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EFFECT OF ACUTE MORPHINE ADMINISTRATION ON HORMONAL MODULATION AND THEIR RELATION TO MALE REPRODUCTION

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Abstract

Twenty-four adult male Sprague-Dawley rats weighing 130-140 grams were used in this study. In experiment I. twelve intact adult sexually active male rats.were sub-divided into two equal groups. In group A, six animals were injected with 0.2ml saline 60 min. before experimentation, while, in group B, animals were injected with 10mg/kg. b. w morphine sulphate 60 min. before experimentation. In experiment II, twelve male rats were used to study the effect of acute morphine administration in castrated male rat (castrated two weeks earlier). Animals were subdivided into two equal groups. In group A, animals were injected with 0.2 ml saline 60 min. before starting the experimentation, while, in group B, animals were injected with 10 mg/kg b. w. morphine sulphate 60 min. before experimentation. In both experiments, exploratory behaviour parameters (sniffing, rearing and investigating movement) were recorded for both latency and frequency, sexual behaviour parameters (mounting, intromission and eiaculation) were also recorded. At the end of 30 minute-behavioural test, all animals were sacrificed and trunk blood samples were collected. LH and FSH and prolactine were assayed in blood serum. Epididymal spermatozoa were collected and examined for spem concentration (million / ml) sperm viability (%), sperm motility (%) and abnormal forms (%).

The obtained results indicated that, in experiment I, acute morphine administration resulted in a non-significant ($P<0.05$) changes in all of the exploratory behaviour, inhibition in all parameters of sexual behaviour, significant ($P<0.01$) decrease in LH and FSH, and significant ($P<0.01$) increase in prolactin level. There was a significant ($P<0.01$) decrease in sperm viability and motility, while, there were non-significant changes in sperm concentration and abnormal forms. In experiment II, acute morphine administration in castrated male rats resulted in a non-significant change on the exploratory behaviour; a complete inhibition of the sexual behaviour, and non-significant change in LH, FSH and prolactin level. It is concluded that, acute morphine administration affects significantly male reproduction, while, castration does not enhance the effect of morphine administration.

INTRODUCTION

The effect of opioids on different aspects of sexual function may be due to central mechanisms that may underline these effects which had not yet been determined. However, the role of opioids in the modulation of hormonal or neurotransmitter systems that may subserve different sexual aspects has been examined only within the last decade (Pfaus & Gorzalka, 1987 and Agmo & Paredes, 1988). Acute morphine administration has been claimed to increase or does not affect brain endogenous opioids peptides (EOPS) (Smyth, 1983).

The present study is an attempt to clarify the effect of acute administration of morphine on gonadotropins and prolactin levels, and its relation to male sexual performance on both sexually active male rats.

MATERIALS AND METHODS

Twenty-four adult male Sprague-Dawley rats weighing 130-140 grams were used in this study. Animals were fed mixed cereal diet together with green fodder and dried skimmed milk (waynforth, 1980).

Experiment I

Twelve intact male rats were used to study the effect of acute morphine administration in sexually active male rats. Animals were subdivided into two equal groups: Group (A) control group, six intact adult sexually active male rats were injected i.m. with 0.2ml saline 60 minutes before experimental conduction. Group (B), six intact adult sexually active male rats injected i.m. with 10mg/kg b.w. morphine sulphate 60min. before conduction of experiment. The dose of morphine was decided according to Wells (1968) and Agmo & Paredes (1988).

In both groups, after 60 minutes of injection, exploratory behaviour parameters (sniffing, rearing and investigating movement) were recorded for both latency (the time elapsed from the beginning of the experiment until end of acting performance by the rate (expressed in seconds) and frequency (the number of acts performed by the rat per 30 minutes). According to Clark *et al.* (1988), sexual behaviour parameters (mounting intromission and ejaculation) were also recorded for both latency (expressed in minutes) and frequency (Meyerson *et al.*, 1988). At the end of 30 minute-behavioural test, animals of both groups were sacrificed, and trunk blood samples were collected; serum was separated and kept at 20^{o}C until hormonal assay. LH and FSH hormones were assayed by direct RIA according to the method of Davidson and Henry (1974), and prolactin was assayed following the method of Djursing (1981).

