Biochemical Pharmacology, Vol. 35, No. 2, pp. 307–310, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

INHIBITION OF BRAIN HISTAMINE METABOLISM BY METOPRINE

LINDSAY B. HOUGH,* JAI K. KHANDELWAL and JACK PETER GREEN
Department of Pharmacology, Mount Sinai School of Medicine of the City University of New York, New York, NY 10029, U.S.A.

(*Received* 9 *February* 1985; *accepted* 12 *June* 1985)

Abstract—To study the extent to which histamine methylation accounts for the biosynthesis of histamine metabolites in brain, the effects of the histamine methyltransferase (HMT) inhibitor metoprine were determined on the whole brain levels of *tele*-methylhistamine (*t*-MH), its oxidative metabolite *tele*-methylimidazoleacetic acid (*t*-MIAA), and brain HMT activity in albino rats. Metoprine (5–30 mg/kg) reduced brain *t*-MH levels by about 75% and caused a dose-dependent reduction (70–90%) in HMT activity 4 hr after administration. Furthermore, the levels of *t*-MH remaining in each brain after metoprine treatment were significantly positively correlated with the remaining HMT activity of that brain after all doses of drug. Although brain *t*-MIAA levels were reduced by only 30% 4 hr after metoprine administration, the levels were reduced by about 75% 12 hr after the drug, similar to the reduction in *t*-MH levels. These findings support previous suggestions that *t*-MH and *t*-MIAA in brain arise from brain histamine metabolism, and that brain *t*-MH synthesis is equivalent to histamine methylation.

It now seems clear that histamine (HA) in the central nervous system is synthesized and stored in neurons [1, 2] as well as in mast cells [3], and it may function as a messenger in both systems. Although HA biotransformation can occur by at least two different enzymatic mechanisms (oxidation and methylation), HA in mammalian brain appears to be almost exclusively methylated [4]. The product of HA methylation (*tele*-methylhistamine, *t*-MH) is present in brain in concentrations similar to those of HA [5], is distributed in brain regions in a manner similar to that of HA [6], and is associated with pools of HA with rapid turnover and release, most likely those of histaminergic neurons [7]. Brain *t*-MH is metabolized by type B monoamine oxidase (MAO), and MAO inhibitors increase endogenous brain *t*-MH levels [8].

Recently, we [7, 9] and others [10] have used the rate of accumulation of brain *t*-MH after MAO inhibition as an approximation of brain HA turnover rates. Among the assumptions necessary for this estimate to be valid [7, 9] is that brain *t*-MH be formed only by the methylation of HA, and not by any other metabolic pathway: *t*-MH synthesis must be equivalent to HA methylation. Although substantial evidence suggests that this is so [4], another potential source of *t*-MH was pointed out by Schwartz *et al.* [11], who showed that labeled *tele*-methylhistidine could be decarboxylated *in vivo* to form *t*-MH. If this pathway were to contribute to brain *t*-MH synthesis, then the measurement of *t*-MH synthesis rates might not be a suitable method for measuring HA turnover. To study the importance of HA methylation for the biosynthesis of HA metabolites, we examined the extent to which the drug metoprine, an inhibitor of histamine methyltransferase (HMT, [12]), can deplete the levels of *t*-MH and its oxidative metabolite *tele*-methylimidazoleacetic acid (*t*-MIAA) in brain.

METHODS

Male Sprague–Dawley albino rats weighing 225–300 g were maintained in 12 hr light–dark cycles and used for all experiments. Metoprine was a gift of Dr. C. Nichol, Wellcome Research Laboratories, Research Triangle Park, NC. The drug was dissolved in 10% aqueous lactic acid (100 mg/ml) and diluted with saline (1:10), to a final concentration of 10 mg/ml in 1% lactic acid. Appropriate volumes of this solution or the control solution (1% lactic acid in saline) were administered by intraperitoneal injection, according to the doses and times stated.

