MORPHINE INHIBITS TESTOSTERONE SECRETION FROM RAT LEYDIG CELLS IN VITRO

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ABSTRACT

Objective: To study the effect of morphine on in vitro production of testicular T from un-stimulated or LH stimulated Leydig cells.

Study design: Experimental study.

Place and Duration: Biological and Biomedical Sciences Department, Aga Khan University Karachi.

Materials and Methods: De-capsulated testes from two rats were subjected to enzymatic dispersion for each experiment. Cells were pre-incubated in the incubation medium to remove endogenous Testosterone Thereafter, 80K cells were used for each treatment in 200 µl of suspension. Incubation medium contained either no treatment (control) or different concentrations of morphine (5x10-5 -10-9 M). Leydig cells were also incubated with different doses of LH (16-500 µIU) and morphine. Naloxone, an opioid receptor antagonist (10-6 M), was used to determine if morphine induced effects on the Leydig cells could be reversed. Incubations were carried out for three hours in a shaking water bath at 34^0C. Reaction was stopped by placing the tubes in a water bath at 60^0C, and thereafter the tubes were transferred to a tray containing ice before freezing. Testosterone was measured directly in the incubation medium by radioimmunoassay.

Results: Morphine reduced Testosterone production from Leydig cells at all concentrations tested (p<0.01). There was a dose dependent response to LH stimulation by the Leydig cells and testosterone levels were significantly different from control (p<0.01) and incubation with morphine in the presence of LH significantly reduced T levels (p<0.01). Inhibition of opioid receptors with naloxone significantly attenuated morphine induced inhibition of T (p<0.01).

Conclusion: These in vitro results show that the morphine inhibits T secretion directly at the testis level.

Key words: Morphine, testes, testosterone secretion, rat

INTRODUCTION

Regulation of the steroid secretion from the gonads in the male and female is under complex regulatory mechanisms that include the feedback actions of gonadal steroids hormones and release of many neurotransmitters at the level of hypothalamus and the gonads. Among the neurotransmitters that affect the release of gonadal secretions are endogenous opioid peptides like enkephalin, dynorphin, beta endorphins etc.

If morphine decreases LH secretion then long term abuse of this drug may result in decrease in gonadal functions. Moreover, it will be interesting to investigate that if morphine has any direct effect on gonadal functions. In the present study Leydig cells from rat testes have been used in an in vitro setup to investigate effect of morphine on the testosterone secretion. Opioid peptides have been localized in a variety of peripheral tissues like placenta, thyroid gland, pancreas, gastrointestinal tract, in the reproductive tract of male and female and in the testis of rats[1]. Since the discovery of endogenous opioids peptides and opioid receptors in the brain, there has been considerable interest in their possible role in various physiological and pharmacological processes, especially in the modulation of reproductive functions through its effects on the hypothalamo-pituitary-gonadal axis. Intrathecal administration of opiods for pain management in patients with intractable non-malignant pain, causes hypogonadism by reducing the levels of gonadotrophins[2,3]. These endogenous opioid peptides form an important constituent of neuro-endocrine mechanism which affect body

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weight and the development of the testis[4,5]. It has been shown that opiates cause their effect through hypothalamic –pituitary-gonadal axis rather than through local gonadal mechanism[6]. Opioids have also been known to play the role of a potentially important paracrine regulator of testicular steroidogenesis in stress[7]. Studies with naloxone suggest that β endorphin may play an important role in steroidogenesis and also regulate seminiferous tubule transport[1]. The inhibitory effect of opioids depends on concentration of sex steroids[8]. Plasma and CNS sex hormone levels have also been shown to be affected by opioid treatment[9]. Glial cells have also been shown to decrease aromatase activity in response to morphine treatment[10]. Some in vivo and in vitro studies show that the endogenous opioid peptide (morphine) inhibits the reproductive function acting via the CNS. Endogenous opioid peptide has been shown to inhibit gonadotrophin secretion in rodents and humans via GnRH inhibition and inhibit pituitary function by interacting with specific binding sites on the gonadotrophs[11-13]. This LH inhibition leads to decrease in LH dependent testicular steroidogenesis[14-18] and spermatogenesis[19,20]. This hypothesis has been supported by other studies that have reported that opioid exert little or no effect on the pituitary or gonads[21-23]. Moreover, numerous in vitro Leydig cell culture studies also have also shown that opioid peptides have no effect on basal and stimulated testosterone production[21,23-25]. In contrast, only one in vivo study[26] supported the hypothesis that opioids inhibit testosterone secretion through direct effects on the testis. This report is also in consistence with other in vivo evidences that endogenous opioid peptide inhibit testosterone secretion[27-32].

However, despite the well-known role of opioids in modulating testicular steroidogenesis, conflicting results have been reported. This is because of interfering regulatory factors affecting this process in an in vivo set up. In vitro models are extremely useful in assessing the direct effects of drugs on testicular function. In order to investigate that there is any significant effect of morphine on testosterone production from Leydig cells, we have studied the effects of morphine and naloxone on testosterone production from rat Leydig cells in vitro.

