ELSEVIER

European Journal of Pharmacology 371 (1999) 43–49

ejp

www.elsevier.nl/locate/ejphar

Participation of the opioid system in the regulation of prolactin secretion in androgenized rats: effect of ovarian steroids

Marta Soaje [1], Ricardo P. Deis [*]

Laboratorio de Reproducción y Lactancia, CRICYT-CONICET, Casilla de Correos 855, 5500 Mendoza, Argentina

Received 26 November 1998; received in revised form 25 February 1999; accepted 2 March 1999

Abstract

We examined the role of the opioid system on the regulation of prolactin secretion in neonatally androgenized rats and evaluated the participation of ovarian steroids in this regulation. Androgenized rats exhibited an increase of prolactin secretion with higher serum circulating levels in the afternoon (1800) than in the morning (1000). The administration of the opioid antagonist naloxone (2 mg/kg, 30 min before decapitation) reduced serum prolactin levels in both groups. To identify the opioid receptor subtypes involved in this regulation, opioid agonists were administered i.c.v. 15 min before the decapitation (1000). The μ-opioid receptor agonist DAMGO ([D-Ala^2, NMe-Phe^4, Gly^5-ol]-enkephalin) caused a significant increase in serum prolactin concentration. The selective κ-opioid receptor agonist U-50, 488H (*trans*-(±)-3,4-dichloro-*N*-[2(1-pyrrolidinyl)-cyclohexyl]-benzene acetamide methane sulfonate salt) induced a small but significant increase in serum prolactin levels but no effect was observed after administration of the δ-opioid agonist DPDPE ([D-Pen^2, D-Pen^5]-enkephalin). The role of oestradiol and the opioid system in the continuous secretion of prolactin was also study. Chronic gonadectomy (3–4 weeks) reduced serum prolactin concentrations measured at 1000 but the administration of naloxone had no effect. Three days of oestrogen treatment (2 μg/rat in oil) restored serum prolactin levels compared with ovariectomized animals and this effect was abolished by naloxone treatment. Interestingly, acute ovariectomy or administration of tamoxifen to intact androgenized rats did not prevent the continuous secretion of prolactin observed in these animals and naloxone treatment reduced serum prolactin levels in both groups of rats. We also examine the participation of adrenal progesterone and the endogenous opioid peptides on the regulation of prolactin levels in androgenized rats. After adrenalectomy, no changes in serum prolactin levels (1000) were observed compared with the control animal and naloxone treatment significantly reduced circulating prolactin levels. Progesterone treatment to intact androgenized rats significantly increased prolactin levels and the administration of naloxone blocked the stimulatory effect of the steroid. These results suggest that the opioid system play a role in the regulation of prolactin secretion in androgenized rats modulated by the persistence of oestrogen action. Moreover, the presence or absence of progesterone did not modify the regulation of prolactin secretion by the opioids. The μ- and κ-opioid receptor subtypes are the ones involved in the modulation of pituitary prolactin secretion.

Keywords: Naloxone; Opioid peptide; Opioid receptor, specific; Steroid, ovarian; Prolactin; Androgenized rat

1. Introduction

Neonatal administration of androgens to rats modifies the mechanisms that regulate hormone secretion by the pituitary. Female androgenized rats are characterised by having an ovulatory failure, a persistent vaginal oestrous and elevated serum concentrations of oestrogen and prolactin (Barraclough, 1961). Also in these rats, there are changes in the functionality of the noradrenergic (Lookingland et al., 1982) and dopaminergic systems (Demarest et al., 1981).

Endogenous opioid peptides play a role in the regulation of prolactin secretion in rats during pro-oestrous (Ieri et al., 1980), stress (Rossier et al., 1979), pregnancy (Sagrillo and Voogt, 1991; Soaje and Deis, 1994, 1997) and lactation (Ferland et al., 1978; Selmanoff and Gregerson, 1986). Although a direct effect of opioids at the level of the anterior pituitary gland cannot be ruled out completely (Enjalbert et al., 1979), it has been accepted that endogenous opioid peptides act at central level in the regulation of prolactin secretion (Buydens et al., 1986; Dobson and Brown, 1988; Soaje and Deis, 1994). The

* Corresponding author. Tel.: +54-261-4286112; Fax: +54-261-4273976; E-mail: cricyt@lanet.com.ar

[1] E-mail: m.soaje@lab.cricyt.edu.ar.

