Histamine H_3 Receptor-Mediated Inhibition of Excitatory Synaptic Transmission in the Rat Dentate Gyrus In Vivo

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ABSTRACT—We investigated the effects of histamine H_3-receptor ligands on hippocampal synaptic transmission by using anesthetized rats in vivo. The medial perforant path was stimulated, and the population excitatory postsynaptic potential (pEPSP) and population spike were recorded from the granule cell layer of the dentate gyrus. Intracerebroventricular injection of the H_3-receptor agonist (*R*)-α-methylhistamine decreased both the pEPSP and population spike, while H_3-receptor antagonists, clobenpropit and thioperamide, increased both the pEPSP and population spike. These results suggest that the histaminergic system plays a role in inhibition of hippocampal synaptic excitation via the H_3 receptor.

Keywords: Histamine H_3 receptor, Excitatory synaptic transmission, Dentate gyrus

Histamine plays many important roles as a neuromodulator or neurotransmitter in the central nervous system (CNS), and histamine receptors are classified into three subtypes, i.e., H_1, H_2 and H_3 receptors. The functions of classical H_1 and H_2 receptors have been well studied, while the role of H_3 receptors in the brain have just been focused on in recent years. Presynaptic H_3 receptors, as autoreceptors, regulate the release and synthesis of histamine. H_3 receptors are also present on the nerve endings of serotonergic, cholinergic, noradrenergic or dopaminergic neurons, and they modulate the release of these neurotransmitters as heteroreceptors (1).

The hippocampus is involved in learning and memory. Since H_3 receptors are present in this brain region at relatively high density (2, 3), H_3-receptor ligands may affect hippocampal-dependent memory processing. In fact, the H_3-receptor antagonist thioperamide has been reported to improve learning impairment in mice (4, 5) and to increase the release of histamine and acetylcholine in the hippocampus (6). H_3-receptor antagonists could be useful for CNS disorders such as dementia. However, there has been no report examining the effects of H_3-receptor ligands on hippocampal synaptic transmission in vivo. In the present study, therefore, we investigated the effects of the selective H_3-receptor agonist (*R*)-α-methylhistamine and antagonists, clobenpropit and thioperamide, on excitatory synaptic transmission in the dentate gyrus of anesthetized rats in vivo.

Recording of evoked potential was made as described in our previous paper (7). Briefly, male Wistar rats (7- to 9-weeks old) were anesthetized with a combination of urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.) and then fixed in a stereotaxic frame. A bipolar stimulating electrode was stereotaxically placed in the left entorhinal cortex to stimulate the medial perforant path, and the evoked potential was extracellularly recorded from the granule cell layer of the ipsilateral, dorsal dentate gyrus. A single test stimulus (0.08-msec duration) was applied at intervals of 30 sec, and the stimulus intensity was usually set a level that evoked a population spike of 50% of the maximum. To check the stimulus-response relationship of the evoked potentials, the stimulus current was stepwise increased in the range of 20–800 μA. The rising slope of the population excitatory synaptic potential (pEPSP) was measured to estimate dendritic synaptic depolarization, and the amplitude of the population spike was measured to estimate cell firing (Fig. 1A). The cannula for intracerebroventricular (i.c.v.) injection was placed in the contralateral ventricle (0.8-mm posterior to bregma, 1.5-mm lateral to midline, 3.7-mm ventral to dura). After stable baseline values were obtained for 30 min, the vehicle saline or the drug solution was injected in a volume of 5 μl using a microsyringe with an injection time for 2.5 min.

