9-Amino-1,2,3,4-Tetrahydroacridine Is a Potent Inhibitor of Histamine N-Methyltransferase

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ABSTRACT—The effect of 9-amino-1,2,3,4-tetrahydroacridine (THA) on histamine N-methyltransferase (HMT), an enzyme catalyzing the methylation of histamine to form tele-methylhistamine in the brain, was studied in vitro using a partially purified enzyme preparation from bovine brain and in vivo in the mouse brain. THA inhibited the HMT activity in competitive and non-competitive mixed type manners with respect to histamine. The $K_i$ and $K_i'$ values were 75 nM and 1.2 $\mu$M, respectively. The $IC_{50}$ values for THA, 9-aminoacridine and physostigmine in the inhibition of HMT determined at fixed concentrations of histamine (20 $\mu$M) and S-adenosylmethionine (50 $\mu$M) were 0.2, 0.37 and 20 $\mu$M, respectively. Neostigmine exhibited only 15% inhibition even at a concentration of 100 $\mu$M. THA (2-10 mg/kg, s.c.) dose-dependently inhibited HMT in the mouse brain. The inhibition of HMT by THA (10 mg/kg) was marked at 30 and 60 min after treatment, but disappeared by 120 min after. THA (10 mg/kg) significantly increased the histamine level and decreased the tele-methylhistamine level in the mouse brain. These results indicate that THA is a potent inhibitor of HMT.

Alzheimer's disease, which causes presenile dementia, is of unknown pathogenesis but characterized by some neurochemical and anatomical changes in specific brain regions. In particular, cholinergic neuron defects have been extensively studied (1-3), and the therapeutic utilization of cholinergic agents for this disease has been tested. In addition to the cholinergic defects in the brain, it has been reported that the cell bodies of monoaminergic neurons in the brainstem, whose fibers diffuse project to cortical areas, contain neurofibrillary tangles (4-7). Furthermore, Saper and German (8) demonstrated neurofibrillary degeneration in tuberomammillary nuclei of the hypothalamus. Since histaminergic neurons are located in this hypothalamic region and project fibers diffusely to the cerebral cortex (9), these neurons may also undergo some degenerative changes (10).

Although physostigmine produces only moderate improvement in patients with Alzheimer's disease, 9-amino-1,2,3,4-tetrahydroacridine (THA) has been reported to be useful in long term palliative treatment (11). This suggests that THA has some therapeutic potential in addition to anticholinesterase activity. THA is structurally similar to some antimalarial drugs such as quinacrine and amodiaquine, which are strong inhibitors of histamine N-methyltransferase (HMT) (12), an enzyme catalyzing the methylation of the imidazole ring of histamine by S-adenosylmethionine to form tele-methylhistamine. HMT has been purified from rat kidney (13-15) and guinea pig brain (16). Recently, we
have also purified HMT from bovine brain by ammonium sulfate precipitation and some DEAE-cellulose chromatographies (17). The present study was designed to examine the effect of THA on HMT activity of partially purified HMT from bovine brain. The in vivo effect of THA both on the HMT activity and on the histamine and tele-methylhistamine levels were also investigated in the mouse brain.

MATERIALS AND METHODS

In vitro experiments

HMT was partially purified from bovine brain obtained from a slaughterhouse. Whole bovine brain (400 g) was homogenized with 1.6 l of 30 mM sodium phosphate buffer (pH 7.4) containing 1 mM dithiothreitol and 1% polyethylene glycol (buffer A). The homogenate was centrifuged at 36,000 × g at 4°C for 1 hr. Ammonium sulfate was added to the supernatant to achieve 40% saturation. After centrifugation, ammonium sulfate was added to the resultant supernatant to achieve 75% saturation. The suspension was centrifuged again, and the precipitate was dissolved in 60 ml of buffer A and centrifuged overnight against 2 l of buffer A with two changes of the buffer. The dialyzed sample was applied to a DEAE-cellulose column (3 × 12 cm) which had been equilibrated with buffer A. The active fractions which passed through this column were concentrated by an ultrafiltration cell (UHP-43K, Advantec, Tokyo, Japan), using a YM-10 Diaflo membrane (Amicon). The concentrate was dialyzed overnight against 2 l of 10 mM sodium phosphate buffer (pH 7.5) containing 1 mM dithiothreitol and 1% polyethylene glycol (buffer B) with two changes of the buffer. The dialyzed sample was applied to a DEAE-cellulose column (2.5 × 11 cm) equilibrated with buffer B and eluted with a linear gradient of NaCl (0–40 mM) in 1 l of buffer B. The active fractions were pooled and concentrated to 27 ml as described above and used as the enzyme preparation for the HMT assay. A 220-fold purification was achieved by these procedures, and the specific activity of the enzyme preparation was 14.8 nmol/min/mg protein.

