The Pharmaceutical and Chemical Journal, 2017, 4(5):66-72

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Evaluation of the Effects of Morphine on Sex Hormones in Wistar Rats

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Abstract Introduction and Objective: Morphine is one of the most important alkaloids of the opioid family and its most effective compound. Morphine is a very potent analgesic and narcotic drug. Considering the widespread use of morphine and its derivatives, its effects on hormones, especially sex hormones, are very important. The aim of this study was to evaluate the effect of morphine on sex hormones and its effectiveness differences according to sex.

Materials and Methods: This applied study was performed on 38 adult Wistar rats. The serum levels of 17-beta-estradiol, progesterone, and testosterone were measured using biochemical kits. Statistical tests, t-test, paired t-test, and ANOVA were used to analyze the results.

Discussion: Our findings showed that the level of testosterone decreased significantly during 1 week after morphine use when compared to the control group. The decrease in the testosterone level continued to the 4th week with continuing the use of morphine. Our results showed that the hormonal levels did not change significantly between the 1st and the 2nd, and between the 1st and the 3rd week.

The level of progesterone decreased significantly in the 2nd week compared to the 1st week ($P<0.002$); however, the comparison between the 2nd and the 1st week was not significant by sex. No significant relationship was observed between morphine use and the level of estradiol.

Conclusion: The level of testosterone decreased significantly following the use of morphine. The decrease is definitely associated with decreased libido and fertility in the male sex. The level of progesterone decreased significantly with the use of morphine, which may be associated with the pregnancy status and fetal preservation during pregnancy.

Keywords Morphine, Testosterone, Progesterone, Estradiol

Introduction

Morphine is one of the most important alkaloids of the opioid family and its most effective compound. Morphine is a very potent analgesic and narcotic drug [1, 2]. This drug undergoes biotransformation in the kidneys, digestive tract, and liver and is excreted through the kidneys [3, 4]. Its mechanism of action is through affecting the central nervous system (CNS); it decreases the pain sensation and produces an analgesic effect. Tolerate morphine treatment depends on the physical and psychological conditions of the patient [5, 6]. Human and animal studies have shown that morphine is converted to morphinone 10 by morphine 6-dehydrogenase and after conjugation and is mainly excreted via the bile [2]. Morphine is available in three forms of oral (pill and capsule), injection (IV, SC, IM, and peritoneal), and suppository although it is possible to use it through inhalation, as well [7]. The use of morphine is recommended for reducing the pain in hospitalized patients and cardiac patients; pain associated with sickle cell anemia wound, cancer, and renal stone; surgical and post surgical pain; severe chronic pain; and severe low back pain. Morphine is also administered as a adjuvant in anesthesia and to control cough and diarrhea [8-11].

The side effects of morphine are different in different tissues; for example, it leads to inhibition of stimulation of the nervous system, causing changes in the body temperature and drowsiness [12] or lowers the blood pressure in cerebral vasculature and increases the ICP in the cardiovascular system [13]. Considering the wide spread use of morphine and its derivatives, its effects on hormones, especially sex hormones, are very important. This compound may have significant effects on sex hormones, especially estrogen, progesterone, and testosterone. According to a review of the literature, most studies have shown the devastating effects of morphine on hormones. Many sexual and psychiatric diseases have been attributed to the direct or indirect use of morphine [1, 4-6, 11, 14]. Since morphine and its derivatives are used in medicine and the scientists have not been able to a compound with a similar efficacy without the side effects of morphine, determining the efficacy of morphine can be the first step toward finding a way to decrease its side effects. The aim of this study was to evaluate the effect of morphine on sex hormones and different efficacy of morphine according to gender.

Materials and Methods

This applied study was performed on 38 adult Wistar rats (19 male and 19 female) weighing 18-20 gr as animal models. The rats were purchased from Pasteur Institute, Karaj, Iran. The animals were kept at 23±2 ºC in 12:12 h light/dark cycles and were fed condensed food for rodents (Pars Dam Tehran Co.).

Male and female rats were kept separately. The rats were randomly divided into two groups:

Group 1: Twenty-six rats (13 female and 13 male) that received morphine 5mg daily for 4 weeks intraperitoneally. In this group, 6 rats (3 male and 3 female) were sacrificed with high dose pentobarbital every week. Eight rats were sacrificed in the last week.

Group 2: Twelve rats (6 male and 6 female) were used as controls. Twenty-four hours after the last injection, 2ml blood was taken from the animal's heart.

Serum was separated from the blood and stored at -70 ºC which was then used for the measurement of the indexes.

