MODULATORY ACTION OF TESTOSTERONE ON MORPHINE-INDUCED CHANGES OF MICE BRAIN PROLACTIN

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SUMMARY: Interactions between sex hormones and opioid system in the brain may play a functional role in the regulation of pituitary hormone release in animals. In this study the effect of chronic administration of morphine on the brain levels of prolactin in gonadectomized and testosterone treated mice were examined. Castration of male animals caused a reduction of about 90% in the brain level of prolactin as compared to that of intact animals. Moreover administration of increasing doses of morphine (40 to 100 mg/kg) for a period of 9 days resulted in a reduction of 30% in the prolactin level in both intact and gonadectomized mice. However administration of testosterone to gonadectomized morphine treated animals resulted in a significant increase (about 40%) in brain prolactin level as compared with control values.

It is suggested that testosterone at its physiological concentration may not be involved in modulatory action of morphine on prolactin secretion, whereas there is a significant interaction between testosterone and opiates for modulation of prolactin secretion when the testosterone concentration is higher than physiological level.

Key Words: Gonadectomize, testosterone, morphine, prolactin, neuroendocrine.

INTRODUCTION

Several lines of evidence indicate that endogenous opiate-like peptides might be involved in mechanisms of release of pituitary hormones (1-3). Recent studies by infusion of long-acting analogues of metencephalin have demonstrated that prolaction secretion increased immediately after the opiate administration (1,4). Moreover, it has been demonstrated that acute and chronic treatment with opioids depress serum testosterone level and disrupt the function of sex organs in male animals (3). It is also well known that the sex steroids in general and testosterone in particular are metabolized to hydroxylated metabolites in the brain and anterior pituitary (4-6). These findings suggest that testosterone might play a physiological role in the neuroendocrine regulation in male animals. In order to determine if the opiate involvement on the prolactin secretion is affected by testosterone, we have examined the effects of chronic administration of morphine on the brain levels of prolactin in gonadectomized and testosterone treated mice. The results do not reveal any marked differences between the effects of morphine on the brain level of prolactin in gonadectomized and intact male mice.

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MATERIALS AND METHODS

Animals and treatment

Male adult Albino mice (Pasteur Institute, Tehran, Iran) with a body weight of 25-35 g were used for this study. Six groups each of five animals were kept in separate cages with natural lighting at a room temperature of 21±1°C, with water and food ad libitum. To study the chronic effects of morphine a sulfate derivative solution was administered subcutaneously (s.c.) twice daily (8 am. and 5 pm.). Doses of morphine increased from 40 mg/kg (day 1) to

100mg/kg (day 9) as described by Heron and Shinitzky (7). The effect of testosterone was examined in gonadectomized mice, morphine treated intact mice and morphine treated gonadectomized mice. In these experiments testosterone propionate (250 mg/kg) in almond oil was injected i.p. 1 h before each injection of morphine in saline (in groups that did not receive morphine). Control animals were injected with the same volume of almond oil 1 h before injection of saline.

The animals were killed by decapitation and the brains were removed and homogenized immediately in ice cold 15 mM sodium phosphate buffer (pH 7.4) containing 0.1% Triton X100 in an ice bath using a glass-Teflon homogeniser to give 1: 6(W/V) homogenate. This was centrifuged at 10000 g for 10 min at 4°C. The pellet was discarded and the supernatant was aliquoted and frozen at -20°C until assayed.

Prolaction levels in the samples were assayed in duplicate by RIA Kits with ^{125}I-tracer (Diagnostic Product Corporation), which used according to the manufacturer's instructions. The interassay coefficient of variation for the assay performed using 10 replicates of pooled serum was 0.62%. All samples were assayed in a single run and therefore no interassay variability was determined. Results are expressed as ng/mg supernatant protein. The concentration of protein in the supernatant was measured by the method of Lowry *et al.* (8) using bovine serum albumin as standard.

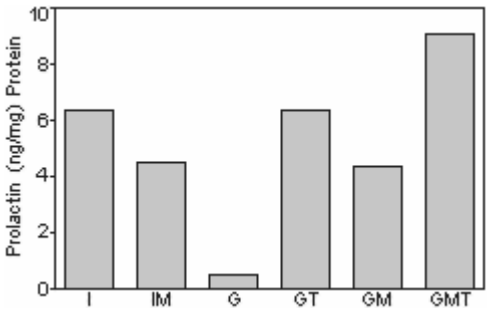
RESULTS AND DISCUSSION

Figure 1 shows the effects produced by indicated doses of morphine and testosterone on the levels of prolactin in the brain of intact and gonadectomized mice. Increasing doses of morphine (40 to 100 mg/kg) for a period of 9 days decreased prolactin levels of the brain by about 30% ($p<0.05$). Similar reduction in the levels of the hormone was also observed in the brain of morphine

Table 1: Prolactin level in the brain of intact and gonadectomized mice after treatment with morphine and/or testosterone. For more details see text. Results represent the mean values ± SEM.

Treatment	Prolactin Conc. (ng/mg)	P value
Intact	6.4 ± 0.3	
+ Morphin (IM)	4.4 ± 0.12	0.05
Gondectomized (G)	0.4 ± 0.02	0.005
G + Testosterone (GT)	6.3 ± 0.25	0.05
G + Morphin (GM)	4.3 ± 0.1	0.05
GM + Testosterone (GMT)	9.3 ± 0.35	0.05

Figure 1: Effect of morphine and testosterone on brain prolactin content of intact and gonadectomized mice. Intact mice (I), morphine treated intact mice (IM), gonadectomized mice (G), gonadectomized testosterone treated mice (GT), gonadectomized morphine treated mice (GM), gonadectomized morphine + testosterone treated mice (GMT), The results represent the mean values of four separate experiments carried out in duplicates, with SEM less than 10%. Results are expressed as ng/mg protein of the brain supernatant.



treated gonadectomized mice ($p<0.05$). However, the reduction in the hormone level of the gonadectomized animal that did not receive morphine was significantly greater than that in morphine treated animals (90%) ($p<0.005$). As shown in Figure 1 administration of testosterone propionate (250 mg/kg) to gonadectomized animals reversed the reduction in brain prolactin level to the control values. However injection of testosterone to morphine treated gonadectomized animals caused about 40% increase in brain prolactin level compared with control values ($p<0.05$). The finding that in intact animals gonadectomy led to the depression of brain prolactin level is in agreement with the results obtained from serum prolactin determinations. Thus it has been demonstrated that prolactin secretion into the blood may be stimulated by endogenous (4,10) or exogenous opioids (2,11). The decrease in prolactin level of the brain suggests that morphine increases the rate of prolactin release from the storage vesicles. Moreover, morphine caused a similar reduction in the level of hormone in the gonadectomized animals ($p<0.05$) (Table 1). It is reasonable to suggest that testosterone at it's physiological concentrations may not be involved in modulatory action of morphine on prolactin secretion. However, morphine in testosterone treated animals increased the prolactin concentration of the brain.

These results suggest that testosterone in higher than physiological concentrations may play an important role in modulator action of opiates on prolactin secretion.

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