Physiology & Behavior 110–111 (2013) 80–86

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Gonadal ERα/β, AR and TRPV1 gene expression: Modulation by pain and morphine treatment in male and female rats

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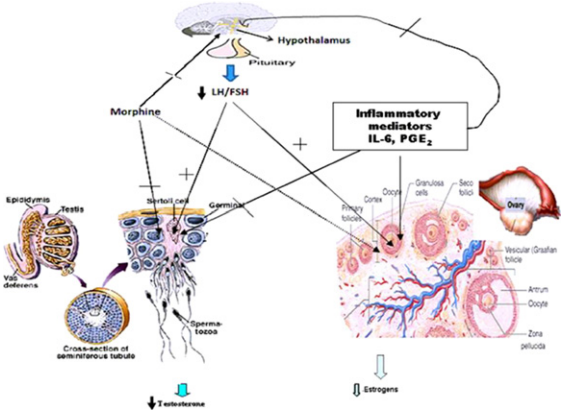
HIGHLIGHTS

- Data indicates a bidirectional relationship among gonadal hormones and pain therapy.
- Morphine decreases testosterone in males, the effect being intense and permanent.
- Estradiol, testosterone and their receptors can be significantly changed by morphine.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 May 2012
Received in revised form 27 July 2012
Accepted 13 December 2012

Keywords:
Androgen receptor
Estrogen receptor
TRPV1
Formalin test
Morphine

ABSTRACT

The results of several studies strongly indicate a bidirectional relationship among gonadal hormones and pain. While gonadal hormones play a key role in pain modulation, they have been found to be affected by pain therapies in different experimental and clinical conditions. However, the effects of pain and pain therapy on the gonads are still not clear. In this study, we determined the long-lasting (72 h) effects of inflammatory pain (formalin test) and/or morphine on estrogen receptor (ER), androgen receptor (AR) and TRPV1 gene expression in the rat testis and ovary. The animals were divided into groups: animals receiving no treatment, animals exposed only to the experimental procedure (control group), animals receiving no pain but morphine (sham/morphine), animals receiving pain and morphine (formalin/morphine), and animals receiving only formalin (formalin/saline). Testosterone (T) and estradiol (E) were determined in the plasma at the end of the testing.
In the sham/morphine rats, there were increases of ERα, ERβ, AR and TRPV1 mRNA expression in the ovary; in the testis, ERα and ERβ mRNA expression were reduced while AR and TRPV1 expression were unaffected

Abbreviations: ERα, estrogen receptor-alpha; ERβ, estrogen receptor-beta; AR, androgen receptor; TRPV1, transient receptor potential vanilloid-1; RT-PCR, real-time reverse transcription-polymerase chain reaction; mRNA, messenger ribonucleic acid; E2, 17β-estradiol; T, testosterone; LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; MOR, μ-opioid receptor; P450arom, aromatase enzyme.

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0031-9384/$ – see front matter
http://dx.doi.org/10.1016/j.physbeh.2012.12.014

by treatment. T and E plasma levels were increased in morphine-treated female rats, while T levels were greatly reduced in morphine-treated and formalin-treated males.
In conclusion, both testicular and ovarian ER (ERα and ERβ) and ovarian AR and TRPV1 gene expression appear to be affected by morphine treatment, suggesting long-lasting interactions among opioids and gonads.

1. Introduction

Several studies have highlighted a higher prevalence of chronic pain states and greater pain sensitivity in women than in men [1–4]. Gonadal hormones are considered factors influencing pain perception [5] as well as the response to opioid analgesia [6]. The link between gonadal hormones and pain is also represented by the strong influence exerted by painkillers on gonadal hormones. Opioids such as morphine, commonly used for the alleviation of severe pain, were shown to affect the hypothalamus–pituitary–gonadal axis [7,8], increase prolactin secretion and inhibit LH release, resulting in a dramatic alteration of the hormone ratio [9].

Morphine can induce significant decreases in testosterone (T) levels in both men and experimental male animals [10–12], the effect being intense and permanent throughout treatment duration. However, other body structures were shown to be affected by morphine treatment, including the testis and liver [12]. Indeed, morphine influences T catabolism by increasing 5-alpha reductase mRNA expression in the liver and aromatase mRNA expression in the brain and gonads [12]. On the whole, these data may explain the rapid disappearance of T from the blood after opioid administration.

Sex hormones are mainly synthesized in the testis and ovary, and the gonads themselves are target-tissues for sex hormones, which are mainly involved in the regulation of spermatogenesis and ovulation processes [13,14]. The principal biological effects of androgens and estrogens are mediated by the binding to androgen receptors (AR) and estrogen receptors (ER) [15]. AR and ER are also present in the gonads and show differential cellular distribution in the testis and ovary.