MATERIALS AND METHODS

Two Sprague Dawley male rats (weight 218.5±24.2g) were used for each experiment. These rats were obtained from AKU animal facility, where they were maintained under standard conditions of 14-hour light and 10-hour dark cycle.

Leydig cells Incubation

Direct effect of morphine on the Leydig cells testosterone production was studied by challenging Leydig cells with morphine in vitro. Leydig cell were obtained from rats killed by decapitation using a guillotine. Testis were removed immediately and decapsulated. Leydig cells were isolated as described previously[33]. Briefly, Leydig cells were pre-incubated for 1 hr, the media were replaced with either fresh medium or medium containing increasing dose of morphine (10^{-9} – 10^{-6} M) alone and in combination with naloxone (10^{-6} M) in triplicate. In addition, in order to test the ability of morphine to modulate LH stimulated testosterone secretion, samples were challenged with either different doses LH (16 – 500μIU) alone or in combination with different doses of morphine (10^{-9}-10^{-6} M). After 3 hrs incubation reaction was stopped by dipping the tubes in water bath at 60°C for 10 min. Samples were kept frozen until testosterone was measured by a highly specific radioimmunoassay.

Radioimmunoassay

Testosterone was measured in the incubation medium directly by a highly sensitive RIA according to WHO protocol, using 3H labeled testosterone, as tracer and a highly specific antiserum for testosterone from Guildhay UK. Serum and testicular testosterone was measured in extracted samples in duplicate, whereas RIA reagents were directly added to tubes containing incubation medium. After addition of all the reagents, tubes were incubated for 30 min at 4°C. The bound and

unbound fractions were separated by the addition of 0.1 % charcoal. Radioactivity was measured in a liquid scintillation counter. Testosterone concentration was calculated by Logit – log transformation[34].

The sensitivity of testosterone assay was 0.0125 ng and the intra-assay coefficient of variation was < 10%. The levels of testosterone in the media were expressed as ng/ml.

Statistical Analysis

Data are expressed as mean±SEM. Results from Leydig cell incubations were analyzed for statistically significant differences using a one-way ANOVA by using SPSS, followed by Turkey's test. A value of $p<0.05$ was considered significant.

RESULTS

Treatment with morphine was able to inhibit both basal and stimulated testicular testosterone secretion from Leydig cells in vitro. However, this inhibition was not morphine dose dependent. Stimulation of Leydig cells with LH showed a dose dependent response. Morphine was also able to significantly inhibit the LH stimulation at all doses tested. Treatment of Leydig cells with naloxone reversed the inhibitory effect of morphine and enhanced maximal testosterone secretion in both basal and LH stimulated production of testosterone. (Table-1)

Table 1 shows effect of treatment of Leydig cells with different doses of morphine 10^{-9}–10^{-6}M. Treatment with morphine significantly ($p<0.05$) inhibited basal testosterone release at all the doses. However, magnitude of this inhibition was not dose dependent in a graded manner. Inhibition with morphine was blocked by naloxone 10-6 M. This naloxane block was significant only at the morphine dose of 10^{-8}($p<0.05$).

Figure 1 shows the LH stimulated testosterone secretion from Leydig cells. LH doses ranging from 31.25µIU to 500µIU were used. There was a dose dependent increase in the Leydig cells response. At all LH concentrations it showed significant ($p<0.05$) difference between control and LH treatments.

Table 2 shows effect of fixed dose of morphine (10-6 M) on LH (62.5-500 µIU) stimulated testosterone secretion. Morphine significantly inhibited LH stimulated testosterone secretion at 250 ($p<0.05$) and 500µIU ($p<0.05$). At both of these LH doses effect of morphine was significantly ($p<0.05$) attenuated by naloxone treatment (Table 3).

Figure 2 shows effect of different doses of morphine on the LH stimulated testosterone secretion. Compared with LH alone morphine significantly ($p<0.05$) inhibited testosterone secretion from the Leydig cells both at 10^{-6} and 10^{-9} M concentrations. Addition of naloxone to

Table-1: effect of different doses of morphine and naloxone on invivo testosterone secretion by the leyding cells.

	MEAN T ng/ml	SEM ng/ml
Control	0.67991	0.00737
Mor10-9	0.50248	0.00385
Mor 10-9+Nal 10-6	0.49918	0.01425
Mor10-8	0.44718	0.00586
Mor 10-8+Nal10-6	0.58619	0.04114
Mor10-6	0.43384	0.0058
Mor 10-6+Nal10-6	0.51095	0.02368
Mor10-5	0.48114	0.00623
Mor 10-5+Nal 10-6	0.55891	0.01922

Table-2: Effect of different doses of lh with fixed doses of morphine and nalaxone on *in vitro* testosterone secretion by the leydig cells

	Mean T ng/ml	SEM ng/ml
Control	0.68	0.007
LH16 µIU	0.75	0.080
LH 16+Mor10-6	0.58	0.016
LH 16+Mor10-6+Nal10-6	0.74	0.008
LH 62.5	1.52	0.011
LH 62.5+Mor 10-6	1.51	0.059
LH 62.5+Mor 10-6+Nal10-6	1.55	0.058
LH250	3.267	0.045
LH 250+Mor 10-6	2.399	0.063
LH 250+Mor 10-6+Nal10-6	3.468	0.020
LH500	4.24	0.045
LH 500+Mor 10-6	0.59	0.005
LH 500+Mor 10-6+Nal10-6	3.17	0.239

