



available at [www.sciencedirect.com](http://www.sciencedirect.com)



journal homepage: [www.elsevier.com/locate/psyneuen](http://www.elsevier.com/locate/psyneuen)



## Endocrine consequences of opioid therapy

Anna Maria Aloisi <sup>a,\*</sup>, Caterina Aurilio <sup>b</sup>, Valeria Bachiocco <sup>c</sup>, Giovanni Biasi <sup>d</sup>,  
Paolo Fiorenzani <sup>a</sup>, Maria Caterina Pace <sup>b</sup>, Valentina Paci <sup>f</sup>, Gilberto Pari <sup>f</sup>,  
Giandomenico Passavanti <sup>g</sup>, Laura Ravaioli <sup>f</sup>, Gianfranco Sindaco <sup>f</sup>,  
Renato Vellucci <sup>e</sup>, Ilaria Ceccarelli <sup>a</sup>

<sup>a</sup> University of Siena, Department of Physiology, Neuroscience and Applied Physiology Section, via Aldo Moro 2, 53100 Siena, Italy

<sup>b</sup> Second University of Naples, Department of Anesthesia, Surgical and Emergency Sciences, Piazza Miraglia 2, 80100 Napoli, Italy

<sup>c</sup> Department of Anesthesia and Intensive Care Unit, S. Orsola Hospital, Via Massarenti 9, 40125, Bologna, Italy

<sup>d</sup> Rheumatology Unit, Santa Maria alle Scotte Hospital, Viale Morgagni, 53100 Siena, Italy

<sup>e</sup> Palliative Care and Pain Therapy Unit, Careggi University Hospital, Viale Morgagni 85, 50134 Firenze, Italy

<sup>f</sup> Pain Medicine Center, Villa Serena Hospital and Advanced Algology Research Unit, 47100 Forlì, Italy

<sup>g</sup> Urological Unit, Misericordia Hospital, 58100 Grosseto, Italy

Received 25 March 2009; received in revised form 17 May 2009; accepted 19 May 2009

### KEYWORDS

Pain;  
Opioids;  
Gonadal hormones;  
Hypogonadism

**Summary** Gonadal hormones are known to be affected by morphine and other opioids. In this paper, we summarize data collected in recent years which clearly indicate that the opioid-induced effects on steroid hormones depend on the opioid used and in some cases on the sex of the subject. Indeed morphine is able to reduce hormones like testosterone and cortisol in both male and female subjects in just a few hours, probably acting directly on peripheral glands. These depressant effects of morphine on hormones are also present in the treatment of surgical pain and are quickly reversible once opioid administration is suspended. Similar actions were also found to occur in experimental animals and in vitro in glial cells, further confirming the morphine-induced reduction of testosterone cell content. Testosterone and its metabolites are well known substances involved in the development and maintenance of the brain and all body structures. Thus when treating pain with opioids, their effects on hypothalamo–pituitary–gonadal and hypothalamo–pituitary–adrenal-related hormones must be considered and, where possible, hormone replacement therapy should be started.

© 2009 Elsevier Ltd. All rights reserved.

### 1. Introduction

In the treatment of pain and particularly of moderate and severe chronic pain, opioids remain one of the most effective and widely used therapies (Rasor and Harris, 2005). In spite of the increase in available pain treatments, opioids are still a first choice for postoperative pain relief and for relief of

\* Corresponding author. Tel.: +39 0577 234103;  
fax: +39 0577 234037.

E-mail address: [aloi@unisi.it](mailto:aloi@unisi.it) (A.M. Aloisi).

many other painful conditions requiring long-term treatment, including cancer. However too often patients achieve little or no pain relief because of the numerous side effects that limit their intake of medication.

Opioid-induced side effects often start immediately and sometimes last for the duration of treatment. OPIAD, i.e. opioid-induced androgen deficiency, is one of the most constant syndromes. That exposure to opioids decreases gonadal hormones in humans, as well as in experimental animals treated with different opioids, is a widely acknowledged fact, given the number of papers in the literature (Cicero et al., 1976; Abs et al., 2000; Daniell, 2002; Roberts et al., 2002; Rajagopal et al., 2003; Amini and Ahmadiani, 2005; Aloisi et al., 2005; Ceccarelli et al., 2006). Nevertheless, this aspect is seldom considered in patients regularly taking opioids as analgesics.

Morphine-induced hypogonadism in men is dramatic, since testosterone levels are decreased enormously, reaching castration levels (<1 ng/ml) in a few hours after a single opioid administration (Aloisi et al., 2005), and unlike other opioid-induced side effects this condition persists throughout the treatment. The reduction in gonadal hormones during opioid intake has been described in both sexes and is associated in men with a reduction in libido and potency (Daniell et al., 2006) and in females with amenorrhea (Tutak and Doleys, 1996; Daniell, 2008). In addition to its influence on sexual interest and function, hypogonadism induces many other physiological changes, in particular fatigue, muscle wasting, osteoporosis and changes in pain.

