Actions of angiotensin II and dopamine in the medial preoptic area on prolactin secretion

Cristiane M. Leite[2], Giscard José R. Machado[1], Rita Cássia M. Dornelles[1] and Celso R. Franci[2]

[1]Departamento de Ciências Básicas, Faculdade de Odontologia de Araçatuba
Universidade Estadual Paulista, Araçatuba, SP, Brazil
[2]Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900 - Campus USP
14049-900, Ribeirão Preto, SP, Brazil

Corresponding author:
Celso Rodrigues Franci
Fax: +55-16-3633 0017
 Phone +55-16-3602 3022
 E-mail: crfranci@fmrp.usp.br

Short title: **Actions of Ang II and DA on PRL secretion**

SUMMARY

Dopamine (DA) is recognized as primary regulator of PRL secretion and the angiotensin II (Ang II) has been recognized as one brain inhibitory factor of this secretion. In this work, estrogen-primed or unprimed ovariectomized rats were submitted to the microinjection of saline or Ang II after previous microinjection of saline or of DA antagonist (haloperidol, sulpiride or SCH) both in the medial preoptic area (MPOA). The study of these interactions showed that: 1) estrogen-induced PRL secretion is mediated by Ang II and by DA actions in the MPOA, i.e., very high plasma PRL would be prevented by inhibitory action of Ang II, while very low levels would be prevented in part by stimulatory action of DA through D_2 receptors; 2) the inhibitory action of Ang II depends on estrogen and it is mediated in part by inhibitory action of DA through D_1 receptors and in other part by inhibition of stimulatory action of DA through D_2 receptors.

Keywords: prolactin, medial preoptic area, angiotensin II, dopaminergic antagonists, estrogen

INTRODUCTION

The control of prolactin (PRL) secretion depends on the balance between the action of releasing (PRF) and inhibiting (PIF) factors. Dopamine (DA) has been recognized as the primary regulator of PRL synthesis and release. However, other hypothalamic, systemic and local factors act as inhibitors and stimulators (Ben-Jonathan and Hnasko 2001; Freeman et al. 2000). These factors include gamma aminobutyric acid (Schally et al. 1977), neuropeptide Y (Rettori et al. 1990; Silveira and Franci 1999), atrial natriuretic peptide (Franci et al. 1992; Samson et al. 1988), oxytocin (Samson et al. 1986) and angiotensin II (Ang II) (Franci et al. 1997; Steele et al. 1981), among other.

The rostral group of neurons in A_{14} nucleus, a periventricular structure, is responsible by DA innervation of the medial preoptic area (MPOA). This suggests that the MPOA as part of the incerto-hypothalamic dopamine system may have involvement in neuroendocrine mechanisms [Björhlund et al. 1975; Day et al.1980; Lindvall et al.1984). The periventricular preoptic area neurons project heavily to the arcuate nucleus and median eminence (Conrad and Pfaff 1975). Studies that used transections techniques showed the interaction between preoptic area and medial basal hypothalamus, which contains the PRL-regulating tuberoinfundibular dopaminergic (TIDA) neurons (Arai and Yamanouchi 1975; Carrer and Taleisnick 1970). Basal plasma PRL was higher in deafferentated rats that showed persistent estrus relative to the deafferentated and sham deafferentated rats that showed regular cycle. This may indicate that preoptic area neurons participate in the tonic hypothalamic inhibition of basal PRL secretion or the deafferantation disinhibited PRL-releasing factor pathways (Jakubowski et al. 1988). Preoptic - anterior hypothalamic area neurons that facilitate PRL secretion may either stimulate the secretion of some PRF or inhibit the secretion of some PIF into the hypophysial portal vasculature (Day et al. 1982).

Tissue extracts from this area have been reported exhibit PRF activity in vitro (Krulich et al. 1971).

Hormonal state of an intact female influences the DA activity in the MPOA (Matuszewich et al. 2000). Estrogen stimulates the PRL secretion when is implanted into the preoptic area (Pan and Gala 1985).