Table 3: _____

	Mean T ng/ml	SEM ng/ml
Control	0.68	0.007
Control LH 500µL	4.237	0.045
LH 500+Mor10-9	0.581	0.007
LH00+Mor10-9+Nal 10-6	3.612	0.108
LH 500+Mor10-6	0.594	0.005
LH 500+Mor 10-6+ Nal 10-6	3.174	0.239

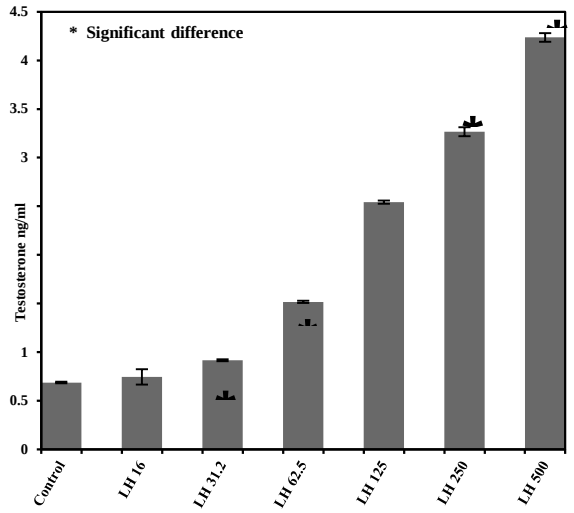
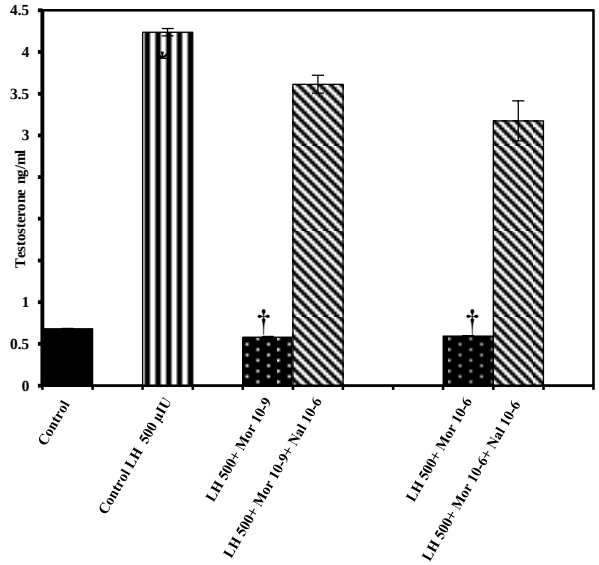


Fig. 1: LH Dose Response Curve of Rat Leydig Cells



the incubation medium reversed morphine induced inhibition of the LH stimulated testosterone secretion (p<0.05).

DISCUSSION

Our data provides evidence for a direct inhibitory role of morphine in the control of basal and stimulated testicular testosterone production in adult rats, in vitro. We also report that morphine blocks the LH stimulated release of testosterone in this specie. Although this effect has been reported in an in vitro set up but in vivo, complex interactions between various systems and cell types that regulate steroidogenesis are also involved which do not necessarily reflect the direct action of these compounds. Our results show that morphine induced inhibition of steroidogenesis can be reversed by treatment with naloxone, suggesting that morphine acts on the testes through opioid receptors in the testes. Furthermore, morphine also suppressed testosterone secretion from LH stimulated Leydig cells. Naloxone was also able to reverse morphine induced inhibition and restore the dose related steroidogenesis pattern of LH. Evidence that inhibitory effect of morphine can be reversed by the opioid antagonist suggests that Leydig cells have opioids receptors. This study supports the hypothesis that both endogenous and exogenous opioids affect the reproduction by modulating the testicular secretion.

Our results are contrary to the more widely accepted hypothesis that opioids do not act on the Leydig cells directly[22,29]. In another study culture of Leydig cells with β-endorphin or naloxone manifested no significant changes of un-stimulated or hCG-stimulated testosterone secretion in 20 or 60 day old rats[23]. However, studies with naloxone have suggested that beta-endorphin may have an important role to play in steroidogenesis and may have a role in regulating transport of luminal material[1].

Our results are consistent with the previous in vivo evidence[28]. In this study, investigators directly injected naloxone into the rat testes which resulted in increase in serum testosterone levels. Later it was observed that morphine exerts effects on the testicular

functions which are independent of their action on LH and that both endogenous and exogenous opioids disrupt two major aspects of testicular function i.e. testosterone secretion and interstitial fluid formation[26].

CONCLUSIONS

Our results indicate that morphine exerts a dose dependent effect directly at the level of testis besides its indirect effect and this effect may be reversed by an opioid antagonist. They also suggest that opioid- induced changes in testosterone secretion may explain the well-established effects of opioids on reproductive endocrinology and function in the male.

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