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BRIEF COMMUNICATION

Attenuating Effect of Serotonin Receptor Antagonists on Impairment of Mealtime-Associated Activity Rhythm in Old Rats

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SHIBATA, S., M. ONO, Y. MINAMOTO, S. WATANABE. *Attenuating effect of serotonin receptor antagonists on impairment of mealtime-associated activity rhythm in old rats.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 541-544, 1995. -- In the present study, we examined attenuating effect of serotonin (5-HT) receptor antagonists on the impairment of the time perception presented by daily scheduled feeding in old rats. When feeding was restricted to a single meal at a fixed time of day (1300-1700 h) for six consecutive days, young rats exhibited intense locomotor activity from 1-3 h before feeding time. Intense locomotor activity was observed between 1200 and 1700 h in young animals even on the fasting day (day 7) (mealtime-associated activity). However, this mealtime-associated activity was impaired in 24-mo-old rats. Daily injections of 5-HT_2 receptor antagonists, mianserin or ritanserin, or a 5-HT_3 receptor antagonist, Y25130, at 1700 h for 6 consecutive days significantly and dose-dependently attenuated the impairment of mealtime-associated activity on the fasting day in old rats without affecting the food intake. Our results suggest that the blockade of 5-HT_2 and/or 5-HT_3 receptors attenuates impairment of the manifestation of mealtime-associated anticipatory activity related to temporal learning in old rats.

Food anticipation Aging Activity Circadian rhythm Learning

TREATMENT of rodents with serotonin (5-HT) receptor antagonists generally produces a memory-enhancing effect (1,22), but there are also data to the contrary (2,10). Despite these inconsistencies, the preponderance of evidence shows that stimulation of 5-HT activity in the brain impairs, whereas impedance of its activity enhances, learning and memory [see (11) for a review]. Recent findings have suggested that 5-HT_2 and 5-HT_3 receptor antagonists have memory-enhancing properties (1,6). Biochemical studies have shown an age-related increase in the turnover of 5-HT (5,13) and also a reduction in 5-HT receptors in rat brains (4). Evidence indicates that 5-HT turnover is increased and the number of 5-HT receptors is reduced as a result of aging. Thus,investigation of selective 5-HT_2 or 5-HT_3 receptor antagonists may be worthwhile in drug treatment studies of old rats with a age-related decline in learning and memory performance.

If feeding is restricted to a single meal scheduled at a fixed time for several consecutive days, rats develop intense locomotor activity around mealtime; this intense activity is observed even on fasting days (mealtime-associated activity) (20). An age-related decline was observed in this mealtime-associated activity rhythm, suggesting impairment of temporal learning with aging in rats (12,20). Therefore, in the present study we investigated whether 5-HT_2 receptor antagonists attenuate

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this impairment of mealtime-associated activity rhythm in old rats.

METHOD

Animals

Seven-week-old male Wistar rats were purchased from Kyudo Animal Inc. (Fukuoka, Japan) and maintained in temperature-controlled animal quarters (22–24°C) with food and water ad lib. We used 11-week-old rats as young animals and 24-mo-old as elderly ones. The animals were housed in groups with a 12 L : 12 D cycle (lights on 0700 h) until the experiment started.

Procedure

Each animal was housed individually in a plastic cage (30 × 40 × 20 cm). Motor activity was measured using an area sensor (Omron F5B, Japan), and the activity count (number of movements) was recorded and printed out at 1-h intervals (Intelligence Printer; Muromachi Kikai, Japan). After the animals fasted for 24 h, restricted feeding was carried out for 6 consecutive days. During this period, animals were allowed access to food for 4 h from 1300 h (6 h after lights on). Food was withheld again on day 7, and 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.

Data Analysis

Results are expressed as the mean ± SEM. Activity levels were fairly consistent for each individual animal, although they differed between groups. Therefore, mealtime-associated anticipation (percent counts per hour) was defined as [(activity count during 1200–1700 h/total activity count) × 100/5] on the fasting day. The significance of differences between groups was determined with Student's *t*-test or one-way analysis of variance followed by Duncan's test.

