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Inhibition of histamine-*N*-methyltransferase (HNMT) by fragments of 9-amino-1,2,3,4-tetrahydroacridine (tacrine) and by β-carbolines

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Abstract—Histamine-*N*-methyltransferase (HNMT), the major enzyme for the metabolism of histamine in rat brain, is potently inhibited by 9-amino-1,2,3,4-tetrahydroacridine (tacrine). Structural fragments of tacrine were less potent inhibitors of rat brain HNMT than was tacrine itself. Harmaline and a number of other β-carbolines inhibited HNMT with IC_{50} values in the range of 1–10 μM. HNMT inhibition by harmaline was competitive with respect to both substrates, *S*-adenosylmethionine and histamine ($K_i = 1.4$ μM). These findings are discussed in the context of mechanisms for HNMT inhibition.

Histamine *N*-methyltransferase (HNMT,* EC 2.1.1.8) was detected in mammalian brain [1] prior to the discovery of a population of histamine-containing neurons in the rat hypothalamus [2, 3]. HNMT catalyzes the methylation of histamine and a few close analogs [4]. Because N-methylation is the predominant route for histamine metabolism in brain [5], potent HNMT inhibitors can produce elevated cerebral histamine levels, as has been demonstrated for metoprine [6], and 9-amino-1,2,3,4-tetrahydroacridine (tacrine [7]). Tacrine was known previously to be an acetylcholinesterase inhibitor, which led to the proposal that it might alleviate Alzheimer's dementia [for review, see Ref. 8]. However, potentiation of histaminergic transmission may be an additional aspect of the cerebral pharmacology of tacrine.

In the present study, a series of tacrine fragments were screened for inhibition of rat brain HNMT to eludicate the structural requirements for this class of HNMT inhibitors. Consideration of these requirements suggested that β-carbolines might also be inhibitors. Therefore, the interaction between β-carbolines and rat brain HNMT was studied.

Materials and Methods

Tacrine, histamine HCl, and 3- and 4-aminopyridine were from Sigma. 9-Aminoacridine HCl was from Fluka. Quinidine, 4-aminoquinaldine, 6- and 8-aminoquinoline, and 8-amino-tetrahydroquinoline were from Aldrich. The β-carboline compounds harmalol HCl-$2H_2O$, harmine HCl-$2H_2O$, harmaline, harmane, norharman, and 3-amino-1-methyl-5*H*-pyrido[3,4-*b*]indole were also from Aldrich. *S*-Adenosylmethionine (SAM) *p*-toluenesulfonate was from Sigma and [^{3}H]SAM (10 Ci/mmol) was from New England Nuclear.

Inhibition of HNMT was determined by an *in vitro* assay employing rat brain HNMT, prepared by ammonium sulfate fractionation as described previously [7]. In brief, 20 μg rat brain protein was incubated for 30 min at 37° in the presence of histamine (6.7 μM), [^{3}H]SAM (10 μM, 0.2 μCi) and various concentrations of the inhibitors in a final volume of 200 μL sodium phosphate (100 mM, pH 7.4). Reactions were terminated by the addition of 150 μL sodium tetraborate (2.5 M, pH 11). The labeled product was extracted into 5 mL toluene/isoamyl alcohol (3:1, v/v), and back-extracted into 250 μL potassium phosphate buffer (1.0 M, pH 7.1), which was then removed for liquid scintillation counting. Assays were conducted in triplicate, and IC_{50} values were calculated by linear interpolation of logit-log plots.

The kinetics of the inhibition of HNMT by harmaline, a prototype of the β-carbolines, were determined by double-reciprocal analysis [9]. Triplicate assays were conducted in the presence of harmaline (0, 0.5, 1.0, or 1.5 μM), in which either SAM was fixed at 10 μM while histamine varied from 1 to 10 μM, or histamine was fixed at 6.7 μM and SAM varied from 1 to 10 μM. K_i was determined by the method of Dixon [10] and by the expression $K^{[I]}/K_m = \{1 + [I]/K_i\}$ [11], where $K^{[I]}$ is the apparent K_m in the presence of the inhibitor at concentration [I].

