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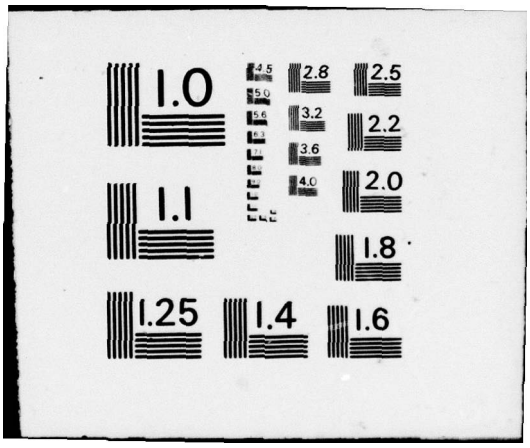
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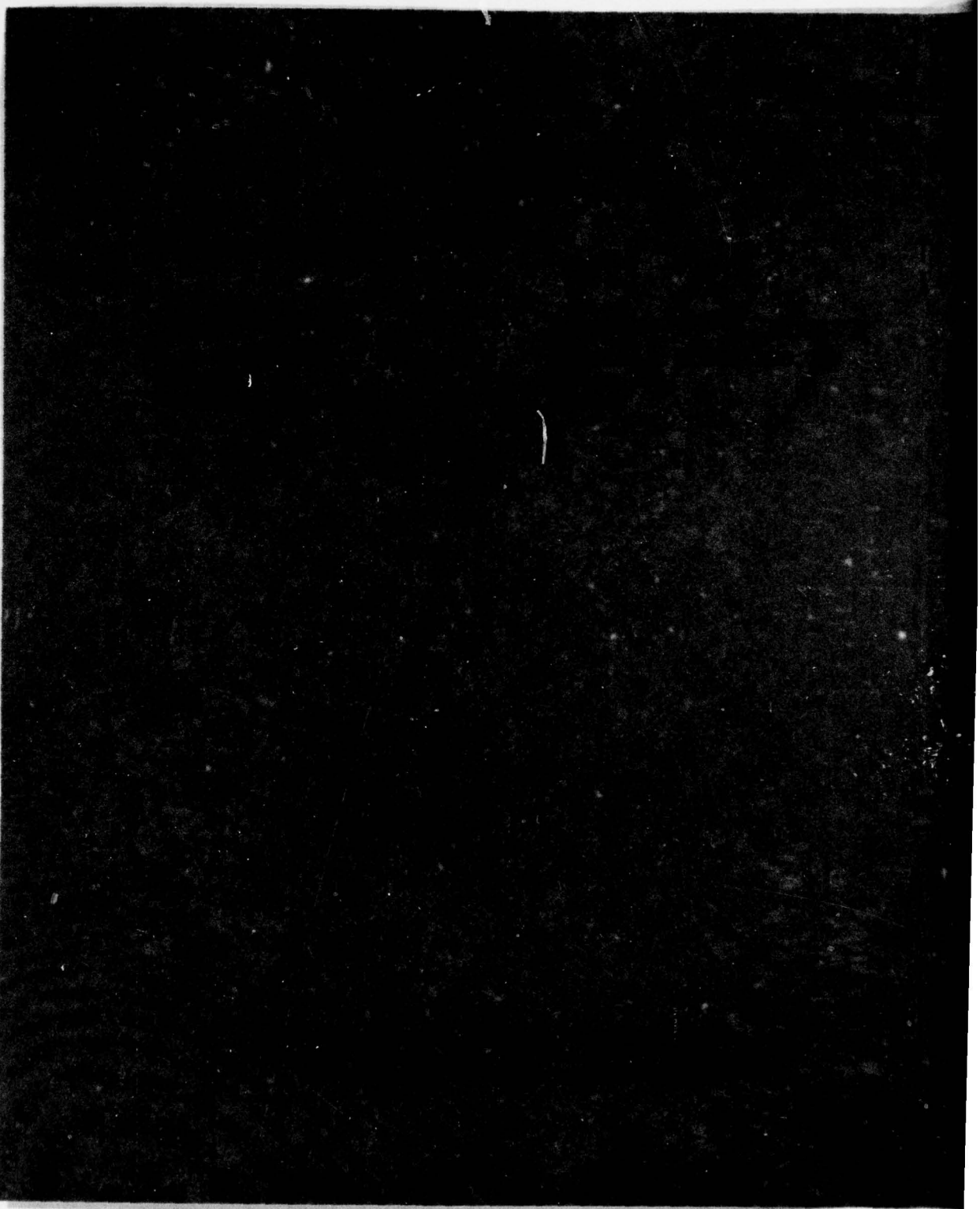