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00152-1

opioid peptides mediate their physiological effects by acting on three distinct opioid receptors named the μ, κ and δ, which have been recently cloned in different tissues and cell lines (Evans et al., 1992; Chen et al., 1993; Meng et al., 1993). Many studies have implicated the opioid receptor subtypes μ and κ in the stimulation of prolactin release (Krulich et al., 1986; Leadem and Yagenova, 1987; Kapoor and Willoughby, 1990). On the other hand, it has been established that steroids can influence both hypothalamic endogenous content of opioid peptides and its binding to the respective receptors (Wilkinson et al., 1985; Bridges and Ronsheim, 1987; Weiland and Wise, 1990). In turn, the ovarian steroid environment markedly alters the opiate regulation of prolactin secretion (Limonta et al., 1987; Singh et al., 1992; Maggi et al., 1993; Soaje and Deis, 1997). Therefore, it is clear that the role of the opioid system in the control of prolactin secretion depends on the endocrine milieu.

In the present study, we examined the role of the opioid system on the regulation of prolactin secretion in neonatally androgenized rats and evaluated the participation of ovarian steroids in this regulation.

2. Materials and methods

2.1. Animals

Female rats (200–250 g) bred in our laboratory and originally derived from the Wistar strain were used. Androgenization was done by injecting s.c. 50 μg of testosterone propionate (Schering, Argentina) to 3-day-old female pups. All neonates were returned to their respective dams after treatment. The litters were adjusted to six or eight pups per dam and were weaned from their dams at approximately 22 days of age. The experiments were conducted when the androgenized rats were 3–4 months of age. The animals were kept in a light (lights on from 0600 to 2000) and temperature (22 ± 2°C)-controlled room; rat chow (Cargill, Argentina) and tap water was available ad libitum. Vaginal smears were taken daily and only androgenized rats in constant oestrous during at least 1 week of sampling were used.

2.2. Surgical procedures

A group of intact androgenized rats was surgically implanted with cannulas into the right lateral ventricle 3 days before the intracerebroventricular (i.c.v.) drugs injection. This procedure was done between 0900 and 1200. Androgenized rats were positioned in a stereotaxic frame and a stainless-steel guide cannula was inserted into the lateral ventricle according to the coordinate system of Paxinos and Watson (1986) (M/L 1.5 mm, A/P −0.4 mm relative to bregma, 4 mm, relative to dura). Cannulas were fixed to the skull using dental acrylic and sealed until the time of drug injection. Placement of cannulas was verified histologically and only those animals with the cannula located in the lateral ventricle were included in the study.

Groups of androgenized rats were ovariectomized through two dorsolateral incisions and used either two days (acute ovariectomy) or 3–4 weeks (chronic ovariectomy) after surgery respectively. Another group of androgenized rats was adrenalectomized through two dorsolateral incisions 20 h before the experiment. The adrenalectomized rats were provided with an ample supply of 0.9% (w/v) NaCl as drinking water after operation. All surgical procedures were done under ether anaesthesia.

2.3. Experimental procedures

2.3.1. Experiment 1

This experiment was done to determine serum prolactin concentration at 1000 and at 1800 in neonatally androgenized rats and to establish the role of the opioid system on prolactin secretion in these animals. The opioid antagonist naloxone (Sigma, St. Louis, MO, USA) was administered to androgenized rats (2 mg/kg i.p. at 0930 or 1730). The animals were decapitated 30 min after treatment. Control animals were injected with saline. Blood samples obtained by decapitation were allowed to clot at room temperature and serum was separated and stored frozen (−30°C) until assayed for prolactin.

2.3.2. Experiment 2

To identify the opioid receptor subtypes involved in the regulation of prolactin secretion in androgenized rats, opioid agonists or saline used as vehicle were administered i.c.v. in a volume of 5 μl/rat. On the day of the experiment, the μ-opioid agonist DAMGO ([D-Ala^2, NMe-Phe^4, Gly^5-ol]-enkephalin; Sigma) 5 μg/rat, the κ-opioid agonist U50-488H (*trans*-(±)-3,4-dichloro-*N*-[2(1-pyrrolidinyl)-cyclohexyl]-benzene acetamide methane sulfonate salt; Upjohn) 20 μg/rat and the δ-opioid agonist DPDPE ([D-Pen^2, D-Pen^5]-enkephalin, Sigma) 20 μg/rat were administered 15 min before decapitation. The drugs were injected using a 10 μl Hamilton microsyringe (at approximately 2.5 μl/min) connected to a 30-gauge stainless-steel injector that protruded 1 mm beyond the tip of the guide cannula into the right lateral ventricle. Serum samples were obtained by decapitation at 1000.