- A 17-beta-estradiol kit purchased from Dia Metra Co. (Lot No.DeM003-10) was used for the measurement of 17-beta-estradiol. The ELISA method was used for measurement. 17 betaestradiol (antigen) in the sample competes with 17-beta-estradiol conjugated with horseradish peroxidase to bind to limited number of anti 17-beta-estradiol antibody covered on the microplate (as the solid phase). The intensity of the color is reversely associated with the concentration of 17-beta-estradiol in the sample [unit: pg/ml)
- A progesterone kit purchased from Dia Metra Co. (Lot No.DKO006) was used to measure the level of progesterone using the direct ELISA method. The principles of this method are similar to the method used for the measurement of 17-beta-estradiol [unit: ng/ml).
- A testosterone kit purchased from Dia Metra Co. (Lot No.DCM002-10) was used to measure testosterone using the direct ELISA method. Testosterone binds to sex hormone binding globulin (SHBG) and other proteins like albumin. The unbound testosterone (less than 1% of total testosterone) is known as free testosterone. The chemical formulation of this method allows the release of testosterone from binding proteins. Therefore, this method measures the concentration of total testosterone (bound + free). The principles of the method are similar to previous methods.

T test, paired t test, ANOVA, Mann Whitney, Wilcoxon, and Kriskal Wallis were used for analysis.

Results

Table 1: Correlation of the serum levels of testosterone, progesterone, and estradiol in the first and second week

Number of Rats	Condition	P Value
53	The level of testosterone in the first week and in the second week	0/162
53	The level of progesterone in the first week and in the second week	* 0/001
53	The level of estradiol in the first week and in the second week	0/441

This evaluation showed that progesterone decreased significantly after receiving morphine in the second week as compared to the first week.

Table 2: Correlation of the serum levels of testosterone, progesterone, and estradiol in male rats in the first and second week

Number of Rats	Condition	P Value
21	The level of testosterone in the first week and in the second week	0/345
21	The level of progesterone in the first week and in the second week	0/02
21	The level of estradiol in the first week and in the second week	0/003

The evaluation showed that none of the hormones changed significantly in male rats after receiving morphine in the second week as compared to the first week.

Table 3: Correlation of the serum levels of testosterone, progesterone, and estradiol in female rats in the first and second week

Number of Rats	Condition	P Value
26	The level of testosterone in the first week and in the second week	0/122
23	The level of progesterone in the first week and in the second week	0/351
23	The level of estradiol in the first week and in the second week	0/413

The evaluation showed that none of the hormones changed significantly in female rats after receiving morphine in the second week as compared to the first week.

Table 4: Correlation of the serum levels of testosterone, progesterone, and estradiol between groups in the first and second measurement

Condition	F	P Value
The level of progesterone in the first measurement	0/142	0/131
The level of estradiol in the first measurement	0/102	0/311
The level of testosterone in the second measurement	2/015	0/511
The level of progesterone in the second measurement	2/010	0/444
The level of estradiol in the second measurement	2/144	0/522

Table 5: Correlation of the serum levels of testosterone, progesterone, and estradiol in the first and second weeks of measurement in female rats

Condition	F	P Value
The level of testosterone in the first measurement	0/341	0/302
The level of progesterone in the first measurement	0/531	0/114
The level of estradiol in the first measurement	0/333	0/643
The level of testosterone in the second measurement	0/302	0/634
The level of progesterone in the second measurement	3/262	0/004*

Evaluations showed that the serum concentration of progesterone correlated significantly with an increase in the use of morphine in female rats.

Table 6: Correlation of the serum levels of testosterone, progesterone, and estradiol in the first and second weeks of measurement in male rats

Condition	F	P Value
The level of testosterone in the second measurement	2/531	0/503
The level of progesterone in the second measurement	3/101	*0/001
The level of estradiol in the second measurement	2/114	0/533
The level of testosterone in the second measurement	2/531	0/503

Evaluations showed that the serum concentration of progesterone correlated significantly with an increase in the use of morphine in male rats.

Table 7: Comparison of the serum levels of testosterone, progesterone, and estradiol between the two groups of rats (after 2 weeks)

	P Value
Testosterone	0/530
Progesterone	*0/000
Estradiol	0/501

The use of morphine was significantly associated with the serum level of progesterone.

