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# Actions of morphine on histamine dynamics in the mouse brain: a strain comparison

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The existence of brain histamine (HA) in both neurons and mast cells within the central nervous system is now well established [1–3]. Brain HA is synthesized by histidine decarboxylation [4] and methylated by HA methyltransferase (HMT) [5], producing *tele*-methylhistamine (t-MH), a substrate for monoamine oxidase (MAO) [6, 7]. After irreversible inhibition of MAO by pargyline, the rate of accumulation of t-MH has been used to estimate brain HA turnover rate [8–11], a parameter thought to be an index of histaminergic neuronal activity under basal conditions [12].

Recent studies suggest that brain HA may mediate some central actions of morphine (MOR). For example, a series of brain-penetrating $H_2$ antagonists inhibited MOR antinociception with a potency that paralleled their affinity at the $H_2$-receptor [13]. Although acute MOR has no effect on the brain levels of HA and t-MH [14–16], Nishibori *et al.* [16] found that MOR increases HA turnover in the ddY mouse brain, consistent with a MOR-induced release of HA. Further results suggest that this effect occurs by activation of *mu* opiate receptors and facilitation of HA release from nerve endings [17]. To compare specific opiate responses to opiate-induced changes in brain HA metabolism, we have presently studied the effect of MOR on brain HA dynamics in three strains of mice known to vary in their responses to opiates [18, 19].

Male mice [Swiss–Webster (SW), C57/BL6 (C57) and DBA/2J (DBA), Taconic Farms, Germantown, NY] weighing between 20 and 30 g were housed in groups of five to six animals per cage in 12-hr light–dark cycles with food and water freely available. Three to four hours into the light cycle of the animals, groups of four to eight mice received pargyline hydrochloride (65 mg/kg, i.p., 10 mL/kg or saline vehicle), immediately followed by a specified dose of MOR sulfate (s.c. or saline vehicle), and were killed by decapitation at the specified intervals. Whole brains were removed and homogenized in 5 vol. of ice-cold deionized water with aliquots (0.1 mL and 0.35 mL) taken for HA and t-MH analysis respectively. Samples were analyzed for HA by the single isotope radioenzymatic assay, and for t-MH by automated gas chromatography–mass spectrometry as previously described [20]. Separate brains were assayed for HMT activity [20].

Table 1 summarizes the baseline value for brain levels of HA, t-MH, HMT activity, and HA turnover rates in three strains of mice. Surprisingly, C57 and DBA whole brain HA

Table 1. Whole brain levels of HA, its metabolite t-MH, HMT activity, and estimated basal HA turnover rates in three strains of mice

| Strain | HA (ng/g) | t-MH (ng/g) | HMT activity (μmol/g/hr) | HA turnover rate (pmol/g/min) |
|---|---|---|---|---|
| SW | 56.8 ± 4.4 (8) | 127.6 ± 11.3 (6) | 1.30 ± 0.02 (4) | 5.7 ± 0.7 (12) |
| C57 | 83.7 ± 6.2* (11) | 171.9 ± 7.4† (14) | 1.44 ± 0.04‡ (3) | 5.2 ± 0.9 (14) |
| DBA | 88.8 ± 3.1† (25) | 143.1 ± 3.3 (25) | 1.49 ± 0.02* (3) | 8.8 ± 1.0‡ (7) |

Animals received pargyline (65 mg/kg, or saline, i.p.) and were decapitated 60 min later. HA turnover rates were determined as molar t-MH levels in the presence of pargyline minus mean control t-MH levels in the absence of pargyline, corrected for treatment time (60 min). Values are means ± SE; the sample size is given in parentheses.

*–‡ Significantly different from SW values: * $P < 0.01$, † $P < 0.005$, and ‡ $P < 0.05$.

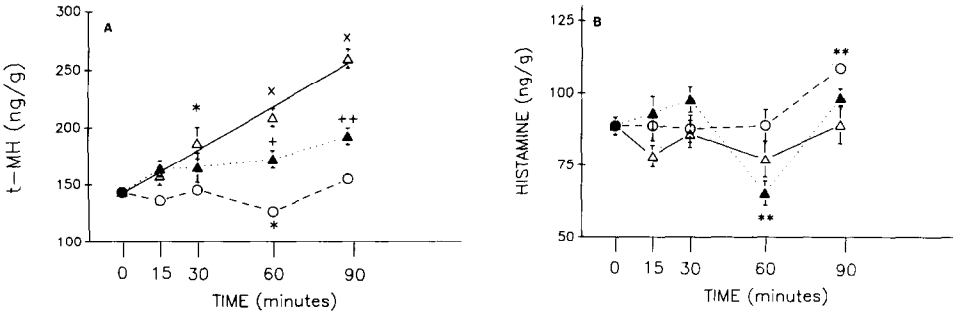


Fig. 1. Time course of action of MOR and pargyline on brain levels of t-MH (A) and HA (B) in DBA mice. Mice received i.p. injections of either pargyline (65 mg/kg) or saline vehicle, immediately followed by s.c. injections of either MOR sulfate (10 mg/kg) or saline vehicle and were killed at the times indicated (abscissa). Whole brains were immediately removed and homogenized, and aliquots were taken for determination of t-MH and HA levels (ordinate, mean ± SE, N = 6–25 animals). Saline–saline treatment had no significant effect on t-MH or HA levels at any time; therefore these values were pooled and represent time 0. Key: (△) pargyline–saline; (○) saline–MOR; and (▲) pargyline–MOR; * $P < 0.05$, ** $P < 0.01$, and $^{\times}P = 0.0001$ relative to the saline–saline control by *t*-test; and $^{+}P < 0.01$ and $^{++}P = 0.0001$ relative to the pargyline–saline group at the same time.

