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Int. J. Devl Neuroscience 23 (2005) 621–626

INTERNATIONAL JOURNAL of DEVELOPMENTAL NEUROSCIENCE

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In vivo evidence for an increase in 5alpha-reductase activity in the rat central nervous system following morphine exposure

Hossein Amini, Abolhassan Ahmadiani *

Department of Pharmacology, Neuroscience Research Center, Shaheed Beheshti University of Medical Sciences, P.O. Box 19835-355, Tehran, Iran

Received 27 April 2005; received in revised form 5 July 2005; accepted 6 July 2005

Abstract

In the present study, the effects of acute and chronic morphine exposure on testosterone concentrations in the central nervous system (CNS) and serum were investigated in rats. Acute morphine administration (5 mg/kg, sc) reduced significantly testosterone levels in serum and spinal cord but not in the brain. Following chronic morphine administration (orally for 21 days), the brain testosterone was also significantly reduced as well as serum and spinal cord. Since, the decrease in testosterone levels following morphine exposure was more obvious in the CNS than serum, we suggested that it cannot be caused by only a direct decline in testosterone levels in periphery, and an increased local metabolism of testosterone in the CNS might be attributed in these effects. This hypothesis was supported with the findings that pretreatment with finasteride, a 5alpha-reductase inhibitor (5 mg/kg, sc) blocked testosterone elimination from the CNS following morphine exposure. Moreover, the serum concentration of 5alpha-reduced metabolites of testosterone, dihydrotestosterone and 3alpha-diol glucuronide was increased significantly following chronic morphine exposure, but not after co-treatment with finasteride. These results suggest that morphine exposure increase the CNS activity of 5alpha-reductase, which is an important metabolizing enzyme for testosterone.

Keywords: Morphine; Testosterone; 5Alpha-reductase; Finasteride; Dihydrotestosterone; 3Alpha-diol glucuronide

1. Introduction

It is well known that chronic morphine exposure decrease serum testosterone (Barraclough and Sawyer, 1955; Cicero et al., 1976; Morley, 1981; Millan and Herz, 1985; Yilmaz et al., 1999; Abs et al., 2000; Rajagopal et al., 2003), but the exact underlying mechanisms have not fully elucidated and need further investigations.

Morphine may affect testicular testosterone formation by inhibiting LH secretion, which is centrally mediated through inhibition of hypothalamic GnRH release (Blank and Roberts, 1982; Drouva et al., 1981; Mehmanesh et al., 1988), although additional effects at the hypophyseal level may also contribute (Blank et al., 1986; Kalra et al., 1988). Castration of male rats affects morphine ability to suppress serum LH. While, it has been shown that morphine was apparently more effective than testosterone in lowering serum LH in the initial stages of castration, but it was completely ineffective in long-term castrated rats (Cicero et al., 1980, 1982; Bhanot and Wilkinson, 1983). Both the delayed loss of the response to naloxone after castration (Masotto and Negro-Vilar, 1988) and lack of effect of castration (Cicero et al., 1982) on naloxone-induced increase in serum LH levels have been reported. Morphine induces supersensitivity to the effects of naloxone on LH (Cicero et al., 1983). Moreover, morphine and naloxone exert age-dependent effects on secretion of LH. It has been shown that morphine suppresses LH secretion at very early stages of development, well before puberty, whereas naloxone not only does not increase LH, but also does not reverse the inhibitory effect of morphine on LH in prepubescent male rats (Cicero et al., 1993). Naloxone treatment was found to elevate plasma FSH levels but not plasma LH levels in immature pigs (Trudeau et al., 1988).

* Corresponding author. Fax: +98 21 22403154.
E-mail address: aahmadiani@yahoo.com (A. Ahmadiani).

0736-5748/$30.00
doi:10.1016/j.ijdevneu.2005.07.001

There is also another possibility that morphine-induced decrease in serum testosterone may be due to its direct effect on leydig cell function (Gerendai et al., 1984). It has been reported that intratesticular injections of naloxone increases serum testosterone levels without increasing LH (Cicero et al., 1989), and morphine suppresses serum testosterone after pretreatment with human chorionic gonadotropin, which reverse morphine's suppression of LH (Adams et al., 1993). Similarly, it has been shown that in hypophysectomized rats, β-endorphin decreases testosterone levels (Chandrashekar and Bartke, 1992). It has been also reported that opioid peptides synthesized in the testis are components of the intratesticular regulatory system and that local opioid actions are modulated by testicular nerves (Gerendai, 1991).