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I.c.v. injection of the vehicle saline produced no change in field potentials, while i.c.v. injection of histamine (50 nmol) significantly reduced both the pEPSP slope and population spike amplitude (Fig. 1A). The reduction in the pEPSP and population spike was parallel and reached a maximum 15–20 min after injection. The inhibitory effect of histamine was not affected by the concomitant injection of the H_1-receptor antagonist diphenhydramine nor the H_2-receptor antagonist ranitidine, but attenuated by the H_3-receptor antagonist clobenpropit. Population spike amplitudes 15–20 min after injection were 76.1±5.4% (n=5) in the group injected with 50 nmol histamine alone, 71.0±4.1% (n=5) in the group injected with 50 nmol histamine plus 100 nmol diphenhydramine, 68.0±8.3% (n=5) in the group injected with 50 nmol histamine plus 100 nmol ranitidine, and 95.3±6.0% (n=5) in the group injected with 50 nmol histamine plus 25 nmol clobenpropit. Similar to histamine, the selective H_3-receptor agonist (*R*)-α-methylhistamine (50 nmol, i.c.v.) significantly reduced both the pEPSP slope and population spike amplitude. The reduction in the pEPSP and population spike reached a maximum 30–40 min after injection (Fig. 1A). The effect of (*R*)-α-methylhistamine was dose-dependent in the range of 10–100 nmol (Fig. 1B). These results suggest that H_3-receptor stimulation leads to inhibition of hippocampal synaptic excitation. It is unclear why histamine-induced reduction in synaptic potentials was transient compared to that induced by (*R*)-α-methylhistamine. It may be due to differences in the metabolic rate between histamine and (*R*)-α-methylhistamine.

To examine if endogenous histamine participates in the modulation of hippocampal synaptic transmission via the H_3 receptor, the effects of H_3-receptor antagonists alone were investigated. I.c.v. injection of the H_3-receptor antagonist clobenpropit (50 nmol) produced a significant increase in the pEPSP and population spike (Fig. 2A). Both the pEPSP and population spike were maximally increased 30–40 min after injection, and then they gradually returned to the level before injection. The effect of clobenpropit was dose-dependent in the range of 10–100 nmol (Fig. 2B). Similarly, another H_3-receptor antagonist thioperamide (100 nmol, i.c.v.) produced an increase in field potentials (Fig. 2B). Unlike the H_3-receptor antagonists, the H_1-receptor antagonist diphenhydramine (100 nmol, i.c.v.) or H_2-receptor antagonists, cimetidine (100 nmol, i.c.v.) or ranitidine (100 nmol, i.c.v.), produced no change in field potentials (data not shown, n=5). We have previously confirmed that i.c.v. injection of cimetidine at 100 nmol is effective in inhibiting the induction of long-term potentiation in the dentate gyrus of anesthetized rats (8). These results suggest that the endogenous histaminergic system plays a role in inhibition

