cyclase activity, did not induce any substantial change in the extent of platelet aggregation. These compounds are likely to be devoid of any effect on adenylate cyclase as they do not possess OH groups, in the 1 and 9 positions, which have been shown to be necessary for the activation of the adenylate cyclase [13].

It therefore appears that forskolin is a potent inhibitor of platelet aggregation induced by either epinephrine, ADP or ionophore 23187. Two reports have recently also pointed out that forskolin could prevent platelet aggregation in response to ADP, arachidonic acid and collagen [14, 15]. Since forskolin is a very hydrophobic drug, it was reasonable to question the direct marked stimulation brought about by the drug on adenylate cyclase; it could have acted in the aggregatory process by merely altering the physiological properties of the plasma membrane. This hypothesis appeared to be unlikely for two main reasons: (i) Ca^{2+} ions inhibit the effects of forskolin on both adenylate cyclase activity and platelet aggregation and (ii) the three hydrophobic analogs of forskolin tested were inactive both on the adenylate cyclase activity of platelet membranes and on platelet aggregation. These data, therefore, further confirm the role of cyclic AMP in regulating platelet aggregation [16, 17]. Since forskolin is a potentially useful drug clinically because of its inotropic and hypotensive properties [18, 19], it is of interest that it also possesses marked antiaggregating properties.

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Effects of pargyline on *tele*-methylhistamine and histamine in rat brain

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Recent studies of histamine (HA) in brain have focused on the identification and characterization of HA-synthesizing pathways [1], HA receptors [2] and HA metabolites [3–5]. Although HA is metabolized by at least two distinct enzymatic systems in non-neural tissue [6], HA in brain is metabolized almost exclusively by methylation [7, 8].

Tele-Methylhistamine (*t*-MH, see Ref. 9 for nomenclature), the product of HA methylation, has been identified and measured in brain, in other animal tissues, and in human fluids by combined gas chromatography–mass spectrometry (GC–MS) [5, 10–12]. Brain *t*-MH levels may be a sensitive indicator of histaminergic activity, since no uptake mechanism for HA has been discovered, at least not in rat brain [13, 14]. Lesion studies [15, 16] support this hypothesis, implying that HA methylation may occur outside of HA-synthesizing fibers, and suggest that *t*-MH formation may be dependent on neuronal HA release. The similarity in the regional distributions of *t*-MH and HA [4, 5] also suggest that brain *t*-MH levels may be a useful indicator of histaminergic activity.

Several studies have indicated that *t*-MH is metabolized by monoamine oxidase (MAO) in brain. Waldmeier *et al.* [17] showed that type B, but not type A MAO inhibitors increased labeled *t*-MH formation after intracisternal

administration of labeled HA. Hough and Domino [3] demonstrated the direct oxidative deamination of labeled *t*-MH by type B MAO in brain homogenates and showed that MAO inhibitors increase endogenous brain *t*-MH levels. We have measured HA and *t*-MH levels in rat brain at various times after administration of the MAO inhibitor, pargyline, to characterize the dynamics of endogenous HA methylation in brain.

Male and female Sprague–Dawley albino rats (15 weeks old, maintained in 12 hr light–dark cycles) received pargyline hydrochloride (75 mg of salt/kg, i.p.) or saline. Four hours into the light cycle, they were decapitated at room temperature, and the brains were rapidly removed and homogenized in ice-cold distilled water. Homogenates were diluted with either 0.1 N sodium phosphate buffer, pH 7.9 (to measure HA), or 0.8 N perchloric acid (to measure *t*-MH).

HA was measured by a previously described single isotope radioenzymatic method [18] with rat kidney histamine methyltransferase [19]. *t*-MH was measured by our recently improved GC–MS method [5]. The method is based on the extraction and derivatization of brain homogenates, with *pros*-methylhistamine as internal standard; this substance was not found in brain homogenates from pargyline or saline-treated animals. MAO of whole brain homogenates was measured with [^{14}C]phenylethylamine as substrate (95 μM) by incubation and organic extraction, as previously described [3]. Student's *t*-test was used to evaluate differences in brain amine levels.

Rat brain *t*-MH levels increased linearly for the first 4 hr after pargyline administration and remained 3-fold above control levels for 12 hr (Fig. 1). No sex differences were observed in the HA or *t*-MH content of these brains. Brain HA levels were slightly increased for 1 and 2 hr after pargyline and then returned to control levels (Fig. 1).

Pargyline was shown previously to produce dose-dependent increases in endogenous rat brain *t*-MH levels 12 hr after its administration [3]. Our present results (Fig. 1) show a similar elevation after a single large dose of pargyline; these *t*-MH levels were achieved within 4 hr after drug administration, suggesting a rapid rate of endogenous *t*-MH formation.

The rate of increase of *t*-MH after pargyline [33 ng or 0.26 nmole/(g·hr)] is an estimate of the synthesis rate of endogenous *t*-MH and probably reflects HA turnover. The rate constant and half-life of whole brain HA can be estimated by methods similar to those used for estimating serotonin turnover after pargyline treatment, according to

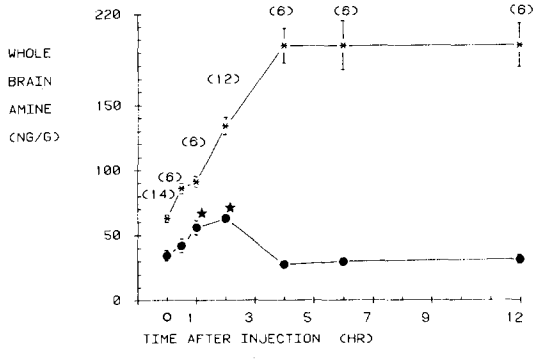

Fig. 1. Effect of pargyline on whole rat brain HA and *t*-MH. Animals received pargyline hydrochloride (75 mg salt/kg, i.p.) and were killed after the times shown. Saline-treated animals represent time zero. Results are plotted as the means ± S.E.M. for *t*-MH (*) and HA (●) for the number of animals in parentheses. Pargyline treatment produced significant ($P < 0.01$) increases in brain *t*-MH levels at all times studied. Significant ($P < 0.01$) elevations in HA levels are indicated by stars.