2.3.3. Experiment 3

This experiment was performed to the effect of oestradiol and the opioid system on prolactin secretion in androgenized rats. Acute ovariectomized rats were treated with naloxone (2 mg/kg, i.p.) or saline at 0930. Chronic ovariectomized rats were previously injected s.c. at 1700 with oestradiol-benzoate (2 μg/rat in oil, Schering, Ar-

gentine) or vehicle during three consecutive days respectively. On the fourth day either saline or the opioid antagonist, naloxone (2 mg/kg) were administered i.p. at 0900. Ovariectomized rats (acute or chronic), injected with oil and saline were used as control. Another group of intact androgenized was treated with the antioestrogen tamoxifen citrate (Gador, Argentina).This drug was dissolved in 0.14 mol NaCl, 0.5% (v/v) Tween 80 and administered per Os in a dose of 500 μg/kg body mass at 2000 the day before and at 0800 on the day of the experiment. Naloxone or saline were injected at 0930. All animals were decapitated at 1000 and blood samples were obtained.

2.3.4. Experiment 4

This experiment was performed to determine the effect of progesterone and the opioid system in the continuous secretion of prolactin in androgenized rats. Adrenalectomized animals were treated with either naloxone or saline at 0930 and decapitated 30 min after. A group of intact androgenized rats was injected s.c. with two doses of progesterone (5 mg/rat in oil, Schering, Argentina) at 2000 the day before and at 0800 on the day of the experiment. Control animals were treated with oil. The rats were decapitated at 1000, 30 min after naloxone or saline administration.

2.4. Prolactin determination

Prolactin was measured by a double-antibody radioimmunoassay using materials generously provided by the NIDDK's National Hormone and Pituitary Program. Prolactin was radioiodinated using the chloramine T method (Niswender et al., 1969) and purified by passage through Sephadex G-75 and polyacrylamide agarose (ACA 54; LKB, Bromma, Sweden) columns. The results are expressed in terms of the rat prolactin RP-3 standard preparation. The sensitivity of the assay was 1 ng/ml serum and the inter- and intra-assay coefficients of variation were less than 10%.

2.5. Statistics

Statistical evaluations were performed using Student's *t*-test to assay significant differences between means of two groups. One-way analysis of variance followed by Tukey's test was used for multiple comparisons. A value of $P < 0.05$ was considered statistically significant (Snedecor and Cochran, 1967).

3. Results

3.1. Experiment 1: serum prolactin concentrations in intact-androgenized rats: effect of naloxone treatment

Serum prolactin levels measured in the afternoon (1800) were significantly higher ($P < 0.05$) than the values obtained in the morning (1000) and treatment with the opioid antagonist naloxone induced a significant decrease in serum prolactin levels in both groups of androgenized rats (Fig. 1).

3.2. Experiment 2: effect of the intracerebroventricular administration of different opioid agonists on prolactin secretion in intact androgenized rats

Intracerebroventricular administration of the μ-agonist DAMGO caused a significant increase in serum prolactin concentration measured at 1000. The selective κ-opioid agonist U-50, 488H was able to induce a small but significant increase in serum prolactin concentration but no effect was observed after administration of the δ-opioid agonist DPDPE (Fig. 2).

3.3. Experiment 3: serum prolactin concentrations in ovariectomized–androgenized rats: effect of oestrogen and naloxone treatment

Androgenized rats have polyfollicular ovaries but not corpora lutea, therefore, ovariectomy in these rats prevents oestrogen action. Chronic gonadectomy significantly ($P < 0.01$) reduced serum prolactin concentrations while the administration of naloxone had no effect. After three days of oestrogen treatment an increase in serum prolactin concentration measured at 1000 was observed when compared with ovariectomized animals. This increased was prevented by treatment with naloxone (Fig. 3a). Interestingly, acute ovariectomy or administration of tamoxifen to intact androgenized rats did not prevent the continuous secretion of prolactin observed in these animals and naloxone treatment reduced serum prolactin levels in both groups of rats (Fig. 3b).

