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Effects of Thioperamide, a Histamine H_3 Antagonist, on the Step-Through Passive Avoidance Response and Histidine Decarboxylase Activity in Senescence-Accelerated Mice

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MEGURO, K.-I., K. YANAI, N. SAKAI, E. SAKURAI, K. MAEYAMA, H. SASAKI AND T. WATANABE. *Effects of thioperamide, a histamine H_3 antagonist, on the step-through passive avoidance response and histidine decarboxylase activity in senescence-accelerated mice.* PHARMACOL BIOCHEM BEHAV **50**(3) 321-325, 1995.—The effect of thioperamide, a histamine H_3 receptor antagonist, on learning and memory was studied in the senescence-accelerated mice-prone strain (SAM-P/8) and normal-rate aging strain (SAM-R/1). In a passive avoidance test, SAM-P/8 mice of 12 months showed significant impairment of learning and memory compared with SAM-R/1 mice of the same age. Thioperamide significantly improved the response latency in SAM-P/8 mice when injected intraperitoneally at a dose of 15 mg/kg. The histidine decarboxylase (HDC) activity in the forebrain was significantly lower in SAM-P/8 mice than in SAM-R/1 mice. Thioperamide administration significantly potentiated HDC activity in the forebrain of SAM-P/8 mice as well as improving learning and memory. These results suggest that central histaminergic neurons may be involved in learning and memory impairment of SAM-P/8 mice, although other possibilities are not ruled out.

Histamine Histamine H_3 receptor Thioperamide Brain histamine content Acquisition memory Passive avoidance Brain

IMMUNOHISTOCHEMICAL studies have demonstrated the existence of histaminergic neurons in the brain, which are concentrated in the tuberomammillary nucleus of the posterior hypothalamus, and which project efferent varicose fibers to almost all parts of the brain (13,23). Consistent with its wide-ranging output, the histaminergic neuron system regulates various activities of the brain, such as the arousal state, brain energy metabolism, locomotor activity, neuroendocrine, autonomic and vestibular functions, feeding, drinking, sexual behavior, and analgesia (20,24). Recent studies also revealed that endogenous histamine plays an important role in learning and memory (7).

The senescence-accelerated mouse (SAM), a murine model of accelerated aging, showed an early onset and irreversible advancement of senescence (12). There are two strains of SAM, SAM-P and SAM-R, respectively, which are prone and resistant to accelerated senescence. SAM-P/8, a substrain of SAM-P, shows marked age-accelerated decrease in life span, deterioration of the skin and hair, amyloid deposition, and a remarkable age-accelerated deterioration in learning passive avoidance, whereas a SAM-R/1, a substrain of SAM-R, does not exhibit these characteristics appreciably, showing a normal process of development and aging. Recently, we measured the level of histamine and activity of histidine decarboxylase

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(HDC, L-histidine carboxylyase, E.C.4.1.1.22) in SAM, and found that the central histaminergic neuron system was attenuated in SAM-P/8 mice, whereas the mast cell-derived histamine content of their brain increased with age (11).

Besides postsynaptic H_1 and H_2 receptors, presynaptic H_3 receptors have been proposed to control the synthesis and release of neuronal histamine (2,20,21). Thioperamide is a histamine H_3 receptor antagonist that increases the release of neuronal histamine and the activity of HDC in the brain. In this article we report the effects of thioperamide on learning and memory, measured by a passive avoidance test, and the HDC activity in SAM.

METHOD

Animals

SAM-P/8 and SAM-R/1 mice were bred under conventional conditions. The two strains of SAM used in this study were brought to our laboratory at about 2 months of age and kept at 24 ± 2°C, with commercial diet and tap water ad lib. Forty-six aged male SAM-P/8 mice (12–16 months) and 41 aged SAM-R/1 mice (12–17 months) were used in our passive avoidance experiments. Previous studies showed that age-related differences between the grading scores of senescence and behavioral performances of SAM-P/8 and SAM-R/1 mice were marked after 6 months of age. The SAM-P/8 mice used in our experiments showed some characteristic features of accelerated senescence. Young (4–6 weeks) and old (11–18 months) groups of both strains of mice were used in binding experiments.