MPOA shows angiotensin II (Ang II) - stained cell bodies (Lind, et al. 1985), angiotensinogen mRNA, immunoreactive angiotensinogen, angiotensin converting enzyme, immunoreactive angiotensin II, angiotensin II binding sites (Bunnemann et al. 1993) and AT_1 receptors (Phillips et al. 1993). Intracerebroventricular microinjection of Ang II decreases (Myers and Steele, 1991) while of specific antiserum against Ang II increases plasma PRL (Franci et al. 1997) in estrogen-primed ovariectomized rats. Intracerebroventricular microinjection of Ang II excites a large proportion of neurons in the preoptic - anterior hypothalamic area, increasing the neuronal discharge frequency (Gronan and York 1978) and facilitating the release of norepinephrine and DA (Quadri et al. 1991; Summers and Phillips 1983). Microinjection of Ang II into the MPOA decreased plasma PRL in estrogen-primed ovariectomized rats. This response was blocked by losartan, an AT_1 receptor antagonist (Dornelles and Franci 1998a), but it was not altered by alpha- or beta-adrenergic antagonists (Dornelles and Franci 1998b).

Considering that: 1) Ang II and DA act on PRL secretion; 2) the influence of estrogen on Ang II and DA activity in the preoptic area; we aimed to verify in this work, if the Ang II action in the MPOA on PRL secretion would be mediated by D_1 and / or D_2 receptors as well, if the presence or absence of estrogen would modify this putative interaction.

MATERIAL AND METHODS

Animals

Adult female Wistar rats weighing 180-200g were kept in a light- and temperature-controlled environment (lights on from 7:00 to 19:00 h, $22 \pm 2 °C$), with free access to water and food.

Surgery and treatments

All rats were ovariectomized (OVX), and 14 days later a unilateral stainless-steel cannula was implanted into the MPOA at the following coordinates: AP = 2.2; L = ±0.8; V = -7.9 using a stereotaxic instrument (Kopf, USA). The guide cannula was fixed to the skull with two screws and dental cement (Simplex Dental, Brazil). A mandril was used to prevent obstruction of the cannula. Animals were returned to individual cages after surgery carried out under sodium thiopental anesthesia (50 mg/kg, i.p.; Abbott Laboratories, USA). An antibiotic (veterinary pentabiotic, Wyeth Ayerst, USA; 0.2 ml/rat) was injected intramuscularly following the two surgeries. One week after stereotaxic surgery, the animals were subcutaneously injected with estradiol benzoate (25 µg/0.5 ml vegetable oil; Schering-Kenilworth, USA; BE group) or vehicle oil (0.5 ml, OV group) for 3 days. On day 3, 24 h before the experiment, the animals were anesthetized i.p. with 1 ml of tribromoethanol (Aldrich Chem., USA) 2.5% in saline /100g b.w. for insertion of the intra-atrial catheter (tubing with a 0.020 x 0.037 diameter; Dow Corning, USA) through the jugular vein.

Experimental procedures

On the day of the experiment, between 8:30 to 9:00 a.m., an extension of polyethylene tubing (PE 50) filled with heparin solution (1:40) in 0.9% NaCl was attached to the distal end of the jugular cannula. After 30 minutes, heparinized blood samples (0.8 ml) were collected from the external jugular vein at the following intervals: -20 (basal bleeding), 0, 10, 20, 30 and 60 minutes, while the animal was freely moving in the cage. The volume of all samples was replaced immediately after each bleeding with an equivalent volume of saline (0.15 M NaCl). Plasma was separated by centrifugation at 4°C and stored at -20°C until the time for PRL measurement.

Saline (0.15 M NaCl), Ang II (Sigma, USA), haloperidol (D_1 / D_2 receptor antagonist; RBI, USA), sulpiride (D_2 receptor antagonist; RBI) or SCH 23390 (D_1 receptor antagonist; RBI) solutions were injected in a volume of 1 µl during one minute with a Hamilton syringe connected by a polyethylene tubing (PE-10) and injecting needle filled with the solution to be injected. The injections into the MPOA were carried out 10 minutes (NaCl, haloperidol, sulpiride or SCH) and 20 minutes (NaCl or Ang II) after basal bleeding (-20 minutes). The largeness of doses used for microinjections of drugs was based in literature references: 100 pmol of Ang II (Dornelles and Franci, 1998a, b), 5 µg of haloperidol (Weiss and Ettenberg, 1986), 10 µg of sulpiride (Morutto and Phillips, 1997) and 10 µg of SCH 23390 (Moses et al, 1995). A blood sample was withdrawn immediately after the second microinjection (time zero). At the end of the experiment, the brains were removed and fixed in 10% formalin for histological analysis to confirm the localization of the cannula in the MPOA through frozen sections. Only animals with the confirmation of cannula placement in the MPOA (near 90%) were included for hormonal

measurement. The tips of the cannulas did reach the bearings of the MPOA in that region near median line as shown in schematic map (figure 1) adapted from Paxinos and Watson (1997).