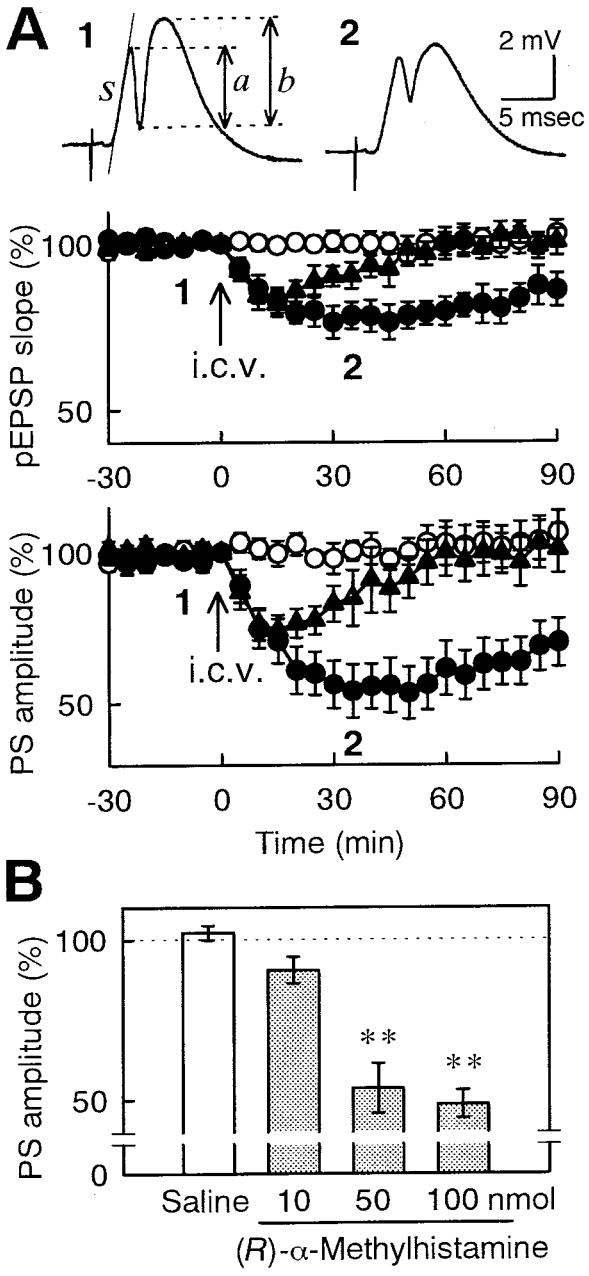

Fig. 1. The effects of histamine and the selective H_3-receptor agonist (*R*)-α-methylhistamine on field potentials in the dentate gyrus of anesthetized rats. A: Time course of changes in the slope of pEPSP (upper) and the amplitude of population spike (PS, lower) before and after i.c.v. injection of saline (○, n=6), 50 nmol histamine (▲, n=5) or 50 nmol (*R*)-α-methylhistamine (●, n=6). Insets are representative field potentials 10 min before (1) and 35 min after (2) injection of (*R*)-α-methylhistamine. The slope of pEPSP was measured on the rising phase (*s*), and the amplitude of population spike was defined as $(a+b)/2$. The pEPSP slopes and the population spike amplitudes were expressed as the percentage of baseline values immediately before injection (time 0). B: Dose-dependent effect of (*R*)-α-methylhistamine. The ordinate indicates the mean amplitude of population spike 30–40 min after injection of saline or (*R*)-α-methylhistamine (10–100 nmol). All data are the mean±S.E.M. values of six separate observations. **$P<0.01$ vs saline group, Duncan's multiple range test.

of hippocampal synaptic excitation via the H_3 receptor.

To examine if H_3-receptor agonists and antagonists affect neuronal excitability, their effects on the EPSP-spike (E-S) relationship were analyzed in detail. As shown in Fig. 3A, field potentials were evoked at various stimulus intensities (20−800 μA), and then the E-S curve was

