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EFFECTS OF THE HISTAMINE H_3 RECEPTOR LIGANDS THIOPERAMIDE AND (R)-α-METHYLHISTAMINE ON HISTIDINE DECARBOXYLASE ACTIVITY OF MOUSE BRAIN

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The effects of the histamine H_3 receptor ligands thioperamide and (R)-α-methylhistamine on the histidine decarboxylase (HDC) activity and histamine content of mouse brain were examined. Thioperamide, a histamine H_3 antagonist, significantly increased the HDC activity in the brain of ddY, W/W^v and ICR mice 2-6 hr after its intraperitoneal (i.p.) injection. On the other hand, (R)-α-methylhistamine, a histamine H_3-receptor agonist, caused no significant change in the HDC activity. The whole brain histamine content of ddY mice decreased significantly to 60-70 % of the control level 2-8 hr after injection of thioperamide (25 mg/kg, i.p), but then increased to 90 % of the control level 10 hr after the injection. These in vivo results showed that blockade of the presynaptic histamine H_3-receptor, which causes release of presynaptic histamine, increased the HDC activity.

Recently, the presence of histamine as a neurotransmitter or neuromodulator in the CNS has been established (1-6), and the presence of the presynaptic H_3 receptor has been proved by the development of the specific antagonist and agonist, thioperamide and (R)-α-methylhistamine, respectively (7,8). Arrang et al. showed that histamine formation was increased by thioperamide and decreased by (R)-α-methylhistamine in slices of rat brain (8). However, since the effects of H_3-receptor ligands on the activity of histidine decarboxylase (HDC, L-histidine carboxylyase, E.C.4.1.1.22) have not been studied, the possible in vivo relationship between intrinsic histamine release and HDC activity is unknown.

Abbreviation used: HDC, histidine decarboxylase, L-histidine carboxylyase, E.C.4.1.1.22.

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We previously reported the effects of thioperamide on the behavior and brain histamine content of W/W^v mice, which were shown to be mast-cell-deficient by Kitamura et al. (9): At lower doses (12.5 and 25 mg/kg, i.p.), thioperamide induced increased locomotor activity of the mice, possibly mediated by H_1 and/or H_2-receptors, with concomitant decrease in the brain histamine level, 1 hr after injection (10). Subsequently, we reported that thioperamide increased the HDC activity in the hypothalamus of W/W^v mice (11). Based on these results, we assumed the existence of a mechanism for up-regulation of intrinsic histamine synthesis in the posterior hypothalamus. To confirm this assumption, we measured the HDC activity in the brain of three strains of mice, ddY, W/W^v and ICR mice, after injections of thioperamide and (R)-α-methylhistamine and also measured the brain histamine content of ddY mice after thioperamide injection.

MATERIALS AND METHODS

Animals and Drug Treatment

Male ddY, W/W^v and ICR mice of 5 weeks old were purchased from Funabashi Farm (Funabashi, Chiba, Japan). They were housed at a constant temperature of 22 ± 2 °C with controlled lighting (7:00-19:00) and had free access to food and water. Thioperamide was dissolved in 1 M HCl, diluted and adjusted to pH 7 with sodium bicarbonate, while (R)-α-methylhistamine was dissolved in saline. These drugs were injected intraperitoneally (i.p.) during light period. At the indicated times, mice were killed by decapitation and their brains were quickly removed and stored at -80°C until use.

Measurement of HDC Activity of Mouse Brain

The HDC activity of mouse brain was measured as described by Watanabe et al. (12). Briefly, brains were homogenized in 10 volumes of HDC solution [100 mM potassium phosphate, pH 6.8, 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate, 1 % polyethyleneglycol (average molecular weight 300) and 100 μg/ml phenylmethanesulfonylfluoride] in a Polytron homogenizer (Kinematica, Lucern, Switzerland) at a maximum setting for two 10-sec periods in an ice bath. The homogenate was centrifuged at 10,000 x g for 30 min and the supernatant was dialyzed against HDC solution overnight. The HDC reaction was started by adding 0.5 mM L-histidine (final concentration) and after 4 hr the histamine produced was measured by the HPLC-fluorescence method described by Yamatoadani et al. (13). Shortly, histamine was separated by the HPLC system, postlabelled with o-phthalaldehyde and detected fluorometrically with an FS-8010 fluorometer (Tosoh, Tokyo, Japan) using excitation and emission wavelengths of 360 and 450 nm, respectively.