3.4. Experiment 4: effect of progesterone and naloxone treatment on serum prolactin levels in androgenized rats

Since androgenized rats have no corpora lutea, progesterone levels in these rats are mostly of adrenal origin

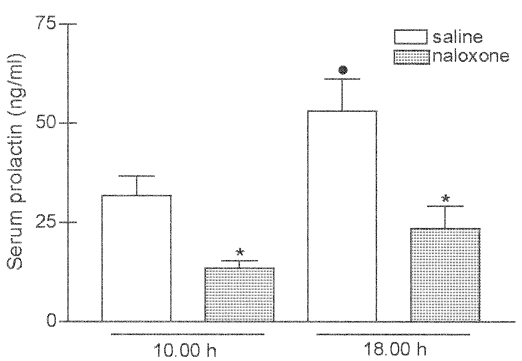

Fig. 1. Effect of saline or naloxone administration on serum prolactin concentration at 1000 or 1800 in androgenized rats. Results are means ± S.E.M of groups of 8 to 12 animals in each experimental group. *$P < 0.05$ compared with saline-injected group (Student's *t*-test); ●$P < 0.05$ compared with the respective value at 1000 (one-way analysis of variance followed by Tukey's test).

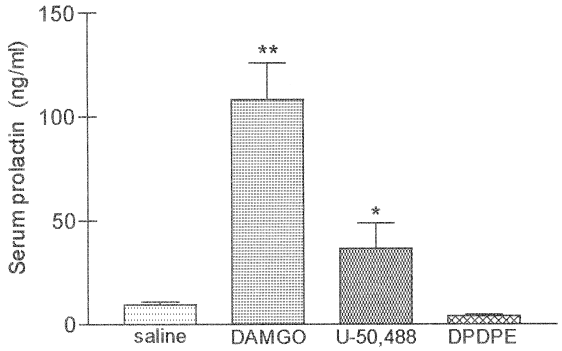

Fig. 2. Effect of opioid agonists on serum prolactin concentration at 1000 in androgenized rat. Animals were injected i.c.v. with either vehicle (saline) or DAMGO or U-50, 488 or DPDPE 15 min before decapitation. Results are means ± S.E.M of groups of seven to nine animals in each experimental group. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the control group (vehicle) (Student's *t*-test).

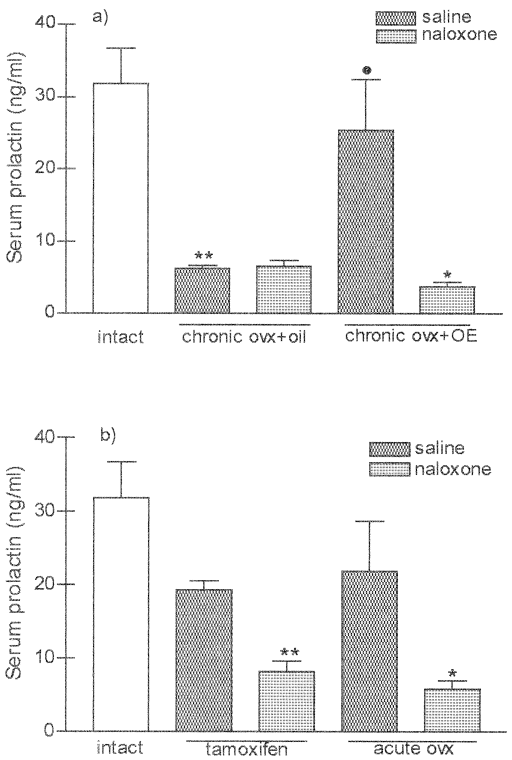

Fig. 3. Effect of oestrogen and naloxone treatment on serum prolactin concentrations (1000) in ovariectomized–androgenized rats. (a) Animals after chronic ovariectomy (chronic OVX) pre-treated with either vehicle (oil) or oestradiol (OE) were injected with either saline or naloxone at 0930, respectively. Results are means ± S.E.M of groups of seven to nine animals in each experimental group. $^{**}P < 0.01$ compared with intact + oil + sal rats; $^{\bullet}P < 0.05$ compared with chronic ovx + oil + saline; $^{*}P < 0.05$ compared with chronic ovx + OE + saline (one-way analysis of variance followed by Tukey's test). (b) Intact androgenized rats pre-treated with tamoxifen and acute ovariectomized (acute OVX) rats were injected with either saline or naloxone at 0930, respectively. Results are means ± S.E.M of groups of seven to nine animals in each experimental group. $^{**}P < 0.01$ compared with tamoxifen + naloxone rats; $^{*}P < 0.05$ compared with acute ovx + naloxone (one-way analysis of variance followed by Tukey's test).

