Effects of Nociceptive Stimuli on Brain Histamine Dynamics

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Abstract—The effects of nociceptive stimuli on the metabolism of brain histamine (HA) were examined, because of a previous observation that the exposure of mice to electric foot shock increased the brain HA turnover. In mice, the exposure to tail pinch markedly increased the brain level of \textit{tле}-methylhistamine (t-MH), a predominant metabolite of brain HA, while the level of HA was not changed. The HA turnover, measured either by the accumulation of t-MH after pargyline injection or by the HA depletion after the treatment with \textit{α}-fluoromethylhistidine, a specific inhibitor of histidine decarboxylase, was enhanced by the exposure to tail pinch, like the enhancement produced by the exposure to foot shock. The exposure of rats to tail pinch increased the t-MH level in the telencephalon and the midbrain. Other types of noxious stimuli, such as placing mice on a hot plate or subjecting mice to acetic acid-induced writhing, also significantly elevated the level of t-MH but not that of HA in the mouse brain. These results suggest that nociceptive stimuli produce an increase in the brain HA turnover.

Although there have been many reports concerning the effects of various types of stress on the brain metabolism of histamine (HA), a putative neurotransmitter in mammalian brain (1, 2), the results that have been obtained are extremely inconsistent. Restraint and/or exposure to cold temperatures have been reported to induce a decrease (3, 4), an increase (5) or no change (6) in the HA level. Blowing air onto the backs of rats has been shown to produce an increase in the brain HA level (7). The HA turnover, as measured by the conversion of $^3$H-histidine to $^3$H-HA, increased in the rat hypothalamus with exposure to cold and restraint stress (3). However, it is lowered in the mouse brain after exposure to restraint stress (8). Therefore, the response of the brain histaminergic (HAergic) systems to stressful situations may depend largely on the type of stress and on the animal species.

Brain HA is almost exclusively metabolized to \textit{tłe}-methylhistamine (t-MH) (9), which is further deaminated to \textit{tłe}-methylimidazole-acetic acid by type B monoamine oxidase (10). Following an injection of pargyline, the t-MH level increases linearly for up to 2–4 hr (11–13). Therefore, it is possible to estimate the HA turnover from the rate of t-MH accumulation after pargyline administration. The non-radioisotopic estimation of HA turnover is also possible by measuring the rate of HA depletion after the inhibition of histidine decarboxylase by \textit{α}-fluoromethylhistidine (\textit{α}-FMH) (14), a highly specific inhibitor of this enzyme (15).

Using the above methods, we reported that the exposure of mice to electrical foot shock produced an enhancement of brain HA turnover (16). To determine if nociceptive stimuli are involved in the activation of brain HAergic systems, we examined the effects of other types of nociceptive stimuli, such as tail pinch, on the brain HA dynamics and compared them with that of the foot shock.

Materials and Methods

Animals and experimental procedure: Male ddY mice, weighing 25–30 g and male Wistar rats weighing 200–250 g (Seiwa Experimental Animals, Fukuoka, Japan), were used. They were housed in groups and
given free access to food and water. The animals were subjected to the following noxious stimuli: 1) Foot shock: An electric shock (1 mA AC, 0.25 sec in duration) was delivered at 15 sec intervals over either 60 or 90 min periods to mice by placing them on the copper-wire grid floor of a plastic chamber. 2) Tail pinch: The tail was pinched with forceps 1 cm (in the case of mice) or 3 cm (in the case of rats) from its root for a 10 sec in duration. The pinch was given at 5 min intervals over a 15–120 min period at the threshold pressure for pain (biting or attacking the forceps). 3) Hot plate: Mice were placed on a plate heated to a temperature of 50°C for 2 min. This procedure was repeated 4 times every 15 min. 4) Writhing: Acetic acid (0.7%) was injected i.p. in a volume of 0.1 ml/10 g of body weight. α-FMH hydrochloride was kindly donated by Dr. J. Kollonitsch of Merck, Sharp & Dohme Research Laboratories (Rahway, NJ). Pargyline hydrochloride was obtained from Sigma (St. Louis, MO). All drugs were dissolved in 0.9% saline solution. α-FMH (50 mg/kg) or pargyline (65 mg/kg) was injected i.p. immediately before the exposure to foot shock or tail pinch. The doses of drugs were expressed as the respective free base.

