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EFFECT OF CHRONIC ADMINISTRATION OF MORPHINE ON MONOAMINE OXIDASE ACTIVITY IN DISCRETE REGIONS OF THE BRAIN OF RATS

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Abstract—The effect of chronic administration of morphine on the activity of monoamine oxidase in specific regions of the brain of rats has been investigated. It was found that, shortly after the last administration of morphine, brain monoamine oxidase (EC 1.4.3.4) was drastically reduced in rats which had been chronically treated with morphine and which had exhibited a hyperactivity syndrome manifested by compulsive gnawing and spasmodic jumping. The lowest values were seen at approximately 30 min and they returned to nearly normal levels by 6 hr after the last injection. In contrast, no significant changes were observed in the activity of this enzyme in animals that did not exhibit this syndrome after morphine administration.

Morphine increases the rate of catecholamine synthesis in the brain of rats and mice [1, 2] suggesting that catecholamine-containing neurons may be involved in some of the effects of morphine on the function of the central nervous system. After repeated administration of morphine or levorphanol to mice, tolerance and cross-tolerance develops to the effects of these narcotics on brain catecholamine synthesis [3]. Furthermore, the development of tolerance to and physical dependence on morphine is not associated with an increase in the activity of brain tyrosine hydroxylase (tyrosine 3-hydroxylase, EC 1.14.3.a) [4]. On the basis of these findings it has been suggested that morphine does not enhance the biosynthesis of catecholamines by a direct effect on tyrosine hydroxylase [3, 4].

The possible role of serotonin in the pharmacologic actions of morphine has been the subject of considerable interest. Evidence on the effects of morphine on this neurotransmitter has been circumstantial and contradictory [5–8], but more recent findings confirm that serotonin is involved, in, at least, some of the effects of morphine and that its turnover rate increases after chronic administration of the drug [9, 10]. Several investigators [11, 12] have reported that narcotic analgesics induce locomotor hyperactivity in mice and that this effect is associated with changes in brain biogenic amines [12, 13]. We have observed that during the development of tolerance to the drug, a number of rats showed an activation reaction which was manifested by hyperactivity, spasmodic jumping and compulsive gnawing within the first 20–30 min after the last administration of morphine, while other rats did not exhibit this hyperactivity reaction. This study was set up to determine if, and to what extent, the activity of brain monoamine oxidase (monoamine: oxygen oxidoreductase, EC 1.4.3.4) is influenced by chronic administration of morphine to these two types of rats. Monoamine oxidase (MAO) activity was measured in hypothalamus, hippocampus, thalamus, cerebellum and cerebral cortex. These brain areas were selected for study because of (1) known effects on catecholamine synthesis in these areas [1, 3]; (2) their involvement in the development of tolerance [14]; and/or (3) evidence that they may be involved in the regulation of a number of brain functions affected by morphine [1].

MATERIALS AND METHODS

Materials. Chemicals utilized in this study were obtained from Sigma Co., St. Louis, Mo. Morphine sulfate (15 mg/ml) was obtained from Eli Lilly Co., Indianapolis, Ind.

Animals. A total number of 120 Sprague–Dawley (AFRRI colony) male rats from 9 to 10 weeks old, weighing 240–260 g, were used. The animals were kept in a temperature-controlled room at 22 ± 0.5°, and were individually housed in cages with free access to food (Wayne Lab Blox) and water. Of these animals, 85 were used for morphine administration and the rest served as controls.

Morphine administration. The experimental animals were given morphine by injecting the drug, 40 mg/kg body weight of morphine sulfate/injection, i.p., twice daily for 8 days. Control animals were injected with sterile physiologic saline in volumes corresponding to those of the morphine solution. After day 6 or 7 of morphine administration, a number of rats (approximately 40 per cent of the experimental animals) exhibited an activation response which was manifested by hyperactivity, spasmodic jumping and compulsive gnawing ("responsive" rats) for the first 20–30 min after each injection of the drug. The remaining rats did not show this hyperactivity reaction ("unresponsive" rats). Of the 85 experimental animals, 28 responsive rats and 42 unresponsive rats were used in this study. The remaining 15 rats could not meet required criteria and were not used. The experimental animals were divided into seven groups of 4 animals each for the responsive and seven groups of 6 rats each for the unresponsive animals. Control animals were divided into seven groups of 5 animals each.