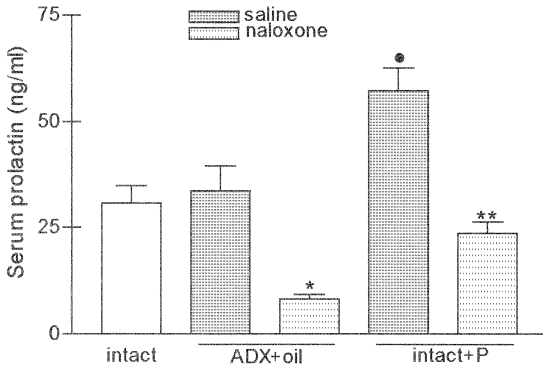

Fig. 4. Effect of progesterone and naloxone treatment on serum prolactin levels (1000) in androgenized rats. Adrenalectomized rats (ADX) were injected with either saline or naloxone at 0930, respectively. Intact androgenized rats pre-treated with either vehicle (oil) or progesterone (P) were injected with either saline or naloxone at 0930, respectively. Results are means ± S.E.M of groups of seven to nine animals in each experimental group. $^{*}P < 0.05$ compared with ADX + oil + saline; $^{\bullet}P < 0.05$ compared with intact + oil + saline; $^{**}P < 0.01$ compared with intact + P + saline (one-way analysis of variance followed by Tukey's test).

(Jahn and Deis, 1986; Deis et al., 1989). When adrenalectomy was performed serum prolactin concentrations measured at 1000 were similar to control values and naloxone treatment significantly reduced serum prolactin levels as in the intact control group. Progesterone treatment to intact androgenized rats significantly increased circulating prolactin levels that was significantly reduced by naloxone (Fig. 4).

4. Discussion

Neonatally female rats treated with androgens is a very interesting model to study the neuromodulatory mechanisms that regulate the increased and constant prolactin secretion observed in these rats. It has been shown that neonatal exposure to androgens modifies the activity of the tuberoinfundibular dopaminergic neurones, being this alteration responsible at least in part of changes in the pattern of prolactin secretion (Demarest et al., 1981). Our results showed that androgenized female rats exhibit an increase of prolactin secretion with higher circulating levels in the afternoon than in the morning. This may suggest that the region generating the prolactin rhythm is not affected by androgenization as observed by others (Yogev and Terkel, 1980; Vaticón et al., 1985). Since serum prolactin concentration was reduced by treatment with the opioid antagonist naloxone in the morning as well as in the afternoon, prolactin secretion seems to be stimulated by the endogenous opioid peptides in these androgenized animals. Other investigators have examined the role of the opioid system on the regulation of the prolactin release in androgenized rats. In agreement with our data, Limonta et al. (1989) reported that an acute injection of naloxone significantly decreases serum levels of prolactin in normal

males and in androgenized females. Moreover, Petersen and Barraclough (1986) demonstrated that prepubertal androgen treatment does not affect the ability of morphine to induce the release of prolactin.

Several studies have implicated the opioid receptor subtypes μ and κ in the stimulation of prolactin release in rats (Krulich et al., 1986; Leadem and Yagenova, 1987; Kapoor and Willoughby, 1990). Using i.c.v. administration of the specific μ-, κ- and δ-opioid agonists, we demonstrate that the μ and κ opioid receptor subtypes are involved in the induction of prolactin release by opioid peptides in androgenized rats. It has been demonstrated that the high oestrogen milieu described in androgenized rats may be responsible for the elevated serum levels of prolactin (Ratner and Peake, 1974; Lookingland et al., 1982). Moreover, oestrogen environment may also influence the action of the opioid system (Limonta et al., 1987; Singh et al., 1992; Soaje and Deis, 1997). Previous reports showed that oestrogen stimulates prolactin secretion in normal ovariectomized animals by acting on the hypothalamic–pituitary axis (Caligaris and Taleisnik, 1976; Raymond et al., 1978; Carón et al., 1994). Oestrogen treatment is also able to restore the elevated serum prolactin levels in androgenized rats after ovariectomy (Lookingland et al., 1982). The presents results confirm this statement since chronic gonadectomy reduced serum prolactin levels and prevented the effect of the opioid system on prolactin secretion. Interestingly, oestrogen treatment restored serum prolactin levels and its regulation by the opioid peptides. The major focus of this study was the responsiveness of androgenized rats to acute ovariectomy or acute tamoxifen treatment. Both treatments were incapable to prevent the participation of the opioid system in the regulation of prolactin secretion previously induced by oestrogen action. It may be considered that an acute fall in circulating oestrogen levels is not sufficient to abolish the high serum prolactin concentration and the participation of the opioid system on prolactin secretion. Most probably, in androgenized rats, a prolonged effect of oestrogen is still present after acute ovariectomy sufficient to maintain activated the opioid system and consequently sustain high levels of prolactin in circulation. This prolonged action of oestrogen was also shown in pregnant rats in which the blockade of the oestrogen receptor by tamoxifen on days 14–15 of pregnancy prevented the inhibitory action of the opioid system on day 19. However, no effect was observed when tamoxifen was administered on day 18 of pregnancy (Soaje and Deis, 1997). On the other hand, it is well known that a single dose of oestrogen given to chronically ovariectomized rats, is more effective on prolactin release three days after administration (Caligaris and Taleisnik, 1976; Carón et al., 1994). Thus, after a chronic gonadectomy a previous sensitisation of the hypothalamus pituitary axis by oestrogen may be necessary to restore the elevated serum prolactin levels and the activation of the opioid system in these animals.