**Determination of HA and t-MH:** The animals were decapitated following the exposure to various noxious stimuli, and the brains, excluding the cerebellum, were immediately removed. Since the t-MH level in the brain shows a circadian variation (17), the decapitation was performed between 12:00–2:00 P.M. In some experiments, brains were placed on ice and divided into 4 regions including the telencephalon, midbrain, thalamus, and hypothalamus. The tissues were homogenized in 2–5 ml of 0.4 N perchloric acid containing an appropriate amount of pros-methylhistamine as an internal standard. The HA and t-MH contents were determined simultaneously by the method used by Tsuruta et al. (18), as modified by Oishi et al. (19), using high performance liquid chromatography with fluorescence detection. For multiple comparisons, the data were first analyzed by one-way analysis of variance. Then, the significances of the intergroup difference were evaluated by Dunnett’s test. The data, concerning the regional effects of the exposure to tail pinch on the brain amine contents, were analyzed by the two-tailed Student’s t-test. The data concerning the effects of the exposure to tail pinch or foot shock on the rate of t-MH accumulation after pargyline and on the rate of HA depletion after α-FMH were analyzed by the two-way analysis of variance to determine the interaction of two factors (drug treatment and exposure to noxious stimuli).

**Results**

**Effect of the exposure to tail pinch on the levels of HA and t-MH in the mouse brain:** As shown in Fig. 1, the steady-state level of t-MH in the mouse brain increased significantly with the exposure to tail pinch. A significant increase in the t-MH level was already observed 15 min after starting the tail pinch test. As the period of exposure to tail pinch increased, the increase in the t-MH level became more marked. On the other hand, the HA level remained unchanged despite the exposure to tail pinch.

**Effects of the exposure to tail pinch or foot shock on the HA turnover in the mouse brain:** To determine if the elevation of the t-MH level induced by tail pinch resulted from an enhancement of the HA turnover, we examined the effect of the exposure of mice to tail pinch for 60 min on the HA turnover, which was estimated either by pargyline-induced t-MH accumulation or by α-FMH-induced HA depletion, and compared the result with that of foot shock. The t-MH level in the control group increased by 104.8 ng/g during the 90 min following the pargyline administration (Fig. 2A). The exposure to foot shock or tail pinch significantly increased the t-MH level in the pargyline treated mice, thereby suggesting an enhancement of HA turnover. However, since the steady-state t-MH level was also significantly increased by the exposure to foot shock or tail pinch, the pargyline-induced t-MH accumulation was only 132.0 and 128.0 ng/g in the foot shock- and the pinch-subjected group, respectively. These values may represent underestimation and no significant interactions were shown between the pargyline or saline treatment and the exposure to noxious stimuli. When the
Fig. 1. Effect of the exposure to tail pinch on the levels of HA and t-MH in the mouse brain. The HA and t-MH contents were determined after the exposure to tail pinch for 15–120 min. Each point represents the mean±S.E.M. of 6 animals. *P<0.05, **P<0.01, compared to values before the exposure to tail pinch (0 min).

Fig. 2. Effects of the exposure to foot shock or tail pinch on A) the pargyline-induced t-MH accumulation and B) the α-FMH-induced HA depletion in the mouse brain. Pargyline (65 mg/kg, i.p.) or α-FMH (50 mg/kg, i.p.) was administered immediately before the exposure to foot shock or tail pinch. Mice were decapitated 90 min and 60 min after the injection of pargyline and α-FMH, respectively. Each column represents the mean±S.E.M. of 5–6 animals. *P<0.05, **P<0.01, compared to the corresponding control group. †P<0.01, compared to the saline-treated control group.

animals were treated with α-FMH, the HA level in the control group decreased by 41.6% during the 60 min period following the administration (Fig. 2B). The HA level in foot shock- and tail pinch-subjected groups decreased by 54.8 and 62.6%, respectively, during the same period of time after the administration of α-FMH. There were significant interactions (P<0.05) between the treatment with saline or α-FMH and the exposure to
Regional effects of the exposure to tail pinch on the levels of HA and t-MH in the rat brain: Since the increase in the steady-state t-MH level may have resulted from an enhancement of HA turnover, we examined the effect of the exposure to tail pinch on the steady-state levels of HA and t-MH in different regions of the rat brain. When the rats were exposed to tail pinch for 60 min, a significant rise of the t-MH level was observed in the telencephalon and the midbrain, but not in either the thalamus or the hypothalamus (Fig. 3).