Measurement of Histamine Content of Mouse Brain

Brains were homogenized in 2.6 ml of 3 % perchloric acid containing 5 mM EDTA-Na_2 in a Polytron, and the homogenate was centrifuged at 12,000 x g for 20 min. The histamine in the resulting supernatnat was then measured by the HPLC-fluorescence method as described above.

Statistics

Data were analyzed by Student's t-test and values of $P<0.05$ were taken as significant.

RESULTS

Effect of H_3-Ligands on the HDC Activity of Mouse Brain

As shown in Fig. 1A and B, thioperamide (25 mg/kg, i.p.) significantly increased the HDC activity in the brain of ddY and W/W^v mice, respectively, 2 hr after its administration, and this increased activity persisted until at least 6 hr after treatment. Thioperamide also increased the HDC activity in the brain of ICR mice 6 hrs after its administration at 25 mg/kg (data not shown). On the contrary, (R)-α-methylhistamine had no significant effect on the HDC activity of ddY mouse brain (data not shown). As shown in Table 1, 3 hr after injection of thioperamide (6.25, 12.5 and 25 mg/kg, i.p.) the HDC activity was significantly increased in both the cortex and midbrain of ddY and W/W^v mice, consistent with previous results (11).

Effect of H_3-Ligands on the Histamine Content of Mouse Brain

As shown in Fig. 2, the whole brain histamine content of ddY mice was significantly decreased 2 hr after injection of thioperamide (25 mg/kg, i.p.),

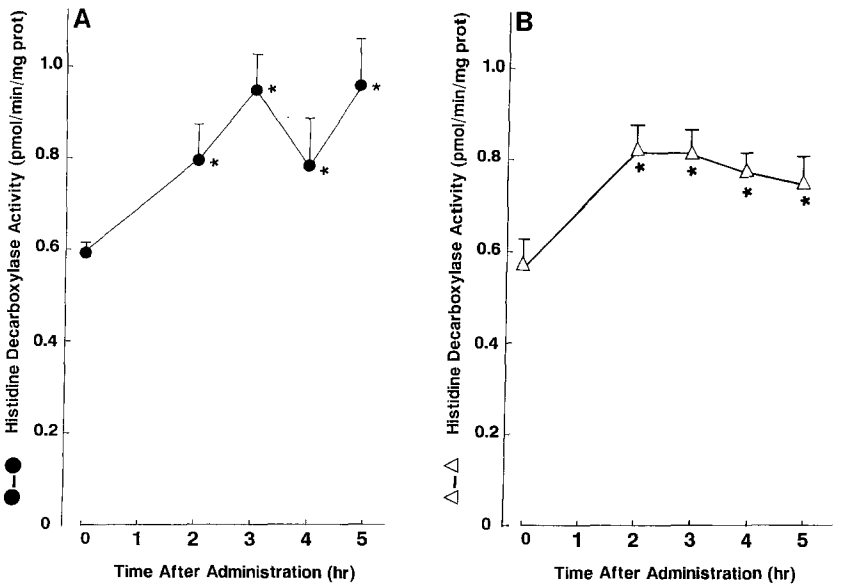

Fig. 1. Time course of increase in HDC activity induced by thioperamide. ddY (A) and W/W^v (B) mice were treated i.p. with 25 mg/kg of thioperamide and killed by decapitation at the indicated times. Their brains were quickly taken out and HDC activity was measured as described in Materials and Methods. *$P<0.05$.

