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EFFECT OF MORPHINE ON MOUSE BRAIN ATPase ACTIVITIES

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The effect of morphine on well-recognized ATPases involved in the active ion transport (1) and in oxidative phosphorylation (2,3) is not clearly understood. Ghosh and Ghosh (4) showed that, in the absence of Na^+, K^+ and Mg^{2+} ions in the reaction medium, morphine inhibits the total ATPase activity in rat brain microsomes. Kaku et al. (5) showed a 23 per cent inhibition of mouse brain synaptosomal Mg^{2+}, Ca^{2+} ATPase in naive animals but not in morphinized mice. On the other hand, Jain et al. (6) have reported stimulation of mouse brain $Na^+ - K^+$ - and Mg^{2+}-dependent ATPase activity. Because of the above discrepancy, we have studied the effects of morphine on three ATPase activities in mouse brain after acute and chronic administration of the drug and during naloxone-precipitated withdrawal.

Male ICR mice (weighing 23-26 g) obtained from Charles River, Wilmington, Mass., U.S.A., were used. All the chemicals used for the enzyme assay were obtained from Sigma Chemical Co., St. Louis, Mo. For acute treatment of morphine, 10 or 30 mg/kg was injected s.c. and 30 min later the mice were sacrificed. For chronic treatment, mice were rendered tolerant and physically dependent by the pellet implantation technique (7). A specially formulated pellet containing 75 mg of morphine base was implanted in the lower back s.c. for 72 hr. The control mice in the acute group received saline and in the chronic group the control mice received placebo pellets. Various doses of naloxone hydrochloride were administered 10 min prior to the sacrifice of the animals.

Mouse brain tissue was homogenized and fractionated as described by Koch (8). The homogenate was centrifuged at 900 g for 10 min (A-fraction) to remove nuclei and cellular debris. The supernatant was then centrifuged at 13,000 g for 20 min (B-fraction) and the pellet was resuspended in sucrose solution, divided into small aliquots and quickly frozen in liquid nitrogen for ATPase assay. The frozen samples were stored at -20° until used for ATPase assay. This B-fraction contained nerve ending particles and mitochondria (8). The presence of 1 mM EDTA in the sucrose solution in which our samples were suspended eliminated the effect of tissue calcium upon the ATPase activities. ATPase activities were

determined essentially according to the enzymatic procedure described previously (3,9).

A 3-ml reaction mixture contained: 4.3 mM ATP, 5 mM Mg^{2+}, 100 mM Na^{+}, 20 mM K^{+}, 135 mM imidazole-Cl buffer (pH 7.5), 0.2 mM NADH, 0.5 mM phosphoenol pyruvate, 0.02% bovine serum albumin, approximately 9 units of pyruvate kinase and 12 units of lactic acid dehydrogenase. A 20-µl (enzyme) tissue preparation was used with a protein content of 20-30 µg. Absorbance changes in the reaction mixture were measured at 340 nm using a Beckman Acta III recording spectrophotometer with temperature controlled at 37°. The change in O.D. at 340 nm over a period of 10 min was used in calculating the specific activity. Enzyme activities were expressed as µmoles Pi mg^{-1} protein hr^{-1}. Total ATPase activity was measured with Mg^{2+}, Na^{+} and K^{+} present in the reaction mixture. Mg^{2+} ATPase activity was measured in the presence of 1 mM ouabain, which is a specific inhibitor of Na^{+} - K^{+} ATPase (10). Na^{+} - K^{+} ATPase activity was obtained by determining the difference between total ATPase activity and Mg^{2+} ATPase activity. Mg^{2+} ATPase was further delineated into oligomycin-sensitive and insensitive ATPase activities by adding 5 x 10^{-6} M oligomycin (based upon a combined mol. wt of 401.20 from 15% oligomycin A and 85% oligomycin B) in ethanol to the reaction mixture. Proteins were determined by the method of Lowry et al. (11). The specific activities in controls as well as in treated groups were the mean of three separate fractions (N = 3). Each fraction was assayed 2-3 times and the average was taken. The mean of three determinations was used for checking the statistical significance between control and treated pairs. Student's t-test was employed in calculating the P values as indicated in the tables.

Table 1. Effect of acute morphine administration and naloxone on ATPase activities in mouse brain

	Specific activity ± S.E.M. (µmoles Pi mg^{-1} protein hr^{-1})		
	Na^{+} - K^{+}	Mg^{2+} ATPase	
		Oligomycin	
Treatment	ATPase	Sensitive	Insensitive
Saline	29.0 ± 1.29	8.62 ± 0.38	10.8 ± 0.36
Morphine Sulfate (10 mg/kg)	31.5 ± 0.93 (NS)*	8.67 ± 0.68 (NS)	11.8 ± 1.14 (NS)
Morphine Sulfate (30 mg/kg)	37.1 ± 0.98 (P < 0.0005)	9.20 ± 0.37 (NS)	13.6 ± 0.28 (P < 0.0005)
Naloxone (5 mg/kg)	28.0 ± 1.10	8.87 ± 0.54	10.3 ± 0.75
Naloxone + Morphine Sulfate+	23.9 ± 1.56 (P < 0.01)	6.92 ± 0.43 (P < 0.005)	10.3 ± 1.08 (NS)
Morphine Sulfate + Naloxone‡	28.0 ± 0.70 (NS)	7.74 ± 0.43 (P < 0.05)	10.6 ± 0.34 (NS)

*Not significant.