Several hypotheses have been proposed to explain the pathogenesis. One of them considers the inhibition by opioids of releasing factor secretion in the hypothalamus (Pimpinelli et al., 2006), while another suggests a direct inhibitory action in the pituitary via specific binding sites on the gonadotrophs (Fabbri et al., 1989). Other hypotheses have also been tested (Jordan et al., 1996; Amini and Ahmadiani, 2005).

The importance of considering testosterone in the study of pain is underlined by clinical and experimental evidence that testosterone-depleted subjects and/or those with low testosterone levels present high pain levels. In particular an inverse relationship was found between plasma testosterone and work-related neck and shoulder disorders in female workers (Kaergaard et al., 2000). Other evidence of an analgesic effect of androgens is the clinical finding that gonadal and adrenal androgen levels (testosterone and DHT) are lower in both female and male rheumatoid arthritis patients than in controls. Interestingly, androgen administration induces a significant improvement of clinical symptoms, probably through their inhibitory action on the immune system (Morales et al., 1994; English et al., 2000). Moreover in male rats, testosterone has a protective role in adjuvant-induced arthritis (Harbuz et al., 1995). We showed that when supraphysiological levels of testosterone were administered to male and female rats, the formalin-induced licking (longer in female than male controls) decreased only in females; interestingly, there was no decrease in flexing or jerking behavior (Aloisi et al., 2004), suggesting that a high level of testosterone did not affect the nociceptive input (jerking and flexing were unchanged) but did induce a 'male-like' response in females with regard to licking, the most complex supraspinal formalin-induced response. In another experiment aimed at evaluating the long-term effect of a painful

stimulus in rats, it was confirmed that male gonadal hormones have an inhibitory, adaptive effect on the behavioral and neuronal responses to repeated nociceptive stimulation (Ceccarelli et al., 2003). This was demonstrated by the fact that in intact male rats (but not in gonadectomized rats) there was adaptation to repetition of the stimulus (three times with one week in between) at both the neuronal (c-Fos) and behavioral (formalin-induced licking) levels. Therefore we can hypothesize that the higher behavioral and neuronal effects observed in response to nociceptive stimulation can be attributed to a 'female-like system' in these animals.

The purpose of this review is to carefully consider the time course and morphine-induced modulation of testosterone metabolism. We present a series of experiments carried out in different patient populations, in experimental animals and in vitro, dealing with the response of hypothalamo-pituitary-adrenal/hypothalamo-pituitary-gonadal-related hormones to the actions of different opioids. For the human experiments, all procedures were conducted in accordance with the Helsinki Declaration and with the adequate understanding and written consent of the subjects. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering, reduce the number of animals used and utilize alternatives to in vivo techniques.

### 1.1. Effects of i.t. morphine administration in men and women (Table 1)

To evaluate the immediate effects of morphine spinal administration on steroid hormones and their time course, we considered male and female patients implanted with an epidural catheter due to persistent severe pain. This technique allows the immediate and safe infusion of small quantities of opioids, thus reducing many side effects. The daily dose of morphine was 0.9 mg/die and the overall administration time was 15 days. The patients, mostly women, remained in the clinic after implantation; the period of hospitalization allowed their state of health to be closely monitored. The following hormones were considered: LH, FSH, testosterone, free testosterone, cortisol. Blood was collected and analyzed on Days 0, 1, 2, 15 (last day of administration) and 16 (the day after withdrawal of morphine).

In both men and women, total testosterone, free testosterone and cortisol blood levels were greatly reduced from the baseline levels (from Day 1 to Day 15). By contrast, gonadotropins tended to minimally increase from the baseline level in men and to decrease in women. In all subjects the values had returned to pre-treatment levels on Day 16.

These findings clearly show that morphine administration (including epidural administration) immediately affects gonadal hormones and cortisol, irrespective of gender. The levels of all these hormones progressively fell starting from the first day of treatment and then recovered 24 h after its suspension. However analysis of the hormone behavior suggested different morphine target points in the two sexes. While hypothalamo-pituitary axis inhibition can be excluded in men due to the lack of gonadotropin decrease, hypothalamo-pituitary involvement in women is suggested by the decrease (even if small) of gonadotropin levels. Thus a direct action of opioids on the testis and/or an increase in testos-

**Table 1** Hormone plasma levels in male and female patients suffering chronic pain, implanted with an epidural catheter to administer a solution containing morphine for 15 days. Blood was collected before the beginning of treatment (baseline) and after 1, 2 and 15 days of treatment (Day 1, Day 2 and Day 15, respectively) and 24 h after opioid withdrawal (Day 16).

