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EFFECT OF MORPHINE SULPHATE ON THE STRESS RESPONSE TO IMMOBILIZATION IN RATS

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ABSTRACT

The effects of peripheral administration of morphine on some haematological parameters and hormone secretion (prolactin and cortisol) were investigated in non-stressed and immobilized rats. Intramuscular injection of morphine sulphate at a dose of 15 mg/Kg body weight, decreased red blood corpuscles (RBCs) count and packed cell volume (PCV) and increased plasma prolactin concentration. Immobilization of rats for 4 hours daily for 4 successive days, induced a marked decrease in PCV value and double fold increase in plasma prolactin and cortisol concentrations. Pretreatment of immobilized rats with morphine sulphate caused a significant decrease in RBCs count, PCV, haemoglobin and plasma cortisol concentration. It was concluded that administration of morphine sulphate to stressed rats adversely affect the haematological picture and partially decrease the adrenal gland response to stressor.

INTRODUCTION

The opioid agonists inhibit lutenizing hormone (LH) secretion and stimulate the prolactin release in rats[1,2], subhuman primates[3] and sheep[4].

Peripheral administration of morphine suppressed LH and prolactin release in postpartum sows[5], while intraventricular administration of morphine in gilts decreased LH and increased prolactin concentrations[6]. Moreover, intravenous injection of morphine has been shown to stimulate growth hormone (GH) release in rats[7].

An increase in adrenal corticosteroid secretion, as reflected by increased plasma corticosteroid concentrations has been widely used as an index of stress in many species including rats[8]. Stress is well known to increase plasma cortisol and prolactin levels in several animal species[9-11].

However, few studies have been conducted to determine the effects of opioids and their agonists on hormone secretion and the Haematological picture under stress conditions.

The Following experiments were conducted to examine the effects of morphine on prolactin and cortisol secretion and the haematological picture in immobilized stressor and non immobilized rats.

MATERIALS AND METHODS

Twenty four non-pregnant female albino rats weighing 180-200 g were divided into 4 groups (6 rats each) and they were housed in plastic cages. They were provided with balanced food and water *ad libitum*.

Animals in group I were i.m. injected with 0.5 ml saline for 4 successive days and considered as control. Rats in group II were injected with morphine sulphate (Misr Pharmaceutical Company) at a dose of 15 mg/kg b.wt. for 4 successive days. Morphine was suspended in saline and the dose was injected in 0.5 ml volume per rat. Rats of the 3rd group were immobilized on their back for 4 hours daily for 4 successive days by ligation of the fore and hind legs tightly by a cotton thread. Rats of the 4th group were pretreated with morphine sulphate (15 mg/kg b.wt. daily for 4 successive days) thirty minutes before immobilization as in group III.

Twenty four hours after each treatment, rats were sacrificed and blood samples were collected for haematological study and plasma was separated and stored at -20°C until used for glucose and hormone analysis.

Red blood corpuscles count (RBCs) and total leukocytic count (WBCs) were determined by using the double improved Neubauer chamber[12], while haemoglobin (Hb%) was determined using the acid haematin method[13], and packed cell volume (PCV) was estimated in double capillary tube preparations using microhaematocrit centrifuge.

Plasma cortisol and prolactin levels were determined by RIA kits (Diagnostic Product Corp., Los Angeles, California, USA). Glucose was

measured using glucose Kits (Glucofix, Menorine Diagnostics, Italy).

Data were statistically analyzed by analysis of variance (ANOVA) and least significant difference method was used to detect the differences between means[14].

RESULTS

Intramuscular administration of morphine sulphate in a dose of 15 mg/kg b. wt. to non-immobilized rats (non stressed) for 4 days resulted in a significant ($P < 0.05$) decrease in RBCs count and PCV, while plasma prolactin level was significantly ($P < 0.05$) increased after morphine treatment (Table 1).

Immobilization of rats (stressed) for 4 hours daily for 4 successive days resulted in a sharp decrease in PCV (Table 1). While a marked elevation in plasma prolactin and cortisol concentrations (approximately double - fold increase) was occurred (Table 1).

Pretreatment of rats with morphine 30 minutes before immobilization resulted in a marked decrease in RBCs count, PCV and Hb% levels (Table 1). Plasma cortisol level was decreased compared to its level in the immobilized rats only (Table 1).

WBCs count and plasma glucose level were unaffected in all groups (Table 1).

DISCUSSION

Peripheral administration of morphine induced a non significant decrease in RBCs count and PCV, while, a significant hyperprolactinemia was observed. Th slight reduction of RBCs count and PCV could be attributed to the increased water retention (haemodilution), since morphine has been found to increase the release of the antidiuretic hormone[15]. The hyperprolactinemic effect of morphine is consistent with earlier studies [1,16]. Several investigators reported that central and peripheral administration of morphine increased prolactin and growth hormone concentrations, while it decreased LH levels[4,6,7&17]. The rise in prolactin after morphine could be attributed to its

Table 1. Effect of morphine sulphate on some haematological and hormonal parameters in stressed and non stressed rats.