†Naloxone, 5 mg/kg, was injected, immediately followed by 30 mg/kg, s.c., morphine sulfate administration.

‡ Naloxone, 5 mg/kg, s.c., was administered 15 min after the injection of morphine sulfate, 30 mg/kg, s.c.

In the in vitro experiment, up to 1 mM morphine sulfate added to the reaction mixture showed no effects on all three types of ATPase activities determined (results not shown). However, as shown in Table 1, in mice receiving morphine, 30 mg/kg, s.c., 30 min prior to the sacrifice, both $Na^+ - K^+$-stimulated and oligomycin-insensitive Mg^{2+} ATPase activities in brain fraction were significantly increased by more than 25 per cent in comparison with those of the control group receiving saline. This increase in ATPase activities by morphine was antagonized by naloxone. The administration of naloxone, 5 mg/kg, s.c., alone has no effect on ATPase activities. However, it is interesting to note that in mice receiving naloxone (5 mg/kg, s.c.) immediately followed by morphine sulfate (30 mg/kg, s.c.) $Na^+ - K^+$-stimulated and oligomycin-sensitive Mg^{2+} ATPases were significantly inhibited instead.

Table 2. Effect of chronic administration of morphine on brain ATPase activities in the mouse

	Specific activities ± S.E.M. (μmoles Pi mg^{-1} protein hr^{-1})		
	$Na^+ - K^+$	Mg^{2+} ATPase	
		Oligomycin	
Treatment	ATPase	Sensitive	Insensitive
Normal	32.4 ± 2.13	9.18 ± 0.67	11.8 ± 0.93
Placebo	31.8 ± 0.60	9.43 ± 0.72	12.4 ± 0.36
Morphine	49.7 ± 4.28 ($P < 0.01$)	11.13 ± 0.67 (NS)	17.1 ± 0.96 ($P < 0.01$)

In mice rendered tolerant to morphine by s.c. implantation of a morphine pellet for 3 days, the treatment conveys about a 10-fold tolerance to the antinociceptive response to morphine (7,12,13). Brain $Na^+ - K^+$ and oligomycin-insensitive Mg^{2+} ATPase activities at this time point were further significantly increased to 54 and 44 per cent, respectively (Table 2), as compared to control animals implanted with placebo pellets. Brain $Na^+ - K^+$ and oligomycin-insensitive Mg^{2+} ATPase activities in morphine-tolerant animals were about 2-fold higher than those of the animals acutely treated with morphine.

In mice rendered dependent on morphine by pellet implantation, an injection of naloxone, ranging from 0.05 to 2 mg/kg, s.c., invariably provoked precipitated withdrawal signs within 2-10 min. Measurement of brain ATPase activities at 10 min after the administration of different doses of naloxone revealed that naloxone prevented the stimulation of ATPase

activities induced by chronic morphine administration. The decrease in brain levels of $Na^+ - K^+$ ATPase activity in morphine-dependent animals affected by naloxone was dependent on the dose of naloxone. Figure 1 shows the brain ATPase activities at 10 min after the injection of various doses of naloxone in morphine-dependent animals; only 0.05 mg/kg was required to produce a significant effect of $Na^+ - K^+$ ATPase activity over control ($P < 0.0005$). Doses higher than the 0.1 mg/kg dose, that is, 0.5 and 2 mg/kg, did not produce a further reduction of $Na^+ - K^+$ ATPase activity. Mg^{2+} ATPase activities, on the other hand, did not exhibit a dose-dependent response with naloxone administration (Fig. 1).

Our preliminary studies indicate that both dopamine and norepinephrine enhance the $Na^+ - K^+$ ATPase activity in naive but not in morphine tolerant-dependent mouse brain. The dopamine stimulatory effect was not observed in chronic morphine-treated animals. These findings provide an incentive to seek a possible relationship between morphine, neurotransmitters and ATPase systems.

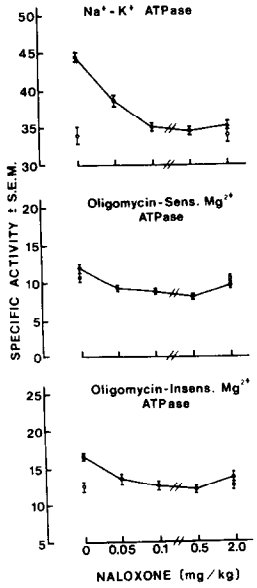

Fig. 1. Effect of naloxone-precipitated withdrawal on morphine-dependent brain ATPase activities. Each value represents the mean of four (N = 4) brain fractions. Each fraction was assayed 2-3 times and the average was taken. Open circles represent the control specific activities in normal mouse brain without and with naloxone treatment.

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