Passive Avoidance

The step-through passive avoidance response was examined between 2100 and 2200 h every day. The apparatus consisted of two compartments; one was illuminated with 60 W (150 × 100 mm with a height of 100 mm to top of chamber) and the other was dark. The compartments were separated by a guillotine door (20 × 20 mm). When the mice were placed into the illuminated safe compartment, they tended to escape to the dark compartment through the door and stand on a grid floor. When all four paws were on the grid, a scrambled constant current (0.3 mA) and constant voltage (50 V, 50 Hz) foot shock was delivered to the grid for 3 s. The mice could escape from the shock only by stepping back into the safe illuminated compartment. Then the mice were returned to their home cages. Passive avoidance learning was repeated on the second, third, fourth, and fifth days in the same way as in the first trial, and the response latency for entering the dark compartment was measured (17). Thioperamide (7.5 or 15 mg/kg as free base) dissolved in 0.2 ml of saline was administered IP 60 min before each trial of passive avoidance. The latency of mice that did not move into the dark compartment during the observation period was calculated to be 300 s.

Determination of Brain HDC Activity

Mice were killed by decapitation 3 h after the treatment of thioperamide on the last day; their brains were quickly removed and divided on ice into three regions: the forebrain (including the cortex, hippocampus, and striatum) and midbrain (the thalamus, hypothalamus, and colliculi).

HDC activities of forebrains and midbrains were measured as described previously (11). Briefly, brains were homogenized in 10 vol. of HDC solution (100 mM potassium phosphate, pH 6.8, 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5′-phosphate, 1% polyethyleneglycol, and 100 μg/ml phenylmethanesulfonylfluoride) in a Polytron homogenizer (Kinematica, Lucern, Switzerland) at a maximum setting for 20 s on ice. The homogenate was centrifuged at 10,000 × *g* for 30 min, and the supernatant was dialyzed against the HDC solution overnight. The HDC reaction was started by adding 0.5 mM L-histidine. The histamine produced during incubation (3 h) at 37°C was measured by the HPLC fluorescence method. Histamine was separated by a cation-exchange column, labeled with *o*-phthalaldehyde, and detected fluorometrically with a fluorometer (Tosoh, Tokyo, FS-8010) using excitation and emission wavelengths of 360 and 450 nm, respectively.

*[^{3}H](*R*)α-Methylhistamine Binding to Histamine H_3 Receptors in Brains of SAM-P/8 and -R/1 Mice*

[^{3}H](*R*)α-Methylhistamine binding was measured by the method described by Arrang et al. (2). Forebrains of SAM-P/8 and -R/1 mice were homogenized in 40 vol. of 50 mM Na^+-K^+ phosphate buffer, pH 7.5, and the homogenate was centrifuged at 50,000 × *g* for 20 min. The precipitate was resuspended in fresh buffer and centrifuged as before, and the final pellet was resuspended in the original volume of ice-cold buffer by homogenization. Binding was assayed in triplicate with 1 nM [^{3}H](*R*)α-methylhistamine. Incubation with [^{3}H](*R*)α-methylhistamine was carried out at 25°C for 60 min, and the reaction was terminated by addition of 5 ml of ice-cold buffer and rapid filtration on a glass fiber filter (GF/B, Whatman, Maidstone, UK). The filter was washed three times with 5 ml of cold buffer, and the radioactivity trapped on the filter was counted in 10 ml of Aquasol 2 with a scintillation counter. Specific binding was defined as the radioactivity bound after subtraction of nonspecific binding, determined in the presence of 5 μM thioperamide. Protein concentration was estimated

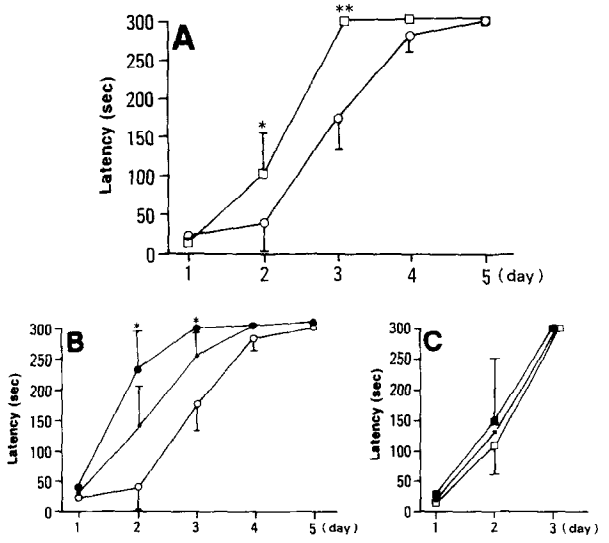

FIG. 1. Passive avoidance experiments in SAM. (A) Mean step-through latencies in SAM-P/8 (○, n = 23) and SAM-R/1 (□, n = 17) mice treated with saline. (B) Mean step-through latencies of SAM-P/8 mice treated with 7.5 (•, n = 11), 15 mg/kg thioperamide (●, n = 12), and saline (○, n = 23) are shown. (C) Mean step-through latencies of SAM-R/1 mice treated with 7.5 (■, n = 11), 15 mg/kg thioperamide (■, n = 13), and saline (□, n = 17) are illustrated. Data are for 5 consecutive days of training. Vertical bars indicate SD .*$p < 0.05$; **$p < 0.01$.