Sex/age	Hormones	Baseline	Day 1	Day 2	Day 15	Day 16
Females/65.5 years, N = 15	LH (mIU/ml)	19.60 ± 4.66	21.66 ± 3.83	19.63 ± 3.47	17.27 ± 5.2	19.80 ± 2.78
	FSH (mIU/ml)	48.51 ± 6.72	44.66 ± 6.21	41.18 ± 5.49	40.93 ± 5.0	52.42 ± 6.07
	Estradiol (pg/ml)	12.73 ± 1.76	11.90 ± 1.64	9.63 ± 0.95	8.45 ± 0.32	12.0 ± 1.16
	Testosterone (ng/ml)	0.41 ± 0.07	0.23 ± 0.03	0.17 ± 0.02	0.17 ± 0.03	0.30 ± 0.07
	Free T (pg/ml)	1.1 ± 0.18	0.60 ± 0.11	0.50 ± 0.09	0.42 ± 0.09	0.90 ± 0.16
	Cortisol (ng/ml)	155.1 ± 13.2	59.0 ± 30.4	54.72 ± 29.06	49.2 ± 20.5	145.72 ± 24.8
Males/68.7 years, N = 4	LH (mIU/ml)	2.63 ± 1.37	3.13 ± 0.28	4.6 ± 2.91	4.9 ± 3.91	2.66 ± 0.60
	FSH (mIU/ml)	4.63 ± 0.98	4.53 ± 0.77	5.55 ± 1.65	6.0 ± 2.91	4.63 ± 0.55
	Estradiol (pg/ml)	24.66 ± 1.33	19.0 ± 3.0	13.0 ± 3.0	9.5 ± 0.49	18.66 ± 2.73
	Testosterone (ng/ml)	4.13 ± 0.49	2.30 ± 0.16	1.4 ± 0.90	1.45 ± 1.15	2.66 ± 0.27
	Free T (pg/ml)	5.70 ± 0.1.67	2.63 ± 0.41	1.80 ± 0.80	1.60 ± 0.9	4.2 ± 1.10
	Cortisol (ng/ml)	142.7 ± 30.8	11.0 ± 3.26	12.0 ± 1.3	10 ± 5.01	119 ± 88.93

terone metabolism has to be hypothesized to explain the testosterone reduction in men (Jenab and Morris, 2000; Amini and Ahmadiani, 2005).

## 1.2. Effect of long-term administration of transdermal buprenorphine in men and women (Table 2)

Male and females treated with transdermal buprenorphine (35 µg/h every 72 h) for acute/persistent pain were included in this study, the principal aim of which was to observe the modifications of the HPG and HPA axes. Estradiol, total testosterone, free testosterone, DHT, cortisol and steroid hormone binding globuline (SHBG) plasma levels were measured at baseline and after 1, 3 and 6 months of treatment.

In the females, all hormones showed slight changes during the observation period that did not become significant except for total testosterone, which was *increased* at 3 months. In the males, there were no significant differences for any hormones

except free testosterone, which appeared to have decreased after 3 months. In both sexes the HPA axis was *not* inhibited since cortisol progressively increased during treatment.

SHBG is a rarely measured protein which indicates the possibility of testosterone being transported in the blood. The bound testosterone is the unavailable fraction, although it remains in equilibrium with the unbound form (free testosterone). Thus the higher the SHBG concentration, the lower the possibility of testosterone being taken up by the cells. SHBG is affected by several factors, including age. In this study, its level did not change significantly in either sex. However in the males, the SHBG peak at 3 months corresponded to the lowest free testosterone values.

Buprenorphine is an old opioid that has recently gained a new role in pain therapy due to its patch formulation. Its characteristics (partial µ agonist and κ antagonist) differ from those of morphine (µ and κ agonist). In this study, its efficacy was also different from that of morphine, since the blood androgen deficiency was not present. From a clinical

**Table 2** Hormone plasma levels in male and female patients suffering chronic pain and receiving transdermal buprenorphine (35 µg/h patch, every 72 h) for 6 months. Blood was collected before the beginning of treatment (baseline) and after 1, 3 and 6 months of treatment.

Sex/age	Hormones	Baseline, N = 28	1 month, N = 23	3 months, N = 20	6 months, N = 18
Females/58.7 years	Estradiol (pg/ml)	32.6 ± 13.3	18.7 ± 6.61	25.9 ± 15.92	12.31 ± 3.20
	Testosterone (ng/ml)	0.20 ± 0.02	0.16 ± 0.03	0.24 ± 0.02*	0.19 ± 0.02
	Free T (pg/ml)	1.26 ± 0.11	0.96 ± 0.10	1.0 ± 0.10	0.92 ± 0.11
	DHT (pg/ml)	30.47 ± 3.53	23.89 ± 4.51	22.03 ± 4.50	40.01 ± 23.66
	Cortisol (ng/ml)	121.1 ± 9.38	142.3 ± 22.06	166.8 ± 11.18**	184.9 ± 20.39**
	SHBG (nmol/l)	80.38 ± 8.95	102.68 ± 12.79	122.69 ± 21.16	86.47 ± 19.12
Males/61.4 years	Hormones	Baseline, N = 12	1 month, N = 10	3 months, N = 5	6 months, N = 5
	Estradiol (pg/ml)	14.47 ± 1.62	11.71 ± 0.87	12.79 ± 3.43	4.75 ± 1.29
	Testosterone (ng/ml)	2.92 ± 0.36	2.20 ± 0.40	2.00 ± 0.37	1.68 ± 0.28
	Free T (pg/ml)	12.02 ± 1.18	5.89 ± 1.05†	5.46 ± 1.11	5.09 ± 0.80
	DHT (pg/ml)	753.6 ± 170	550.25 ± 114.4	439.9 ± 154.23	126.4 ± 20.2
	Cortisol (ng/ml)	168.4 ± 11.0	150.45 ± 23.2	164.2 ± 19.03	163.21 ± 15.3
SHBG (nmol/l)	56.98 ± 6.95			27.18 ± 16.8	

\*  $p < 0.04$  vs baseline.