Animals were decapitated 5–7 hr into the light cycle, and whole brains were weighed and homogenized in 5 vol. of ice-cold deionized water for 15 sec in a Polytron homogenizer. Aliquots (0.1 ml) were taken for the assay of HMT, performed according to the radioisotopic method of Taylor and Snyder [13], with a HA concentration of 20 μM; the remainder of each homogenate was extracted and assayed for *t*-MH and *t*-MIAA by our combined automated gas chromatographic–mass spectometric method recently described [14].

RESULTS

All doses of metoprine studied reduced rat whole brain *t*-MH levels by about 75% 4 hr after administration (Fig. 1). There was no obvious dose–effect relationship, as the *t*-MH levels remaining after a

* Author to whom all correspondence should be addressed. Present address: Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

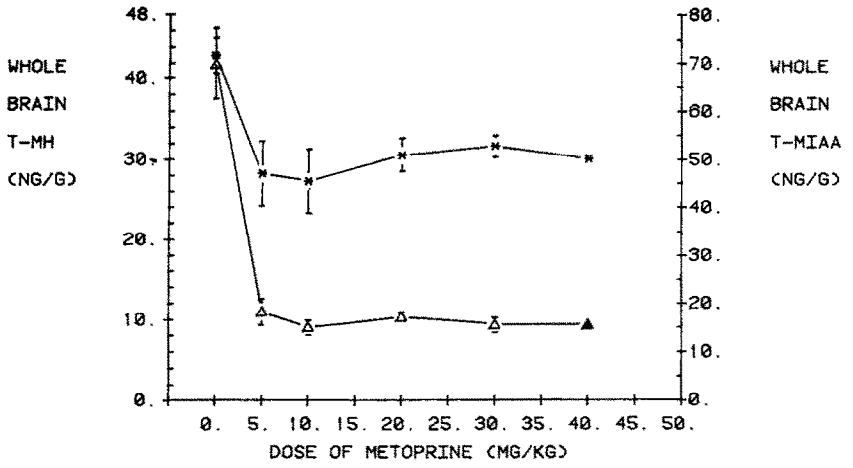

Fig. 1. Effect of metoprine on whole brain levels of *t*-MH (left axis, triangles) and *t*-MIAA (right axis, asterisks). Rats received the doses of metoprine shown (i.p.) and were decapitated 4 hr later. Controls are shown as zero time. Points indicate the mean ± S.E.M. for four animals, except for the 20 mg/kg values (N = 3). Only one animal survived the 40 mg/kg dose, whose values are shown.

dose of 5 mg/kg were not significantly different from those remaining after a dose of 30 mg/kg (Fig. 1). After 30 mg/kg and lower doses, there were no adverse effects observed, but only one animal out of four survived the 40 mg/kg dose of drug. Metoprine also lowered whole brain *t*-MIAA levels in the same animals, but only by about 30% (Fig. 1).

HMT activity was also determined in homogenates of the brains from animals treated with metoprine, and was inhibited by all doses of drug (Fig. 2). The inhibition ranged from 70 to 90% after these doses and resembled the pattern observed for *t*-MH (Fig. 2).

To explore further the effects of metoprine on the HA metabolite levels, the effect of metoprine (30 mg/kg) was determined at intervals from 2 to 24 hr after administration (Fig. 3). Metoprine reduced whole brain *t*-MH levels by about 70% throughout this period (Fig. 3), similar to the effects observed after 4 hr (Fig. 1). The depletion of *t*-MIAA occurred much more slowly, 12 hr being required to achieve maximum depletion (Fig. 3). Twelve to eighteen hours after metoprine administration, both *t*-MH and *t*-MIAA were depleted by about 70% (Fig. 3).

DISCUSSION

We studied the effect of metoprine on rat brain HA metabolite levels to test the hypothesis that all of brain *t*-MH arises from the action of HMT, a necessary assumption for our HA turnover methods [7, 9]. This hypothesis would be most easily tested with a drug capable of irreversibly and completely inhibiting HMT, after which no metabolites should be detected. Metoprine is not an irreversible inhibitor of HMT, so that the decline in HA metabolites cannot be used to estimate HA turnover. However,