Radioimmunoassay (RIA)

RIA for the measurement of plasma PRL was performed using a kit from the National Hormonal and Peptide Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDKD, USA). The lower detectable amount of PRL RP_3 standard was 0.2 ng/ml, the interassay coefficient of variation was 11.7%, and the intra-assay coefficient of variation was 5.5%.

Statistical analysis

The data were analyzed statistically by analysis of variance for repeated measures, followed by Tukey's multiple range test, using a computer software (SAS, USA). The level of significance was set at $P<0.05$. Results are expressed as means ± standard error.

RESULTS

The basal plasma PRL at -20 minutes was around 10 ng / ml in the several unprimed ovariectomized groups (figure 2) and 40 ng / ml in the several estrogen primed ovariectomized groups (figure 3). The difference between unprimed ovariectomized groups and estrogen primed ovariectomized groups was significant ($p < 0.001$) and it indicates the known stimulatory action of estrogen on PRL secretion.

Microinjection of sulpiride (D_2 receptor antagonist) in the MPOA decreased plasma PRL in estrogen-primed (F= 22.987 for critical level of 2.353, figure 3.4) and unprimed (F=

5.216 for critical level of 2.331, figure 2.4) ovariectomized rats while microinjections of saline (figures 3.1 and 2.2), haloperidol (a D_1 / D_2 receptor antagonist, figures 2.2 and 3.2) or SCH (D1 receptor antagonist, figures 2.3 and 3.3) in the MPOA did not change plasma PRL. The decrease of plasma PRL was significant at 30 and 60 minutes in estrogen- primed (figure 3.4) and at 20, 30 and 60 minutes in unprimed- ovariectomized rats (figure 2.4) that received sulpiride microinjection when compared with the control groups that received just microinjections of saline.

The figures 3.1 and 2.1 show that combined microinjections of Ang II with saline in the MPOA decreased the plasma PRL, respectively, in estrogen-primed (F= 213.67 for critical level of 2.315) and unprimed ovariectomized (F= 5.216 for critical level of 2.331) rats. This diminishing was significant at 10, 20, 30 and 60 minutes in estrogen- primed (figure 3.1) and at 60 minutes in unprimed- ovariectomized rats (figure 2.1) that received Ang II microinjection when compared with the respective control groups that received just microinjections of saline.

The combined microinjections of haloperidol (figure 2.2) or SCH (figure 2.3) with AngII in the MPOA did not change the plasma PRL in unprimed ovariectomized rats. However, plasma PRL decreased in the group that received the combined microinjections of sulpiride with Ang II (F= 10.65 for critical level of 2.331, figure 2.4). This diminishing was significant at 10 and 20 minutes in the group submitted to the microinjection of sulpiride combined with Ang II when compared with the group submitted to the microinjection of NaCl combined with Ang II (figure 2.1)

The combined microinjections of haloperidol with Ang II (figure 3.2) in the MPOA in estrogen primed ovariectomized rats did not change the plasma PRL. However, there was change of plasma PRL in the group that received combined microinjections of

sulpiride with AngII (F= 97.744 for critical level of 2.315, figure3.4) or SCH with Ang II (F= 115.412 for critical level of 2.315, figure 3.3). Plasma PRL at 20, 30 and 60 minutes in the group submitted to the microinjection of sulpiride combined with Ang II (figure 3.4), at 30 and 60 minutes in the group submitted to the microinjection of SCH combined with Ang II (figure 3.3) or at 10, 20, 30 and 60 minutes in the group submitted to the microinjection of haloperidol combined with Ang II (figure 3.2) was significantly higher than in the group submitted to the microinjection of NaCl combined with Ang II (figure 3.1).

DISCUSSION

Our results (Figs. 2.1 and 3.1) are in agreement with a known stimulatory action of estrogen on PRL secretion which involves: a) a direct effect on the pituitary to induce synthesis, storage and release of PRL (Maurer and Gorski 1977; Vician et al. 1979); b) stimulation and inhibition of hypothalamic releasing and inhibiting factors of PRL secretion, respectively (Demarest et al. 1984; Pilotte et al. 1984); c) altered sensitivity of the pituitary to the regulating factors of PRL secretion (Rayomond, et al. 1978).