\*\*  $p < 0.001$  vs baseline.

**Table 3** Testosterone and estradiol plasma levels in men subjected to urological surgery and implanted with an i.v. catheter filled with morphine or morphine plus fentanyl. Blood was collected before the beginning of treatment (baseline) and 24 and 72 h after surgery.

Treatment	Hormones	Baseline	24 h	72 h
Morphine	testosterone (ng/ml)	4.94 ± 0.27 (N = 25)	1.83* ± 0.14 (N = 25)	4.65 (N = 2)
	estradiol (pg/ml)	27.2 ± 5.6 (N = 9)	16.7* ± 3.7 (N = 9)	
Morphine + fentanyl	testosterone (ng/ml)	4.04 ± 0.20 (N = 4)	0.93* ± 0.11 (N = 4)	1.81 (N = 1)

\*  $p < 0.01$  vs baseline.

perspective this means that buprenorphine therapy may be carried out for several months without creating the hypogonadism induced by morphine.

### 1.3. Effect of surgical analgesia by i.v. opioid administration in men (Table 3)

Pain in surgical patients is often managed with i.v. opioids. In this study we measured the testosterone and estradiol levels in the blood of a sample of male patients who had undergone surgery and were treated with morphine (20 mg/24 h) in order to evaluate the changes in these hormones in these specific conditions.

The values reported in Table 3 show that the testosterone levels were very low 24 h after the operation, particularly when morphine and fentanyl were used. The estradiol levels were also lowered by the treatment. In particular, the testosterone levels of men treated with morphine alone for the management of transvescical prostateadenectomy were significantly reduced after 24 h and returned to normal 3 days later; other patients who underwent more invasive surgery such as nephrectomy and treated with morphine and fentanyl showed a greater and more long-lasting effect than that reported above. In confirmation of this finding, one patient in this group presented low testosterone levels also on the third day. Taken together, these findings support the results we observed on the effects of opioids.

### 1.4. Effect of morphine administration on plasma and brain testosterone levels in male and female rats (Fig. 1)

Male and female rats were injected subcutaneously with morphine to study the effects of this opioid on testosterone

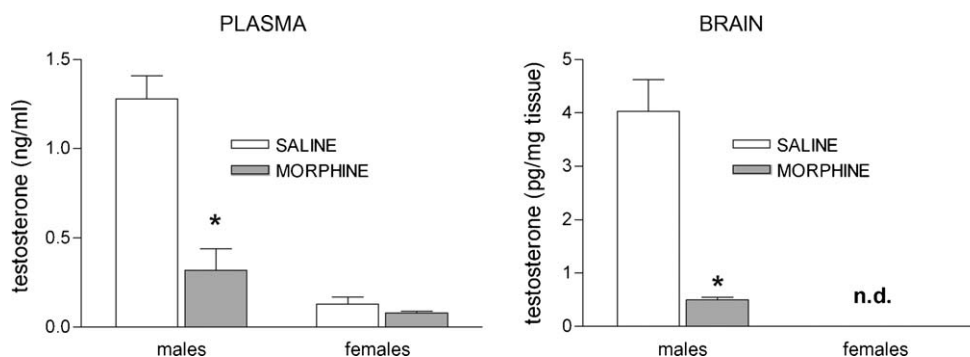
levels in the plasma and brain. To determine the modification after a single s.c. injection of morphine, male and female rats were treated with a dose of 5 mg/kg and blood and brain tissue samples were collected after 4 h.

As shown in Fig. 1, testosterone decreased in the blood of both female and male rats. Testosterone levels in the brain fell significantly in the males while the hormone did not reach detectable levels in either the morphine-treated or saline-treated females.

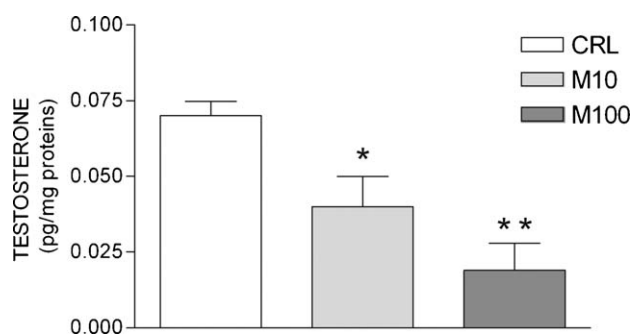
### 1.5. Effect of morphine on testosterone levels in rat C6 glioma cells (Figs. 2–4)

A series of experiments was carried out to determine whether testosterone levels in rat C6 glioma cells were affected by morphine administration and whether this effect could be modified by adding drugs to the culture medium that interfere with testosterone degradation by acting on different enzymes: anastrozole, able to block the enzyme aromatase, or finasteride, able to block 5 $\alpha$ -reductase. Moreover, to evaluate the effect of morphine on aromatase activity and the possible interaction between morphine and anastrozole, we measured aromatase activity in pools of cells treated with anastrozole, morphine (10  $\mu$ M) and the association of these two substances. Aromatase activity was expressed as percentage of controls.

