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ACUTE AND CHRONIC MORPHINE TREATMENT AND THE HYDROXYLATION OF [1-^{14}C]-L-TYROSINE IN THE MOUSE BRAIN

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Tyrosine hydroxylase activity in slices of caudate nucleus was increased by morphine (100 mg/kg i.p.) administered to naive mice. During chronic treatment with morphine tolerance developed to this effect and 36 h after the final chronic morphine injection there was a decrease in enzyme activity in this area. There was no change in tyrosine hydroxylase activity in slices of diencephalon, brainstem or parietal cortex from either naive or morphine tolerant mice. Therefore, changes in tyrosine hydroxylase activity, measured *in vitro*, could account for the changes in dopamine synthesis, but not noradrenaline synthesis, produced by morphine *in vivo*.

Introduction

In the present study, the effect of morphine on the hydroxylation of [^{14}C]-tyrosine, which is a measure of the activity of the rate-limiting step in catecholamine biosynthesis (Nagatsu, Levitt & Udenfriend, 1964), was investigated to determine whether changes in the activity of this biosynthetic step parallel the changes in cerebral catecholamine synthesis reported to occur after the administration of morphine in mice (Smith, Villarreal, Bednarczyk & Sheldon, 1970; Smith, Sheldon, Bednarczyk & Villarreal, 1972) and rats (Clouet & Ratner, 1970).

Methods

Albino Swiss-Webster mice (25-30 g) were decapitated, and their brains removed. Tyrosine hydroxylase activity was measured in whole brain homogenates and in brain slices. For the homogenates, tyrosine hydroxylase activity was measured using the method of Waymire, Bjur & Weiner (1971). The principle of this method is the collection of [^{14}C]-CO_2 evolved by the coupled decarboxylation of [^{14}C]-L-dihydroxyphenylalanine formed from [1-^{14}C]-L tyrosine. The protein content of the samples was measured by the method of Lowry, Rosebrough, Farr & Randall (1951). Tyrosine hydroxylase activity of whole brain homogenates is expressed as nmol [^{14}C]-CO_2 evolved per g brain protein and per min of incubation.

In order to study the tyrosine hydroxylase activity in brain slices, four slices (about 0.5 mm thick) were cut from the diencephalon (10.07 ± 0.28 mg combined wet weight ± s.e. mean), brainstem (12.64 ± 0.24 mg), caudate nucleus (13.50 ± 0.22 mg) and parietal cortex (18.14 ± 0.31 mg). The four slices from each area were placed in 0.9 ml of Krebs-Ringer phosphate buffer (pH 7.4) of the following composition (mM): NaCl, 121; KCl, 4.9; $CaCl_2.2H_2O$, 1.3; $MgSO_4.7H_2O$, 1.2; Na_2HPO_4, 12.8; NaH_2PO_4, 3.0; glucose 20. Also dissolved in this solution were 2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine hydrochloride, 1 mM and 2-mercaptoethanol, 25 mM. Tyrosine hydroxylase activity in slices was measured by a modification of the method of Weiner, Cloutier, Bjur & Pfeffer (1972). At the end of a 15 min pre-incubation period, 0.1 ml of [1-^{14}C]-L-tyrosine (1.0 μCi/incubate) was added to give a concentration of 10^{-4} M. The [^{14}C]-CO_2 evolved during the next 20 min was collected in 0.2 N NCS organic tissue solubilizer and its radioactivity was counted in a liquid scintillation spectrometer. Tyrosine hydroxylase activity of brain slices is expressed as pmol [^{14}C]-CO_2 evolved per g brain and per min of incubation.

Results

Hydroxylation of tyrosine was measured in homogenates of whole brain where any localized inhibition of the enzyme had been removed by disruption. No significant change (t test; $P > 0.05$) in the hydroxylation of tyrosine was found 70 min after the acute injection of morphine (3, 10, 30, 100 or 300 mg/kg i.p.). Similarly, no change was seen when mice were given the same test doses of morphine 6 h after chronic morphine treatment (100 mg/kg i.p. at 6 h intervals for nine injections). When given either 0.9% w/v NaCl solution (saline) or morphine (30 mg/kg) at 18, 36, 54, or

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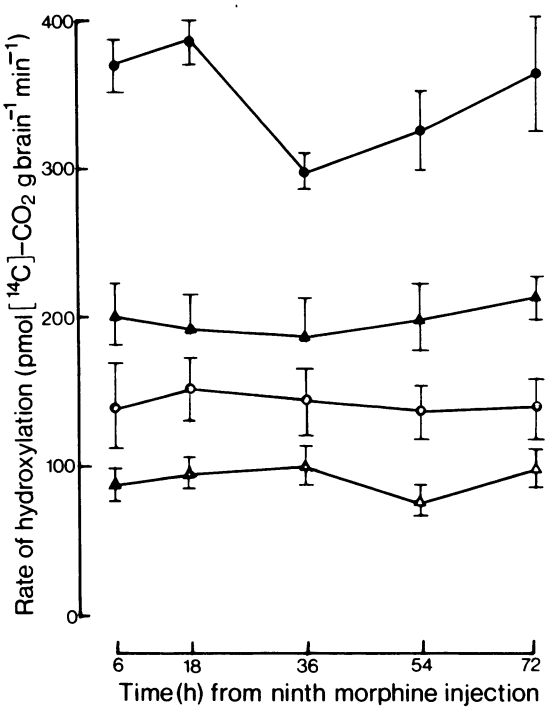

