Effects of GABA-Mimetic Drugs on Turnover of Histamine in the Mouse Brain

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Abstract—The effect of GABA-mimetics on histaminergic activity in the mouse brain was investigated. Systemic administration of muscimol (2 and 5 mg/kg), THIP (5–15 mg/kg) and aminooxyacetic acid (AOAA) (25 mg/kg) but not baclofen (2.5–15 mg/kg) inhibited the pargyline (65 mg/kg)-induced accumulation of *tele*-methylhistamine (t-MH). There was no regional difference in the inhibitory effect of muscimol on the t-MH accumulation by pargyline treatment. Muscimol and AOAA also inhibited decrease in the histamine (HA) level induced by α -fluoromethylhistidine (50 mg/kg), a specific inhibitor of histidine decarboxylase. These results suggest that these GABA-mimetics reduce the HA turnover in the mouse brain.

Histaminergic (HAergic) neurons have been histochemically visualized in rats using antibodies against histidine decarboxylase (1) and histamine (HA) (2, 3). The cell bodies of HAergic neurons are present only in the posterior hypothalamic area, whereas the HAergic fibers occupy many areas of the brain in various densities (1, 3). Neurochemical studies of the turnover of HA in the brain provided data on the regional HAergic activity (4–6). The regulation of the activity of HAergic neurons by other neurotransmitters or neuromodulators has not been determined, although the K⁺-induced release of ³H-HA from slices of the rat cerebral cortex was reported to be under negative feedback control, through presynaptic autoreceptors (7).

γ -Aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the mammalian central nervous system and GABAergic mechanisms are involved in the regulation of the activity of monoaminergic systems in some brain regions (8). Since GABA administered peripherally cannot penetrate the blood-brain barrier, several different

GABA-mimetics have been used in behavioral and neurochemical studies (9). We investigated the effects of an inhibitor of GABA transaminase (GABA-T), aminooxyacetic acid (AOAA), and GABA agonists such as muscimol, 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol (THIP) and baclofen on the HA turnover in the mouse brain.

Materials and Methods

Animals and experimental procedure: Male ddY mice weighing 24–32 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used. To examine the effect of the GABA-mimetics on the pargyline (65 mg/kg, i.p.)-induced accumulation of *tele*-methylhistamine (t-MH), AOAA (25 mg/kg, i.p.) was administered 90 min before pargyline treatment, whereas muscimol (0.5–5.0 mg/kg), THIP (1–15 mg/kg) and baclofen (2.5–15 mg/kg) were administered i.p. immediately before pargyline treatment. In these experiments, the mice were decapitated 120 min after pargyline treatment. When necessary, the brain was dissected into three brain regions: the telencephalon, hypothalamus, and brain stem (including thalamus). In the

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experiments on GABA-receptor antagonism, bicuculline and picrotoxin were given twice: i.p. at 10 min before and s.c. at 60 min after pargyline treatment. Bicuculline was first given in a dose of 2 mg/kg and then 1.5 mg/kg was given. The first and the second doses of picrotoxin were 4 and 2 mg/kg, respectively. The rate of disappearance of HA was determined after inhibition of histidine decarboxylase by α -fluoromethylhistidine (α -FMH) (50 mg/kg, i.p.). The mice were decapitated 90 min after α -FMH treatment. AOAA (25 mg/kg, i.p.) and muscimol (2 mg/kg, i.p.) were administered 90 min and immediately before α -FMH treatment, respectively.

Determination of HA and t-MH: The HA and t-MH contents in the brain, excluding cerebellum, were simultaneously determined using high performance liquid chromatography with fluorescence detection, as previously described (10). Statistical significance was evaluated by the two-tailed Student's *t*-test.

Results

Effect of GABA-mimetics on the pargyline-induced accumulation of t-MH in the mouse brain: AOAA (25 mg/kg) and muscimol (1 and 2 mg/kg) administered alone had no significant effect on either the HA or the t-MH level in the mouse brain up to 4 and 2 hr after injection, respectively. The turnover of HA in the brain was determined, based on the linear accumulation of t-MH, after pargyline treatment (4–6). As shown in Tables 1 and 2, pargyline increased the t-MH level by about 110 ng/g during 2 hr after injection, with no change in the HA level. The pargyline-induced

accumulation of t-MH in AOAA-pretreated mice was as low as 35% of the control value in the saline-treated group (Table 1). Muscimol and THIP also significantly inhibited the pargyline-induced accumulation of t-MH in a dose-dependent manner (Table 2). The intensities of the inhibitory effect of muscimol were not different among three brain regions: the telencephalon, hypothalamus and brain stem (Fig. 1). In contrast to the effects of muscimol and THIP, another class of GABA-receptor agonist baclofen (11) had no influence on the t-MH accumulation by pargyline (Table 2). Systemic administration of bicuculline and picrotoxin failed to reverse the inhibitory effect of AOAA, muscimol (2 mg/kg) and THIP (10 mg/kg) on the pargyline-induced accumulation of t-MH (Table 3).