The HMT activity was determined as previously described (18). In brief, the reaction mixture (1 ml) was comprised of 50 mM sodium phosphate buffer, pH 7.4, containing 100 μM histamine, 50 μM S-adenosylmethionine, 10 μM pargyline and 10 μl of the enzyme preparation. After a 10-min preincubation, the reaction was performed for 15 min at 37°C and stopped by the addition of 0.25 ml of 1 M NaOH. The tele-methylhistamine formed was assayed according to the method of Tsuruta et al. (19). The HMT activity was expressed as nmol tele-methylhistamine formed/min/mg protein. The protein concentration was determined by the method of Lowry et al. (20) using bovine serum albumin as the standard.

Kinetic studies were carried out at histamine concentrations ranging from 10 to 50 μM, four THA concentrations between 0.05 and 0.4 μM, and a fixed concentration of S-adenosylmethionine (50 μM). Kinetic parameters for the inhibition by THA were determined by the methods of Lineweaver and Burk (21) and Dixon (22). The IC50 values were determined by plotting the % inhibition of HMT activity by drugs against the log of the drug concentrations, at the fixed concentrations of histamine (20 μM) and S-adenosylmethionine (50 μM).

In vivo experiments

Male ddY mice weighing about 30 g (Seiwa Experimental Animals, Fukuoka, Japan) were housed in a room at 22 ± 2°C, and supplied with food and water ad libitum.

For the determination of HMT activity in the brain, the whole brain excluding the cerebellum was homogenized in 1 ml of ice-cold 0.01 M sodium phosphate buffer, pH 7.9. After centrifugation at 10,000 × g for 20 min at 4°C, 200 μl of the supernatant was used for the reaction. The reaction mixture contained 10 μM histamine, 50 μM S-adenosylmethionine and 10 μM pargyline in a total volume of 250 μl.
For the determination of histamine and tele-methylhistamine levels, mice were decapitated and the whole brain, excluding the cerebellum, was rapidly removed. The histamine and tele-methylhistamine contents were simultaneously determined by the method of Tsuruta et al. (23) with a slight modification (24), using high-performance liquid chromatography with fluorescence detection.

The significance of differences was evaluated by analysis of variance followed by Dunnett's test.

Chemicals and drugs
Histamine dihydrochloride was obtained from Wako Pure Chemical Industries (Osaka, Japan). Tele-methylhistamine dihydrochloride and pros-methylhistamine dihydrochloride were purchased from Calbiochem-Behring (San Diego, CA, U.S.A.); pargyline hydrochloride and S-adenosyl-L-methionine iodide were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); dithiothreitol, ammonium sulfate and polyethylene glycol (average molecular weight 300) were from Nacalai Tesque; DEAE-cellulose (DE-52) was from Whatman (Maidstone, England). All other chemicals used were at least of a guaranteed reagent grade and were obtained from Wako Pure Chemical Industries and Nacalai Tesque. The drugs used were: THA hydrochloride (Aldrich Chemical Co., Milwaukee, WI, U.S.A.), 9-aminoacridine hydrochloride, neostigmine bromide (Nacalai Tesque) and physostigmine sulfate (Wako Pure Chemical Industries).