a one-compartment open model [20]. The rate constant for brain HA methylation [k, calculated as the *t*-MH synthesis rate, 0.26 nmole/g·hr, divided by the control HA levels, 34.6 ng or 0.31 nmole/g] is 0.84 hr^{-1}. From this value, the brain HA half-life (0.693/k) is 0.83 hr or about 50 min. This estimate is in excellent agreement with that of Pollard *et al.* [21], who found the half-life of whole brain HA to be 46 min after intraventricular administration of labeled histidine. Dismukes and Snyder [22] performed similar isotopic experiments and obtained very similar data, but assumed that only a portion of the labeled histidine was available for histamine synthesis; this kinetic model estimated the rat brain HA half-life to be less than 1 min [22]. Such discrepancy emphasizes the importance of the choice and verification of kinetic models. Present methods do not permit validation of either model, but the close agreement between the results of Pollard *et al.* [21] and our findings is noteworthy. Earlier studies [8] estimated the rat brain HA half-life to be 1–3 hr.

Our method for estimating HA turnover does not require the characterization of precursor pools, or the use of intraventricular techniques, but does rest on several assumptions: (1) that brain HA and *t*-MH are in a single compartment, (2) that all brain HA is methylated and subsequently oxidized, (3) that *t*-MH is formed only from HA, (4) that *t*-MH oxidation is immediately and completely inhibited by pargyline, and (5) that HA levels are not changed by pargyline.

Brain HA is probably not in a single compartment, since it is stored in both nerve endings and mast cells (see Ref. 1). However, the extent to which mast cells contribute to adult rat whole brain HA levels is uncertain. It is clear that nearly all brain HA is methylated [7, 8, 17, 23]. Oxidation appears to be the major metabolic fate of brain *t*-MH, since only labeled *tele*-methylimidazoleacetic acid was found after incubation of labeled *t*-MH with brain homogenates [3]; also, in unpublished studies we have been unable to identify acid-labile conjugates of *t*-MH in rat brain. *t*-MH in some tissues may be formed from *tele*-methylhistidine by decarboxylation [24], but the importance of this pathway as a source of brain *t*-MH remains to be established.

Whole brain MAO activity, assessed with phenylethylamine as substrate, was 99% inhibited 0.5, 1 and 2 hr after this dose of pargyline (not shown). Previous work has shown that phenylethylamine oxidation and *t*-MH oxidation are nearly indistinguishable by both biochemical and pharmacological criteria [3]. These findings strongly suggest that pargyline treatment completely inactivated endogenous *t*-MH catabolism. Finally, pargyline slightly increased brain HA levels (Fig. 1); this increase could cause a slight underestimate of the rate of endogenous *t*-MH formation.

The fact that pargyline increases brain HA levels does not mean that HA is a substrate for MAO (see Ref. 25). Rather, these results suggest that the elevated HA levels are a consequence of the increased levels of *t*-MH, known to inhibit brain histamine methyltransferase both *in vitro* [26] and *in vivo* [7]. The latter study found that intracisternal administration of *t*-MH increased brain HA levels. Whatever the mechanism, these results suggest that brain HA content is highly regulated and is sensitive to changes in either its own levels or those of its metabolite. Such a regulatory mechanism has been suggested for peripheral HA content [27], as well as for brain [7]; a simple HA-feedback inhibition of histidine decarboxylase seems not to be the mechansim [7].

Since several days are required to synthesize new MAO after its inactivation by pargyline [28], the plateau in *t*-MH levels 4 hr after pargyline (Fig. 1) suggests that *t*-MH synthesis (and thus HA methylation) has been reduced to the rate of *t*-MH exodus from brain. If so, then HA turnover is probably still low 4–12 hr after pargyline (perhaps remaining depressed for days), despite the return of brain HA levels to control values. Earlier studies [29, 30]

reported that MAO inhibitors had no effect on rat brain HA levels; another study found that an MAO inhibitor decreased labeled HA metabolism in brain [23]. Our results indicate that both findings may be correct, since HA content is only slightly altered by pargyline for a short time, yet HA turnover may be decreased even after brain HA content returns to control values (Fig. 1). Thus, MAO inhibitors may have long-term effects on the brain histaminergic system. This hypothesis, which requires further study, may be relevant to the actions of other antidepressants on brain HA receptors [31, 32].

In summary, rapid and complete inhibition of brain MAO produced linear increases in brain *t*-MH levels from 30 min to 4 hr after drug treatment at a rate of 0.26 nmole/g·hr, resulting in a 3-fold increase which persisted for at least 12 hr. HA levels were slightly elevated 1 and 2 hr after drug administration but quickly returned to control levels, suggestive of sensitive regulatory mechanisms in brain. Although the slight change in HA levels precludes steady-state assumptions, the rate of increase in brain *t*-MH levels after MAO inhibition provides a novel estimate of the half-life of endogenous brain HA (50 min). Despite the transient effect of pargyline on brain HA content, the effect of pargyline on brain *t*-MH levels suggests that MAO inhibitors may produce long-term alterations in brain histaminergic dynamics.

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