Effect of AOAA and muscimol on the α -FMH-induced decrease in the HA level in the mouse brain: α -FMH (50 mg/kg), a specific inhibitor of histidine decarboxylase, reduced the HA level to 45% of the initial level 90 min after administration. The treatment with either AOAA (25 mg/kg) or muscimol (2 mg/kg) significantly inhibited the decrease in the HA level induced by α -FMH (Table 4).

Discussion

The turnover of HA in the brain was determined from the pargyline-induced linear accumulation of t-MH (4–6), a predominant metabolite of HA in the brain (12, 13). The regional turnover rates of HA were demonstrated to have a good correlation with the steady-state t-MH levels (4–6); however, the reduction in the turnover of HA by the

Table 1. Effect of AOAA on HA and t-MH levels in the brain of pargyline-treated mice

Treatments	HA (ng/g)	t-MH (ng/g)
Saline	51.4 $\pm$ 4.7	120.5 $\pm$ 12.5
AOAA	46.2 $\pm$ 3.4	102.4 $\pm$ 8.0
Saline+Pargyline	46.6 $\pm$ 4.1	227.5 $\pm$ 12.4*
AOAA+Pargyline	39.9 $\pm$ 2.6	140.3 $\pm$ 11.9†

In the first and the second groups, AOAA (25 mg/kg, i.p.) or saline was administered 90 min before decapitation. In the lower two groups, pargyline (65 mg/kg, i.p.) was administered 90 min after AOAA (25 mg/kg, i.p.) or saline, and the mice were decapitated 120 min thereafter. The results are the means $\pm$ S.E.M. of 6 or 7 animals. **P*<0.001, as compared with the saline control group. †*P*<0.001 as compared with the saline+pargyline group.

Table 2. Effect of muscimol, THIP and (-)-baclofen on HA and t-MH levels in the brain of pargyline-treated mice

Treatments	HA (ng/g)	t-MH (ng/g)
Saline	47.1±2.6	118.5± 7.4
Pargyline	50.5±4.0	226.4± 7.9
Muscimol (0.5 mg/kg)+Pargyline	48.0±1.5	254.9±19.6
Muscimol (1.0 mg/kg)+Pargyline	54.4±5.6	205.9±17.7
Muscimol (2.0 mg/kg)+Pargyline	49.5±3.6	162.6±11.1**
Muscimol (5.0 mg/kg)+Pargyline	50.8±1.6	163.2± 6.1***
Saline	47.6±3.0	116.7± 4.0
Pargyline	49.4±4.3	231.7±10.1
THIP (1 mg/kg)+Pargyline	51.9±4.5	234.5±12.2
THIP (5 mg/kg)+Pargyline	56.6±1.9	200.9±12.4*
THIP (10 mg/kg)+Pargyline	56.9±2.5	176.2± 8.9***
THIP (15 mg/kg)+Pargyline	51.1±4.9	178.3±12.4***
Saline	48.9±2.3	88.1± 7.5
Pargyline	43.8±2.7	213.6± 6.1
(-)-Baclofen (2.5 mg/kg)+Pargyline	47.9±4.3	188.8±11.4
(-)-Baclofen (5.0 mg/kg)+Pargyline	46.9±3.7	217.0±12.3
(-)-Baclofen (10 mg/kg)+Pargyline	43.8±2.3	225.6± 7.8
(-)-Baclofen (15 mg/kg)+Pargyline	49.0±3.8	224.0±20.5

Muscimol, THIP and (-)-baclofen were administered i.p. immediately before pargyline (65 mg/kg, i.p.) injection. Mice were decapitated 120 min after pargyline treatment. The results are the means±S.E.M. of 5 or 6 animals. *P<0.05, **P<0.01, ***P<0.001 as compared with the corresponding pargyline group.

Fig. 1. Effect of muscimol on the pargyline-induced accumulation of t-MH in different brain regions of the mouse. Muscimol (i.p.) was administered to mice immediately before pargyline treatment (i.p.). The mice were decapitated 120 min after pargyline. The results are the means±S.E.M. of 5–7 animals. *P<0.001 as compared with the saline-saline group. †P<0.05, ††P<0.01 as compared with the saline-pargyline group.

