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Barbiturate-induced inhibition of rat brain histamine turnover

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Histamine (HA) is now believed to function as a CNS transmitter or modulator [1–4]. Neurons containing HA and its biosynthetic enzyme have been shown recently to emanate from hypothalamic tuberal and caudal magnocellular perikarya, with widespread projections to telencephalon, diencephalon, brain stem, and spinal cord [5–7]. These neurons are suspected of contributing to the control of arousal, body temperature, hormone release, sympathetic activity, and pain perception [4].

HA turnover rates in brain are thought to be indices of the activity of histaminergic neurons. Based on the fact that HA metabolism in brain occurs nearly exclusively by methylation [8], the accumulation of the HA metabolite *tele*-methylhistamine (*t*-MH) after complete inhibition of monoamine oxidase has been used to estimate HA turnover [9–11]. Studies with this method found that HA turnover rates vary enormously among different rat brain regions, with the highest values in the hypothalamus; such results are in good agreement with turnover rates found with an earlier isotopic method, which required intraventricular injections [12].

Recent studies with this method have demonstrated that several classes of centrally-acting drugs modulate brain HA turnover. Thus, ethanol [13] and Δ^9-tetrahydrocannabinol [14] both decrease brain HA turnover, whereas morphine [15] and phencyclidine [16] increase it. Several CNS depressants were found to decrease rat whole brain HA turnover, as estimated with the intraventricular labeled precursor method [17]. In a subsequent report using the same method [12], thiopental reduced the estimated HA turnover in both cortex and hypothalamus. Because of the possible role of brain HA in arousal mechanisms, and to document further the similarities between methods for measuring HA turnover, the effects of a hypnotic dose of pentobarbital have been determined on HA levels, *t*-MH levels, and HA turnover rates in nine regions of rat brain.

Methods

Male Sprague–Dawley rats (225–300 g, Taconic Farms, Germantown, NY) were maintained in 12-hr light–dark cycles, and received one of the following four combinations of two separate i.p. injections: saline/saline, pargyline hydrochloride (75 mg salt/kg)/saline, saline/sodium pentobarbital (50 mg/kg, injectable formulation, Abbott Laboratories), or pargyline/pentobarbital (same doses). Four hours later (4 hr into the light cycle), the animals were decapitated at room temperature, and their brains were removed rapidly and dissected on ice. Tissues were homogenized in 5–10 vol. of ice-cold deionized water for 15 sec in a Polytron homogenizer, and then diluted with an equal part of either 0.1 N sodum phosphate buffer (pH 7.9) (to measure HA) or an equal part of 0.8 N perchloric acid (to measure *t*-MH). A separate experiment verified that the pentobarbital vehicle (saline containing 40% propylene glycol and 10% ethanol) had no effect on whole brain HA or *t*-MH levels, or on HA turnover rates. Pentobarbital-treated animals slept for approximately the first 2.5 hr of the 4-hr post-injection interval. Previous studies [9, 10] have shown that the rate of pargyline-induced accumulation of *t*-MH is constant for the first 4 hr, hence the choice of this interval.

HA was measured by a previously described single isotope radioenzymatic method [18]. Incubations were performed at 0°, with partially purified rat kidney histamine methyltransferase as the enzyme source [19]. Each biological sample was assayed in triplicate plus another triplicate containing exogenous HA as an internal standard to determine recovery. HA levels are corrected for recovery, which ranged from 71 to 105%.

t-MH was measured by combined gas chromatography–mass spectrometry (GC–MS) according to the method developed and described previously [20]. Trideuterated *t*-MH was synthesized from deuterated dimethylsulfate and *N*-acetyl-histamine, as described for non-deuterated *t*-MH [21], and used as an internal standard. Derivatized *t*-MH and its deuterated analog were separated by capillary gas chromatography with a cross-linked 5% phenyl methyl silicone column (0.2 mm 25 m, 0.33 μm film thickness) on a Hewlett–Packard 5890A gas chromatograph in the splitless mode. Selected ion monitoring was performed with a Hewlett–Packard 5970A mass selective detector and a 9836C data station.

