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MODIFICATION OF SEROTONIN METABOLISM IN RAT BRAIN AFTER ACUTE OR CHRONIC ADMINISTRATION OF MORPHINE*

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Abstract—The steady state concentration of 5-hydroxyindoleacetic acid (5-HIAA) was elevated in rat brain for at least 4 hr after administration of a single dose of morphine sulfate (30 mg/kg, s.c.), and for more than 40 hr after subcutaneous implantation of pellets of morphine alkaloid. The concentration of 5-HIAA returned to normal 3 days after pellet implantation at a rate that paralleled the development of tolerance to the analgesic and other overt actions of morphine. Morphine did not modify the steady state concentration of serotonin under any of the treatment conditions. The turnover of brain serotonin was increased significantly during the 90-min period following a single injection of morphine sulfate (30 mg/kg, s.c.), as indicated by an increased rate of accumulation of 5-HIAA after blockade of efflux of 5-HIAA by probenecid in morphine-treated animals. As judged either by the rate of accumulation of 5-HIAA after administration of probenecid, or by the rate of accumulation of serotonin after treatment with pargyline, an increase in turnover of serotonin was evident in brains of tolerant rats 72 hr after pellet implantation. The rate of efflux of 5-HIAA from the brain was the same in control and morphine-tolerant rats. These results indicate that changes in brain serotonin metabolism are associated with both the acute effects of morphine and with morphine tolerance.

THE POSSIBLE role of serotonin in mediating both the acute pharmacologic responses to morphine and the adaptive processes leading to tolerance and physical dependence after repeated administration of morphine has recently been the subject of considerable interest.[1] Several studies showing marked reduction of morphine-induced analgesia,[2,3] hypothermia[4,5] and of the spontaneous motor depressant effect[6] after depletion of serotonin suggest that the presence of this amine is necessary for morphine to elicit these central depressant actions. Furthermore, Yarborough *et al.*[7,8] have shown that a single dose of morphine increases the rate of turnover of serotonin in brain. Tolerance to morphine, and the development of physical dependence were reported by Way, Loh and Shen[9–12] to be associated with an increase in the rate of turnover of serotonin in the brains of mice. Moreover, these investigators[9–12] found that tolerance and the development of physical dependence could be blocked by depletion of brain serotonin with *para*-chlorophenylalanine. Recent reports by other investigators[13–19], who have failed to corroborate these findings, have added to the present debate regarding the participation of serotonin in the development of tolerance and physical dependence to morphine. This paper presents data indicating that serotonin metabolism is altered in the brains of rats after acute administration of morphine, and that tolerance develops to the acute effects of morphine on serotonin metabolism concurrently with tolerance to the overt pharmacological effects of morphine.

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EXPERIMENTAL

Male Holtzman rats, 140–220 g, were used in all experiments. Rats were sacrificed by decapitation between 6 and 9 a.m., and the brains were removed and stored under dry ice until assayed. Morphine sulfate (30 mg/kg, s.c.) and pargyline HCl (75 mg/kg, i.p.) were administered in isotonic saline. Probenecid, which was injected intraperitoneally (200 mg/kg), was prepared by dissolving the acid in 0·1 N NaOH and then adjusting the pH of the resulting solution to 7·4 with 0·1 M phosphate buffer. Pellets of morphine alkaloid were prepared in a steel die (9 mm, i.d.) in a Carver laboratory press. The powder was compressed, together with 0·1 ml of distilled water, at a pressure of 5000 lb. These pellets, each 120 mg when dry, were implanted subcutaneously in the dorsal cervical region of rats under light ether anesthesia. Sham-operated animals were used as controls.

The turnover rate of brain serotonin was estimated from the regression coefficients for the accumulation of serotonin or of 5-HIAA induced by administration of pargyline or probenecid, respectively, as described previously.[20] The rate of efflux of 5-HIAA from brain was assessed by measuring the rate of decrease in 5-HIAA concentration induced by administration of pargyline.[20] In these turnover experiments, groups of five or six animals were killed at times up to 60 min after the administration of pargyline, or 90 min after the administration of probenecid. Animals treated either by injection of the morphine vehicle or by sham implantation of pellets were used as the zero-time controls.