Parameters	Control	Morphine	Immobilized	Immobilized + Morphine
RBCs/mm^3 (x 10^6)	$6.44 \pm 0..13^a$	6.04 ± 0.15^a	6.26 ± 0.11^a	5.02 ± 0.25^c
WBCs/mm^3 (x 10^3)	11.05 ± 0.53^a	11.25 ± 0.49^a	11.08 ± 0.71^a	12.22 ± 0.62^a
PCV (%)	43.70 ± 0.70^a	41.50 ± 1.00^a	35.75 ± 0.40^c	30.20 ± 1.20^d
Hb (mg%)	13.40 ± 0.30^a	12.70 ± 0.40^a	13.60 ± 0.30^a	10.80 ± 0.30^b
Glucose (mg%)	101.80 ± 2.10^a	105.30 ± 2.60^a	104.40 ± 3.10^a	104.30 ± 2.20^a
Prolactin (ng/ml)	3.51 ± 0.19^a	5.47 ± 0.13^b	7.68 ± 0.18^c	7.99 ± 0.11^c
Cortisol (ng/ml)	5.32 ± 0.23^a	5.16 ± 0.17^a	11.09 ± 0.32^b	8.76 ± 0.28^c

Means ± S.E. n= 6 rats.

a, b, c and d Mean having the same superscript letter are non significantly different from each other, otherwise they are significantly different from each other ($P < 0.05$).

effect at the hypothalamic or pituitary level. Morphine is known to have a number of diverse effects on brain metabolism through its potent effect on dopamine and serotonin turnover in the brain[7]. Acute administration of morphine (20 mg/kg b.wt.) was also reported to cause a significant elevation in serotonin (prolactin releasing factors) concentration in the hypothalamus of rats[18].

Immobilization of rats, adversely affect the PCV value. The decrease in PCV value was not due to the decrease in RBCs count since the RBCs count was not significantly decreased. The release of endogenous opioid under stress condition might increase the release of antidiuretic hormone[11] which subsequently caused water retention and decreased PCV. Immobilization of rats caused approximately a double-fold increase in prolactin and cortisol levels. This result was similar to those reported in several animal species in response to different stress conditions[8,10,19&20]. Generally, stressors activate the hypothalamic-pituitary-adrenal axis. However, complex hormonal changes in other systems (e.g. catecholamines, prolactin, growth hormone, glucagon, insulin, thyroid hormones, melatonin, endorphins, ..etc) are occurring simultaneously[9].

Pretreatment of rats with morphine before immobilization, induced a marked reduction in RBCs count, PCV and Hb% values. This is a pronounced indication that if morphine is administered to stressed animals, the haematological picture is severely affected. The exact mechanism of these effects was not clearly understood, however, it has been reported that when the cardiovascular system is stressed, morphine produces hypotension due to peripheral arterial and venous vasodilatation due to release of histamine and depression of vasomotor stabilizing mechanisms[15]. This effect of morphine might partially explain the adverse effect of morphine on the haematological picture of stressed animals. The increase in the ADH is also due to morphine administration and the subsequent haemodilution could be considered[15].

Morphine induced approximately 30% reduction in cortisol level, while it has no effect on prolactin levels when administered to immobilized rats. In view of these observations, it seemed possible that morphine could partially suppress the hypothalamic pituitary-adrenal axis in stressed animals. It seems conceivable that morphine does not affect this axis in non-stressed animals since cortisol level was not changed significantly in rats treated with morphine. The mechanism of action of morphine in suppression of this axis in stressed rats is not fully clear. However, morphine is known to have a number of effects on the hypothalamus metabolism specially the mono-amines and the hypothalamic releasing factors[5-7].

In conclusion, morphine could partially suppresses the hypothalamic-pituitary-adrenal axis in stressed rats, but it adversely affect the haematological parameters.

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تأثير كبريتات المورفين على الفئران المجهدة عن طريق التثبيت بالربط

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تم فى هذا البحث دراسة التأثيرات الناشئة عن حقن كبريتات المورفين على بعض صور الدم وكذا إفراز هرمونى البرولاكتين والكورتيزول فى الفئران الغير مجهدة والمجهدة عن طريق التثبيت بالاربطة. ولقد أدى حقن المورفين فى العضل فى جرعة قدرها ١٥مجم/كجم من وزن الفئران الحية الى نقص عدد كريات الدم الحمراء وكذا حجم خلايا الدم الحمراء المرصوصة وكذا زاد تركيز هرمون البرولاكتين فى بلازما الدم.

كما أدى تثبيت الفئران عن طريق الأربطة لمدة ٤ ساعات يوميا ولمدة أربعة أيام الى ضعف نقص واضح فى حجم خلايا الدم الحمراء المرصوصة وزاد تركيز هرمونى البرولاكتين والكورتيزول إلى الضعف. ولقد أدى علاج الفئران بالمورفين قبل إجهادها الى نقص عدد كريات الدم الحمراء وحجم خلايا الدم الحمراء المرصوصة والهيموجلوبين وتركيز هرمون الكورتيزول فى البلازما.

واستخلص من هذه الدراسة أن حقن المورفين فى الفئران المجهدة أدى إلى حدوث أثار ضاة على صورة الدم كما قلل جزئياً من استجابة الغدة الكظرية لهذا الإجهاد.