In the testis, AR are expressed in Sertoli, Leydig, peritubular myoid and vascular smooth muscle cells, spermatogonia and spermatocytes [16,17]. Moreover, both ER forms (ERα and ERβ) are expressed in spermatocytes, elongating spermatids, Sertoli cells and Leydig cells [18]. Multiple cell types in the testis, as well as Leydig cells, Sertoli cells, and germ cells (from pachytene spermatocytes to elongated spermatids) express aromatase, supplying high concentrations of E in the testis [19,20]. Indeed, estrogens play an important role in spermatogenesis and testicular functions.

In the ovary, estrogens and ER are essential for normal follicular maturation and are expressed in distinct cell types [21,22]. ERβ is expressed in the granulosa cells of primary, secondary and mature follicles but not in the germinal epithelium or in thecal, luteal or interstitial cells. ERα protein is found in the germinal epithelium and the interstitial and thecal cells. AR protein is present in the oocyte, granulosa cells and theca cells of preantral and antral follicles [23]. Moreover, testosterone secreted by theca cells plays an essential role in follicular growth, maturation, atresia and luteinization [24].

In addition to ER and AR, transient receptor potential vanilloid 1 (TRPV1) is involved in cell responses to internal and external environmental factors, including hormones and inflammatory mediators [25]. TRPV1 is principally expressed in the CNS and PNS, keratinocytes, hepatocytes, granulocytes, macrophages and testis [26,27], while TRPV1 functions in the ovary are described only in in vitro transfected artificial cultures [28].

The aim of the present study was to determine the effects of inflammatory pain and morphine treatment (alone and in combination) on ER, AR and TRPV1 gene expression in the rat ovary and testis and on circulating E and T levels.

2. Materials and methods

2.1. Animals

Male and female age-matched Sprague–Dawley rats (Harlan Italy, Milan, Italy), weighing 240–280 g and 220–250 g respectively, were used in the experiment. Animals were kept in separate gender groups and were housed two per cage in Plexiglas cages (35 × 23 × 18 cm) divided by a Perspex wall with holes for total isolation and to avoid interaction.

The rooms where the animals were housed were kept under controlled illumination (12/12 h light/darkness cycle; light off at 07:00 a.m.) and environmental conditions (ambient temperature 23 ± 1 °C; humidity 60 ± 10%). Prior to the experiments, the animals were kept in the animal house for at least 8–10 days with free access to food and water. Tests were carried out between 10:00 a.m. and 4:00 p.m. during the darkness phase, the active period of the animals; females were tested during the diestrus phase of the estrous cycle.

The experimental procedure was approved by the Ethics Committee of the University of Siena. In all experiments, we closely adhered to the guidelines for the handling of laboratory animals laid down by the European Communities Council Directive (86/609/EEC) and the Ethical Guidelines for investigation of experimental pain in conscious animals issued by the ad-hoc Committee of the International Association for the study of pain [29]. All measures were taken to minimize the discomfort of the animals and to reduce the number of animals used.

2.2. Experimental procedure

To study the effects of inflammatory pain (formalin test [30]) and/or long-term morphine administration, we randomly divided all the animals into groups depending on Pain treatment (sham, formalin) and drug treatment (saline, morphine). On Day 1, animals belonging to the Formalin groups (F) received a formalin injection (10%, 50 μl) in the dorsal part of the hind paw, while Sham (S) animals were pricked with a syringe needle in the dorsal surface of the right hind paw without the injection of any substance. One hour later, each subject received the first morphine (M) or saline (S) injection (in a mean volume of about 220 μl) subcutaneously in the back. The second morphine or saline injection was performed after 6 h. Morphine treatment was also performed on Day 2 (morning and evening) and Day 3 (morning). Immediately after each treatment, the rats were returned to their home cage. Animals present in the same cage received the same treatments.

The following five groups were obtained for each sex:

1. *Control* (Ctr): without treatment, left in the home cage (n = 4 females, n = 4 males);
2. *Sham − saline* (SS): treatment with sham injection and saline (n = 4 females, n = 4 males);
3. *Sham − morphine* (SM): treatment with sham injection and morphine (n = 4 females, n = 4 males);
4. *Formalin − saline* (FS): treatment with formalin injection and saline (n = 4 females, n = 4 males);
5. *Formalin–morphine* (FM): treatment with formalin injection and morphine (n = 4 females, n = 4 males);