Fig. 1 Effect of withdrawal from morphine on tyrosine hydroxylase activity in mouse brain slices from the caudate nucleus (●), diencephalon (▲), brainstem (○), and parietal cortex (△). Ordinates: rate of hydroxylation of [1-^{14}C]-L-tyrosine in pmol [^{14}C]-CO_2 g brain^{-1} minute^{-1}. Abscissae: time in hours from ninth morphine injection. Each point represents the mean of six determinations. Vertical bars represent standard errors of the means.

72 h after the final chronic morphine injection there was no change in the hydroxylation of tyrosine in whole brain homogenates.

Changes in the activity of an enzyme may occur *in vivo* as a result of changes in the local concentration of inhibitors and where the structural integrity of the tissue is essential to demonstrate such changes. Therefore, tyrosine hydroxylase activity was measured in brain slices. The activity of the enzyme in slices from the diencephalon, brainstem and parietal cortex of either naive or chronically morphine-treated animals was unaltered by morphine, 3-300 mg/kg. However, morphine, 100 mg/kg, did significantly increase ($P < 0.05$) the activity of tyrosine hydroxylase in the caudate nucleus (366.9 ± 12.8 to 421.9 ± 21.3; mean ± s.e.). After nine injections of morphine this effect disappeared. Thirty-six hours after abrupt withdrawal from morphine there was a significant decrease ($P < 0.005$) in the tyrosine hydroxylase activity of caudate slices (Figure 1). In contrast, there were no changes in the activity of this enzyme in slices from other brain areas over a 72 h period of withdrawal. When naloxone, 3 mg/kg, was given to mice 6 h after their final chronic morphine injection, slices from the four brain areas showed no alteration in tyrosine hydroxylase activity when studied 70 min after the injection of the antagonist.

Discussion

The present series of experiments was designed to determine whether changes in tyrosine hydroxylase activity, measured *in vitro*, could explain the changes in catecholamine synthesis produced by morphine in the mouse brain *in vivo*. For example, acute injections of morphine increase the synthesis of dopamine and noradrenaline in the mouse brain *in vivo* (Smith *et al.*, 1970; 1972). The present experiments suggest that the hydroxylation of tyrosine in whole brain homogenates does not change after the acute administration of morphine. Fukui, Shiomi & Takagi (1972) also found unchanged tyrosine hydroxylase activity when morphine, 10^{-5} to 10^{-3} M, was added *in vitro* to mouse brain homogenates. Smith *et al.* (1972) reported increased incorporation *in vivo* of [^{14}C]-tyrosine into [^{14}C]-catecholamines in the cerebral cortex, diencephalon, striatum, brainstem and cerebellum of the mouse. However, in the present study, the activity of tyrosine hydroxylase was increased only in slices from the caudate nucleus after the acute administration of morphine.

After the repeated administration of morphine, tolerance developed to the effects of this drug on [^{14}C]-catecholamine synthesis in the mouse brain (Smith *et al.*, 1972). The present results suggest that during chronic morphine treatment tolerance developed to the effect of morphine on the activity of tyrosine hydroxylase in brain slices from the caudate nucleus. After chronic morphine treatment, Reis, Hess & Azmitia (1970) reported increased tyrosine hydroxylase activity in homogenates of rat caudate nucleus in contrast with the present findings using homogenates of whole mouse brain.

Rosenman & Smith (1972) reported a decrease in catecholamine synthesis in the mouse brain on withdrawal from morphine. This effect on synthesis was at a maximum 36 h after the final morphine injection. At this time, a significant decrease in tyrosine hydroxylase activity was seen in brain slices from the caudate nucleus (Figure 1). This decrease in activity could explain the decrease in dopamine synthesis *in vivo* during withdrawal.

The activity of the enzyme was unaltered in slices from the diencephalon, brainstem or parietal cortex, areas in which noradrenaline is the most abundant catecholamine. In rats withdrawn from morphine for 48 h, there was a decreased depletion of dopamine after α-methyl-*p*-tyrosine (Gunne, Jonsson & Fuxe, 1969). This suggests decreased release and possibly a decreased rate of dopamine turnover and synthesis, in agreement with the present findings. Naloxone administration to mice which had chronically received morphine precipitated a withdrawal syndrome, which included jumping, diarrhoea and piloerection, but did not decrease tyrosine hydroxylase activity in slices from the caudate nucleus. This suggests a difference between the withdrawal syndrome produced on termination of drug treatment and that elicited by a narcotic antagonist.

In conclusion, the assay of tyrosine hydroxylase activity *in vitro* did not produce results which could account for the changes in noradrenaline synthesis produced by morphine *in vivo*. However, the changes in tyrosine hydroxylase activity seen in the caudate nucleus could partly account for the changes in dopamine synthesis *in vivo* following acute and chronic morphine treatment.

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