Mean HA turnover rates for each region (expressed as pmol/g·hr) were calculated as the mean *t*-MH levels after pargyline treatment (pmol/g) minus saline levels for that region, corrected for the 4-hr pretreatment [9, 10]. Both regional HA levels and *t*-MH levels were analyzed separately by analysis of variance (ANOVA) with repeated measures (grouping factors [GF] = pargyline and barbiturate; within factor [WF] = brain regions) with the BMDPC program 2V [22]. Pairwise comparisons were made with Student's *t*-test, as permitted by the ANOVAs.

Results and discussion

ANOVA of the *t*-MH data (Fig. 1A) revealed highly significant main effects of pargyline, pentobarbital, and brain regions ($P < 0.0001$), as well as significant interactions of all four interaction terms ($P < 0.0001$). In the absence of pentobarbital, pargyline increased *t*-MH levels in all brain regions ($P < 0.001$ or better, by *t*-test); the magnitude of the effect in each region was proportional to the control *t*-MH levels in that region (Fig. 1A), as found previously [10]. When the HA levels were analyzed by the same ANOVA, a significant effect of regions ($P < 0.0001$), but not of the other factors, was observed, confirming [10, 11] that pargyline has no effect on HA levels in any brain region (Fig. 1B). Control turnover rates (i.e. in the absence of pentobarbital) calculated from the *t*-MH data ranged from 22.5 to 539.2 pmol/g·hr for cerebellum and hypothalamus respectively (Table 1). The present values for rat brain HA turnover are somewhat lower than comparable values found previously, although the values are well correlated ($r = 0.97$ between the present turnover rates and previous values [10]). As the present study was performed with rats from a different supplier than used previously, the source of the animal may explain this difference (e.g. see Ref. 23).

Pentobarbital had no significant effect on regional *t*-MH levels in the absence of pargyline (Fig. 1A), determined by a separate ANOVA of the *t*-MH data excluding pargyline-treated animals (GF = barbiturate, WF = region), and by *t*-tests. Pentobarbital also had no effect on HA levels, either in the presence or absence of pargyline (Fig. 1B). These results are in agreement with Pollard *et al.* [17], who also found that barbiturate treatment had no effect on brain HA levels, although an earlier study [24] found that pentobarbital increased HA levels in striatum. In the latter study, HA was measured by the fluorimetric method, which can give spurious results, although it is also possible that the diurnal factors considered in this study [24] might explain the discrepancy. In the mouse, pentobarbital apparently increases whole brain HA levels, as measured by a radioenzymatic method [25].