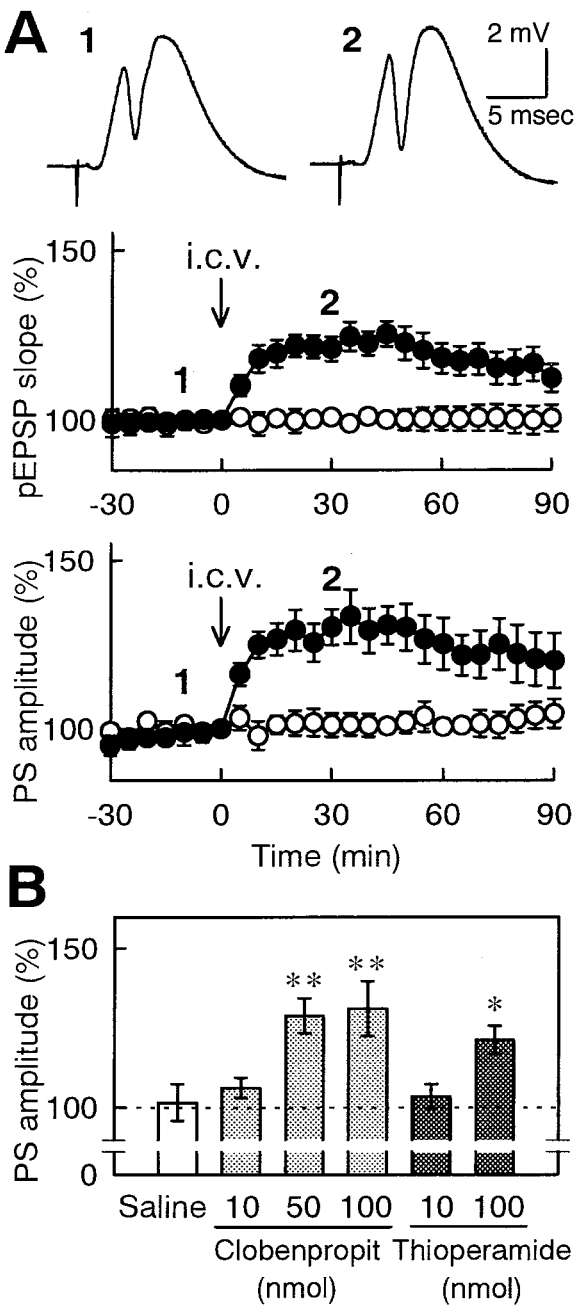

Fig. 2. The effects of H_3-receptor antagonists on field potentials in the dentate gyrus of anesthetized rats. A: Time course of changes in pEPSP slope (upper) and population spike amplitude before and after i.c.v. injection of saline (○, n=5) or 50 nmol clobenpropit (●, n=5). Insets are representative records at the times denoted by the numbers. B: Dose-dependent effect of clobenpropit and thioperamide. The experimental protocol and representation of graphs are as in Fig. 1. All data are the mean±S.E.M. (n=5). *P<0.05, **P<0.01 vs saline group, Duncan's multiple range test.

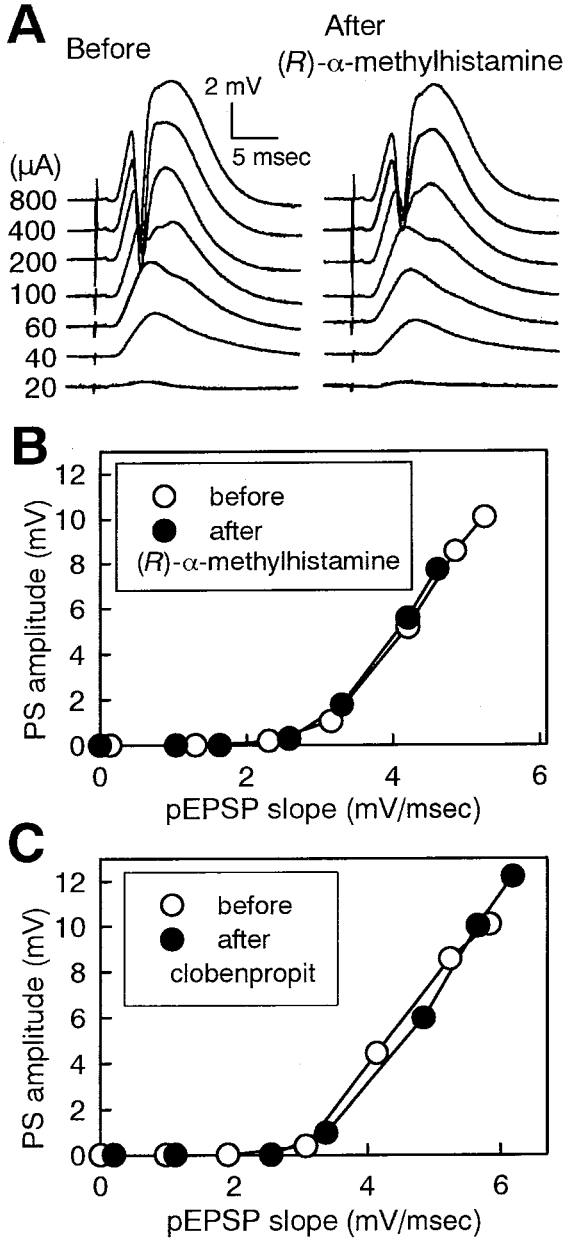

Fig. 3. The effects of (*R*)-α-methylhistamine and clobenpropit on the E-S relationship of field potentials in the dentate gyrus of anesthetized rats. A: Sample records of field potentials evoked at various stimulus intensities immediately before (left) and 30 min after i.c.v. injection of 50 nmol (*R*)-α-methylhistamine (right). B and C: The E-S curve immediately before and 30 min after i.c.v. injection of 50 nmol (*R*)-α-methylhistamine (B) or 50 nmol clobenpropit (C). Field potentials were evoked at seven stimulus intensities (20, 40, 60, 100, 200, 400 and 800 μA), and the pEPSP slope was plotted against population spike amplitude at each stimulus intensity. Each point represents the mean value from four separate observations. For clarity, S.E.M.s are omitted.