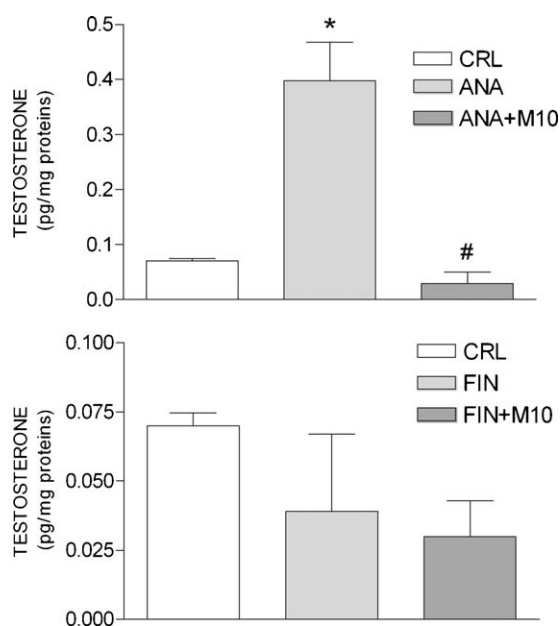
As shown in Fig. 2, testosterone was measurable in the glioma cells and its level was strongly reduced by morphine addition in a dose-dependent way. In other groups of glial cells the addition of anastrozole and finasteride had different effects on the testosterone concentration. As shown in Fig. 3A, anastrozole significantly increased testosterone concentration with respect to controls, an effect completely negated by the contemporary administration of morphine. In



**Fig. 1** Testosterone levels in plasma (left) and brain (right) in male and female Sprague–Dawley rats 4 h after s.c. treatment with saline or morphine (5 mg/kg). Testosterone was not detected in the female brain. \* $p < 0.01$  vs saline.



**Fig. 2** Testosterone levels in control (CRL) and morphine-treated rat C6 glioma cells. M10: morphine 10  $\mu$ M. M100: morphine 100  $\mu$ M. Data are mean  $\pm$  SEM. \* $p$  < 0.01 and \*\* $p$  < 0.001 vs CRL.



**Fig. 3** Cellular testosterone levels in: (A): control (CRL), anastrozole (ANA) and ANA + morphine (M10: 10  $\mu$ M); (B): control (CRL), finasteride (FIN) and FIN + morphine (M10: 10  $\mu$ M). Data are mean  $\pm$  SEM. \* $p$  < 0.01 vs CRL; # $p$  < 0.01 vs ANA.

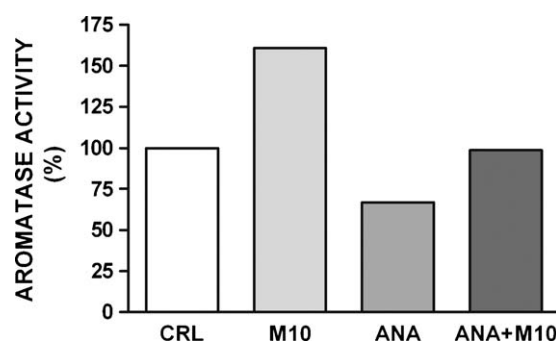
contrast, finasteride (Fig. 3B) did not significantly alter the testosterone levels.

Morphine treatment induced a 66.8% increase in aromatase activity (Fig. 4), whereas anastrozole induced a 33% decrease; the association of anastrozole and morphine blocked both effects.

Therefore our data provide evidence that glial cells contain low but detectable levels of testosterone and that morphine significantly decreases the cellular levels of testosterone.

## 2. Discussion

The experiments presented in this paper show the differing abilities of opioids to affect hormones. The hypogonadism induced by morphine and its effect on neuronal testosterone are clinically relevant: although opioids remain the most



**Fig. 4** Aromatase activity in control (CRL), morphine (M10: 10  $\mu$ M), anastrozole (ANA) and ANA + M10 groups. Data, from a pool of cells, are expressed as percentage of control activity.

important group of drugs commonly used in pain relief, physicians need to be aware of all their long-term consequences.

Opioids are generally cheap and their various formulations make them easy and appropriate to use for any painful condition. For example, fentanyl is a fast opioid often used during surgery while buprenorphine, which needs more time to achieve its effect, is more indicated for long-term treatment. However morphine remains the reference point of all studies on opioids.