Effects of various noxious stimuli on the levels of HA and t-MH in the mouse brain: To further confirm that noxious stimulation causes an activation of brain HAergic systems, we examined the effects of other types of noxious stimuli, such as placing mice on a hot plate or subjecting mice to acetic acid-induced writhing, on the levels of HA and t-MH in the mouse brain. All the noxious stimuli tested significantly elevated the level of t-MH but not that of HA (Fig. 4).

Discussion

In our previous study, the exposure of mice to electric foot shock induced an enhance-
ment of the HA turnover in the brain, which was accompanied by a marked increase in the t-MH level (16). In the present study, the exposure to tail pinch increased the brain t-MH level. Both the pargyline-induced t-MH accumulation and α-FMH-induced HA depletion were enhanced by the exposure to tail pinch to extents similar to those produced by the exposure to foot shock. Therefore, the increase in the t-MH level induced by the exposure to tail pinch seems to be due to an enhancement of brain HA turnover. Like foot shock or tail pinch, other types of nociceptive stimuli, such as heat stress on a hot plate and i.p. injection of acetic acid, increased the t-MH level in the mouse brain, thereby suggesting that nociceptive stimuli facilitate brain HA turnover. On the other hand, the HA level was not changed by any type of nociceptive stimuli tested in the mouse brain. This is consistent with our previous results, in which phencyclidine (20) and opioids (21), strong enhancers of brain HA turnover, had no influence on the steady-state level of HA. Regulatory mechanisms may be set in motion rapidly to maintain the HA level within a relatively narrow range.

Significant increases in the t-MH level were observed in the telencephalon and the midbrain of rats exposed to tail pinch. However, the levels in the thalamus and the hypothalamus were not altered. This is consistent with the effect of the exposure to foot shock. These results suggest that activation of the HAergic systems by nociceptive information occurs predominantly in the midbrain and the telencephalic regions.

Immunohistochemical studies, using fluorescent antibodies against HA (22) or its synthesizing enzyme histidine decarboxylase (23), have shown the distribution of HAergic neurons in the mammalian brain. The cell bodies of HA containing neurons are located only in the caudal hypothalamus but their fibers are widely distributed throughout the brain. The density of HAergic fibers is highest in the hypothalamus. The turnover rate of HA is also highest in the hypothalamus in the mammalian species studied (12, 13, 24). The reason why the enhancement of HA turnover by the exposure to tail pinch was not observed in the hypothalamus is not clear at present.

The nociceptive information seems to enhance the activity of HAergic nerve terminals in some restricted brain areas outside the hypothalamus.

The increase in HA turnover in the mouse brain induced by electric foot shock is attenuated by systemic administration of naloxone (25). Therefore, it is likely that the exposure to foot shock causes an activation of endogenous opioid systems, which in turn increases the HAergic outflow. Indeed, opioids enhance the HA turnover by acting on the opioid µ receptors and increasing the release of HA from nerve endings (21). Various noxious stimuli reportedly cause an activation of endogenous opioid systems by facilitating the release of met-enkephalin (26) and β-endorphin (27). Taken together, these results suggest that nociceptive stimuli increase the HA turnover partly by releasing endogenous opioid peptides.

HA injected intracerebroventricularly (28) or into the midbrain raphe region (29) produces analgesia. In addition, foot shock-induced analgesia is attenuated by HA H2 antagonists, such as cimetidine and ranitidine (30). Therefore, the activation of the HAergic systems, as suggested in the present study by various nociceptive stimuli, may be involved in antinociception, although the precise physiological roles of endogenous HA in the brain still remain to be determined.

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