Assessment of tolerance. The degree of tolerance to morphine was determined by the hot plate technique of Eddy and Leimbach [15] as modified by Johannesson and Woods [16].

Preparation of enzyme systems. At the end of day 8, one group of the responsive and one group of the unresponsive rats were sacrificed just before receiving the last morphine injection and were used as baseline controls. The other groups were given the last morphine dose and were sacrificed at 5, 15, 30 and 60 min and 6 hr or 24 hr after the last injection. Experimental animals as well as saline controls were sacrificed by decapitation and their heads instantly frozen in liquid nitrogen in a Dewar flask. The heads were later removed from the liquid nitrogen and stored at −90° until the time of assay. Storage at this temperature for as long as 5 days was found to cause no detectable loss in enzymatic activity. Enzymatic activity determinations were performed within 24 hr after sacrificing the animals. Rapid freezing of the rats' heads in liquid nitrogen usually resulted in bilateral splitting of the skull and brain, facilitating removal of the brain areas under investigation. The frozen heads were partially thawed in a cold room, kept at 2–3°, and the thalamus, hypothalamus, cerebral cortex, cerebellum and hippocampus were dissected out and homogenized in 10 vol. of 0.25 M sucrose containing 0.001 M $MgCl_2$ using glass homogenizers of the Potter–Elvehjem type with Teflon pestle, kept in crushed ice. Each of the homogenates was centrifuged (Sorvall (RC-2B)) at 1,500 *g* for 10 min and the resultant supernatant fluid was centrifuged at 10,000 *g* for 30 min. After carefully decanting the supernatant fluid, the mitochondrial pellet was suspended in an equal volume of homogenization medium and was used for the assay. No monoamine oxidase activity was found to be present in the 10,000 *g* supernatant.

Monoamine oxidase. Monoamine oxidase activity was assayed by a modification of the method of Weissbach *et al.* [17]. The assay mixture contained 0.05 M Tris–HCl buffer, pH 7.4, 0.22 mM kynuramine dihydrobromide, 0.08 mM $MgCl_2$ and the enzyme preparation (0.6 to 1 mg protein). The final volume of the incubation mixture was made up to 3 ml with water and the reaction was stopped by the addition of 0.2 ml of 0.5 M NaOH and 0.4 ml of 10% $ZnSO_4$. The mixture was then shaken, heated in a boiling water bath for 5 min, and centrifuged at 10,000 *g* for 10 min. The concentration of the reaction product 4-hydroxyquinoline was determined in the supernatant spectrophotometrically by measuring the absorbance (appearance of the peak) at 330 nm [18]. A blank cuvette was prepared by replacing kynuramine with water. When various, increasing concentrations of 4-hydroxyquinoline were used as standards, the height of the peak at 330 nm was found to be directly proportional to the amount of 4-hydroxyquinoline present in the solution [18]. Measuring the increase in absorbance at 330 nm instead of decrease at 360 nm [17], at least a 3- to 4-fold increase in the sensitivity of the reaction can be achieved. Enzymic activities were expressed per mg of protein. Protein determinations were performed according to the method of Lowry *et al.* [19].

Data presentation. Data are presented as the mean ± standard error. Values are expressed as μmoles 4-hydroxyquinoline produced/90 min/mg of protein. Student's two-tail *t*-test was used for statistical analysis.

RESULTS

The effects of chronic administration of morphine on MAO activity in rats showing the hyperactivity reaction (responsive rats) are presented in Table 1. Within minutes after the last administration of the drug, MAO activity decreased in all brain areas investigated, reaching lowest levels at approximately 15–30 min post-injection. In rats sacrificed at 60 min, the morphine-induced changes in MAO activity were less pronounced and this activity was found to return to nearly normal levels in animals sacrificed at 6 or 24 hr after the last morphine injection.

Table 2 shows the results obtained with rats, chronically treated with morphine, which did not exhibit the hyperactivity reaction (unresponsive rats). In contrast to the results with the responsive animals, no appreciable changes or some increases in brain MAO activity occurred.