Epididymal spermatozoa were collected and examined for sperm concentration (million/ml), sperm viability and motility. Abnormal forms were determined from film stained with eosin & negrosin (Blom, 1983).

Experiment II

Twelve male rats were used to study the effect of acute morphine administration in castrated male rats. Animals were subdivided into two equal groups. Group I. Control castrated group, six castrated rats (operated upon two weeks earlier) were injected i.m. with 0.2 ml saline. Group II. six male castrated rats (castrated two weeks earlier) were injected i.m. with 10 mg/kg b.w. morphine sulphate.

After 60 minutes of injection, both exploratory and sexual behaviour parameters were recorded, blood samples were collected for hormonal assay as in experiment I.

Statistical analysis

Student "t" test was applied to compare between the treated and the control groups in each experiment (Snedecor and Cochran, 1967).

RESULTS

Experiment I

Acute morphine adminstration (10 mg/kg b.w. i/m) resulted in a statistically non-significant changes ($P<0.05$) in all of exploratory behaviour (Sniffing, rearing and investigating movement) in both latecy and frequency (Table 1).

Table 1. Effect of morphine treatment on exploratory behaviour in male rats.

Parameters	Group A (control)		Group B (treated)	
	Latency (sec.)	Frequency	Latency (sec.)	Frequency
Sniffing	2.00±0.00	51.00±0.58	2.00±0.00	50.00±0.58
Rearing	7.00±0.26	54.00±0.37	7.00±0.26	55.00±0.67
Investigating Mov.	150.00±0.37	8.00±0.26	146.00±0.58	9.00±0.37

Effects of acute morphine administration on musculine sexual behaviour are shown in Table 2. There was a statistically significant ($P<0.01$) inhibition in all parameters of sexual behaviour.

Table 2. Effect of morphine treatment on sexual behaviour of male rats.

Parameters	Group A (control)		Group B (treated)	
	Latency (min)	Frequency	Latency (min)	Frequency
Mounting	5.23±0.07	6.83±0.31	1.80±1.14*	2.5±1.59
Intromission	5.55±0.11	8.83±0.31	1.83±1.17*	1.17±0.17
Ejaculation	14.90±0.42	---	5.15±3.26*	---

* Significant at level P<0.01

Serum FSH and LH levels were decreased significantly (P<0.01) in treated group more than those of the control groups as shown in Table 3, while, the prolacting level was increased significantly (P<0.01).

Table 3. Effects of acute morphine administration on serum hormonal levels.

Hormone	Group A (control)	Group B (treated)
FSH	2.5 ± 0.13	0.61 ± 0.02
LH	2.3 ± 0.13	0.80 ± 0.04
Prolactin	5.8 ± 0.21	10.5 ± 0.19

Effects of acute morphine administration on seminal parameters are shown in Table 4. There were significant inhibition in both sperm viability and motility (P<0.01), while, there were non-significant (P<0.05) changes in sperm count and abnormal forms.

Table 4. Effects of morphine treatment on seminal parameters of male rats.

Characters	Group A (control)	Group B (treated)
Spern cell count	90.33 ± 1.44	85.83 ± 2.56
Viability %	79.33 ± 1.26	13.33 ± 1.10*
Motility %	75.83 ± 1.23	11.67 ± 0.68*
Abnormal forms %	16.50 ± 0.62	14.67 ± 0.62

* Significant at level P<0.05

Experiment II

Effect of acute morphine in castrated male rats on the exploratory behaviour parameters are tabulated in Table 5.

Table 5. Effect of morphine treatment on exploratory behaviour of castrated male rats.

Parameters	Group I (control)		Group II (treated)	
	Latency (sec.)	Frequency	Latency (sec.)	Frequency
Mounting	2.00 ± 0.00	48.17 ± 0.40	2.00 ± 0.00*	49.00 ± 0.37
Intromission	8.00 ± 0.37	54.00 ± 0.58	6.00 ± 0.00*	54.00 ± 0.37
Ejaculation	144.00 ± 0.93	8.00 ± 0.37	145.00 ± 0.54	8.00 ± 0.20

* Significant at level P<0.01

Acute morphine adminsistration at a dose rate of 10 mg /kg b.w. resulted in complete inhibition of sexual behaviour parameters compared to control group (Table 6).