levels were approximately 50% higher than the comparable SW value (84 and 89 ng/g, respectively, vs 57 ng/g, Table 1). These strain differences were confirmed in separate, preliminary experiments measuring HA by an HPLC method [21]; SW, C57 and DBA whole brain HA levels were [ng/g, mean ± SE (N)]: 45.3 ± 4.2 (8), 78.4 ± 5.1 (5) and 72.3 ± 4.4 (6). Whole brain levels of HA in rodents in general are in the 40–60 ng/g range [22].

DBA (but not C57) HA turnover rates were also much higher than comparable SW values (Table 1). The higher HA levels and higher turnover rates in DBA are consistent with a higher density of histaminergic neurons in this strain. However, brain t-MH levels were much higher in C57 than in the other two strains, a pattern not consistent with their respective turnover rates (Table 1). C57 and DBA brain HMT activities were not different from each other (consistent with previous studies [23]), and both were higher than SW values. Since the elevated HA levels in C57 were not accompanied by elevated HA turnover rates in this strain, the underlying explanation for the elevated brain HA levels in one mouse strain may not account for the elevated levels observed in another. A brain HA turnover rate of 5.2 pmol/g/min has been reported for the ddY mouse [24], similar to our SW and C57 results (Table 1).

In DBA mice, pargyline produced a linear accumulation of whole brain t-MH from 30 to 90 min after injection (Fig. 1A), with no effects on brain HA levels (Fig. 1B), yielding the above turnover rate (Table 1). In saline-treated mice, MOR (10 mg/kg) caused a 12% reduction in t-MH levels after 60 min (Fig. 1A) with no effect on HA levels at this time, although an unexpected increase in HA levels was observed after 90 min (Fig. 1B). In the presence of pargyline, the MOR-induced reductions in t-MH were 18 and 26% at 60 and 90 min, respectively, compared to pargyline treatment alone. Pargyline changed HA levels 60 min after MOR treatment (Fig. 1B), violating the steady-state assumption, and indicating that the characterization of the actions of MOR on brain HA metabolism cannot be interpreted as MOR-induced changes in HA turnover, but rather as its effects on t-MH synthesis rates [8].

Because t-MH synthesis in DBA mouse brain was suppressed by a single dose of MOR during the first hour after its administration, this time interval was used to perform complete dose–response studies with MOR in three strains of mice. In DBA, MOR lowered brain t-MH levels (Fig. 2A) and suppressed brain t-MH synthesis (Fig. 2A). Since brain t-MH synthesis is thought to be dependent upon neuronal HA release [3, 9], the MOR-induced inhibition of brain t-MH synthesis is most likely to be mediated by an inhibition of the release of neuronal HA. Combinations of MOR and pargyline, but neither alone, tended to decrease slightly DBA HA levels (not shown). Several other CNS-acting drugs also decrease brain t-MH synthesis, with no effects on HA levels [25–28].

In SW mice, MOR alone failed to change significantly t-MH levels at any dose, but suppressed t-MH synthesis (Fig. 2B). SW HA levels were decreased slightly by higher doses of MOR, but were unaffected by combinations of MOR and pargyline (not shown). IN C57 mice, analysis of variance (ANOVA) indicated a slight general reduction in t-MH levels by MOR treatment ($P < 0.02$, see legend of Fig. 2), although Student's *t*-tests showed that no dose of MOR significantly changed C57 brain t-MH levels either in the presence or absence of pargyline treatment (Fig. 2C). Pargyline did not change significantly HA levels in saline-treated C57 mice, although several combinations of pargyline and MOR unexpectedly lowered HA levels (not shown).

ANOVA (two factors: MOR, strain) of the combined turnover data from Fig. 2 found significant ($P < 0.001$) main effects of MOR, strain, and a MOR by strain interaction. Thus, MOR reduced t-MH syntheses rates in all three strains. Furthermore, ANOVA of all combinations of pairs of strains showed the MOR effect to persist, and that the effect was greater in DBA than in the other two strains, which did not differ from each other. The ability of MOR to inhibit histaminergic neurons in each strain of mouse could be related to the basal HA turnover rate of that strain. Thus, MOR was most effective in inhibiting t-MH synthesis in DBA mice, which had the highest basal HA turnover rate (Table 1). Alternatively, the varied effects of MOR on t-MH synthesis in different strains of mice could be due to differences in the number and/or type of brain opiate receptors [29*].