constructed by plotting the population spike amplitude against pEPSP slope at each stimulus intensity. If a test drug leads to a change in the firing characteristics of postsynaptic neurons, it should be seen as a shift of the E-S curve (9). Following i.c.v. injection of (*R*)-α-methylhistamine (50 nmol), both the pEPSP and population spike were reduced, but there was no shift in the E-S curve (Fig. 3B). Similarly, clobenpropit (50 nmol, i.c.v.) produced an increase in the pEPSP and population spike, but no shift in the E-S curve (Fig. 3C). These results indicate that H_3-receptor ligands do not affect the firing characteristics of postsynaptic neurons.

It has previously been reported that blockade of H_3 autoreceptors enhanced the release of histamine in the hippocampus (6) and that histamine increased the population spike in rat hippocampal slices in vitro (10, 11). Finally, we therefore investigated the influences of H_1- and H_2-receptor antagonists on clobenpropit-induced enhancement of field potentials. However, the effect of clobenpropit was not significantly affected by the concomitant injection of the H_1-receptor antagonist diphenhydramine or H_2-receptor antagonists, cimetidine or ranitidine. Population spike amplitudes 30–40 min after injection were 130.8±5.0% (n=5) in the group injected with 50 nmol clobenpropit alone, 135.1±7.9% (n=5) in the group injected with 50 nmol clobenpropit plus 100 nmol diphenhydramine, 137.9±8.6% (n=5) in the group injected with 50 nmol clobenpropit plus 100 nmol cimetidine, and 134.7±4.3% (n=4) in the group injected with 50 nmol clobenpropit plus 100 nmol ranitidine. Clobenpropit-induced enhancement of field potentials is unlikely to result from an increase in histamine release.

Although the cellular mechanism underlying H_3-receptor-mediated inhibition of hippocampal synaptic excitation is unknown, several possibilities can be argued. First, H_3-receptor agonists and antagonists produced a parallel change in the pEPSP and population spike without changing the E-S relationship, indicating that H_3 receptors modulate dendritic synaptic transmission, but not cell firing. H_3 receptors may regulate the release of glutamate from presynaptic terminals or the activity of glutamate receptors on postsynaptic membranes. Second, clobenpropit-induced enhancement of field potentials was not blocked by H_1- nor H_2-receptor antagonists, indicating that the clobenpropit-induced effect does not result from blockade of histamine autoreceptors. Clobenpropit may affect the release of neurotransmitters other than histamine through blockade of H_3 heteroreceptors. In our in vivo experiment, it is also possible that H_3 receptor ligands act not only the hippocampus but also on other brain regions that have neural connections to the hippocampus. Further investigations are underway in our laboratory to test these possibilities.

Histamine has been reported to enhance *N*-methyl-D-aspartate (NMDA)-receptor-mediated responses in hippocampal neurons (12, 13). However, NMDA receptors do not contribute appreciably to excitatory synaptic responses evoked by low-frequency stimulation under physiological conditions because Mg^{2+} blocks the cation influx through the NMDA receptor-associated channel in a voltage-dependent manner (14). In fact, we have confirmed that basal synaptic potentials in the dentate gyrus of anesthetized rats are not changed by blocking NMDA receptors with NMDA-receptor antagonists (7) or by potentiating NMDA receptors with polyamines (15). Therefore, modulation of NMDA receptors is unlikely to be involved in the effect of histamine observed in the present study.

In conclusion, we have demonstrated for the first time that the H_3 receptor plays a role in inhibition of hippocampal synaptic excitation in vivo. Previous behavioral studies have reported that H_3 receptor antagonists are effective in improving learning impairment of mice (4, 5). It will be interesting to examine if the enhancement of hippocampal synaptic excitation by H_3 receptor antagonists underlies their effect on learning and memory.

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