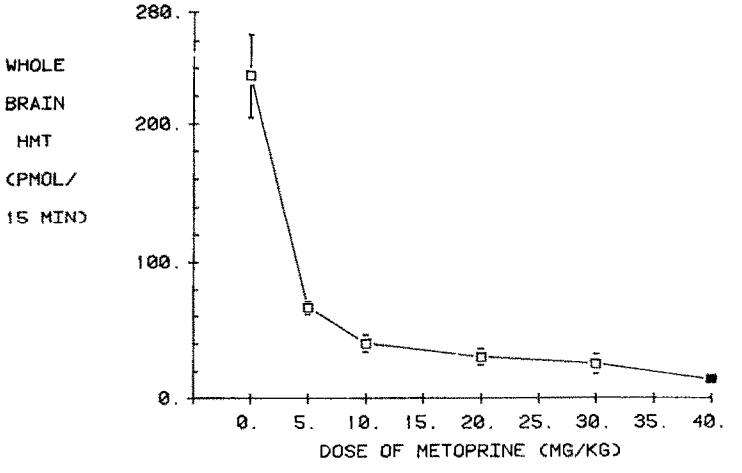

Fig. 2. Effect of metoprine on whole brain HMT activity. The experiment and the results are as described in Fig. 1. Activity is expressed as pmoles of *t*-MH formed during the 15-min incubation. Each tube contained 2 mg protein.

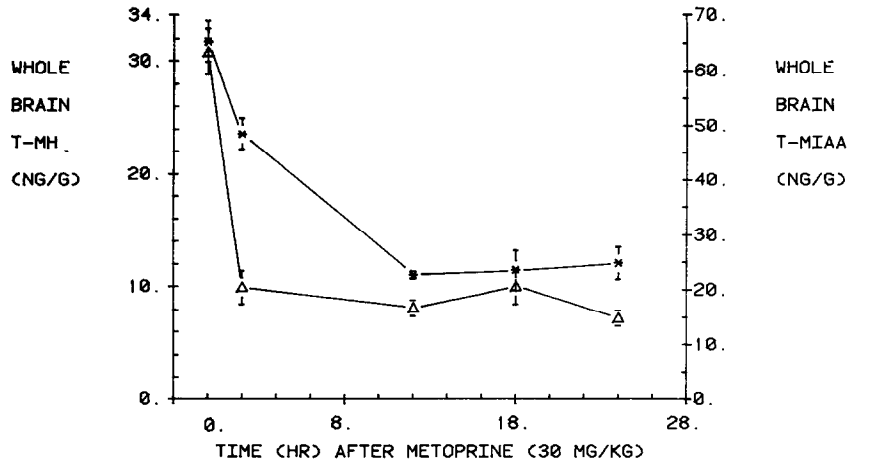

Fig. 3. Time–action curve for metoprine on whole brain *t*-MH (left axis, triangles) and *t*-MIAA (right axis, asterisks). Animals received metoprine (30 mg/kg, i.p.), and were decapitated after the times indicated. Shown are the mean ± S.E.M. for four animals per group.

metoprine can still be used to assess the importance of HMT in *t*-MH synthesis. This drug penetrates rat brain after peripheral administration, and persists there for several days, inducing a long-lasting elevation in brain HA levels [12]. HA levels are maximally elevated 4–5 hr after an oral dose of 10 mg/kg [12].

The specificity of metoprine remains unstudied, but if the action of metoprine on the brain HA system is limited to inhibition of HMT, then our results indicate that at least 75% of whole brain *t*-MH is synthesized by HMT (Figs. 1 and 3). This is a lower limit, and it is likely to be even greater. First of all, as the inhibition is competitive, it may not be possible to remove all of brain *t*-MH, due to the accumulation of brain HA [12]. For the same reason, the estimates of HMT activity in homogenates after metoprine (Fig. 2) must be regarded as lower limits of inhibition. Second, the *t*-MH in brain that remains after high doses of metoprine may arise from HMT, but be present in a compartment with a much slower turnover rate, a hypothesis that might be testable with chronic metoprine. If so, this finding would also require modification of the turnover methods that use *t*-MH measurements. Presently, there are no data on compartmentation of *t*-MH in brain, but brain *t*-MH may exist in more than one compartment, because HMT is likely to be in both presynaptic and postsynaptic elements [15], as well as in glia [16]. Perhaps most convincing that nearly all of *t*-MH arises from HMT is that we found a significant correlation between the *t*-MH remaining in brain after a dose of metoprine and the HMT activity of the same brain ($r = 0.88$, $P < 0.01$, $N = 19$, individual data points whose means are shown in Figs. 1 and 2). This implies that it is the remaining HMT activity that is the major determinant of the remaining *t*-MH levels. However, our results cannot rule out a small contribution from *tele*-methylhistidine decarboxylation.