Table 1. HDC activity in the brain of mice treated with thioperamide

Strain	Region	Dose of thioperamide (mg)	HDC activity (pmol/min/mg prot.)
ddy	Cortex	0.0	0.216 ± 0.016
		6.25	0.264 ± 0.040
		12.5	0.338 ± 0.029*
ddy	Midbrain	0.0	0.607 ± 0.101
		6.25	0.665 ± 0.056
		12.5	0.664 ± 0.094
		25	0.953 ± 0.075*
W/W^v	Cortex	0.0	0.390 ± 0.059
		25	0.414 ± 0.084
	Midbrain	0.0	0.559 ± 0.056
		25	0.832 ± 0.321*

Mice were injected i.p. with saline or thioperamide and killed by decapitation 3 hr later. Their brains were quickly taken out and HDC activity was measured as described in Materials and Methods. *$P < 0.05$, n= 4.

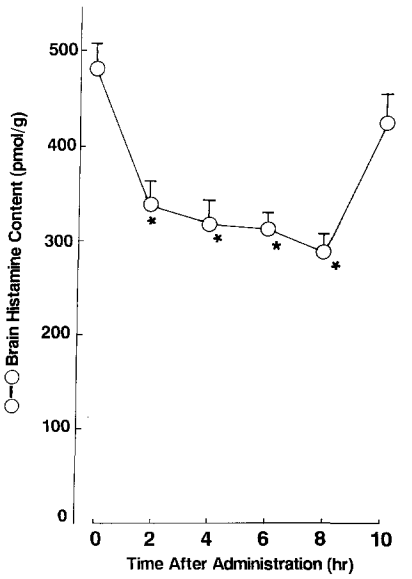

Fig. 2. Time course of changes in brain histamine content of ddY mice after thioperamide injection. ddY mice were treated i.p. with 25 mg/kg of thioperamide and killed by decapitation at the indicated times. Brains were quickly taken out and histamine content was measured as described in Materials and Methods. *$P < 0.05$.

remained at a reduced level until 8 hr after the injection, and then increased to 90 % of the control level after 10 hr.

DISCUSSION

Intrinsic histamine is thought to have an important role in the CNS (1-3). Its release is highly controlled by H_3-receptors (4-8) and it is replenished by a histamine-synthesizing enzyme, HDC, present in the tuberomammillary nucleus of the posterior hypothalamus (14). Several reports have shown a relationship between intrinsic histamine and the circadian rhythm (15-19), but there are only a few reports on the relationship between histamine release and synthesis (20,21). Thioperamide, an H_3-antagonist, has been found to enhance histamine release from histaminergic presynapses, thus affecting the levels of histamine and its metabolites in the brain (22,23) and modifying the activity of the histaminergic neuron system (7,8).

We have reported that thioperamide increased the locomotor activity of W/W^v mice with concomitant decrease of the histamine level and increase of the HDC activity (10,11). These observations were confirmed in two other strains of mice in the present study. In the present study, we followed the change in the brain histamine content for a long period after thioperamide injection. We found that the histamine content of the brain decreased to 60-70 % of that of controls for 6 hr after thioperamide injection, but was restored to 90 % of the control level after 10 hr. Thus, we assumed that the enhancement of brain HDC activity after thioperamide injection was to replenish the histamine in terminals after its presynaptic release.

We found that after thioperamide injection, the brain histamine level of ddY mice decreased, and then the brain HDC activity gradually increased. The increase of histamine 10 hr after thioperamide administration may be caused by up-regulation of HDC activity triggered by decrease of histamine in presynapses. In this experiment, the recovery of the histamine content of the brain was slow, because histamine release induced by thiopermide continued in spite of the up-regulation of the HDC activity. On the contrary, (R)-α-methylhistamine injection caused no significant change in HDC activity, although it caused marked increase in the brain histamine levels (Sakai et al., submitted to Eur. J. Pharmacol.). As it is possibe that a small decrease in HDC activity in the brain caused by (R)-α-methylhistamine would not be detected with our assay system, studies on change in level of HDC mRNA level would be interesting.