Drugs

The drugs used in the present study were Y25130 (+)N-(1-azabicyclo[2.2.2]oct-3ly)-6-chlor-1,4-methyl-3,4-dihydro-2H-1,4-benzoxazine-8 carboxamide hydrochloride) (Yoshitomi, Japan) as a 5-HT_3 receptor antagonist (8), ritanserin (Funakoshi, Japan), and mianserin (Funakoshi, Japan). All drugs were dissolved in physiologic saline and administered intraperitoneally (IP). Control animals were received physiologic saline. Because food intake during feeding might be affected by these drugs (15), all drugs were administered IP at the end of feeding (at 1700 h) for 6 consecutive days. Drug administration was withdrawn on the fasting day (day 7).

RESULTS

Activity levels were fairly consistent for each individual rat, although they differed between young and old groups. For example, the activity counts over 24 h on the fasting day were 1548 ± 138(n = 6) in young free-feeding rats and 684 ± 69 (n = 7) in old free-feeding rats ($p < 0.01$ vs. young; Student's *t*-test); and 1479 ± 161 (n = 4) in young restricted-feeding rats and 784 ± 105 (n = 5) in old restricted-feeding rats ($p < 0.01$ vs. young; Student's *t*-test) (Fig. 1A). Thus, restricted feeding did not affect total activity counts. Activity counts between 1200 and 1700 h on the fasting day were 93 ± 17 in young free-feeding rats, 436 ± 45 in young restricted-feeding rats ($p < 0.01$ vs. young free-feeding rats; Student's *t*-test), and 45 ± 9.6 in old free-feeding rats ($p < 0.05$ vs. young free-feeding rats) and 86 ± 22 in old restricted-feeding rats ($p < 0.01$ vs. young restricted-feeding rats) (Fig. 1B). Anticipatory activity expressed as percent counts per hour was impaired in the old rats (Fig. 1C).

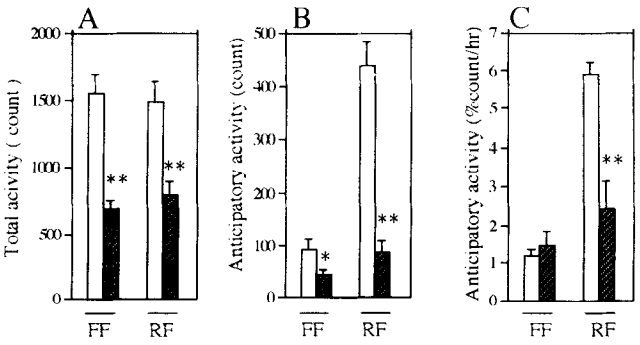

FIG. 1. Absolute levels of total and anticipatory activity on the fasting day in young and old rats. The vertical axis shows the total activity counts (A), activity counts between 1200 and 1700 h (B), and the mealtime-associated anticipatory activity [percent activity per hour (% count/hr] (C) defined as [(activity count during 1200–1700 h/total activity count) × 100/5] on the fasting day. Open column, young rats; stippled column, old rats. FF, free feeding; RF, restricted feeding. *$p < 0.05$; **$p < 0.01$ vs. young rats (Student's *t*-test).

Figure 1 shows the levels of mealtime-associated activity in young and old rats on the fasting day, for a 24-h period. Young rats exhibited an apparent increase in daytime activity as a development of mealtime-associated activity on the fasting day. In contrast, old rats exhibited no mealtime-associated activity on the fasting day, but these animals exhibited normal nocturnal activity increases (Fig. 2). We examined the attenuating effects of 5-HT receptor antagonists on the impairment