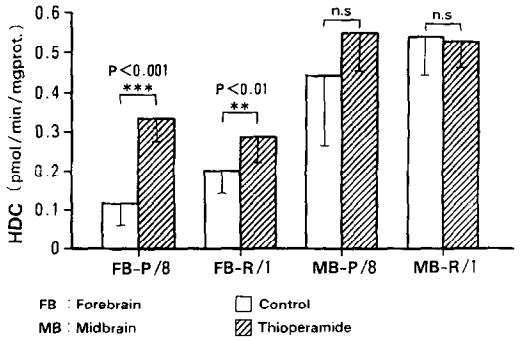

FIG. 2. Histidine decarboxylase (HDC) activities in the forebrains and midbrains of SAM-P/8 and SAM-R/1. The HDC activity in thioperamide-treated mice is shown by hatched columns and that of saline-treated mice by open columns. Vertical bars indicate SD. $^{**}p < 0.01$; $^{***}p < 0.001$. NS, not significant.

by the method of Bradford using bovine serum albumin as a standard (BIO-RAD protein assay kit).

Chemicals

[^{3}H](*R*)α-Methylhistamine (39 Ci/mmol) and thioperamide maleate were generous gifts from Green Cross Corporation (Osaka, Japan) and Sumitomo Pharmaceut. Co (Osaka, Japan), respectively. Other chemicals were of reagent purity and were obtained commercially.

Statistics

The significance of differences in latency of passive avoidance and HDC activity was determined by analysis of variance (ANOVA) followed by Duncan's test.

RESULTS

The mean step-through latency in a passive avoidance test was measured for the aged SAM-P/8 and SAM-R/1 groups treated with 7.5, 15 mg/kg of thioperamide or saline. As shown in Fig. 1, there was no significant difference in the step-through latencies of any groups on the first day, which was the day of training. In acquisition trials, SAM-P/8 treated with saline showed a shorter step-through latency than saline-treated SAM-R/1 (Fig. 1A). Intraperitoneal administration of thioperamide at a dose of 15 mg/kg resulted in significant prolongation of the response latency in SAM-P/8 mice, which showed a marked age-accelerated deterioration in learning tasks of passive avoidance (Fig. 1B). Differences between the SAM-P/8 mice treated with 15 mg/kg thioperamide and saline were observed on the second and third days ($p < 0.05$). In contrast, thioperamide had no significant effect on the response latency of SAM-R/1 at any doses (Fig. 1C). Thioperamide administration at a dose of 7.5 mg/kg slightly improved passive avoidance response latency in SAM-P/8 mice, but the effects were not statistically significant (Fig. 1B).

After the behavioral test, the brain HDC activity of the mice was determined. The mean HDC activities in the brains of SAM treated with 15 mg/kg thioperamide or saline are summarized in Fig. 2. In control mice, the HDC activity of the forebrain of old P/8 mice was significantly lower than that of old R/1 mice ($p < 0.05$). There were no significant differences in the HDC activities of the midbrain of thioperamide- and saline-treated mice in the two strains. However, thioperamide significantly increased the HDC activity in the forebrain of SAM-P/8 ($p < 0.001$) and R/1 ($p < 0.01$) mice. The increase in HDC activity in the forebrain induced by thioperamide was apparently related to the effect of thioperamide on the mean step-through latency.

In mouse forebrain, the maximum binding of [^{3}H](*R*)α-methylhistamine at 25°C was observed at a concentration of approximately 4–8 nM and the half-maximum binding at about 2 nM. Histamine H_3 receptors were distinctly distributed in cerebral cortex, striatum, substantia nigra, ventral pallidum, and olfactory nucleus. The autoradiographic distributions of the specific [^{3}H](*R*)α-methylhistamine binding in brains of old P/8 and R/1 mice were similar (data not shown). The distribution in mouse brain was consistent with that in rat and guinea pig brains (2,8). There was no significant difference in the bindings of [^{3}H](*R*)α-methylhistamine at 1 nM in the forebrains of young and old P/8 and R/1 mice (Table 1).