It has been shown that in the arcuate nucleus the neurons responsible for producing opioid peptides are able to concentrate oestrogens (Morrell et al., 1985), thus a subset of β-endorphin or dynorphin containing neurons in the medial basal hypothalamus accumulates oestradiol (Morrell et al., 1985). Interestingly long-term application of oestrogen in the arcuate nucleus induced a continuous hyperprolactinemia with a simultaneous regulatory–inhibitory influence of the opioid system on the prolactin secretion (Carón and Deis, 1996). In a recent work it was also observed the presence of μ-opioid receptor mRNA expressing cells in the arcuate nucleus of the male rats (Mitchell et al., 1998). Also, it has been well established that steroids can influence both hypothalamic endogenous opioid content and its binding to the respective receptors (Wardlaw et al., 1982; Wilkinson et al., 1985; Bridges and Ronsheim, 1987; Weiland and Wise, 1990; Hammer et al., 1994). Moreover, evidences indicate that a long oestrogen treatment increases receptor densities (Wilkinson et al., 1985; Hammer and Bridges, 1987). Since opioid action in chronic ovariectomized rats was restored after three days of oestrogen treatment, the effect of oestrogen may involve an increase of binding sites to naloxone.

It is well established that androgenized rats have polyfollicular ovaries without having corpora lutea, and they exhibit persistent vaginal estrous and lack cyclic preovulatory luteinizing hormone and follicle-stimulating hormone surges (Barraclough, 1961). Thus, progesterone levels in androgenized rats are mostly from the adrenals which can be a source of significant amounts of the steroid in rats (Resko, 1969; Piva et al., 1973; Deis et al., 1989). Our results obtained in adrenalectomized rats clearly indicate that progesterone from adrenal origin is not involved in the regulation of prolactin secretion in androgenized rats. However, the administration of two doses of progesterone to intact androgenized rats induced a significant increase in serum prolactin levels at 1000, in agreement with previous results demonstrating that high levels of progesterone are able to increase significantly serum prolactin levels in intact androgenized rats (Jahn and Deis, 1986). Since this increase of prolactin levels was blocked by naloxone treatment, the effect of progesterone on prolactin release, seems to be mediate by the opioid system.

In conclusion, in this study we extend earlier results confirming the participation of the opioid system on prolactin release in androgenized rats. Our results demonstrate that the modulatory effect of the opioid system on prolactin release seems to be dependent on the persistence of oestrogen action in acute ovariectomized and tamoxifen treated androgenized rats. The results obtained in adrenalectomized rats demonstrate that the presence or absence of progesterone did not modify the regulation of prolactin secretion by the opioids. Furthermore, considering the location of μ- and κ-opioid receptors at the arcuate nucleus together with the presence of oestradiol receptors, we may suggested that in the modulation of prolactin

release in androgenized rats, participate the opioid system with the necessary presence of oestrogen and most probably through its antidopaminergic effect.

Acknowledgements

The authors offer sincere thanks to Dr. A. Koninckx and Mrs. E.G. de DiNasso for their skillful technical assistance. This work was partially supported by the Agencia Nacional de Promoción Científica y Tecnológica PMT-PICT 0098 and from the Programa Latinoamericano de Capacitación e Investigación en Reproducción Humana (PLACIRH) (PLI-256/96; Re-entry grant PRE-005/96). R.P.D. and M.S. are Argentina. R.P.D. and M.S. are members of CONICET. The authors are indebted to NIADDK's National Hormone and Pituitary Program and to Dr A.F. Parlow for kindly providing the materials for the prolactin radioimmunoassay and to UpJohn (Kalamazoo, MI, USA) for the gift of U50,488H.

References

Barraclough, C.A., 1961. Production of anovulatory sterile rats by single injections of testosterone propionate. Endocrinology 68, 62–67.