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The effect of chronic administration of morphine on the activity of monoamine oxidase in specific regions of the brain of rats has been investigated. It was found that, shortly after the last administration of morphine, brain monoamine oxidase was 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. Lowest values were seen at approximately 30 minutes and

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returned to nearly normal levels by 6 hours 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. The study of morphine-induced changes in brain monoamine oxidase described above provides information on basic mechanisms of function of the mammalian central nervous system. This information is of great value to studies of effects of other toxic agents including ionizing and nonionizing radiation.

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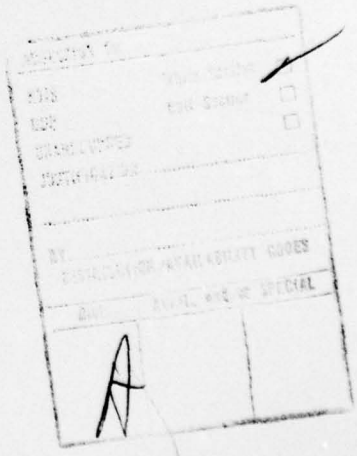

INTRODUCTION

Morphine increases the rate of catecholamine synthesis in the brain of rats and mice[6,22] 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 develop to the effects of these narcotics on brain catecholamine synthesis.[21] Furthermore, the development of tolerance to and physical dependence on morphine is not associated with an increase in the activity of brain tyrosine hydroxylase.[5] 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.[5,21]

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,[4,17,20,25] 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.[11,24] Several investigators[8,19] have reported that narcotic analgesics induce locomotor hyperactivity in mice and that this effect is associated with changes in brain biogenic amines.[3,19] 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 minutes 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, brain monoamine oxidase activity is influenced by chronic administration of morphine to these two types of rats. Monoamine oxidase activity was measured in hypothalamus, hippocampus, thalamus, cerebellum, and cerebral cortex. These brain areas were selected for study because (1) morphine has known effects on catecholamine synthesis in these areas;[6,21] (2) these areas are involved in the development of tolerance;[23] and/or (3) they may also be involved in the regulation of a number of brain functions affected by morphine.[6]

MATERIALS AND METHODS

Materials. Chemicals utilized in this study were obtained from Sigma Chemical Company, St. Louis, Missouri. Morphine sulfate (15 mg/ml) was obtained from Eli Lilly Company, Indianapolis, Indiana.

Animals. A total of 120 Sprague-Dawley (AFRRI colony) male rats 9-10 weeks old, weighing 240-260 grams, were used. The animals were kept in a temperature-controlled room at $22^{\circ} \pm 0.5^{\circ}C$, 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 per 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 the 6th or 7th day of morphine administration, a number of rats (approximately 40 percent of the experimental animals) exhibited an activation response which was manifested by hyperactivity, spasmodic jumping, and compulsive gnawing ("jumping" rats) for the first 20-30 minutes after each injection of the drug. The remaining rats did not show this hyperactivity reaction ("nonjumping" rats). Of the 85 experimental animals, 28 jumping rats and 42 nonjumping 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 four animals each for the jumping and seven groups of six rats each for the nonjumping animals. Control animals were divided into seven groups of five animals each.

Assessment of tolerance. The degree of tolerance to morphine was determined by the hot plate technique of Eddy and Leimbach[7] as modified by Johannesson and Woods.[13] The rats were placed on a hot plate maintained at $55^{\circ} \pm 0.5^{\circ}C$ within a Plexiglas restraining cylinder. The time interval between contact of the paws of the animal with the plate and reaction to the stimulus (licking of fore or hind paws) was used as an index of analgesia. In control animals this occurred with a mean latency of approximately 4-6 seconds. Unresponsive, morphine-injected rats were arbitrarily removed after 60 seconds.

Preparation of enzyme systems. At the end of the 8th day, one group of the jumping and one group of the nonjumping rats were euthanatized just before receiving the last morphine injection and were used as base-line controls. The other groups were given the last morphine dose and were euthanatized at 5, 15, 30, 60 minutes, 6 or 24 hours after the last injection. Experimental animals as well as saline controls were euthanatized by decapitation and their heads were instantly frozen in liquid nitrogen in a Dewar flask. The heads were later removed from the liquid nitrogen and stored at -90°C until the time of assay. Storage at this temperature for as long as 5 days was found to cause no detectable loss in enzymic activity. Enzymic activity determinations were performed within 24 hours after euthanasia of 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°C, and the thalamus, hypothalamus, cerebral cortex, cerebellum and hippocampus were dissected out and homogenized in 10 volumes 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 1500 x g for 10 minutes and the resultant supernatant fluid was centrifuged at 10,000 x g for 30 minutes. 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 x g supernatant.