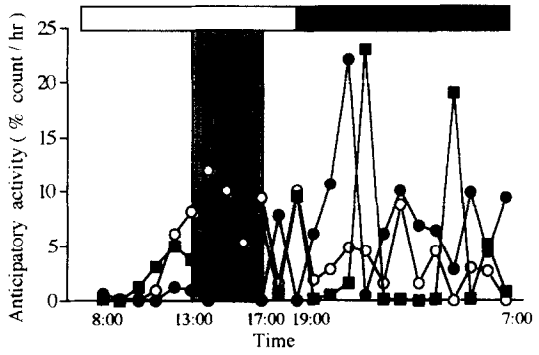

FIG. 2. Representative mealtime-associated activity patterns of young, and saline- or Y25130-treated old rats on the fasting day. The horizontal axis indicates the time of the day. Open bar, light period; solid bar, dark period. The vertical axis indicates the percent activity per hour (%count/hr) defined as [(activity count for 1 h/total activity count) × 100] on the fasting day. Open circles, young rat with saline-only injection; closed circles, old rat with saline-only injection; closed square, old rat with Y25130 (0.5 mg/kg, IP) treatment. Feeding restriction and drug injection at 1700 h were conducted for 6 consecutive days, and treatments were withdrawn on day 7. F, feeding time before fasting day 7.

of mealtime-associated activity in old rats. Old rats that received a single daily injection of Y25130 (0.5 mg/kg, IP) increased the level of mealtime-associated activity on the fasting day (Fig. 2).

Young rats exhibited a mealtime-associated activity on the fasting day (Figs. 1C and 3). In contrast, old rats exhibited no mealtime-associated activity (Fig. 3) ($p < 0.01$, Student's t-test). Daily injections of mianserin [$F(3, 16) = 6.2$, $p < 0.01$], ritanserin [$F(2, 12) = 5.8$, $p < 0.05$], or Y25130 [$F(3, 12) = 5.8$, $p < 0.01$] at 1700 h significantly attenuated the age-induced impairment of the development of mealtime-associated activity in a dose-dependent manner (Fig. 3). However, under a free-feeding schedule, old rats treated with mianserin (20 mg/kg), ritanserin (10 mg/kg), or Y25130 (1 mg/kg) at 1300 h for 6 consecutive days exhibited no activity increase between 1200 and 1700 h on day 7 [1.2 ± 0.1 (n = 3)] percent counts per hour in saline-treated animals [1.1 ± 0.3 (n = 3)] in mianserin-treated animals, [1.4 ± 0.5 (n = 3) in ritanserin-treated animals, and [1.2 ± 0.3 (n = 3) in Y25130-treated animals.

It is well known that appetite is under negative control by 5-HT-ergic activation (19). Considering the possibility that improving the impairment of mealtime-associated activity by 5-HT receptor antagonists might be due to the improvement of appetite, we measured food intake during feeding restriction. The food intake of old rats was significantly lower than that of young rats ($p < 0.01$, Student's t-test) (Fig. 3). However, food intake during feeding restriction in old rat was unaffected by the treatment with mianserin [$F(3, 16) = 2.6$, $p > 0.05$], ritanserin [$F(2, 12) = 0.16$, $p > 0.05$], or Y25130 [$F(3, 12) = 0.17$, $p > 0.05$] (Fig. 3).

DISCUSSION

In the present study, young rats on a restricted feeding schedule for 6 consecutive days exhibited a mealtime-associated activity rhythm on the fasting day. In contrast to young animals, no such mealtime-associated activity rhythm was apparent in 24-mo-old rats. Thus, aging impaired the appearance of this mealtime-associated activity rhythm. The present results demonstrated that 5-HT_3 receptor antagonist Y25130 (8) and 5-HT_2 receptor antagonists, mianserin and ritanserin, attenuated this impairment of mealtime-associated activity in old rats. Treatment of rodents with 5-HT_2 receptor antagonist, ketanserin or mianserin, is reported to produce a memory-enhancing effect in a learned aversive habit test (1). In addition, Chugh et al. (6) found memory-enhancing effects of the 5-HT_3 receptor antagonist, granisetron, in a passive avoidance task. Therefore, the lack of mealtime-associated activity seen in old rats could be due to the increase in 5-HT-ergic activity in aged rats (5,13). This evidence suggests that 5-HT_2 and 5-HT_3 receptor antagonist can improve age-related dysfunction of both spatial perception and time perception.

The destruction of 5-HT neurons and also 5-HT receptor agonists have been reported to influence endogenous rhythms and entrainment [see (15) for review; 21]. In addition, several researchers revealed that aging shortened the free-running period (14) and modified light-induced (18) and feeding-induced entrainment (12,20). Therefore, it is possible that 5-HT receptor antagonists improve the impairment of endogenous rhythms as well as feeding-induced entrainment in aging animals.