Recently, we have reported that serum testosterone is not a predictor of testosterone concentration in the central nervous system (CNS). Moreover, the brain enzyme 5alpha-reductase which metabolizes testosterone, is activated by formalin-induced tonic pain (Amini and Ahmadiani, 2002). The CNS is a target for morphine to induce analgesia and interest in this study was based on the notion that the effect of morphine on testosterone concentration in the CNS had not been studied.

The objective of the present study was to evaluate acute and chronic morphine administration on testosterone levels in the rat CNS and periphery and to investigate possible involved mechanisms.

2. Experimental procedures

2.1. Animals and materials

All experiments were performed using adult male Sprague–Dawley rats (250–300 g), kept on a 12-h dark/12-h light cycle (lights on at 05:00 h) with ad libitum access to food (pellet from Pars Co., Tehran, Iran) and water. Experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1985).

Finasteride was from sigma (St. Louis, MO, USA). Morphine sulfate was kindly donated by Temad Co. (Tehran, Iran). All used solvents and salts were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Drug treatment

For acute morphine exposure, morphine sulfate (5 mg/kg) was dissolved in saline and injected sc in volume of 1 ml/kg, 2 h before decapitation. For chronic morphine exposure, morphine was added to feed water. The first 3 days were started with a concentration of 0.1 mg/ml of morphine followed by 0.2 mg/ml in the second 3 days period and 0.3 mg/ml in the third 3 days period. From the days 9 to 21, a concentration of 0.4 mg/ml morphine was used. From the days 1 to 6, glucose in a concentration of 0.5% was added to feed water to compensate bitter taste of morphine. After 21 days oral morphine treatment, the rats were decapitated while morphine addiction was apparent in all. Finasteride (5 mg/kg) and its vehicle ethanol–caster oil (20:80, v/v) were given in two sc injections at 2 h apart, i.e. 2 and 4 h before decapitation.

2.3. Assay method for steroids

All animals were killed by rapid decapitation between 10:30 and 11:30 h. The brain and spinal cords were rapidly removed and kept frozen at $-20\,^{\circ}C$. Trunk blood was collected and centrifuged at $900 \times g$ for 20 min, after 1 h room temperature. Following centrifugation, serum was separated and kept frozen. Testosterone assays were performed using commercially available RIA kit (Immunotech, Marseilles, France), directly in serum but following extraction from the brain and spinal cords using previously described method (Amini and Ahmadiani, 2002). Serum determination of dihydrotestosterone and 3alpha-diol glucuronide was done using a direct ELISA kit (Diagnostic Biochem Canada, Canada).

2.4. Statistical analysis

Data are presented as mean ± S.E.M. The statistical significance of differences was assessed by analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons tests. Statistical significance was accepted at level of $P < 0.05$.

3. Results

3.1. Effects of morphine exposure and finasteride treatment on testosterone levels in serum and CNS

Testosterone levels in the brain ($F(6, 43) = 4.38$, $P < 0.0015$), spinal cord ($F(6, 42) = 15.77$, $P < 0.0001$) and serum ($F(6, 40) = 3.07$, $P < 0.014$) were significantly different between treatment groups (Fig. 1). A significant reduction of the brain testosterone (97%, $P < 0.01$) was observed after chronic morphine exposure compared to intact group. To determine whether decrease in testosterone levels might be attributed to increased testosterone metabolism via 5alpha-reductase pathway, finasteride (an inhibitor of 5alpha-reductase) was used. Finasteride completely reversed decrease in brain testosterone induced by chronic morphine. In spinal cord, both acute and chronic morphine exposure significantly (90% in comparison to intact group, $P < 0.01$) reduced testosterone levels, which were reversed by finasteride treatment. It was also observed that the injection of finasteride and its