DISCUSSION

Biochemical and pharmacological lines of evidence indicate that cholinergic dysfunction plays an important role in age-related memory disturbances in humans and animals. A number of neurotransmitter and neuropeptide systems in both cortical and subcortical brain regions are compromised in Alzheimer-type dementia. In most cases of Alzheimer's disease, the cholinergic innervation of the cerebral cortex is degenerated, and in some cases, the noradrenergic and serotonergic innervations are also reduced (15). However, little is known about changes in the histaminergic neuron system in normal and abnormal aging, although ontogenic and developmental changes in the histaminergic neuron system in mammalian brain have been studied. In contrast to the metabolites of other aminergic transmitters, the levels of histamine metabolites are known to increase in human cerebrospinal fluid with age (14). Mazurkiewics-Kwilecki and Nsoswah reported that histamine in the brain normally increases with age, but decreases in Alzheimer's disease (10). On the other hand, Caca-

TABLE 1

[^{3}H](*R*)α-METHYLHISTAMINE BINDING TO MEMBRANES OF THE FOREBRAINS OF SAM-P/8 AND R/1 MICE

	Young (4–6 weeks)		Old (12–16 months)	
	SAM-P/8	SAM-R/1	SAM-P/8	SAM-R/1
Binding	27.0 ± 4.8	30.4 ± 4.9	25.5 ± 2.8	29.4 ± 4.9

Values are means ± 1 SD for five–six determinations. Binding of [^{3}H](*R*)α-methylhistamine at 1 nM is expressed as fmol/mg protein (see the Method section).

belos et al. found that its level in the posterior hypothalamus is increased in Alzheimer's disease (3). Immunohistochemical study did not confirm that histamine-immunoreactive neurons in the hypothalamus are altered in Alzheimer's disease (1). To clarify the participation of histamine neurons in abnormal aging, we have used two strategies: a senescence-accelerated mouse (SAM) model in animal experiments (11) and positron emission tomography (PET) with [^{11}C]pyrilamine and [^{11}C]doxepin (potent histamine H_1 receptor antagonists) as probes in human studies (26).

In this study, we examined the effects of thioperamide, a histamine H_3 antagonist, on the response latency times, measured by a passive avoidance test, and HDC activity in a murine model of abnormal aging, SAM-P/8, and a control strain, SAM-R/1. HDC activity is a good marker for the central histaminergic neuron system, as described previously (11). Thioperamide significantly improved the impaired latency times of SAM-P/8 mice to those in the control mice, R/1, at a dose of 15 mg/kg. Sakai et al. (16) reported that locomotor activity of W/W^v mice was about 1.5-fold increased at doses of 10–20 mg/kg (as free base) 60 min after IP injection of thioperamide. Therefore, we chose the two doses of 7.5 and 15 mg/kg in our passive avoidance experiments. The effects of thioperamide on the mean step-through latencies were dose dependent in SAM-P/8 mice as shown in Fig. 1B. Neurochemical analyses at the end of behavioral tasks revealed that the HDC activities in the forebrains of SAM-P/8 and -R/1 were also restored to the levels of young SAM-P/8 and -R/1 mice (11). Thioperamide had no significant effect on HDC activity in the midbrain. This difference in the effects of thioperamide in different brain regions is consistent with the regional distribution of histamine H_3 receptors in the brain (2).

In previous studies, SAM-P/8 mice were found not to show any age-accelerated changes in spatial learning, measured by radial-arm maze performance, or water-filled multiple T-maze and T-maze avoidance, in contrast to a passive avoidance task (6). Moreover, no age-related changes in the concentration of acetylcholine were demonstrated in young and old SAM-P/8 and -R/1 mice (6). These findings suggest that the brain mechanisms involved in spatial learning may be different from those in passive avoidance, and that histamine may be one of the neurotransmitters related to the impairment of latency of SAM-P/8 mice in the passive avoidance test.

The histamine H_3 receptor operates in rodents and humans by inhibiting histamine release and synthesis, and is considered to be autoreceptors expressed in histaminergic terminals as well as perikarya (20,21). However, it is now considered to act as a heteroreceptor in the central and peripheral nervous systems. It also regulates the release of other neurotransmitters such as serotonin, noradrenaline, acetylcholine, and γ-aminobutyric acid (4,5,18,19). Recently, Servos et al. suggested that the sleep–waking and behavioral arousal proposed for histaminergic neuronal function is probably indirect, acting via other neurotransmitter systems (22). Thioperamide is reported to interact with the heme moiety of hemoproteins and bind to non-H_3 receptors in peripheral tissues, in addition to acting through histamine H_3 receptors (9,25). It could be possible that thioperamide affected the memory and learning in SAM via other neuronal systems. Although it is not clear whether the effects of thioperamide observed in this study were direct or indirect on the central histaminergic system, this study suggests that histamine H_3 receptor antagonists could improve learning and memory impairment observed in passive avoidance in parallel with the increase in the locomotor activity. We are now examining the exact mechanisms for the effects of thioperamide on the passive avoidance in SAM-P/8.

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