The dose–response curves for the effect of metoprine on brain *t*-MH and *t*-MIAA levels differed, for *t*-MIAA was not depleted to the same extent as was *t*-MH 4 hr after metoprine (Fig. 1). The time chosen for the dose–response curve (4 hr) was close to the time shown previously to cause the largest increase in brain HA levels [12]. This finding implied that either the time chosen for the dose–response curve was inappropriate, or that *t*-MIAA could be made in brain by mechanisms other than *t*-MH oxidation. The time–action curve of metoprine (Fig. 3) adequately answers the question—the depletion of *t*-MIAA by metoprine is equivalent to that of *t*-MH, but requires 12 hr to occur. This finding is not surprising, since *t*-MH synthesis is immediately inhibited by metoprine, whereas the depletion of *t*-MIAA must occur in the presence of both *t*-MIAA synthesis and *t*-MIAA metabolism.

These findings support the suggestion [7–9] that brain *t*-MH synthesis reflects HA methylation and is a good index to the rate of HA turnover in brain.

Acknowledgements—This work was supported by grants from the National Institute of Mental Health (MH-31805) and from the National Institute on Drug Abuse (DA-03816). Data analyses were performed on the PROPHET computer system, a national resource supported by the Chemical-Biological Information Handling Program, Division of Research Resources, National Institutes of Health. We thank Ms. Kay Su for excellent technical assistance.

REFERENCES

1. T. Watanabe, Y. Tagushi, S. Shiosaki, J. Tanaka, H. Kubota, Y. Terano, M. Tohyama and H. Wada, *Brain Res.* **295**, 13 (1984).
2. P. Panula, H. Y. T. Yang and E. Costa, *Proc. natn. Acad. Sci. U.S.A.* **81**, 2572 (1984).
3. R. C. Goldschmidt, L. B. Hough, S. D. Glick and J. Padawer, *Brain Res.* **323**, 209 (1984).
4. L. B. Hough and J. P. Green, in *Handbook of Neurochemistry* (Ed. A. Lajtha), Vol. 6, p. 145. Plenum Publishing, New York (1984).
5. L. B. Hough, P. L. Stetson and E. F. Domino, *Analyt. Biochem.* **96**, 56 (1979).

6. L. B. Hough and E. F. Domino, *J. Neurochem.* **32**, 1865 (1979).
7. L. B. Hough, J. K. Khandelwal and J. P. Green, *Brain Res.* **291**, 103 (1984).
8. L. B. Hough and E. F. Domino, *J. Pharmac. exp. Ther.* **208**, 422 (1979).
9. L. B. Hough, L. K. Khandelwal and J. P. Green, *Biochem. Pharmac.* **31**, 4074 (1982).
10. R. Oishi, M. Nishibori and K. Saeki, *Life Sci.* **34**, 691 (1984).
11. J. C. Schwartz, C. Rose and H. Caillens, *J. Pharmac. exp. Ther.* **184**, 766 (1973).
12. D. S. Duch, S. W. Bowers, M. Edelstein and C. A. Nichol, in *Transmethylation* (Eds. E. Usdin, R. Borchardt and C. Creveling), p. 287. Elsevier North Holland, New York (1979).
13. K. M. Taylor and S. H. Snyder, *J. Neurochem.* **19**, 1343 (1972).
14. J. K. Khandelwal, L. B. Hough and J. P. Green, *J. Neurochem.* **42**, 519 (1984).
15. S. Bischoff and J. Korf, *Brain Res.* **141**, 375 (1978).
16. M. Garbarg, M. Baudry, P. Brenda and J. C. Schwartz, *Brain Res.* **83**, 538 (1975).