Results

The inhibition of HNMT by tacrine and eight compounds with related structures (Fig. 1A) indicates that tacrine was the most potent inhibitor in the series. Quinidine, an aminoquinoline alkaloid structurally related to quinacrine, was of moderate potency. Full potency of HNMT inhibition required the integrity of the ring system, as well as the correct orientation of the amine-group with respect to the aromatic ring: only compounds containing the 4-aminopyridine structure were good inhibitors.

A variety of β-carbolines were moderately potent inhibitors of HNMT, with IC_{50} values in the range of 2–20 μM (Fig. 1B). The degree of saturation of the 3–4 position (harmine vs harmaline and harmol vs harmalol) was without great effect on the IC_{50}. Nor did HNMT distinguish between 7-hydroxy and 7-methoxy compounds. However, norharman, which lacks the 1-methyl group, was a less potent inhibitor than the other β-carbolines. The least effective inhibitor in the series was 3-amino-1-methyl-5*H*-pyrido-[3,4-*b*]indole, in which the nitrogen is in the 3-position, unlike the β-carbolines (Fig. 1B, see inset).

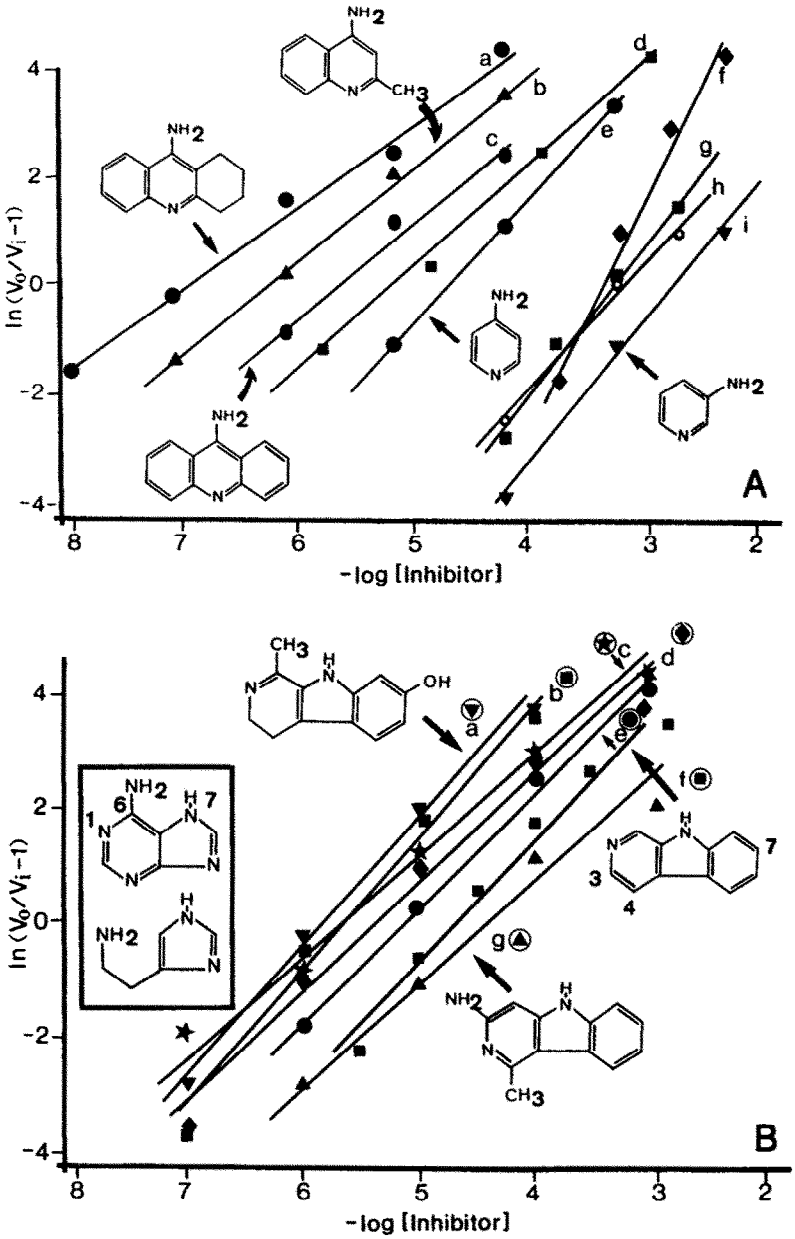
The inhibition of rat brain HNMT by harmaline was competitive with respect to both histamine ($K_i = 1.4 \pm 0.4$ μM, Fig. 2A) and SAM ($K_i = 1.4 \pm 0.2$ μM, Fig. 2B). Analysis of these data by the method of Dixon yielded identical results.

