. Daily injection of BF attenuated the impairment of the mealtime-associated activity rhythm. Treatment with BF was reported to improve the memory deficiency in the passive avoidance test (15). In addition, BF reduced the scopolamine-induced memory impairment in radial maze behavior (8). Therefore, BF is considered to have an activating effect on cholinergic neuron systems.

Biochemical studies have reported that a loss of cholinergic function occurs during aging (3,6,7,16). In addition, BF has been shown to enhance the affinity of muscarinic receptors as well as choline acetyltransferase activity of aging rats (2). Moreover, the decrease in cerebral muscarinic receptor number in gerbil hippocampus, after a transient ischemia, is restored to the normal level by the administration of BF (1). These findings suggest the possibility that the alleviation of impairment of the mealtime-associated activity observed in aged rats that have received BF is at least partly explained by

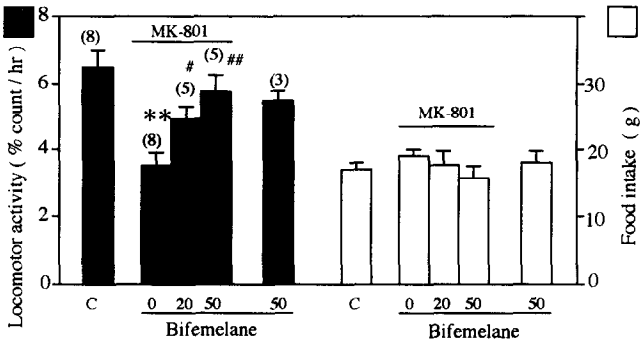

FIG. 5. Effect of bifemelane on the manifestation of mealtime-associated activity and food intake in young MK-801-treated rats. The left vertical axis indicates the mean percentage of activity per hour from 1200–1700 h of unrestricted (ad lib) feeding on the fasting day. The right vertical axis indicates the mean volume of food intake during 1300–1700 h on day 6 after restricted feeding. Feeding restriction and bifemelane (p.o. injection at 1700 h) and/or MK-801 (0.2 mg/kg, IP injection at 1730 h) were conducted for six successive days; treatments were withdrawn on day 7. Numbers in parentheses indicate the number of animals. $**p < 0.01$ vs. saline-treated and $\#p < 0.05$, $\#\#p < 0.01$ vs. saline-treated old rats (Duncan's test).

the activation of cholinergic neuronal systems by this agent. In fact, we recently observed an attenuating effect of cholinergic drugs on the impairment of mealtime-associated activity in aged rats (11). As the daily administration of BF under ad lib feeding did not cause an activity increase during 1200–1700 h, BF-induced attenuation of the impairment of mealtime-associated activity in aged rats is not due to a BF-induced, undefined increase in activity.

Treatment with MK-801 is reported to cause learning and memory deficits in spatial learning performance (14,17). MK-801 is supposed to suppress mealtime-associated activity in young rats by the blockade of NMDA receptors. At present, we do not know the manner in which BF improves mealtime-associated activity in MK-801-treated young rats. The reduction of NMDA receptors has been revealed in the gerbil hippocampus after transient ischemia (4). Postischemic BF treatment almost completely prevented ischemia-induced decreases in NMDA receptors (1). In addition, BF attenuated the reduction of NMDA receptors in aged rats (unpublished observation). Although the precise mechanisms remain to be elucidated, BF may counteract the MK-801-elicited suppression of mealtime-associated activity via activation of NMDA receptor mechanisms.

Food intake during feeding restriction was affected by neither MK-801 nor BF both in aged and young rats. Therefore, the change of mealtime-associated activity by these agents may not be directly related to the feeding motivation of restricted feeding. Actually, we found that aging impaired mealtime-associated activity without reducing the motivating effect of restricted feeding in aging rats (12).

In summary, the present results clearly demonstrated that BF possesses an attenuating effect on the impairment of anticipatory activity rhythm in aged rats as well as MK-801-treated ones, suggesting that BF may have an enhancing effect on learning and memory performance such as spatial and temporal perception.