RESULTS

In vitro experiments
The inhibition of HMT activity by THA is shown in Fig. 1. Lineweaver-Burk plots of histamine methylation by HMT in the presence of various concentrations of THA revealed that THA is a competitive and non-competitive mixed type inhibitor of HMT with respect to histamine. The Kᵢ value (dissociation constant of enzyme-inhibitor complex) determined from the secondary plots (right panel in Fig. 1) of the Lineweaver-Burk plots was 75 nM, and the Kᵦ value (equilibrium constant for dissociation of inhibitor from enzyme-inhibitor-substrate complex) determined from another secondary plots (intercept on the vertical axis vs. THA concentration; figure not shown) of the Lineweaver-Burk plots was 1.2 μM.

Fig. 1. Effect of THA on the HMT activity. Left: Lineweaver-Burk plot with histamine as the substrate at variable concentrations in the absence and presence of THA. The HMT preparation was preincubated in the presence of THA at concentrations of 0 (○), 0.05 (.), 0.1 (■), 0.2 (□) and 0.4 μM (▲), and the enzyme reaction was started by the addition of histamine. The concentration of S-adenosylmethionine was fixed at 50 μM. The initial velocity of the enzyme reaction (V) was expressed in nmol/min/mg protein. Each point is the mean of three determinations. Right: Secondary plot of the Lineweaver-Burk plot. The slopes were plotted against the concentrations of THA. The intercept on the x-axis gives an estimate of Kᵢ.
Figure 2 shows the effects of THA, 9-aminoacridine, physostigmine and neostigmine on HMT activity with the histamine concentration fixed at 20 μM. The IC50 values were 0.20, 0.37 and 20 μM for THA, 9-aminoacridine and physostigmine, respectively. Neostigmine exhibited only 15% inhibition of the enzyme activity even at a concentration of 100 μM.

In vivo experiments

The HMT activity in the mouse brain was significantly decreased at 30 and 60 min after treatment with THA (5 mg/kg, s.c.), but it recovered to approximately the control level by 120 min after treatment (Fig. 3). When determined 30 min after treatment with various doses of THA, the HMT activity was significantly decreased by 27, 48 and 64% by 2, 5 and 10 mg/kg of this compound, respectively (Fig. 4).

Table 1 shows the effect of THA on the histamine and tele-methylhistamine levels in the mouse brain. The histamine levels increased significantly 1 and/or 2 hr after treatment with THA (5 and 10 mg/kg, s.c.), when compared with the saline-treated controls. On the other hand, the tele-methylhistamine levels de-
Table 1. Effect of THA on the histamine and tele-methylhistamine levels in the mouse brain

| Drugs        | Time after treatment (hr) | Levels (ng/g)          |
|--------------|---------------------------|------------------------|
|              |                           | histamine              | tele-methylhistamine   |
| Saline       | 1                         | 39.8 ± 5.1             | 84.4 ± 4.1             |
| THA, 5 mg/kg | 1                         | 57.4 ± 4.1*            | 73.6 ± 5.8             |
| THA, 10 mg/kg| 1                         | 64.0 ± 5.3**           | 59.6 ± 7.2*            |
| Saline       | 2                         | 39.4 ± 3.4             | 72.9 ± 2.5             |
| THA, 5 mg/kg | 2                         | 57.7 ± 5.0*            | 74.7 ± 2.8             |
| THA, 10 mg/kg| 2                         | 52.9 ± 5.1             | 73.4 ± 5.7             |

Mice were treated s.c. with saline or THA and killed 1 or 2 hr later. Each result represents the means ± S.E.M. of five mice. *P < 0.05, **P < 0.01 as compared with the corresponding values in the saline-treated group.

creased significantly 1 hr after treatment with THA (10 mg/kg, s.c.). However, the tele-methylhistamine levels returned to approximately the control level by 2 hr after treatment.