Discussion

HNMT is inhibited by a wide variety of substances: chloropromazine [1], indoleamines [12], histamine H_1 antagonists [13, 14], impromidine, an H_2 partial agonist [15], in addition to other thiourea derivatives [16], and zolantidine, a benzthiazole H_2 antagonist [17]. Among the most potent HNMT inhibitors are metoprine, a diaminopyrimidine [6], quinacrine, an aminoquinoline [18], and tacrine [7].

Attempts have been made to account for the structural diversity of HNMT inhibitors on the basis of common structural elements such as the presence of two nitrogens separated by several carbons [19], or the presence of an ethylamine moiety [14]. The diversity of inhibitors may

* Abbreviations: HNMT, histamine *N*-methyltransferase; SAM, *S*-adenosylmethionine; and SAHC, *S*-adenosyl-L-homocysteine.



Fig, 1. Logit-log analysis of the inhibition of rat brain HNMT by various concentrations of tacrine and related compounds (A, top) and by β-carbolines (B, bottom). Rat brain HNMT was incubated in the presence of histamine (6.7 μM) and [^{3}H]SAM (10 μM). Enzyme activity in the absence of inhibitors (V_0) was 100 pmol/min/mg protein. IC_{50} values calculated by interpolation, are reported for each compound. (A, top): (a) tacrine, 130 nM; (b) 4-aminoquinaldine, 800 nM; (c) 9-aminoacridine, 2.8 μM; (d) quinidine, 8 μM; (e) 4-aminopyridine, 24 μM; (f) 6-aminoquinoline, 540 μM; (g) 8-aminoquinoline, 0.9 mM; (h) 8-amino-tetrahydroquinoline, 1.3 mM; and (i) 3-aminopyridine, 1.9 mM. (B, bottom): (a) harmalol, 1.7 μM; (b) harmol, 2.4 μM; (c) harmine, 1.9 μM; (d) harmaline, 4.4 μM; (e) harmane, 7 μM; (f) norharmane, 23 μM; and (g) 3-amino-1-methyl-5*H*-pyrido[3,4-*b*]indole, 41 μM. Symbols for each data set are illustrated in the cirular insets. The large inset illustrates the structures of the adenine moiety (upper) and histamine (lower).

also be a reflection of the mechanism of catalysis. A ping-pong mechanism, in which SAM binding and methylation of the enzyme are the initial catalytic steps, has been proposed for guinea pig brain HNMT [18]. However, an "ordered Bi-Bi" reaction in which the substrates SAM and histamine bind in succession, followed by the ordered release of methylhistamine and *S*-adenosyl-L-homocysteine (SAHC), was observed for HNMT from mouse brain [13] and rat brain [20]. Furthermore, in a study employing SAM labeled with a chiral methyl group, the transmethylation catalyzed by HNMT from guinea pig brain seemed to occur directly, without methylation of the enzyme [21].