REFERENCES

1. Asanuma, M.; Ogawa, N.; Haba, K.; Hirata, H.; Chou, H.; Mori, A. Effects of bifemelane hydrochloride on loss of *N*-methyl-D-aspartate receptor and muscarinic cholinergic receptor binding in the gerbil hippocampus after transient ischemia. Arch. Int. Pharmacodyn. Ther. 315:16–21; 1992.
2. Egashira, T.; Nagai, T.; Kimba, Y.; Takano, R.; Yamanaka, Y. Effects of bifemelane hydrochloride on various cholinergic makers in cortical and subcortical regions of aged rats. Jpn. J. Pharmacol. 51:211–218; 1989.
3. Gottfries, C. G. Neurochemical aspects on aging and diseases with cognitive impairment. J. Neurosci. Res. 27:541–547; 1990.
4. Haba, K.; Ogawa, N.; Mizukawa, K.; Mori, A. Time course of changes in lipid peroxidation, pre and postsynaptic cholinergic indices. NMDA receptor binding and neuronal death in the gerbil hippocampus following transient ischemia. Brain Res. 540:116–122; 1991.
5. Hirai, S.; Araki, G.; Tohgi, A.; Ohtomo, A.; Okada, J.; Nukada, T. Clinical trial of 4-(o-benzyl-phenoxy)-N-methylbutylamine hydrochloride. Jpn. J. Clin. Med. 60:1745–1752; 1983.
6. Meyer, E. M.; Crews, F. T.; Ofero, D. H.; Larsen, K. Aging decreases the sensitivity of rat cortical synaptosomes to calcium ionophore-induced acetylcholine release. J. Neurochem. 47:1244–1246; 1986.
7. Michalek, H.; Fortuna, S.; Pinter, A. Age-related differences in brain choline acetyltransferase, cholinesterase activity and muscarinic receptor sites in two strains of rats. Neurobiol. Aging 10: 143–148; 1989.
8. Ogawa, N.; Haba, K.; Sora, Y.; Higashida, A.; Sato, H.; Ogawa, S. Comparison of the effects of bifemelane hydrochloride and indeloxazine hydrochloride on scopolamine hydrobromide-induced impairment in radial maze performance. Clin. Ther. 10: 704–711; 1988.
9. Ogawa, N.; Haba, K.; Yoshikawa, H.; Ono, T.; Mizukawa, K. Comparison of the effects of bifemelane hydrochloride, idebenone and indeloxazine hydrochloride on ischemia-induced depletion of brain acetylcholine levels in gerbils. Res. Commun. Chem. Pathol. 61:285–288; 988.
10. Ohara, K.; Motoyasu, A.; Fukazawa, H.; Mizume, A.; Taniyama, J.; Shiozaki, K.; Tamefusa, N.; Nishimura, N.; Matsumoto, H.; Sugitani, T.; Nishimoto, M. Clinical efficacy of bifemelane hydrochloride on cerebrovascular disease and senile dementia. Geriatr. Med. 27:1515–1535; 1989.
11. Ono, M.; Minamoto, Y.; Shibata, S.; Watanabe, S. Attenuating effect of arecoline and physostigmine on an impairment of mealtime-associated activity rhythm in old rats. Physiol. Behav. (in press) 1995.
12. Shibata, S.; Minamoto, Y.; Ono, M.; Watanabe, S. Age-related impairment of food-anticipatory locomotor activity in rats. Physiol. Behav. 55:875–878; 1994.
13. Shibata, S.; Minamoto, Y.; Ono, M.; Watanabe, S. Aging impairs methamphetamine-induced free-running and anticipatory locomotor activity rhythms in rats. Neurosci. Lett. 172:107–110; 1994.
14. Staubli, U.; Thibault, O.; DiLorenzo, M.; Lynch, G. Antagonism of NMDA receptor impairs acquisition but not retention of olfactory memory. Behav. Neurosci. 103:54–60; 1989.
15. Tobe, A.; Yamaguchi, T.; Nagai, R.; Egawa, M. Effects of bifemelane hydrochloride (MCI-2016) on experimental amnesia (passive avoidance failure) in rodents. Jpn J. Pharmacol. 33:775–784; 1983.
16. Waller, S. B.; London, E. D. Choline acetyltransferase activity and muscarinic binding in brain regions of aging Ficher-344 rats. Neurochem. Int. 14:483–490; 1989.
17. Ward, L.; Mason, S. E.; Abraham, W. C. Effects of NMDA antagonists CPP and MK-801 on radial arm maze performance in rats. Pharmacol. Biochem. Behav. 35:785–790; 1990.