In this work, we studied the HDC activity after thioperamide injection to determine whether histamine release is related with intrinsic histamine synthesis. Our results showed a close relationship between decrease in histamine and increase in HDC activity induced by thioperamide: after injection of this drug, the amount of histamine released was synthesized within 10 hr owing to the enhancement of HDC activity.

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REFERENCES

1. Schwartz, J.C., Arrang, J.M., Garbarg, M., Pollard, H., and Ruat, M. (1991) Physiol. Res. 71, 1-51.
2. Watanabe, T. and Wada, H. (Eds.) (1991) Histaminergic Neurons: Morphology and Function, CRC Press, Boca Raton.
3. Wada, H., Inagaki, N., Yamatodani, A., and Watanabe, T. (1991) Trends Neurosci. 14, 415-418.
4. Schwartz, J.C., Arrang, J.M., Garbarg, M., and Pollard, H. (1990) Agents Actions 30, 13-23.
5. Timmerman, H. (1990) J. Med. Chem. 33, 4-11.
6. Hill, S.J. (1990) Pharmacol. Rev. 42, 45-83.
7. Arrang, J.M., Garbarg, M., and Schwartz, J.C. (1983) Nature 302, 832-837.
8. Arrang, J.M., Garbarg, M., Lancelot, J.C., Lecomte, J.M., Pollard, H., Robba, M., Schunack, W., and Schwartz, J.C. (1987) Nature 302, 832-837.
9. Kitamura, Y., Go, S., and Hatanaka, K. (1978) Blood 52, 447-452.
10. Sakai, N., Onodera, K., Maeyama, K., Yanai, K., and Watanabe, T. (1991) Life Sci. 48, 2397-2404.
11. Nimura, T., Maeyama, K., Sakai, N., and Watanabe, T. (1991) Biog. Amines 8, 299-307.
12. Watanabe, T., Nakamura, H., Linag, L.Y., Yamatodani, A., and Wada, H. (1979) Biochem. Pharmacol. 28, 1149-1155.
13. Yamatodani, A., Fukuda, H., Wada, H., Iwaeda, T., and Watanabe, T. (1985) J. Chromatog. 344, 115-123.
14. Watanabe, T., Taguchi, Y., Shiosaka, S., Tanaka, J., Kubota, H., Terano, Y., Tohyama, M., and Wada, H. (1984) Brain Res. 295, 13-25.
15. Monnier, M, Sauer, R., and Hatt, A.M. (1971) Int. Rev. Neurobiol. 12, 165-305.
16. Wada, H., Watanabe, T., Yamatodani, A., Maeyama, K., Itowi, N., Cacabelos, R. Seo, M., Kiyono, S., Nagai, K., and Nakagawa, H. (1985) In Frontiers in Histamine Research (C.R. Ganellin and M.E. Parsons, Eds.), pp. 225-236, Pergamon Press, London.
17. Kiyono, S., Seo, M.L., Shibagaki, M., Watanabe, T., Maeyama, K., and Wada, H. (1985) Physiol. Behav. 34, 615-617.
18. Lin, J.S., Sakai, K., and Jouvet, M. (1988) Neuropharmacology 27, 111-127.
19. Monti, J.M., D'Angelo, L., Jantos, H., and Pazos, S. (1988) J. Neurol. Transmit. 72, 141-146.
20. Gulat-Murray, C., Lafitte, A., Arrang, J.M., and Schwartz, J.C. (1989) J. Neurochem. 52, 248-254.
21. Gulat-Murray, C., Lafitte, A., Arrang, J.M., and Schwartz, J.C. (1989) J. Neurochem. 53, 519-524.
22. Garbarg, M., Trung Toung, M.D., Gross, C., and Schwartz, J.C. (1989) Eur. J. Pharmacol. 164, 1-11.
23. Oishi, R., Itoh, M., Nishibori, M., and Sakai, K. (1982) J. Neurochem. 51, 1388-1392.