Monoamine oxidase. Monoamine oxidase activity was assayed by a modification of the method of Weissbach et al.[26] The assay mixture contained 0.05 M Tris-HCl buffer pH 7.4, 0.22 mM kynuramine-di-hydrobromide, 0.08 mM $MgCl_2$ and the enzyme preparation (0.6-1 mg protein). 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 0.5 N NaOH and 0.4 ml 10 percent $ZnSO_4$. The mixture was then shaken, heated in a boiling water bath for 5 minutes, and centrifuged at 10,000 x g for 10 minutes. 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.[2]

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.[2] By measuring the increase in absorbance at 330 nm instead of decrease at 360 nm,[26] at least a threefold to fourfold increase in the sensitivity of the reaction can be achieved. Enzymic activities were expressed per milligram of protein. Protein determinations were performed according to the method of Lowry et al.[16]

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

RESULTS

The effects of chronic administration of morphine on the activity of monoamine oxidase in rats showing the hyperactivity reaction (jumping rats) are presented in Table 1. Within minutes after the last administration of the drug, monoamine oxidase activity decreased in all brain areas investigated, reaching lowest levels at approximately 15-30 minutes postinjection. In rats euthanatized at [illegible] minutes, the morphine-induced changes in the activity of monoamine oxidase were less pronounced and this activity was found to return to nearly normal levels in animals euthanatized at 6 or 24 hours after the last morphine injection.

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

DISCUSSION

Previous experiments with mice have indicated that brain catecholamines are involved in the mechanism by which morphine-like drugs induce locomotor activity.[19] In addition, agents such as pargyline which prevent the oxidative deamination of catecholamines enhance the locomotor activity-increasing effect of levorphanol in mice,

Table 1. Changes in Monoamine Oxidase Activity in Jumping Rats Chronically Treated with Morphine

Time after last morphine injection	Brain Areas									
	Thalamus		Hypothalamus		Hippocampus		Cerebellum		Cerebral Cortex	
	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	--
Morphinized:										
0 minutes (base-line 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 minutes	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 "	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 "	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 "	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	90.9
6 hours	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 "	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 of 4-hydroxyquinoline per 90 min per mg of protein
Values are means ± S.E.

Table 2. Changes in Monoamine Oxidase Activity in Nonjumping Rats Chronically Treated with Morphine

Time after last Morphine Injection	Brain Areas									
	Thalamus		Hypothalamus		Hippocampus		Cerebellum		Cerebral Cortex	
	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	
Morphinized:										
0 minutes (base-line 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 minutes	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 "	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 "	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 "	0.39±0.04	90.7	0.45±0.03	97.8	0.45±0.03	95.7	0.42±0.02	116.6	0.42±0.05	102.4
6 hours	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 "	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±0.03	119.5

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

whereas reserpine, which depletes brain catecholamines, diminishes it.[12] 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[5] 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.[10] 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,[15] it is very likely that this hyperactivity syndrome is, at least partly, due to a decrease in the activity of brain monoamine oxidase; 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.[1] Our results suggest that these effects might also be due to monoamine oxidase inhibition.

In chronically treated rats which did not exhibit hyperactivity reaction after the last dose of morphine, monoamine oxidase activity either did not change appreciably or it was somewhat increased (Table 2). These observations also tend to support the existence of a relationship between the appearance of the hyperactivity syndrome and decrease in the activity of brain monoamine oxidase. Differences between strains of mice in their sensitivity to opiates and their patterns of running behavior have been observed by other investigators[18] who concluded that strain differences were not due to differences in absorption, metabolism or permeability of the blood-brain barrier, but were rather modulated by different genetic mechanisms. The observation that strains of mice characterized by more spontaneous motor activity have higher brain

amine levels than mice which exhibit much lower motor activity[14] is also consistent with our findings. Monoamine oxidase in the brain of rats exists in several forms with different substrate specificities.[9,27] Experiments are in progress to determine to what extent each of these forms contributes to the observed hyperactivity syndrome.

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