Table 6. Effect of morphine treatment on sexual behaviour of castrated male rats.

Parameters	Group I (control)		Group II (treated)	
	Latency (min.)	Frequency	Latency (min.)	Frequency
Mounting	0.90 ± 0.90*	1.33 ± 1.33*	0.00 ± 0.00*	0.00 ± 0.00*
Intromission	0.95 ± 0.95*	1.67 ± 1.67*	0.00 ± 0.00*	0.00 ± 0.00*
Ejaculation	2.50 ± 2.50*	2.50 ± 2.50*	0.00 ± 0.00*	0.00 ± 0.00*

n = 6 * Significant at level P<0.05

Changes in hormonal level (FSH, LH & PRL) in both groups I & II are tabulated in Table 7. These changes were non-significant.

Table 7. Effects of morphin treatment on levels of castrated male rats.

Hormone	Group A (control)	Group B (treated)
FSH	9.90 ± 0.14	9.70 ± 0.14
LH	10.36 ± 0.22	10.20 ± 0.15
Prolactin	1.40 ± 0.03	1.50 ± 0.06

DISCUSSION

The effects of morphine administration at a dose rate of 10 mg/kg b.w in both sexually active and castrated groups showed that there were no significant changes (P<0.05) in exploratory behaviour parameters, while, the parameters of sexual behaviour were significantly decreased. These results are in agreement with the acute effects of opioids on sexual behaviour in male rats by other investigators (Hetla, 1977; Mumford & Kumar, 1979; Pfaus & Gorzalka, 1987; Agmo & Paredes, 1988).

The inhibitory effects of morphine on male rats sexual behaviour may be due to a direct effect of morphine on central opiod receptors, decreasing sexual motivation (the initiation (the initiation of sexual contact with a female) (Agmo & Paredes, 1988), or may be due to inhibitory effect on gonadotroping releasing hormone (GnRH) (Gabriel *et al.*, 1986 and Masotto & Negro-Vilar, 1988). Meites (1962) and Bruni *et al.* (1977) stated that, acute morphine administration, also, causes hyperprolactinemia which, in turn, can also, inhibit male copulatory behaviour by inhibiting both sexual arousal and erectile functions (Kalra *et al*, 1983 & Doherty *et al.*, 1989).

Complete inhibition of sexual activities of castrated male rat after administration of 10 mg/kg b.w. may be due to elimination of certain permissive or facilitative role played by gonadal hormones antagonizing the inhibitory action of opioids on male sexual behaviour, or it may be due to increased number of opioid receptors in the brain after castration (Hahn & Fishman, 1979 & 1985 and Pfaus & Gorzalka, 1987).

In treated group, there were no significant changes in sperm cell concentration and sperm morphological abnormalities in relation to control group, while, the sperm motility and viability were significaantly decreased. These results were in accordance with those of Davies (1983). These effects may be due to acute hormonal changes (marked decrease in FSH & LH and prolactin increase) which may, in turn, mediate to decrease in serum and intratesticular testosterone levels that affect the physiological functions of accessory sex organs (seminal vesicles and prostate), and may lead to decrease the sperm motility and viability (Ahmed *et al.*, 1987).

The significant decrease in FSH and LH levels were found in line with the results of Van Vugt *et al.* (1984), Gabriel *et al.* (1986), Miller *et al.* (1986) and Kalra *et al.* (1988). The inhibitory effect of acute morphine administration on serum FSH and LH levels of intact rats may be due to inhibitory effect of opioid adminstration on hypothalamic gonadotropin secretion (Masotto & Negro-Vilar, 1988), or due to the testosterone on pituitary cells (Kaynord *et al.*, 1990, Abass *et al.*, 1991 and Salem *et al.*, 1991).

