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Dissociation of morphine tolerance and dependence from brain serotonin synthesis rate in mice

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ALTHOUGH acute and chronic administration of morphine, as well as morphine withdrawal, results in little or no change in brain serotonin (5-HT) levels in animals,[1–5] there is indirect evidence of a role for this brain amine in the action of morphine. Thus, administration of the 5-HT precursor, 5-hydroxytryptophan, has been reported to potentiate effects of morphine,[6,7] while *p*-chlorophenylalanine (pCPA), an inhibitor of 5-HT synthesis at the tryptophan hydroxylase step,[8] is said to antagonize morphine analgesia, tolerance and physical dependence.[9,10] Way *et al.*[10,11] have related morphine tolerance in mice to an increased rate of brain 5-HT turnover. In the present studies, we have measured the activity of tryptophan hydroxylase in whole brains of morphine-tolerant and control mice, and also measured whole brain 5-HT levels in mice treated similarly to those in the studies of Way *et al.*[10,11]

Three groups of male mice (18–30 g) were prepared that were tolerant to and physically dependent on morphine. Two of these groups were N.I.H. random-bred mice implanted for 72 hr with 75-mg morphine pellets of our manufacture (prepared according to the published formulation[10]), or implanted for 72 hr with 75-mg morphine pellets kindly supplied by Dr. Way. The third group consisted of CF-1 mice (Carworth Farms) implanted with morphine pellets from Dr. Way's supply. Control mice were implanted with placebo pellets.

Tolerance to morphine was assured by demonstrating at least a 3-fold increase in the dose of morphine necessary to prolong the reaction time on a hot plate at 55°[12] to 30 sec. or longer in treated animals, when compared to controls. Pellets were removed 72 hr after implantation; 6 hr after pellet removal, mice were given subcutaneous doses of morphine and tested. With 9 mg/kg of morphine, all controls responded by lifting and licking the forepaws in less than 30 sec. No morphine-implanted mouse given up to 30 mg/kg responded within 30 sec. Physical dependence was established by the jumping behavior precipitated by naloxone.[13] Seventy-two hr after morphine pellet implantation, the ED_{50} of naloxone required to elicit jumping within 15 min was 0·035 mg/kg subcutaneously. Placebo-implanted mice did not jump even after administration of 20 mg/kg.

Tryptophan hydroxylase activity was determined by a tritium release assay using 5-tritio-tryptophan as the substrate material.[14] Brains were removed 72 hr after implantation of the morphine or placebo pellet and duplicate samples were assayed from each mouse brain.

The rise of brain serotonin levels after intraperitoneal injection of pargyline hydrochloride, 75 mg/kg, was used as an index of the rate of serotonin synthesis.[15] At 0, 30, 60 and 120 min after pargyline, the mice were sacrificed by decapitation and the brains removed for assay. Whole brain 5-HT was measured by the method of Bogdanski *et al.*[16] with one modification; the final acid extract was reacted with orthopthalaldehyde[17] to increase the sensitivity so that triplicate samples from single mouse brain homogenates could be assayed. Periodic comparisons of assays with and without this modification yielded comparable results.

TABLE 1. MOUSE WHOLE BRAIN TRYPTOPHAN HYDROXYLASE ACTIVITY*

Controls	Morphine-tolerant
0·1017 ± 0·0065 N = 12	0·0953 ± 0·0097 N = 12

* Values are means ± S.E. expressed as millimicromoles of tryptophan hydroxylated per hour per milligram of protein.

TABLE 2. BRAIN SEROTONIN LEVELS IN PLACEBO AND MORPHINE-TREATED MICE AFTER MAO INHIBITION*

Time after pargyline HCl (min)	Brain 5-HT level (μg/g)		Significance of difference
	Placebo	Morphine	
0	0·63 ± 0·03	0·64 & 0·70 (N = 2)	NS
30	0·97 ± 0·02	1·11 ± 0·07	NS
60	1·23 ± 0·09	1·20 ± 0·05	NS
120	1·30 ± 0·04	1·36 ± 0·05	NS

* Values shown are means ± S.E. of 3–14 animals with the exception noted. NS indicates the difference was not statistically significant, i.e. $P > 0·05$. Pargyline HCl was injected intraperitoneally in a dose of 75 mg/kg.

The activity of tryptophan hydroxylase in whole brains of placebo-implanted and morphine implanted N.I.H. mice is presented in Table 1. We could not find any difference in enzyme activity between these two groups. Table 2 presents the concentration of 5-HT in whole brains of placebo-implanted and morphine pellet-implanted mice at various times after monoamine oxidase (MAO) inhibition. No significant differences could be demonstrated between control and morphine-tolerant mice at 0, 30, 60 or 120 min after pargyline administration. This was true whether the data from the three pellet-implanted groups were analyzed separately or pooled. These findings are in contrast to previous reports[10,11] of a 2- to 5-fold greater level of 5-HT in morphine-tolerant animals after injection of pargyline, compared to control animals receiving pargyline alone.

In a separate study, N.I.H. male mice were chronically injected with morphine starting with 15 mg/kg subcutaneously every 8 hr and increasing the dose by 50 per cent daily until 240 mg/kg every 8 hr was reached. Control mice were injected with equivalent volumes of saline. Here too, no significant differences were found in whole brain 5-HT levels between control and morphine-treated animals at 0, 30, 60 and 120 min after pargyline administration.

If morphine tolerance increased the rate of 5-HT synthesis, the activity of tryptophan hydroxylase, the rate-limiting step in this amine's synthesis,[18] might be expected to increase. In addition, reports that pCPA reduced the development of tolerance and physical dependence to morphine and that cycloheximide, an inhibitor of protein synthesis, prevented morphine tolerance and dependence led to the speculation that this enzyme might represent the protein responsible for morphine tolerance and physical dependence.[11] Our findings that tryptophan hydroxylase activity is unchanged in the presence of morphine tolerance refutes this hypothesis.

Finding no difference in enzyme activity, we then attempted to repeat the experiments of Way *et al.*[10,11] on the synthesis of 5-HT *in vivo*, following their experimental design as closely as possible. We were unable to find evidence of a change in 5-HT turnover associated with the development of morphine tolerance in mice.

These findings in mice are similar to the results obtained in the rat by Algeri and Costa,[19] which suggest that, in this species as well, there is no association between morphine tolerance and whole

brain 5-HT turnover. However, Azmitia *et al.*[20] have reported an increase in rat midbrain tryptophan hydroxylase activity associated with chronic morphine administration. Interpretation of the data from this latter report is difficult, since the increase in hydroxylase activity is greater in saline-injected *vs.* untreated controls than in morphine-injected *vs.* saline-injected rats.

Since our most conspicuous deviation from the original experimental design of Way *et al.*[10,11] involves the strain of mice used, the findings of Way *et al.*[10,11] may be peculiar to one or more specific strains of mice. Inasmuch as tolerance to morphine can be demonstrated in an enormous variety of species, we must, therefore, conclude that a mechanism other than one involving brain indoles should be sought to explain the tolerance phenomenon.

During the preparation of this paper, two reports appeared which also could not confirm an increased 5-HT turnover in mice made tolerant to morphine using other methods to measure the turnover of this amine.[21,22] A report by Maruyama *et al.*,[23] however, did show an increased level of brain 5-HT after pargyline in mice made tolerant to morphine.

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