Bridges, R.S., Ronsheim, P.M., 1987. Immunoreactive beta-endorphin concentrations in brain and plasma during pregnancy in rat: possible modulation by progesterone and estradiol. Neuroendocrinology 45, 381–388.

Buydens, P., Velkeniers, B., Goldstein, J., Vanhaelst, L., 1986. Dissociation of the central and peripheral effects of naloxone on PRL secretion. Horm. Metab. Res. 18, 575–576.

Caligaris, L., Taleisnik, S., 1976. The role of hypothalamic afferent in the release of prolactin induced by ovarian steroids. Neuroendocrinology 21, 139–145.

Carón, R.W., Deis, R.P., 1996. Participation of opioid and serotoninergic systems in prolactin secretion induced by hypothalamic action of estradiol. Neuroendocrinology 64, 124–130.

Carón, R.W., Salicioni, A.M., Deis, R.P., 1994. Mifepristone treatment demonstrates the participation of the adrenal glucocorticoids in the regulation of oestrogen-induced prolactin secretion in ovariectomized rats. J. Steroid Biochem. Mol. Biol. 48, 385–389.

Chen, Y., Mestek, A., Liu, J., Hurley, J.A., Yu, L., 1993. Molecular cloning and functional expression a μ-opioid receptor from rat brain. Mol. Pharmacol. 44, 8–12.

Deis, R.P., Leguizamón, E., Jahn, G.A., 1989. Feedback regulation by progesterone of stress-prolactin release in rats. J. Endocrinol. 120, 37–43.

Demarest, K.T., McKay, D.W., Riegle, G.D., Moore, K.E., 1981. Sexual differences in the sensitive tuberoinfundibular dopamine nerve activity induced by neonatal androgen exposure. Neuroendocrinology 32, 108–113.

Dobson, P.R.M., Brown, B.L., 1988. Involvement of the hypothalamus in opiate-stimulated prolactin secretion. Regul. Pept. 20, 305–310.

Enjalbert, A., Ruberg, M., Fiore, L., Arancibia, S., Priam, M., Kordon, C., 1979. Effect of morphine on the dopamine inhibition of prolactin release in vitro. Eur. J. Pharmacol. 53, 211–212.

Evans, C.J., Keith, D.E., Morrison, H., Magendzo, K., Edwards, R.H., 1992. Cloning of a delta opioid receptor by functional expression. Science 258, 1952–1955.

Ferland, L., Kledzik, G.S., Cusan, L., Labrie, F., 1978. Evidence for a role of endorphins in stress and suckling induced prolactin release in the rat. Mol. Cell. Endocrinol. 12, 267–272.

Hammer, R.P., Bridges, R.S., 1987. Preoptic area opioids and opiate receptors increase during pregnancy and decrease during lactation. Brain Res. 420, 48–56.

Hammer, R.P., Zhou, L., Cheung, S., 1994. Gonadal steroid hormones and hypothalamic opioid circuitry. Horm. Behav. 28, 431–437.

Ieri, T., Chen, H.T., Campbell, G.A., Meites, J., 1980. Effects of naloxone and morphine on the surge of prolactin and gonadotropins in the rat. Endocrinology 106, 1568–1570.

Jahn, G.A., Deis, R.P., 1986. Stress-induced prolactin release in female, male and androgenized rats influence of progesterone treatment. J. Endocrinol. 110, 423–428.

Kapoor, R., Willoughby, J.O., 1990. Activation of MU opioid receptors in the pre-optic-anterior hypothalamus releases prolactin in the conscious male rats. Neuropeptides 19, 231–236.

Krulich, L., Koenig, J.I., Conway, S., McCann, S.M., Mayfield, M.A., 1986. Opioid κ receptor agonist and the secretion of prolactin (PRL) and growth hormone (GH) in the rat. Neuroendocrinology 42, 75–81.

Leadem, C.A., Yagenova, S.V., 1987. Effects of specific activation of mu, delta and kappa opioid receptors on the secretion of luteinizing hormone and prolactin in the ovariectomized rat. Neuroendocrinology 45, 109–117.

Limonta, P., Maggi, R., Dondi, D., Martini, D., Piva, F., 1987. Gonadal steroid modulation of brain systems. J. Steroid Biochem. 27, 691–697.

Limonta, P., Dondi, D., Maggi, R., Martini, L., Piva, F., 1989. Neonatal organization of the brain opioid systems controlling prolactin and luteinizing hormone. Endocrinology 124, 681–686.

Lookingland, K.J., Wise, P.M., Barraclough, C.A., 1982. Failure of the hypothalamic noradrenergic system to function in adult androgen-sterilized rats. Biol. Reprod. 27, 268–281.