Serotonin and 5-HIAA were extracted as described previously[21,22] and were measured fluorometrically after reaction with *ortho*-phthalaldehyde.[23]

Analgesia, measured by the tail-flick response method,[24] was expressed as the analgesic index, which is defined as follows: analgesic index $= (T - C)/(10 - C)$, where C = response time of control animals, and T = response time of morphine-treated animals. The thermal stimulus to treated rats was terminated when the response time exceeded 10 sec, approximately twice the control response time. Thus, an analgesic index of 1·0 indicates that none of the animals responded prior to the 10-sec cut-off time.

TABLE 1. EFFECT OF A SINGLE DOSE OF MORPHINE ON THE CONCENTRATIONS OF SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID IN RAT BRAIN

Time after administration of morphine* (hr)	Serotonin concn (ng/g ± S.E.)	5-Hydroxyindoleacetic acid concn (ng/g ± S.E.)
0	541 ± 18	417 ± 15
0·5	497 ± 25	438 ± 12
1·5	524 ± 35	521 ± 25†
3·0	541 ± 27	605 ± 25†
4·0		626 ± 24†
8·0	492 ± 23	454 ± 12

* Groups of four to five rats were sacrificed at various intervals after treatment with morphine sulfate, 30 mg/kg, s.c.

† $P < 0·01$; all others, $P > 0·1$; Student's t-test.

TABLE 2. EFFECTS OF MORPHINE ADMINISTRATION ON THE METABOLISM OF SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID IN RAT BRAIN*

Measurement	Morphine treatment	Control		Morphine		Statistical significance (P)§
		(ng/g/min ± S.E.)†	(min^{-1} ± S.E.)‡	(ng/g/min ± S.E.)†	(min^{-1} ± S.E.)‡	
Probenecid-induced accumulation of 5-HIAA	30 mg/kg, s.c.;	6·18 ± 0·66		8·10 ± 0·67		< 0·001
	Pellet, implanted 72 hr	7·08 ± 0·40		8·49 ± 0·62		< 0·05
Pargyline-induced accumulation of serotonin	Pellet, implanted 72 hr	6·08 ± 1·28		9·42 ± 0·90		< 0·01
Pargyline-induced decrease in 5-HIAA concentration	Pellet, implanted 72 hr		0·0113 ± 0·0015		0·0108 ± 0·0018	> 0·05

* Each value is based on 19–21 rats that were sacrificed at zero time and at three different times up to 60 min after the administration of pargyline, or 90 min after the administration of probenecid.

† Regression coefficients for the rate of accumulation of serotonin or 5-HIAA induced by administration of pargyline HCl (75 mg/kg, i.p.) or probenecid (200 mg/kg, i.p.) respectively.

‡ Rate constant of 5-HIAA loss induced by administration of pargyline HCl (75 mg/kg, i.p.).

§ Student's *t*.

Student's *t*-test was used to analyze the differences between pairs of means and between regression coefficients.[25]

RESULTS

Effect of a single dose of morphine sulfate. Administration of a single dose of morphine sulfate induced a significant increase in the concentration of 5-HIAA in the brain of rats (Table 1). The increase was evident within 1·5 hr after morphine treatment, reached a maximum elevation of 40–50 per cent above the control level between 3 and 4 hr after treatment, and had returned to normal within 8 hr. The concentration of brain serotonin was not altered by a single dose of morphine sulfate (Table 1).

To assess the mechanism for this increase in the concentration of 5-HIAA, rats were treated with probenecid to block the active transport of 5-HIAA from the brain.[20] When rats were treated with morphine sulfate and immediately thereafter with probenecid, the rate of accumulation of 5-HIAA was 31 per cent greater in the brains of morphine-treated rats than in those of control rats (Table 2).