To decide whether a correlation exists between behavioral and MAO activity changes, rats that were chronically treated with morphine and did not exhibit the hyperactivity syndrome (unresponsive rats) were injected with the MAO inhibitor, pargyline (20 mg/kg,

Table 1. Changes in monoamine oxidase activity in responsive rats chronically treated with morphine

	Brain areas									
	Thalamus		Hypothalamus		Hippocampus		Cerebellum		Cerebral cortex	
Time after last morphine injection	Activity*	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Controls	0 49 ± 0.02		0.56 ± 0 03		0.52 ± 0.03		0 42 ± 0 02		0 44 ± 0.04	
Morphine-treated										
0 min (baseline controls)	0.41 ± 0.01†	83.6	0 49 ± 0.03	87.5	0.38 ± 0.04†	73 1	0.36 ± 0 03	85 7	0 42 ± 0 01	95 4
5 min	0.39 ± 0 02†	79.5	0.38 ± 0.02†	67.8	0.39 ± 0 03†	75.0	0.32 ± 0.03†	76 2	0 34 ± 0 02†	77 2
15 min	0.30 ± 0 02†	61.2	0.29 ± 0 02†	51 7	0 40 ± 0.02†	76.9	0.34 ± 0.05	80 9	0.30 ± 0.02†	68 2
30 min	0.29 ± 0.03†	59 1	0 35 ± 0.03†	62 5	0.35 ± 0.02†	67 3	0 29 ± 0 01†	69 0	0 31 ± 0.04†	70.4
60 min	0.38 ± 0 02†	77 5	0.43 ± 0 04†	76.7	0 41 ± 0 04†	78 8	0 32 ± 0.02†	76 2	0.40 ± 0 01	90 0
6 hr	0 41 ± 0 01†	83.6	0 42 ± 0.03†	75.0	0.48 ± 0.02	92 3	0.35 ± 0.03†	83 3	0 37 ± 0 04	84 1
24 hr	0.44 ± 0.03	89 8	0 48 ± 0.05	85 7	0 45 ± 0.04	86 5	0 39 ± 0 04	92 8	0 47 ± 0 03	106 8

* Expressed as μmoles 4-hydroxyquinoline/90 min/mg of protein. Values are means ± S. E.

† Values significantly different from their respective controls ($P < 0.005$).

Table 2. Changes in monoamine oxidase activity in unresponsive rats chronically treated with morphine

	Brain areas									
	Thalamus		Hypothalamus		Hippocampus		Cerebellum		Cerebral cortex	
Time after last morphine injection	Activity*	% of control	Activity	% of control	Activity	% of control	Activity	% of control	Activity	% of control
Controls	0.43 ± 0.03		0.46 ± 0.03		0.47 ± 0.04		0.36 ± 0.03		0.41 ± 0.02	
Morphine-treated										
0 min (baseline controls)	0.40 ± 0.04	93.1	0.39 ± 0.03	84.7	0.44 ± 0.04	93.6	0.33 ± 0.02	91.6	0.45 ± 0.03	109.7
5 min	0.38 ± 0.02	88.4	0.43 ± 0.03	93.4	0.39 ± 0.04	82.9	0.35 ± 0.03	97.2	0.41 ± 0.02	100.0
15 min	0.41 ± 0.04	95.3	0.44 ± 0.04	95.6	0.44 ± 0.02	93.6	0.36 ± 0.03	100.0	0.45 ± 0.04	109.7
30 min	0.47 ± 0.03	109.3	0.49 ± 0.02	106.5	0.42 ± 0.04	89.3	0.38 ± 0.03	105.5	0.48 ± 0.02†	117.1
60 min	0.39 ± 0.04	90.7	0.45 ± 0.03	97.8	0.45 ± 0.03	95.7	0.42 ± 0.02	116.0	0.42 ± 0.05	102.4
6 hr	0.39 ± 0.03	90.7	0.42 ± 0.02	91.3			0.34 ± 0.02	94.4	0.37 ± 0.03	90.2
24 hr	0.44 ± 0.05	102.3	0.50 ± 0.03	108.6	0.41 ± 0.05	87.2	0.37 ± 0.02	102.7	0.49 ± 0.04	119.5

