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Increased serotonin turnover in acutely morphine-treated mice*†

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THE EFFECT of chronic administration of narcotics on cerebral serotonin (5HT) synthesis rates in mice *in vivo* is a subject of current interest and much controversy.[1–8] However, there is relatively little information available on the effect of acute narcotic administration on cerebral 5HT turnover in mice. Way and associates[2,3] stated that, after the administration of a single dose (100 mg/kg) of morphine, the turnover rate of 5HT in these animals was identical to that calculated for saline controls. However, Bowers and Kleber[7] reported a dose-related increase in cerebral 5-hydroxyindoleacetic acid (5HIAA) levels in mice acutely treated with methadone (5–15 mg/kg). An increase in 5HIAA levels may be indicative of an increase in 5HT synthesis.[9–11]

The calculated values for 5HT turnover rates in the brains of rats, derived from both nonisotopic and isotopic data, agree quite well.[12] However, no such comparisons have been made in mice. Indeed, the disagreement as to chronic narcotic effects on 5HT turnover in the mouse may be due to different results obtained through the use of a nonisotopic technique[13] as compared with an isotopic technique of estimation.[5]

We have examined the effects of acute administration of morphine (32 mg/kg, s.c.) on cerebral 5HT turnover in mice using both a nonisotopic[14] and an isotopic[15] method. With both experimental designs, morphine administration resulted in an increase in 5HT turnover.

Male, DBL–ICR mice (Flow Laboratories, Dublin, Va.) weighing 18–25 g were used. The method of Giacalone and Valzelli[16] was employed to measure cerebral 5HIAA. Mice were pretreated with morphine or saline and injected 10 min later with probenecid (200 mg/kg, i.p.). The animals were

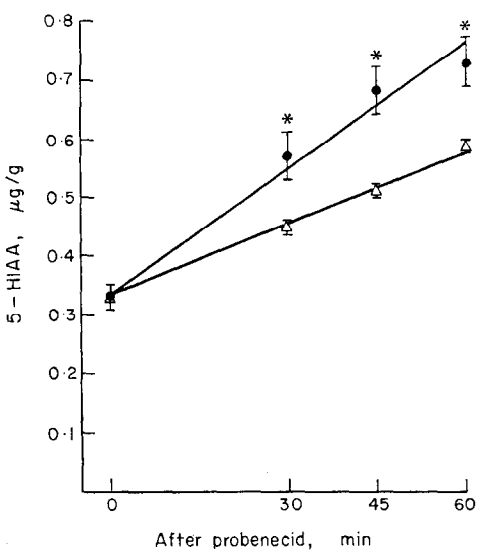

FIG. 1. Accumulation of cerebral 5HIAA after probenecid (200 mg/kg, i.p.) in mice pretreated with 32 mg/kg, s.c., of morphine (closed circles) or saline (open triangles). Animals received morphine or saline 10 min prior to the probenecid. Each point represents the mean ± S.E.M. of determinations made on four samples (three brains/sample). The asterisks indicate significant differences ($P < 0.05$) calculated using a two-tailed Student's *t*-test.

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sacrificed at various intervals thereafter, and the 5HIAA content in three pooled brains was determined. At each time interval, the morphine-pretreated mice exhibited significantly higher levels of 5HIAA (Fig. 1). The rate of accumulation of 5HIAA was 0·40 μg/g/hr in mice receiving morphine as compared with 0·25 μg/g/hr in the saline controls. Furthermore, over the time course of the experiment, the accumulation of 5HIAA in the brains of both control and treated mice was linear, indicating that probenecid had achieved a complete blockade of the acid transport system. The accumulation of 5HIAA after probenecid is considered to be a valid index of 5HT synthesis if the 5HT concentration remains constant. In agreement with previous reports,[2,3] we found that the steady state levels of 5HT were not altered by a single injection of 32 mg/kg of morphine. Thus, these data suggest that 5HT synthesis is enhanced in mice acutely treated with morphine.

In isotopic experiments, mice were injected intravenously with 0·5 mc/kg of L-(G-^{3}H)-tryptophan (5·3 c/m-mole, New England Nuclear, Boston, Mass.) 30 min prior to the administration of morphine or saline. Animals were sacrified at 15, 45 and 90 min after the last injection. Total and labeled 5HT and tryptophan in whole brain (three brains per sample) were isolated by ion-exchange chromatography and estimated by spectrophotofluorometry and scintillation radiometry, according to the method described by Schubert *et al.*[17] Recoveries of known amounts of labeled and unlabeled 5HT and tryptophan carried through the method ranged from 60–80 per cent.

The declines of 5HT and tryptophan specific activities in morphine- and saline-injected mice are shown in Fig. 2. Calculation of the fractional rate constants (K_m) over the 75-min interval and 5HT turnover rates (according to the method of Neff *et al.*[15]) revealed an enhanced rate of 5HT synthesis in mice receiving morphine. The K_m min^{-1} was 0·0119 in the morphine-treated mice and 0·0070 in the saline controls. The 5HT turnover rates were 0·31 μg/g/hr in mice receiving morphine and 0·19 μg/g/hr in animals receiving saline. Interestingly, with both the nonisotopic and isotopic methods, the magnitude of the increase in 5HT turnover induced by morphine was approximately 160 per cent of control.

Previous work from this laboratory presented evidence to suggest that whole-brain 5HT turnover is enhanced in rats receiving a single injection of morphine.[18] The data reported here indicate that a

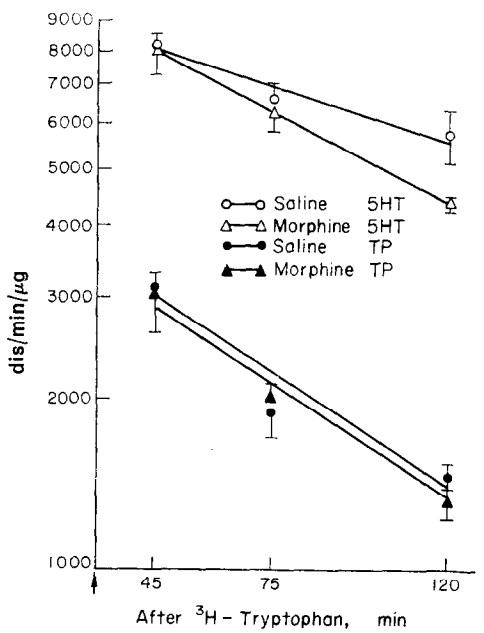

FIG. 2. Decline of serotonin (5HT) and tryptophan (TP) specific activities in brains of mice receiving a single injection of morphine (32 mg/kg, s.c.) or saline. The arrow indicates the time of morphine or saline injection (30 min after labeling with ^{3}H-tryptophan, 0·5 mc/kg, i.v.). Each point is the mean ± S.E.M. of determinations on four to five samples (three brains/sample). The lines connecting the 5HT specific activity points were determined by linear regression. The mean 5HT concentration, which was not altered by morphine, was 0·44 ± 0·06 μg/g.

similar effect occurs in mice. We are unable to explain the conflicting nature of our observations with the conclusions reached by previous investigations.[2,3] The relevance of the increase in 5HT turnover in regard to the pharmacological effects of morphine remains to be elucidated.

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