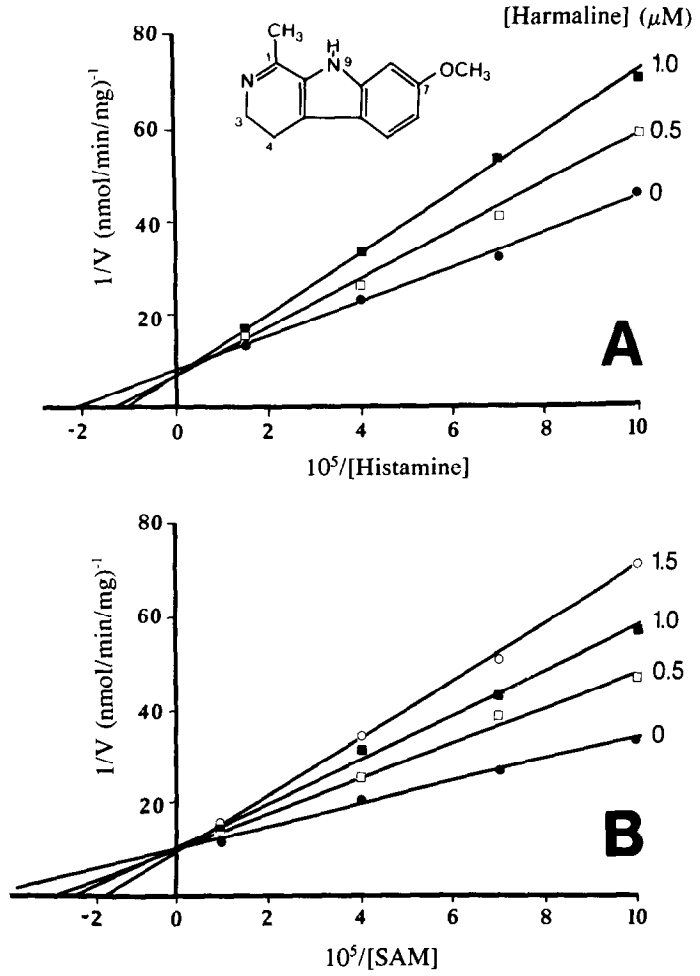


Fig. 2. Double-reciprocal plots of the inhibition of rat brain HNMT by harmaline. Harmaline was included at a final concentration of 0.0, 0.5, 1.0, and 1.5 μM as indicated. (A) Inhibition with respect to histamine, varied from 1 to 10 μM, while SAM was held constant at 10 μM. The structure of harmaline is illustrated. (B) Inhibition with respect to SAM, varied from 1 to 10 μM, while histamine was held constant at 6.7 μM.

The most potent HNMT inhibitors in Fig. 1A, like the very potent HNMT inhibitors amodiaquine and quinacrine, contain the 4-aminopyridine structure. Amodiaquine inhibits guinea pig brain HNMT in a manner which is competitive with respect to histamine, and mixed with respect to SAM [18]. A similar pattern of inhibition of rat brain HNMT has been observed for tacrine [7]. Therefore, the 4-aminopyridine structure may be a pharmacophore capable of binding to the native enzyme and to the HNMT–SAM complex, prior to the binding of histamine.

SAHC is a competitive inhibitor of rat brain HNMT with respect to SAM [20]. Various structural modifications of SAHC indicated the 6-amino group of the adenine moiety (Fig. 1B, inset) to be essential for effective HNMT inhibition [22, 23]. The 4-aminopyridine moiety is structurally analogous to the aminopyrimidinyl portion of adenine. However, several drugs with the 4-aminopyridine structure are mixed inhibitors with respect to SAM, thus binding to HNMT and to the SAM–enzyme complex. Therefore, the 4-aminopyridine and SAM binding sites are unlikely to be identical. Harmaline is a competitive inhibitor of HNMT with respect to both substrates, suggesting that inhibition by harmaline occurs by a different mechanism than inhibition by compounds with the 4-aminopyridine structure.

Harmaline, a hallucinogen which may be extracted from *Peganum harmala* and *Banisteriopsis spp* [24], is a reversible monoamine oxidase-A inhibitor (IC_{50} ~10 nM [25]). Other β-carbolines inhibit the binding to rat brain membranes of benzodiazepine (IC_{50} 10–100 μM [26]) and γ-aminobutyric acid (IC_{50} ~10 μM [27]), or inhibit the high affinity uptake of dopamine (IC_{50} ~10–100 μM [28]). HNMT inhibition by β-carbolines is therefore intermediate in the potency range of previously described pharmacological properties. Given that other HNMT inhibitors can elevate cerebral histamine levels [6, 7], certain of the physiological effects of β-carbolines could be mediated via potentiation of histaminergic transmission in the central nervous system.

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