In this paper, we have described the different approaches we adopted to better evaluate the possible interactions between opioids and the most common steroid hormones. The hypothalamus and the pituitary are well-known targets of various endogenous opioids (i.e. beta-endorphins) which modulate their cellular activity. On the other hand, endorphinergic neurons present in the arcuate nucleus of the hypothalamus are known to be modulated by gonadal hormones. Thus the possibility that opioids modulate the HPA or HPG axis must be considered physiological. However when given to treat pain, morphine and the other opioids disrupt the physiological ratio of opioids to gonadal hormones, altering many of the functions in which opioids and the hormones are involved. This important interaction probably plays a role in the different capacities of the two sexes to develop chronic pain (Aloisi and Bonifazi, 2006). As recently demonstrated in women (Smith et al., 2006), the opioid-binding capacity in the CNS in response to a painful stimulation differs according to the estrogen plasma levels.

In the first study, both males and females subjected to epidural morphine administration showed a significant decrease in testosterone plasma levels. These effects completely disappeared after the interruption of therapy. As already suggested, the decrease in testosterone can be attributed to a peripheral action, i.e. on the testis in males and the adrenals in males and females. Opioid receptors are present in the testis and have a physiological role in testosterone production as well as in spermatogenesis (Fabbri et al., 1989; Jenab and Morris, 2000). Like testosterone, cortisol appears to be quickly affected by opioid administration, supporting a direct action of opioids on the adrenals (Pirnik et al., 2001).

In the second study, buprenorphine-treated subjects suffering acute persistent pain were followed for six months. However this opioid had only limited endocrine effects. At 1,

3 and 6 months after the start of treatment, there were no decreases in any of the hormones in females, while in males only *free* testosterone appeared to be constantly lower than baseline levels, an effect paralleled by the increase of SHBG. Indeed buprenorphine is a synthetic derivative of opioids, which does not induce most of the common side effects described for other opioids (i.e. on immunity, Sacerdote, 2008). The increased testosterone levels in females can probably be attributed to the decrease of pain and the consequent stress conditions.

Post-surgical pain is another important application for opioids. The clear depressant effects of morphine on testosterone are transitory but can interact with the healing process. Indeed both testosterone and estradiol are involved in wound healing through their action on tissue regeneration and immunity (i.e. Engeland et al., 2008). Androgens generally lengthen healing times in skin, whereas estrogens shorten them (see Gilliver and Ashcroft, 2007); yet the results are not unequivocal (see Engeland et al., 2006). Although the effects of gonadal hormones on healing are still not clear, morphine may affect post-surgical patients not only by relieving their pain but also by changing their tissue-repair processes.

Neurons and glial cells produce and store steroids, also called neurosteroids. These cells also take up testosterone or other steroids from the blood to be metabolized and/or used in cell processes. Testosterone was found in the brain of male rats and its levels were drastically decreased just 4 h after a single s.c. injection of morphine (Ceccarelli et al., 2006). In this study, we applied the protocol to both male and female rats to verify the effect in females as well. We found that the low baseline plasma levels of testosterone in females were further decreased while the brain testosterone levels were always below the detection limits, even though the sensitivity of the method was very high. Since we paid great attention to the procedure of 'cleaning' the brain tissues of blood, we conclude that the testosterone measured in the brain was present within the cells. The fact that there were no detectable levels in females suggests that they have no consistent uptake of testosterone, as instead may be presumed to occur in males. This is probably due to the low testosterone plasma levels in females but we cannot exclude that the female brain does not require 'high' levels of testosterone to be aromatized to estradiol since estrogens can be taken up from the blood and their synthesis from testosterone is not as necessary as in males.

Thus the morphine-induced decrease clearly present in the male brain can only be hypothesized in females. The influence of morphine on glial cells was also demonstrated in the *in vitro* preparation: the addition of different concentrations of morphine to C6 glioma cells induced a dose-dependent decrease in cellular levels of testosterone. This decrease can be attributed to a decrease in testosterone synthesis but also to an increase in its metabolism to estradiol (through aromatase) or dehydrotestosterone (DHT, through 5 $\alpha$ -reductase). Interestingly the addition of anastrozole (aromatase inhibitor) to the culture medium caused a significant increase in testosterone levels, suggesting that the aromatase pathway is used considerably in these cells. In contrast, finasteride, which blocks 5 $\alpha$ -reductase, failed to induce significant changes in cellular levels of testosterone. Thus the testosterone present at baseline in glial cells is metabo-

lized by local aromatization to estradiol, while its transformation to DHT does not appear to play a significant role. The direct determination of aromatase activity demonstrated that morphine increased this enzyme, while the addition of anastrozole blocked the increase.

Gonadal hormones are known to affect the homeostatic conditions of the central nervous system (CNS) (Goodman et al., 1996; Behl et al., 1997; Ahlbom et al., 1999; Lee and Pfaff, 2008; Fuller et al., 2007; Fargo et al., 2008; Vegeto et al., 2008). Estrogens protect neurons from oxidative stress-induced death and androgens can rescue specific populations of motoneurons or hippocampal neurons from ontogenetic axotomy-induced death or glucose-deprivation *in vivo*. Thus acute or prolonged deprivation of gonadal hormones can have important long-term consequences.