Maggi, R., Dondi, D., Rovati, G.E., Martini, L., Piva, F., Limonta, P., 1993. Binding characteristic of hypothalamic mu opioid receptors throughout the estrous cycle in the rat. Neuroendocrinology 58, 366–372.

Meng, F., Xie, G.X., Thompson, R.C., Mansour, A., Goldstein, A., Watson, S.J., Akil, H., 1993. Cloning and pharmacological characterization of a rat kappa opioid receptor. Proc. Natl. Acad. Sci. USA 90, 9954–9958.

Mitchell, V., Prévot, V., Beauvillain, J.C., 1998. Distribution and diurnal variations of the μ-opioid receptor expression in the arcuate nucleus of the male rat. Neuroendocrinology 67, 94–100.

Morrell, J.L., McGinty, J.D., Pfaff, D.W., 1985. A subset of β-endorphin or dynorphin containing neurons in the medio basal hypothalamus accumulates estradiol. Neuroendocrinology 41, 417–426.

Niswender, G.D., Chen, C.L., Midgley, A.R. Jr., Meites, J., Ellis, S., 1969. Radioimmunoassay for rat prolactin. Proc. Soc. Exp. Biol. Med. 130, 793–797.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd edn. Academic Press, Sydney.

Petersen, S.L., Barraclough, C.A., 1986. Effect of naloxone and morphine on LH and prolactin release in androgen-sterilized rats. Neuroendocrinology 44, 84–88.

Piva, F., Gagliano, P., Motta, M., Martini, L., 1973. Adrenal progesterone: factors controlling its secretion. Endocrinology 93, 1178–1184.

Ratner, A., Peake, G.T., 1974. Maintenance of hyperprolactinemia by gonadal steroids in androgen-sterilized and spontaneously constant-estrous rats. Proc. Soc. Exp. Biol. Med. 146, 680–683.

Raymond, V., Beaulieu, M., Labrie, F., Boissier, J., 1978. Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. Science 200, 1173–1175.

Resko, J.A., 1969. Endocrine control of adrenal progesterone secretion in the ovariectomized rat. Science 164, 70–71.

Rossier, J., French, E., Rivier, C., Shibasaki, T., Guillemin, R., Bloom, F.E., 1979. Stress-induced release of prolactin: blockade by dexamethasone and naloxone may indicate β-endorphin mediation. Proc. Natl. Acad. Sci. USA 77, 666–669.

Sagrillo, C.A., Voogt, J.L., 1991. Endogenous opioids mediate the nocturnal prolactin surge in the pregnant rat. Endocrinology 129, 925–930.

Selmanoff, M., Gregerson, K.A., 1986. Suckling-induced prolactin release is suppressed by naloxone and stimulated by β-endorphin. Neuroendocrinolgy 42, 255–259.

Singh, M., Millard, W.J., Layden, M.P., Romano, T.M., Simpkins, J.W., 1992. Opiate stimulation of prolactin secretion is reversed by ovarian hormone treatment. Neuroendocrinology 56, 195–203.

Snedecor, G.W., Cochran, W.G., 1967. Statistical Methods, 2nd edn. Iowa State Univ. Press, Ames.

Soaje, M., Deis, R.P., 1994. A modulatory role of endogenous opioids on prolactin secretion at the end of pregnancy in the rat. J. Endocrinol. 140, 97–102.

Soaje, M., Deis, R.P., 1997. Opioidergic regulation of the prolactin secretion during pregnancy: role of ovarian steroids. J. Endocrinol. 154, 99–106.

Vaticón, M.D., Fernández Galaz, M.C., Tejero, A., Aguilar, E., 1985. Alteration of prolactin control in adults rats treated neonatally with sex steroids. J. Endocrinol. 105, 429–433.

Wardlaw, S.L., Thoron, L., Frantz, A.G., 1982. Effects of sex steroids on brain β-endorphin. Brain Res. 245, 327–331.

Weiland, N.G., Wise, P.M., 1990. Estrogen and progesterone regulate opiate receptor densities in multiple brain regions. Endocrinology 126, 804–808.

Wilkinson, M., Brawer, J.R., Wilkinson, D.A., 1985. Gonadal-steroid induced modification of opiate sites in anterior hypothalamus of female rats. Biol. Reprod. 32, 501–506.

Yogev, L., Terkel, J., 1980. Daily rhythm in secretion of prolactin in androgenized rats. J. Endocrinol. 87, 327–332.