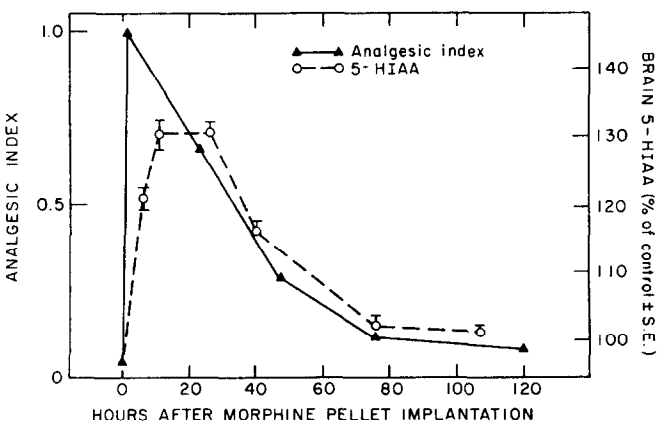

FIG. 1. Analgesic index and concentration of 5-hydroxyindoleacetic acid (5-HIAA) in rat brain after subcutaneous implantation of morphine pellets. Analgesia was assessed by measuring tail-flick response times; five sham-operated rats and six in which morphine pellets had been implanted were used in a repeated measures design. Brain 5-HIAA concentrations are expressed as a percentage of those of the sham-operated controls; four to six animals were used in control and treated groups at each time interval.

Effects of implantation of morphine pellets. To study the relationship between tolerance and serotonin metabolism, pellets of morphine alkaloid were implanted subcutaneously in rats. After implantation of these pellets, rats exhibited marked analgesia, catatonia, and decreased spontaneous motor activity for about 8 hr. These effects gradually subsided and were completely absent by the third day after implantation of the pellet. The analgesic activity and the elevation of 5-HIAA concentration produced by morphine released from the pellet are illustrated in Fig. 1. The maximal analgesic response was observed within 2 hr after implantation of the pellet. At that time, none of the animals that had received pellets responded to the thermal stimulus prior to the cut-off time. Twenty-four hr after implantation, four of five treated animals responded to the stimulus and, but the third day, the response times of treated and control animals were nearly the same. Similarly, the concentration of 5-HIAA in

the brains was significantly elevated (20 per cent, $P < 0.05$) within 7 hr after implantation of the pellet, reached a plateau between 15 and 30 hr, but then subsided at a rate parallel to the decreasing analgesic index. The concentration of 5-HIAA was restored to normal within 3 days after implantation of the pellet.

This gradual loss of pharmacologic activity resulted from the development of tolerance and was not due to a change in the rate of release of morphine from the pellets, because the rate of dissolution of the pellet was constant at 8 mg/day for as long as 9 days after implantation. Furthermore, animals tolerant to the analgesic activity 3 days after implantation of the pellet were also tolerant to the hypothermic action of a single large dose (50 mg/kg, i.p.) of morphine sulfate.

These findings indicate that tolerance develops to the elevated concentration of 5-HIAA induced by acute treatment with morphine. The mechanism for the return of 5-HIAA to normal in tolerant rats may involve a change in the rate of formation of the acid. This possibility was evaluated by measuring the rate of accumulation of serotonin or 5-HIAA induced by administration of pargyline or probenecid,[20] respectively, to morphine-tolerant (72 hr after pellet implantation) and sham-operated control rats. The rates of accumulation of serotonin and 5-HIAA are shown in Table 2. The rate of increase in serotonin was 55 per cent greater in the brains of tolerant rats than in those of sham-operated controls. Similarly, the rate of accumulation of 5-HIAA was faster (15 per cent) in the brains of morphine-tolerant rats treated with probenecid than in the corresponding control animals. These findings suggest that the turnover rate of serotonin and, therefore, the rate of formation of 5-HIAA, are actually greater in tolerant rats. Thus, it is unlikely that 5-HIAA returns to normal levels in tolerant animals because of a decrease in the rate of formation of the acid.

The concentration of 5-HIAA might return to normal in the brains of tolerant rats because of an increase in the rate of transport of the acid from the brain. To test this possibility, the rate of efflux of 5-HIAA from the brain was measured by treating rats with pargyline and measuring the rate of decrease in concentration of brain 5-HIAA.[20] As shown in Table 2, the rate constants for the efflux of 5-HIAA from the brain were the same in tolerant and control rats.

DISCUSSION

Our results clearly indicate that administration of morphine alters the metabolism of serotonin in the brains of rats. This alteration was reflected as an increase in the concentration of 5-HIAA, the principal cerebral metabolite of serotonin, after injection of a single dose of morphine sulfate, or after pellets of the alkaloid had been implanted subcutaneously. This increase in concentration of brain 5-HIAA appears to be caused by an increase in the rate of formation of the acid, because administration of probenecid induced a faster accumulation of 5-HIAA in the brains of rats treated with a single dose of morphine than in the brains of probenecid-treated controls. This finding, which corroborates that of Yarborough *et al.*,[7,8] suggests that acute treatment with morphine increases the turnover rate of serotonin.