Finally, androgens are also good candidates for the modulation of neuronal and behavioral responses to pain. Indeed low androgen levels could be a primary contributor to the incidence of fibromyalgia and to its anti-anabolic features (Dessein et al., 2000). In experimental animals, testosterone decreased long-term behavioral, hormonal and neuronal effects of recurrent pain, while its administration to female rats was analgesic in a model of inflammatory pain (Ceccarelli et al., 2003; Aloisi et al., 2004). Thus if opioids are given to fight pain, their side effects can act in an opposite direction. Indeed, opioid-induced hyperalgesia has been described (Mitra, 2008).

In conclusion, opioid therapy is mandatory in several types of pain, particularly chronic pain. However its administration requires a detailed understanding of all the mechanisms it affects, including gonadal hormone metabolism. All the data we have reported are in agreement with the reports by other authors that morphine (the most widely used opioid) significantly influences testosterone metabolism, also in glial cells. Therefore the prescription of opioid treatment requires great attention to and awareness of these crucial side effects.

## Role of funding source

Funding for this study was provided by University of Siena funds to AMA.

## Conflict of interest

All other authors declare that they have no conflicts of interest.

## Acknowledgements

We thank Prof. Leonida Fusani and Dr. Melinda Maddalena who kindly assisted with the aromatase activity determination and analysis of the data.

## References

- Abs, R., Verhelst, J., Maeyaert, J., Van Buyten, J.P., Opsomer, F., Adriaensen, H., Verlooy, J., Van Havenbergh, T., Smet, M., Van Acker, K., 2000. Endocrine consequences of long-term intrathecal administration of opioids. *J. Clin. Endocrinol. Metab.* 85, 2215–2222.