* Expressed as μmoles 4-hydroxyquinoline/90 min/mg of protein. Values are means ± S. E.
† Value significantly different from its respective control ($P < 0.05$).

i.p.), at approximately 5 min before administration of the last dose of morphine. These rats became behaviorally responsive, i.e. they developed the stereotyped hyperactivity syndrome within 10–20 min after the morphine injection. The syndrome lasted 20–30 min. The animals were sacrificed at approximately 45 min after administration of the drug, while the syndrome was still present, and MAO activity and catecholamine and serotonin level determinations were performed in their brains. Up to a 90 per cent decrease in MAO activity was observed, while the levels of catecholamines and serotonin increased to 130 and 160 per cent, respectively, of controls.

A new series of experiments was conducted to determine whether the decreases in MAO activity observed in responsive rats were due to the presence of an inhibitor metabolite of morphine or to oxygen deficiency in the brain homogenates because of the known respiratory depressant characteristics of morphine. Brain homogenates from morphine-treated rats were either pre-incubated (30 min at 37°) in air before addition of the substrate, or dialyzed (1 hr at 4°) against the homogenization medium. They were then compared for MAO activity to corresponding homogenates that had not been pre-incubated or dialyzed. No significant differences were found.

DISCUSSION

Previous experiments with mice have indicated that brain catecholamines are involved in the mechanism by which morphine-like drugs induce motor activity [12]. In addition, agents, such as pargyline, which prevent the oxidative deamination of catecholamines enhance the locomotor activity-increasing effect of levorphanol in mice, whereas reserpine, which depletes brain catecholamines, diminishes it [20]. In agreement with these results, our rats, which when treated chronically with morphine exhibited the hyperactivity syndrome, had a drastically reduced brain monoamine oxidase activity. Thus, a hyperactivity syndrome results whether the activity of monoamine oxidase was inhibited by monoamine oxidase inhibitors or by chronic administration of morphine. The fact that development of tolerance to morphine is not associated with an increase in the activity of brain tyrosine hydroxylase [4] supports the idea that the hyperactivity syndrome is not linked to an enhanced synthesis of brain catecholamines but rather to a decrease in the rate of their oxidative deamination.

A similar hyperactivity syndrome has been observed in rats after administration of monoamine oxidase inhibitors and tryptophan, and the rate of development of this hyperactivity correlated with the rate of accumulation of brain serotonin [21]. Since chronic administration of morphine produces no change in the activity of the soluble form of brain tryptophan hydroxylase and only minor increase in the activity of the particulate enzyme [22], it is very likely that this hyperactivity syndrome is, at least partly, due to a decrease in brain MAO activity, and this agrees with our results. Compulsive gnawing and hyperexcitability were found to result from direct deposition of morphine-like drugs to the central thalamic region of the brain of rats [23]. Our results suggest that these effects might also be due to MAO inhibition.

The findings [24, 25] that high levels of catecholamines and indolamines in the brains of rats chronically treated with morphine are associated with morphine-induced hyperactivity behavior are in agreement with our results and could be partly explained by our finding that the hyperactivity syndrome is associated with decreased levels of brain monoamine oxidase activity. Furthermore, the observation that intraventricular injection of norepinephrine [25] or direct implantation of dopamine into the corpus striatum of rats [26] elicited hyperactivity behavior is also consistent with our results. It is also of interest to note that chronic administration of methadone to rats induces gnawing and other symptoms of stereotyped behavior and that the suggestion was made that increased dopamine production may be the cause of this behavior [27]. Our observation that when MAO inhibitors were injected in unresponsive rats chronically treated with morphine, these animals exhibited hyperactivity behavior upon administration of a single dose of morphine, supports the idea that a link exists between behavior and brain MAO activity changes.

In chronically treated rats which did not exhibit a hyperactivity reaction after the last dose of morphine (unresponsive rats), monoamine oxidase activity either did not change appreciably or it was somewhat increased (see Table 2). These observations also tend to support the existence of a relationship between the appearance of the hyperactivity syndrome and the decrease in the activity of brain monoamine oxidase. Monoamine oxidase in the brain of the rat exists in several forms with different substrate specificities [28, 29]. Experiments are in progress to determine to what extent each of these forms contributes to the observed hyperactivity syndrome.

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