The increase in concentration of brain 5-HIAA induced by administration of morphine may result from a direct effect of morphine on serotonergic neurons, leading to an increase in the rate of release of serotonin from serotonergic neurons in the brain. Other investigators have shown that an increase in the concentration of

brain 5-HIAA occurs when serotonergic neurons are stimulated by electrodes placed in the midbrain raphe area.[26] Furthermore, a morphine-induced release of serotonin has been demonstrated in peripheral tissue.[27]

We have not excluded the possibility that the increase in brain 5-HIAA could also be an indirect effect of morphine caused by an increase in the concentration of brain tryptophan. The concentration of tryptophan appears to be rate-limiting in the synthesis of serotonin.[28] Thus, if morphine increased the concentration of brain tryptophan, this would be expected to lead to an enhanced synthesis of serotonin. Although administration of lower doses of morphine was reported to have no effect on brain or plasma tryptophan,[29] the effects of morphine on concentration of tryptophan were not determined under our experimental conditions.

The finding of an acute effect of morphine on the metabolism of brain serotonin may indicate that serotonergic neurons participate in mediating some of the acute pharmacologic actions of the drug. This suggestion is consistent with the findings that the hypothermic[4,5] and analgesic[2,3] actions of morphine are diminished when the concentration of serotonin is reduced, either by treatment of animals with *para*-chlorophenylalanine or by production of lesions in the midbrain raphe. This hypothesis would also explain the enhanced toxicity and analgesia caused by treatments that enhance the concentration of serotonin in the brain.[30]

Our finding of a close temporal relationship between the development of tolerance to the elevated concentration of 5-HIAA and to the analgesic and other overt depressant actions of morphine shows that biochemical changes involving serotonin metabolism are associated with the development of tolerance to morphine. However, Bowers and Kleber[31] have reported that chronic administration of morphine to mice resulted in an increase in the concentration of 5-HIAA in brain. Although their findings appear to contradict ours, these investigators induced tolerance and dependence by repeated intraperitoneal administration of morphine, and it is not clear from their report if the mice were sacrificed immediately after injection of morphine, or during withdrawal from the last dose of morphine. In our experiments, withdrawal from morphine was not a variable because tolerance was induced by implantation of pellets. Thus, differences in the method of producing tolerance could account for our conflicting results.

The mechanism for the return of 5-HIAA concentration to normal values in the brains of tolerant rats is not clear from our results. However, we have eliminated the possibility that 5-HIAA returns to normal concentration because of an increase in the rate of transport of the acid from the brain, by demonstrating that the rate of efflux of 5-HIAA from brain was the same in control and morphine-tolerant rats. Furthermore, restoration of concentration of 5-HIAA to pretreatment values cannot be explained on the basis of a decrease in the rate of formation of the acid. On the contrary, our results indicate that the rates of formation of both serotonin and 5-HIAA are accelerated in the brains of morphine-tolerant rats. This finding of an accelerated turnover of serotonin in the brains of tolerant rats confirms that of Way *et al.* in mice.[1,9–12] However, other investigators have failed to obtain a similar increase in turnover, despite the development of tolerance to the overt actions of morphine,[13–19] suggesting that this increase in serotonin turnover may not be necessary for tolerance to occur. We do not know why other investigators have not found an accelerated turnover of serotonin in brains of animals tolerant to morphine.

Hitzemann *et al.*[32] and Cheney and Costa[33] have discussed the possibility that the method of estimating serotonin turnover may lead to erroneous results. Other possible variables include the strain or species of animal, methods of morphine administration, and the degree to which tolerance or physical dependence had developed at the time when serotonin turnover was measured.

The development of tolerance to the increased concentration of 5-HIAA caused by acute treatment with morphine, despite an apparent increase in the rate of turnover of serotonin in tolerant rats, suggests the possibility that serotonin catabolism is altered in the brains of tolerant animals. In support of this suggestion, studies in this laboratory[34] have demonstrated an enhanced rate of accumulation of serotonin-[^{14}C] into brain slices from morphine-tolerant rats.

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