- Ahlbom, E., Grandison, L., Bonfoco, E., Zhivotovsky, B., Ceccatelli, S., 1999. Androgen treatment of neonatal rats decreases susceptibility of cerebellar granule neurons to oxidative stress *in vitro*. *Eur. J. Neurosci.* 11, 1285–1291.
- Aloisi, A.M., Bonifazi, M., 2006. Sex hormones, central nervous system and pain. *Horm. Behav.* 50, 1–7.
- Aloisi, A.M., Pari, G., Ceccarelli, I., Vecchi, I., Ietta, F., Lodi, L., Paulesu, L., 2005. Gender-related effects of chronic non-malignant pain and opioid therapy on plasma levels of macrophage migration inhibitory factor (MIF). *Pain* 115, 142–151.
- Aloisi, A.M., Ceccarelli, I., Fiorenzani, P., De Padova, A.M., Massafra, C., 2004. Testosterone affects formalin-induced responses differently in male and female rats. *Neurosci. Lett.* 361, 262–264.
- Amini, H., Ahmadiani, A., 2005. *In vivo* evidence for an increase in 5 $\alpha$ -reductase activity in the rat central nervous system following morphine exposure. *Int. J. Dev. Neurosci.* 23, 621–626.
- Behl, C., Skutella, T., Lezoulac'h, F., Post, A., Widmann, M., Newton, C.J., Holsboer, F., 1997. Neuroprotection against oxidative stress by estrogens: structure–activity relationship. *Mol. Pharmacol.* 51, 535–541.
- Ceccarelli, I., De Padova, A.M., Fiorenzani, P., Massafra, C., Aloisi, A.M., 2006. Single opioid administration modifies gonadal steroids in both the CNS and plasma of male rats. *Neuroscience* 140, 929–937.
- Ceccarelli, I., Scaramuzzino, A., Massafra, C., Aloisi, A.M., 2003. The behavioral and neuronal effects induced by repetitive nociceptive stimulation are affected by gonadal hormones in male rats. *Pain* 104, 35–47.
- Cicero, T.J., Wilcox, C.E., Bell, R.D., Meyer, E.R., 1976. Acute reductions in serum testosterone levels by narcotics in the male rat: stereospecificity, blockade by naloxone and tolerance. *J. Pharmacol. Exp. Ther.* 198, 340–346.
- Daniell, H.W., 2002. Hypogonadism in men consuming sustained-action oral opioids. *J. Pain* 3, 377–384.
- Daniell, H.W., Ientz, R., Mazer, N.A., 2006. Open-label pilot study of testosterone patch therapy in men with opioid-induced androgen deficiency. *J. Pain* 7, 200–210.
- Daniell, H.W., 2008. Opioid endocrinopathy in women consuming prescribed sustained-action opioids for control of nonmalignant pain. *J. Pain* 9, 28–36.
- Dessein, P.H., Shipton, E.A., Stanwix, A.E., Joffe, B.I., 2000. Neuroendocrine deficiency-mediated development and persistence of pain in fibromyalgia: a promising paradigm? *Pain* 86, 213–215.
- Engeland, C.G., Sabzehei, B., Marucha, P.T., 2008. Sex hormones and mucosal wound healing. *Brain Behav. Immun.* (December) (epub ahead of print).
- Engeland, C.G., Bosch, J.A., Cacioppo, J.T., Marucha, P.T., 2006. Mucosal wound healing: the roles of age and sex. *Arch. Surg.* 141, 1193–1197.
- English, K.M., Steeds, R.P., Jones, T.H., Diver, M.J., Channer, K.S., 2000. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: a randomized, double-blind, placebo-controlled study. *Circulation* 102, 1906–1911.
- Fabbri, A., Jannini, E.A., Gnassi, L., Ulisse, S., Moretti, C., Isidori, A., 1989. Neuroendocrine control of male reproductive function. The opioid system as a model of control at multiple sites. *J. Steroid Biochem.* 32, 145–150.
- Fargo, K.N., Galbiati, M., Foeking, E.M., Poletti, A., Jones, K.J., 2008. Androgen regulation of axon growth and neurite extension in motoneurons. *Horm. Behav.* 53, 716–728.
- Fuller, S.J., Tan, R.S., Martins, R.N., 2007. Androgens in the etiology of Alzheimer's disease in aging men and possible therapeutic interventions. *J. Alzheimer Dis.* 12, 129–142.
- Gilliver, S.C., Ashcroft, G.S., 2007. Sex steroids and cutaneous wound healing: the contrasting influences of estrogens and androgens. *Climacteric* 10, 276–288.
- Goodman, Y., Bruce, A.J., Cheng, M., Mattson, M.P., 1996. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. *J. Neurochem.* 66, 1836–1844.
- Harbuz, M.S., Perveen-Gill, Z., Lightman, S.L., Jessop, D.S., 1995. A protective role for testosterone in adjuvant-induced arthritis. *Br. J. Rheumatol.* 34, 1117–1122.
- Jenab, S., Morris, P.L., 2000. Interleukin-6 regulation of kappa opioid receptor gene expression in primary sertoli cells. *Endocrine* 13, 11–15.
- Jordan, D., Tafani, J.A., Ries, C., Zajac, J.M., Simonnet, G., Martin, D., Kopp, N., Allard, M., 1996. Evidence for multiple opioid receptors in the human posterior pituitary. *J. Neuroendocrinol.* 8, 883–887.
- Lee, A.W., Pfaff, D.W., 2008. Hormone effects on specific and global brain functions. *J. Physiol. Sci.* 58, 213–220.
- Kaergaard, A., Hansen, A.M., Rasmussen, K., Andersen, J.H., 2000. Association between plasma testosterone and work-related neck and shoulder disorders among female workers. *Scand. J. Work Environ. Health* 26, 292–298.
- Mitra, S., 2008. Opioid-induced hyperalgesia: pathophysiology and clinical implications. *J. Opioid Manag.* 4, 123–130.
- Morales, A.J., Nolan, J.J., Nelson, J.C., Yen, S.S., 1994. Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. *J. Clin. Endocrinol. Metab.* 78, 1360–1367.
- Pimpinelli, F., Parenti, M., Guzzi, F., Piva, F., Hokfelt, T., Maggi, R., 2006. Presence of delta opioid receptors on a subset of hypothalamic gonadotropin releasing hormone (GnRH) neurons. *Brain Res.* 1070, 15–23.
- Pirnik, Z., Schwendt, M., Jezova, D., 2001. Single dose of morphine influences plasma corticosterone and gene expression of main NMDA receptor subunit in the adrenal gland but not in the hippocampus. *Endocr. Regul.* 35, 187–193.
- Rajagopal, A., Vassilopoulou-Sellin, R., Palmer, J.L., Kaur, G., Bruera, E., 2003. Hypogonadism and sexual dysfunction in male cancer survivors receiving chronic opioid therapy. *J. Pain Symptom Manag.* 26, 1055–1061.
- Rasor 3rd, J., Harris, G., 2005. Opioid use for moderate to severe pain. *J. Am. Osteopath. Assoc.* 105, S2–S7.
- Roberts, L.J., Finch, P.M., Pullan, P.T., Bhagat, C.I., Price, L.M., 2002. Sex hormone suppression by intrathecal opioids: a prospective study. *Clin. J. Pain* 18, 144–148.
- Sacerdote, P., 2008. Opioid-induced immunosuppression. *Curr. Opin. Support Palliat. Care* 2, 14–18.
- Smith, Y.R., Stohler, C.S., Nichols, T.E., Bueller, J.A., Koeppe, R.A., Zubieta, J.K., 2006. pronociceptive and antinociceptive effects of estradiol through endogenous opioid neurotransmission in women. *J. Neurosci.* 26, 5777–5785.
- Tutak, U., Doleys, D.M., 1996. Intrathecal infusion systems for treatment of chronic low back and leg pain of noncancer origin. *South Med. J.* 89, 295–300.
- Vegeto, E., Benedusi, V., Maggi, A., 2008. Estrogen anti-inflammatory activity in brain: a therapeutic opportunity for menopause and neurodegenerative diseases. *Front. Neuroendocrinol.* 29, 507–519.