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PRELIMINARY COMMUNICATIONS

EFFECT OF MORPHINE ON CYSTEINE OXIDASE ACTIVITY IN THE BRAIN AND REPRODUCTIVE TRACT OF RATS

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The function and structural integrity of the secondary sex organs are quite sensitive to alteration in nutritional state and to plasma levels of testosterone (1). The influence of testosterone on the concentrations of hypotaurine and taurine in the reproductive tract of the rat, guinea pig and mouse has been shown by Kochakian (2), in continuation of his previous studies in which testosterone was shown to induce protein synthesis in many tissues, in addition to the accessory sex organs. Evidence is accumulating (3,4) that chronic morphine administration produces a marked reduction in the wet and dry tissue weights of the secondary sex organs of the male rat; this reduction is associated with a decrease in plasma testosterone levels in blood.

The purpose of this study was to determine whether reduction of testosterone levels in blood, due to morphine pellet implantation, has any effect on the activity of cysteine oxidase (cysteine dioxygenase, EC 1.13.11.20), the enzyme which oxidizes cysteine into cysteine sulfinic acid, a precursor for taurine biosynthesis (5), in the brain and in the secondary sex organs (testes, prostate glands and seminal vesicles); the presence of cysteine oxidase has been shown in the rat brain (6) and in testes (7).

MATERIALS AND METHODS

L-(^{35}S)cysteine HCl was purchased from the Amersham Searle Co., Arlington Heights, IL; Dowex-50W-H^+ and NAD^+ were purchased from the Sigma Chemical Co., St. Louis, MO; and Triton X-100 from Packard Inc., Downers Grove, IL.

Animals, tissue preparation and enzyme assay

Male rats of the Holtzman strain were briefly anesthetized (ether-ethyl chloride, 50:50) and a 1 cm incision was made on the back of the neck. Seventy-five mg morphine (monohydrate) or placebo (lactose) pellets (8) were then inserted under the skin 1-2 cm

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away from the incision and the wound was closed with a wound clip. After 3 days of pellet implantation (2), the rats were decapitated and the brains, testes, seminal vesicles, and prostate glands were removed. The organs were dissected free from connecting tissue and fat, chilled in 0.9% saline, and then blotted, weighed and homogenized with a Teflon pestle tissue homogenizer at 4^0 in 0.05 M sodium phospate buffer, pH 6.8 [containing 0.32 M sucrose and 0.05 mM Fe^{2+} in a ratio of 1:9 (w/v)]. Supernatant fluid was obtained by centrifuging the homogenate at 10,000 g for 20 min at 4^0. Brain homogenate and supernatant fractions of other tissues were used for determination of cysteine oxidase activity by a modified method (7) based on a previous report (6). Protein was determined by the procedure of Lowry et al. (9), using bovine serum albumin as a standard.

RESULTS

The effects of morphine and placebo implantations on rat cysteine oxidase activity are shown in Table 1. In control (placebo) animals, the activity of cysteine oxidase found in brain and testes is in agreement with previous results (6,7). A marked reduction in cysteine oxidase activity was observed in brain, testes and seminal vesicles in the experimental (morphine-implanted) group, in comparison to the control. In the prostate glands the activity was slightly less than in the control, but the difference was statistically insignificant.

TABLE 1. Effect of morphine implantation on cysteine oxidase activity in rat brain and secondary sex organs*

Tissue	Specific activity of cysteine oxidase+	
	Control	Experimental
Brains	1.06 ± 0.14 (4)	0.71 ± 0.05‡ (3)
Prostate glands	0.12 ± 0.05 (3)	0.11 ± 0.02 (4)
Seminal vesicles	0.93 ± 0.18 (3)	0.55 ± 0.12§ (4)
Testes	0.48 ± 0.05 (4)	0.32 ± 0.02‡ (4)

* Each result represents the mean ± standard error of a separate experiment. The numbers in parentheses represent the numbers of animals used. Significance was determined by Student's t-test, using a two-tailed table.

+ Specific activity is expressed in μmoles cysteine sulfinate formed/hr/mg of protein.

‡ $P < 0.01$.

§ $P < 0.05$.

DISCUSSION

The activities of enzymes in tissues may depend on the rate of their synthesis and/or modulation by various effectors. Narcotics have been shown to act directly on the testes to inhibit testosterone synthesis by virtue of their inhibitory effects on RNA and protein synthesis (10). The effects of morphine on decreased testosterone levels, and related effects in narcotic users, however, may be primarily through the hypothalamus pituitary-gonadal axis. The present study indicates that a brief period of morphine implantation results in a significant reduction in the cysteine oxidase activity of the two principal secondary sex organs of the rat, the seminal vesicles and the testes. Under similar conditions, atrophy accompanied by a marked reduction in the secretory material in the seminal vesicles (4), characteristic of starvation or castration (1), has been shown. Such atrophy, however, was not observed in rat testes and brain (4). Therefore, atrophy cannot be the cause of a decrease in cysteine oxidase activity in these organs.

Steroid hormones are known as potent inducers of many enzymes (11), exerting their control either at transcriptional (12) or post-transcriptional (13) levels. Though the vertebrate brain is most susceptible to sex hormones in the perinatal period (14), several studies have shown that sex hormones play a major role in the maturation of the central nervous system (15) and in the maintenance of adult sexual behavior. Nevertheless, administration of gonadal hormones to adults alters neurobehavioral patterns to a lesser extent (16). Castration and testosterone (2) did not change the concentration of taurine in the seminal vesicle, but in prostate glands castration resulted in a 2-fold increase which returned to normal after testosterone treatment. Hypotaurine, a precursor of taurine, however, nearly disappeared in both the prostate and seminal vesicle after castration, and was restored completely or partially to normal by testosterone administration. Therefore, reduction in the level of testosterone in the blood (5,7) in morphine-implanted rats may account for the reduction of cysteine oxidase in these tissues; however, a direct effect of morphine on the synthesis and/or biodegradation of this enzyme cannot be ruled out, at present, in spite of the fact that the influence of testosterone on the concentration of hypotaurine and taurine in the reproductive tract of the rat and other animals has been shown (2).

The results of this study may have significant implications for the human narcotic addict if, of course, a similar reduction in cysteine oxidase activity occurs during narcotic dependence, since taurine has been proposed as a modulator or neurotransmitter in mammalian CNS (17) and its involvement has been suggested in some aspects of the maturation and/or function of the sperm (2). In light of the present study, recent findings of cysteine sulfinic acid and hypotaurine in human adenomatous prostate glands (18) warrant future study of cysteine oxidase in such cases.

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