As regards to serum prolactin levels, there was more increase in prolactin level in treated group than in control group. These results are in agreement with those of Bruni *et al.*, (1977), Van-Vugt & Meites (1980) and Ahmed *et al.* (1989).

This is due to reduction in dopamine release and turnover in hypothalamic tuberoinfunibular dopaminergic neurons which, in turn, cause increase prolactin level (Van Loon *et al.*, 1980 & Forman *et al.*, 1981).

It is concluded that, acute morphine administrationg reduces the sexual behaviour, FSH, LH, sperm motility and viability, however, it increases prolactin level. It does not affect exploratory behaviour, sperm concentration and sperm abnormalities in intact rat. On the other hand, acute morphine administration inhibits sexual behaviour, and it does not affect both exploratory behaviour and FSH, LH and prolactin in castrated rats.

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تأثير الجرعة الحادة من المورفين على الموائمة الهرمونية وعلاقتها بالتكاثر فى الذكور

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تم استخدام فى هذه الدراسة ٢٤ ذكرا من الفئران البيضاء تتراوح اوزانها من ١٣٠ – ١٤٠ جم قسمت الى مجموعتين تحتوى كل منهما على اثنى عشر فأرا.

التجربة الاولى :

تم استخدام اثنى عشر فأرا قسمت بدورها الى مجموعتين متساويتين. حقنت المجموعة (أ) ٠,٢ ملليلتر محلول ملح فسيولوجى منظم، بينما حقنت المجموعة الثانية (ب) ١٠ مجم / كجم من وزن الحيوان سلفات المورفين. هذا وقد تم الحقن فى المجموعتين قبل إجراء التجربة بحوالى ٦٠ دقيقة.

التجربة الثانية:

تم استخدام أثنى عشر فأرا لدراسة تأثير الجرعة الحادة لسلفات المورفين على ذكور الفئران البيضاء المخصية (تم خصى الفئران قبل التجربة بأسبوعين)، ثم قسمت الفئران الى مجموعتين متساويتين ، حقنت المجموعة الاولى (أ) ٠,٢ ملليلتر محلول ملح فسيولوجى منظم بينما حقنت المجموعة الثانية (ب) ١٠ مجم/كجم من وزن الحيوان سلفات المورفين، علماً بأنه تم حقن المجموعتين قبل أجراء التجربة بحوالى ٦٠ دقيقة.

هذا وقد تم أخذ القياسات الآتية عقب كل تجربة وهى : النشاط الجنسى والاستكشافى وبعد ثلاثين دقيقة تم ذبح جميع الحيوانات وقياس محتوى سيرم الدم من الهرمون الحاث للخلايا البينيه للخصية LH والهرمون الحاث لحوصلة جراف FSH وكذا هرمون البرولاكتين وتم أخذ حيامن البربخ وقياس الحيامن (مليون/مم) ونسبة الحركة الامامية ونسبة الاشكال الشاذة للحيامين.

ولقد دلت النتائج بالنسبة للتجربة الاولى على أن تأثير الجرعة الحادة لسلفات المورفين على الفئران السليمة غير معنوى على النشاط الاستكشافى ومثبط لكل ممارسات النشاط الجنسى، علاوه على نقص معنوى فى مستوى الهرمون الحاث للخلايا البينيه LH والهرمون الحاث لحوصلة جراف FSH وكذا زيادة معنوية فى مستوى هرمون البرولاكتين. أما بالنسبة للصفات المورفولوجية والبيولوجية لحيامن البربخ، فقد أدت الجرعة الحادة لسلفات المورفين إلى تثبيط حيوية وحركة الحيامن مع عدم التأثير معنوياً على تركيز الحيامن ونسبة الاشكال الشاذة، أما بالنسبة للتجربة الثانية فلقد كان تأثير الجرعة الحادة لسلفات المورفين على ذكور الفئران المخصية غير معنوى بالنسبة للنشاط الاستكشافى ونسبة الهرمون الحاث للخلايا البينية للخصية LH ونسبة الهرمون الحاث لحوصلة جراف FSH وايضا لمستوى هرمون البرولاكتين، هذا بالإضافة الى أنه قد فقدت الرغبة الجنسية بالكامل.