In the current study, we have been unable to demonstrate a MOR-induced stimulation of brain HA turnover in three strains of mice, despite careful studies of the MOR time

* Garcin F, Mack G, Radouco-Thomas S and Mandel P, Opiate receptor binding differences in inbred strains of mice. *Seventh International Congress of Pharmacology*, 1441, 1978.

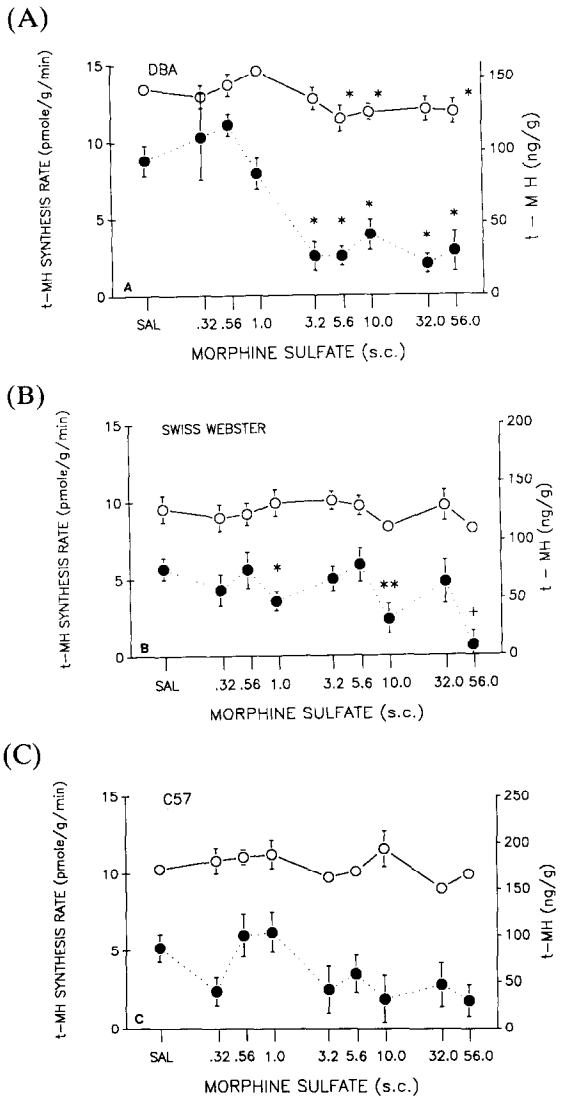


Fig. 2. Dose–response curves for the effects of MOR (abscissa) on brain t-MH levels (○, right ordinate) and on t-MH synthesis rates (●, left ordinate) in DBA (A), SW (B) and C57 (C) mice (mean ± SE, N = 4–14). Pargyline (65 mg/kg, i.p.) or saline was administered immediately prior to the s.c. injection of MOR or saline, and animals were decapitated 60 min later. Brain t-MH levels were determined as described above. Brain t-MH synthesis rates were determined in the presence of pargyline treatment as described in Table 1 for HA turnover. ANOVA of t-MH levels in the presence and absence of pargyline (two factors: pargyline, MOR) revealed significant ($P < 0.0001$) effects of pargyline in all three strains and significant effects ($P < 0.0001$, $P < 0.01$, and $P < 0.02$) in DBA, SW and C57 respectively. * $P = 0.05$, ** $P < 0.01$, and $^{+}$ $P < 0.005$ by *t*-test relative to the respective saline control in the same strain.

course and dose. These results are in contrast to the findings of Nishibori *et al.* [16], who found a MOR-induced increase in brain HA turnover in the ddY mouse. MOR is known to release HA from mast cells [30, 31], and as noted by Nishibori *et al.* [16], it is possible that the MOR-induced increase in brain t-MH synthesis in the ddY mouse is reflective of a mast cell origin. If this is true, then MOR may be capable of simultaneously inhibiting histaminergic neurons and degranulating brain mast cells. Thus, the effect of MOR on brain t-MH synthesis in a given strain could be dictated by the relative proportion of brain HA present in mast cells and neurons.

Our present results show that the effectiveness of MOR in inhibiting brain HA metabolism (i.e. DBA > SW = C57) roughly parallels the order of sensitivity of these strains to MOR antinociception [18, 19]. Furthermore, the kinetics of this inhibition (Fig. 1) and potency of this effect (Fig. 2A) match the time course and potency of MOR antinociception [32]. One interpretation of these findings, that a reduction in brain HA release is important for MOR-induced antinociception, seems consistent with earlier findings showing that inhibition of brain HA synthesis in SW mice potentiates MOR antinociception [15]. Furthermore, brain microinjection studies in rats found HA to induce both analgesia and hyperalgesia, depending on the site of injection [33]. However, such a hypothesis would seem to require significant reductions in brain t-MH synthesis by analgesic doses of MOR in all strains of mice, including C57, whereas only a slight, general suppression was observed in this strain. Clearly, then, it is also possible that MOR-induced changes in brain HA metabolism are unrelated to MOR antinociception. Further studies are needed to resolve the relationship of the actions of MOR on HA metabolism to its pharmacological effects on brain.

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