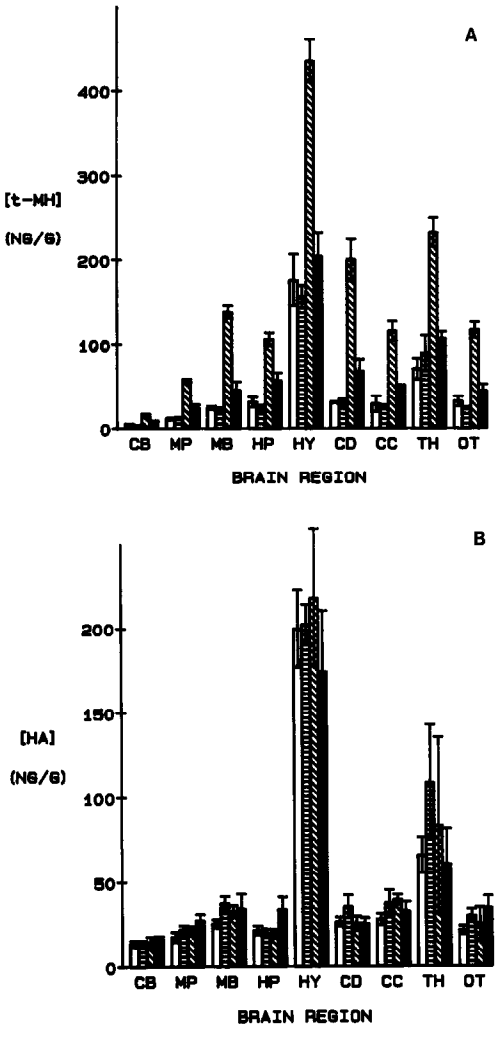

Fig. 1. Effects of sodium pentobarbital (50 mg/kg, horizontal bars), pargyline hydrochloride (75 mg/kg, hatched bars), and both drugs given together (solid bars) on the levels of *t*-MH (the major HA metabolite in brain, top, A), and HA (bottom, B) in nine regions of rat brain, measured 4 hr later. Values are mean ± SEM (N = 3–6). Control animals received saline injections (open bars). The brain regions studied were (left to right) cerebellum, medulla-pons, midbrain, hippocampus, hypothalamus, striatum, cerebral cortex, thalamus, and the remainder of the brain. Effect of pargyline on *t*-MH levels in all regions: $P < 0.001$. Effect of pentobarbital on the pargyline-induced increase: $P < 0.02$.

Even though HA and *t*-MH levels were not changed presently by pentobarbital, this drug dramatically attenuated the pargyline-induced increase in *t*-MH levels in all regions studied ($P < 0.02$ by *t*-test). Calculations of the HA turnover rates in pentobarbital-treated animals (Table 1) revealed drug-induced reductions in turnover from 62 to 85%, with a mean of 76%. These results are in excellent agreement with those of Pollard *et al.* [17], who observed a 47% reduction in whole brain HA turnover after a lower dose of pentobarbital (35 mg/kg) than used presently. The agreement in results obtained with different turnover methods adds evidence for the validity of both.

Table 1. Turnover rates of HA in regions of rat brain: Effect of pentobarbital

Region	Mean HA turnover rate (pmol/g·hr) Control	Pentobarbital	% Reduction
Cerebellum	22.5	8.1	64
Medulla-pons	89.6	25.5	72
Midbrain	230.3	44.3	81
Hippocampus	156.7	59.6	62
Hypothalamus	539.2	79.3	85
Striatum	339.0	74.2	78
Cerebral cortex	177.1	44.3	75
Thalamus	305.5	53.2	83
Remainder	178.1	33.4	81

The effect of pentobarbital (50 mg/kg) was determined on the pargyline-induced increase in brain *t*-MH levels. Turnover rates were calculated from the data of Fig. 1A, as described in Methods. Pentobarbital induced a significant inhibition of HA turnover in all brain regions ($P < 0.02$), indicated by *t*-tests and ANOVA performed on the data of Fig. 1.

The present findings further exemplify the importance of turnover methods for the neurochemical characterization of histaminergic neurons. Thus, pentobarbital induced a dramatic reduction in the turnover of brain HA, but it had no effect on brain HA or *t*-MH levels. These findings also suggest that HA levels are regulated in brain, since rapid compensatory changes in synthesis or metabolism probably accompanied the drug effect, preventing changes in HA levels. Barbiturates probably produce their effects on histaminergic neurons in a manner similar to their depressant actions on other transmitter systems (e.g. Ref. 26), although it is not clear if these results from direct or indirect actions on these neurons [27]. These observations, in turn, provide further evidence that the cellular origin of the rapidly turning over HA in brain is neuronal.

Several observations support the hypothesis that HA is a mediator of CNS arousal [4], including electroencephalographic effects of HA, the circadian rhythm of endogenous HA, the sedative effects of H_1-antagonists, and the recently described effects of an inhibitor of HA biosynthesis [28]. The present findings, that a hypnotic dose of pentobarbital induced a profound, widespread inhibition of brain HA turnover, is also consistent with this hypothesis.

In summary, administration of sodium pentobarbital (50 mg/kg, i.p.) to rats resulted in a 62–85% reduction in HA turnover rates throughout nine brain regions, with no effect on the steady-state levels of either HA or its brain metabolite, *t*-MH. This effect further strengthens the hypothesis that neuronal activity is responsible for HA turnover in brain and is consistent with the proposed role for brain HA in arousal mechanisms.

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