Table 3. Effect of picrotoxin and bicuculline on the inhibition by AOAA, muscimol and THIP of t-MH accumulation

Treatments	t-MH (ng/g)	Δ t-MH (ng/g)
Saline	95.2 $\pm$ 5.3	—
Pargyline	204.4 $\pm$ 9.7*	109.2
Pargyline+AOAA	167.3 $\pm$ 7.3†	72.1
Pargyline+AOAA+Picrotoxin	157.1 $\pm$ 5.2††	61.9
Saline	100.2 $\pm$ 4.6	—
Pargyline	223.5 $\pm$ 7.4*	123.3
Pargyline+Muscimol	160.0 $\pm$ 10.7††	59.8
Pargyline+Muscimol+Picrotoxin	147.0 $\pm$ 8.7††	46.8
Pargyline+Muscimol+Bicuculline	166.7 $\pm$ 4.7††	66.5
Saline	88.4 $\pm$ 4.4	—
Pargyline	189.7 $\pm$ 5.7*	101.3
Pargyline+THIP	157.2 $\pm$ 6.2††	68.8
Pargyline+THIP+Picrotoxin	158.3 $\pm$ 5.9††	69.9
Pargyline+THIP+Bicuculline	145.0 $\pm$ 6.6††	56.6

The doses of AOAA, muscimol and THIP were 25, 2 and 10 mg/kg, i.p., respectively. Picrotoxin and bicuculline were given twice: 10 min before pargyline treatment (65 mg/kg, i.p.), i.p., 4 and 2 mg/kg, respectively, and 60 min after, s.c., 2 and 1.5 mg/kg, respectively. Other procedures were the same as in Tables 1 and 2. The results are the means $\pm$ S.E.M. of 5–10 animals. * P <0.001 as compared with the saline control group. † P <0.05, †† P <0.01 as compared with the pargyline control group.

Table 4. Effect of AOAA and muscimol on α -FMH-induced decrease in HA level

Treatments	HA (ng/g)
Saline	41.7 $\pm$ 2.2
α -FMH	21.1 $\pm$ 2.3**
AOAA+ α -FMH	30.0 $\pm$ 2.8*,†
Muscimol+ α -FMH	31.4 $\pm$ 3.5*,†

AOAA (25 mg/kg, i.p.) and muscimol (2.0 mg/kg, i.p.) were administered 90 min and immediately before α -FMH (50 mg/kg, i.p.), respectively. The HA level was determined 90 min after α -FMH treatment. The results are the means $\pm$ S.E.M. of 6 or 7 animals. * P <0.05, ** P <0.01 as compared with the saline group. † P <0.05 as compared with the α -FMH group.

drug is not sufficiently reflected in the t-MH level as indicated previously (10). The present study also showed that AOAA and muscimol inhibit the pargyline-induced accumulation of t-MH in the mouse brain in spite of the lack of influence on the steady-state levels of HA and t-MH. Another GABA_A agonist THIP exerted the same effect, but the GABA_B agonist baclofen was ineffective. These results suggest that muscimol, THIP and AOAA decrease the HA turnover in the mouse brain. This proposal is supported by the finding that treatment with either AOAA or muscimol significantly inhibited the reduction of HA after the treatment with α -FMH, a specific

inhibitor of histidine decarboxylase (14, 15). Thus, it is unlikely that the AOAA inhibition of the t-MH accumulation is due to an inhibition of the activity of histidine decarboxylase, an enzyme requiring pyridoxal phosphate as a cofactor. There was no difference in the intensities of the inhibitory effect of muscimol on the HA turnover among the three brain regions tested. The doses of GABA_A-agonist effective in decreasing the HA turnover in the present study were not high as compared with doses given in other studies (16, 17). This suggests that the inhibitory effects of these GABA agonists on the activity of HAergic neurons

are mediated by GABA_A receptors. However, the decreases in the HA turnover induced by muscimol, THIP and AOAA were not blocked by systemic administration of either bicuculline or picrotoxin. It has also been reported that the analgesic effects of muscimol and THIP are not antagonized by bicuculline (18, 19). Thus, the *in vivo* antagonism by bicuculline and picrotoxin of some effects of GABA-mimetics may be difficult to observe without inducing convulsions, as suggested by Murray et al. (19).

Recently, Takeda et al. (20) reported that in rats, most histidine decarboxylase-like immunoreactive cells in the caudal, tuberal and postmammillary caudal magnocellular nuclei of the posterior hypothalamus contain glutamate decarboxylase-like immunoreactivity and they suggested the possible coexistence of histamine and GABA. Thus, the regulation of HAergic neuronal activity by GABA-mimetics demonstrated in the present study may be relevant to the function of GABA synthesized in the HAergic neurons.

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