Table 8: Comparison of the correlation of testosterone, progesterone, and estradiol in the first week between the control rats and the rats that received morphine for 1 week

	F	P Value
Testosterone	131/044	* 0/0
Progesterone	10/031	0/004*
Estradiol	2/005	0/533

Table 9: Comparison of the correlation of testosterone, progesterone, and estradiol in the first week between the control rats and the rats that received morphine for 2 weeks

	F	P Value
Testosterone	1/661	0/234
Progesterone	6/415	0/044
Estradiol	0/113	0/535

Table 10: Comparison of the correlation of testosterone, progesterone, and estradiol in the first week between the control rats and the rats that received morphine for 3 weeks

	F	P Value
Testosterone	1/661	0/234
Progesterone	6/415	0/044
Estradiol	0/113	0/535

Table 11: Comparison of the correlation of testosterone, progesterone, and estradiol in the first week between the control rats and the rats that received morphine for 4 weeks

	F	P Value
Testosterone	23/515	0/005*
Progesterone	22/323	0/003*
Estradiol	3/154	0/053*

Table 12: Comparison of the correlation of testosterone, progesterone, and estradiol in the first week between the control rats and the rats that received morphine for 4 weeks

	F	P Value
Testosterone	22/135	0/000*
Progesterone	10/322	0/002*
Estradiol	0/053	0/332

Discussion

Narcotics are a major challenge worldwide. Their frequent or excess use is associated with injury to the person, society, or both [15].

It has been reported that the use of oral narcotics like methadone is associated with hypogonadism in 89% of men and a decrease in estradiol, dihydrotestosterone, LH, and FSH [16]. These damages can be due to the adverse effect of these drugs on gonads and hormones that are involved with libido and reproduction.

It has been shown that the use of morphine as the major alkaloid of opium or narcotic peptides causes disorders in the neuroendocrine regulation of the release of the hormones from the anterior pituitary gland, especially decreased LH secretion [17]. Studies have shown that morphine had inhibitory effects on GnRH. Ghows et al also showed that

the use of morphine decreased LH significantly. They also found that the use of morphine decreased testosterone concentrations significantly [18, 19]. The decrease can be due to a decrease in the cellular function of the testes or decreased LH release [20]. Our study showed that the level of testosterone decreased significantly during one week of morphine use as compared to the control group. The testosterone level continued to decrease with continuing the morphine use to the 4^{th} week. Our evaluations showed that the level of the hormone did not change between the first and the second week, and also between the first and the third week.

The majority of the studies have shown that the reproduction system in the hypothalamic-pituitary- gonadal axis is affected by narcotics. The hypothalamus produces LHRH which causes secondary changes in LH and consequently testosterone. Some studies have shown that the pituitary glands and gonads are not directly affected by narcotics [20, 21]. There is evidence that narcotics change the mRNA level of GnRH and decrease GnRH biosynthesis [22]. Feedback inhibition of LH by gonadal steroids is regulated by narcotic drugs [23].

Ghosian Moghaddam et al showed that the use of morphine was not associated with a significant decrease in progesterone [24].

Sianati et al found that prescribed beforehand of estrogen and progesterone at physiologic amounts had substantial effects on motor responses and weight loss of the rats receiving morphine [25]. No other studies were found in this regard.

Our study showed a significant decrease in the second week as compared to the first week of morphine use ($P<0.002$) but this difference was not significant in rats by gender between the first and the second week.

The level of progesterone showed a significant decrease between the rats that received morphine and control rats ($P=0.00$). This significant decrease was continuously observed from week 1 to week 4. It seems that a significant decrease in morphine is associated with a significant decrease in the production of LH in rats receiving morphine [18].

The decreased LH production and its absence in the luteal phase of the menstruation cycle is associated with a decrease in progesterone production in this phase, which may have marked effects on fertility and libido of the female sex.

Ghosian Moghaddam et al showed that the level of estradiol was significantly associated with morphine use [24].

Our study showed that the level of estradiol did not decrease significantly in the second week versus the first week of morphine use in male and female rats. The decrease was not significant between control rats and the rats that received morphine for 1, 2, and 3 weeks. The relationship was only significant between the control rats and the rats that received morphine for 4 consecutive rats ($P<0.035$).

Ghosian Moghaddam et al showed that the morphine use per se did not decrease FSH and reported that there was no significant relationship between the morphine use and decreased FSH [24]. Our study showed no significant relationship between the morphine use and the level of estradiol. Therefore, since the increase in FSH in the follicular phase during follicular growth leads to an increase in estradiol, it can be concluded that the use of morphine has no significant association with estradiol production.

Conclusion

The level of testosterone decreases significantly following the use of morphine. This decrease is definitely associated with decreased libido and fertility in the male sex. The level of progesterone shows a significant decrease with the use of morphine, which can be associated with gestational conditions and fetal preservation during pregnancy.

Suggestions

More studies are warranted to evaluate the relationship between morphine use and gestation and fetal preservation during pregnancy. It seems that the use of morphine and probably other narcotics decreases fertility and fetal preservation, which should be confirmed in further studies.

Acknowledgements

This study was approved by the Research Deputy of Tehran University of Medical Sciences (project number: 19916-31-01-92). The authors wish to thank the Research Deputy of Tehran University of Medical Sciences.

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