DISCUSSION

Histamine is metabolized through two pathways, i.e., oxidative deamination by diamine oxidase and methylation by HMT (25). In the mammalian brain, however, the methylation is the exclusive route of histamine metabolism (26, 27). HMT is inhibited by several centrally-acting drugs such as chlorpromazine, imipramine and H1 receptor antagonists, although their potencies are low (12, 28, 29). The present study shows that THA is a strong inhibitor of HMT. The determined Ki value of this compound (75 nM) was comparable to those of amodiaquine (10–12 nM), quinacrine (10–100 nM) and metoprine (100 nM) determined using different enzyme preparations (12, 28–30). In our previous experiment using highly purified HMT (8,000-fold) from bovine brain, the Ki values of amodiaquine and metoprine were 7.5 and 58 nM, respectively (17). The present study also showed that 9-aminoacridine is a strong inhibitor of HMT. This suggests that molecules with an aminoacridine moiety inhibit HMT.

The inhibition of HMT by THA was also shown by the present in vivo experiments. However, since the inhibitory effect of THA (5 mg/kg) on HMT activity in the mouse brain disappeared by 2 hr after treatment, the duration of this action may be short. Consistent with this result, THA (10 mg/kg) decreased the tele-methylhistamine level by about 30% 1 hr but not 2 hr after treatment, although THA (5 and 10 mg/kg) increased the histamine levels in the mouse brain by about 50% 1 and 2 hr after treatment. These effects of THA on the histamine and tele-methylhistamine levels in the mouse brain are less marked than those of metoprine (24, 31, 32). The difference may be due to a shorter duration of HMT inhibiting action of THA.

The inhibitory effect of physostigmine was 100 times less potent than THA on the basis of the IC50 values. THA has been reported to be superior to physostigmine as a therapeutic agent for Alzheimer's disease (11). Besides anti-cholinesterase activity (33), THA possesses multiple pharmacological properties including blocking of potassium channels (34–36), inhibition of the uptake or storage of monoamines (37) and inhibition of the binding of ligands to nicotinic and muscarinic receptors (38). In addition to these effects, the present study demonstrated that THA has a strong inhibitory effect on HMT.

With respect to histamine release from slice preparations, it was reported that presynaptic
muscarinic receptors present on histaminergic nerve terminals regulate the release (39). We also found that the systemic administration of physostigmine markedly decreases the histamine turnover rate through the stimulation of muscarinic receptors (40). These findings suggest that treatment with anti-cholinesterases may result in decreased histamine release subsequent to presynaptic inhibition through muscarinic receptors. However, it is unlikely that the THA-induced increase in the histamine levels is due to an inhibition of histamine release, because physostigmine shows no increase in the histamine levels at a dose that shows a marked inhibition of histamine turnover in the mouse brain (40). Anti-cholinesterase THA having strong inhibitory activity on HMT may diminish the above-mentioned inhibitory influence on histaminergic transmission or rather facilitate histaminergic transmission through the increase in histamine concentration in the synaptic cleft by means of retardation of its breakdown. This may in part explain the difference in the therapeutic efficacy between THA and physostigmine for Alzheimer’s disease, since brain histamine has been suggested to have a role in the regulation of arousal level (41, 42) and memory retention (43).

While we were preparing the present paper, Cumming et al. (44) reported the similar results. They showed that THA is a competitive inhibitor of rat brain HMT in vitro with a $K_i$ of 35 nM with respect to histamine and that systemic administration of THA (5 and 10 mg/kg, i.p.) largely inhibits HMT and increases the histamine levels in the rat brain. These results are generally consistent with those obtained in the present study, except that our Lineweaver-Burk plots of histamine methylation by HMT in the presence of various concentrations of THA clearly showed that the inhibition of HMT by THA is a mixed type with respect to histamine (Fig. 1). Although the reason for this discrepancy is not clear, the present experiment seems to be more accurate. In the experiments of Cumming et al. (44), the enzyme preparation used is the 45–70% ammonium sulfate fraction and only two concentrations of THA were used. However, in the present experiment, the enzyme preparation was further purified about 30 times from the 40–75% ammonium sulfate fraction using two different DEAE-cellulose chromatography and four different concentrations of THA were used. It might be also possible that the discrepancy is due to the species difference of the enzyme.

In conclusion, the present study in addition to the results reported by Cumming et al. (44), clearly indicates that THA is a potent inhibitor of HMT.

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