The increased activity in the 5-HT receptor antagonist-injected rats might be because possibly aversive effects of these drugs are repeatedly injected just after scheduled feeding. Under a free-feeding schedule, the daily IP administration of 5-HT receptor antagonists to old rats caused no activity increase between 1200 and 1700 h. This observation suggests that the daily injection of these drugs per se does not induce an increase in activity during the daytime.

It might be prudent to consider that the reduced anticipatory activity in aged rats might not be due to age itself, but rather to an associated change such as the increased body weight in aged male rats. They do not exhibit anticipatory activity because of the great fuel reserves from their increased body fat. This could be examined in the future experiment by determining whether reduced body weight in aged rats show anticipatory activity.

Appetite is negatively controlled by 5-HT-ergic activity [see (19) for review]. However, food intake during feeding restriction was unaffected by 5-HT receptor antagonists. In addition, it was reported that 5-HT_2 receptor antagonists enhance food intake in satiated rats, but not in hungry animals (7), and that 5-HT_3 receptors are not involved in the control of feeding behavior (19). Thus, the improvement of mealtime-associated activity by 5-HT receptor antagonists in old rats may not be related to the improvement of feeding motivation during restricted feeding. In the present experiment, we did not examine the effects of aging and 5-HT receptor antagonists on the overall distribution of feeding. Therefore, we cannot exclude the possibility that 5-HT receptor antagonists improve the impairment of anticipatory activity in aging rats by affecting the distribution of feeding.

The application of 5-HT inhibits the release of acetylcholine from the striatum (9) and hippocampus (16) slices, and this inhibitory effect of 5-HT is seen to be attenuated by 5-HT_2 receptor antagonists (16). In addition, activation of 5-HT_3 receptors can reduce acetylcholine release from the cerebral cortex (3), and 5-HT_3 receptor antagonists may act by facilitating acetylcholine release (3). Thus, the modulation of acetylcholine by 5-HT receptor antagonists may be involved in the neuronal mechanism attenuating the impairment of time

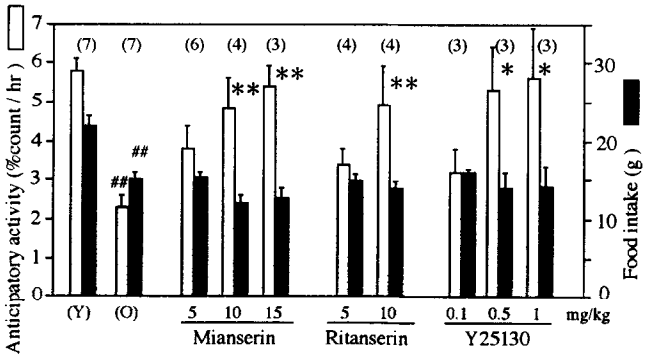

FIG. 3. Effects of serotonin receptor antagonists on impairment of the manifestation of mealtime-associated activity and food intake in old rats. The left vertical axis shows the mealtime-associated anticipatory activity [percent activity per hour (%count/hr)] defined as [(activity count during 1200–1700 h/total activity count) × 100/5] on the fasting day. The right vertical axis shows the volume of food intake between 1300 and 1700 h on day 6 after restricted feeding. Feeding restriction and drug injection at 1700 h were conducted for 6 consecutive days, and treatments were withdrawn on day 7. (O), old rats treated with saline; (Y), young rats treated with saline. Numbers in parentheses indicate the number of animals. ##$p < 0.01$ vs. young rats (Student's t-test). *$p < 0.05$; **$p < 0.01$ vs. saline-treated old rats (Duncan's test).

perception. In fact, we recently reported the attenuating effect of cholinergic drugs on the impairment of mealtime-associated activity in old rats without affecting feeding behavior (17).

In summary, the present study suggests that the blockade of 5-HT_2 and 5-HT_3 produces an attenuating effect on the impairment of time perception in old rats.

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