We observed a significant decrease in plasma PRL after microinjection of sulpiride (D_2 receptor antagonist), but not of SCH (D_1 receptor antagonist) or haloperidol (D_1 / D_2 receptor antagonist) in the MPOA in estrogen-primed (figure 3) or unprimed (figure 2) ovariectomized rats.

The female hormonal state, the copulatory environment and perineal stimulation modulate the activity of DA in the MPOA (Matuszewich et al. 2000). DA content (Crowley et al. 1978) and DA turnover (Wuttke et al. 1981) in the MPOA are lower in dietrus and proestrus than during estrus and metestrus. On the other hand, estrogen significantly reduces the DA turnover in the MPOA in ovariectomized rats (Hiemke et al.

1983). It has been suggested that the estrogen can block the DA neurotransmission in the MPOA at the pos-synaptic level ((Döcke et al. 1987).

How could the DA activity in the preoptic area to influence the TIDA neurons that control the pituitary PRL secretion?

PRL release involves two pathways, one originated from midbrain (ascending) and other one from prefrontal córtex (descending), both projecting to the lateral and medial preoptic area. Then, the common PRL release pathway from the preoptic area to turn, caudally until the anterior hypothalamic area (Tindal and Knaggs 1972), which projects monosynaptically to the arcuate region (Kawakami 1976). Such a path would be well situated to influence the system of DA neurons in the arcuate nucleus and hence to regulate the release of DA into the portal vessels. The stimulation of the rostral periventricular area might act, therefore by inhibiting transmission in dopaminergic neurons, which in turn, inhibit the release of DA from arcuate neurons and permit the release of PRL (Tindal and Knaggs 1972). Furthermore, the rostral periventricular region was found to be an effective site for stimulating prolactin release in the rat (Kawakami et al. 1973).

In situ hybridization studies indicate the presence of dopamine D_1 and D_2 receptors into the preoptic-anterior hypothalamic area (Tokyama and Takatsuji 1998).

Our results suggest a tonically active stimulation of PRL secretion by DA acting in D_2 receptors in the MPOA, via an estrogen-independent mechanism since the effect was observed in estrogen primed and unprimed ovariectomized rats. However, we can rule not out a presynaptic D_2 stimulatory autoreceptor regulation of endogenous DA release. In this case, the blockage of these receptors could induce an increase in DA endogenous that could act exclusively at post-synaptic D_1 receptors to inhibit the PRL release. The effect of haloperidol could be explained by its ability to block the presynaptic D_2 stimulatory

autoreceptors as well the pos-synaptic effect through D_1 receptors. On the basis of recent observations obtained with pharmacological probes more selective for different DA receptor subtypes, it was concluded that a simultaneous activation or inhibition of D_1 and D_2 receptors blocks the actions on TIDA neurons mediated by these receptors (Durham et al., 1998). Thus, it is possible that simultaneous blockade of D_1/D_2 receptors by haloperidol in the MPOA also has suppressed any action of DA on PRL secretion, while the blockade of only D_2 receptors by sulpiride did block the tonically stimulated PRL secretion.

Ang II significantly decreased plasma PRL (figure 3.1) in estrogen-primed ovariectomized rats (from close to 40 ng / ml at –20 minutes to near 10 ng / ml at 60 minutes). This lower level was similar to that found in unprimed ovariectomized rats at –20 minutes (figure 2.1).

The endogenous angiotensin system in the preoptic-hypothalamic region does not seem to be involved in the maintenance of basal PRL secretion, since centrally administered Ang II receptor antagonists or angiotensin convertase inhibitors do not change PRL secretion in female or male rats (Dornelles and Franci 1998a; Myers and Steele 1989, 1991). However, the blockade of the central Ang II system by these compounds greatly facilitates stress- or estradiol-induced PRL secretion [Myers and Steele 1989, 1991; Saavedra 1992). Microinjection of Ang II into the MPOA decreased plasma PRL in estrogen-primed ovariectomized rats. This response was blocked by losartan, an AT_1 receptor antagonist (Dornelles and Franci 1998a), but it was altered not by alpha- or beta-adrenergic antagonists (Dornelles and Franci 1998b). Our data suggest that Ang II may antagonize the stimulatory action of estrogen on PRL secretion and thereby to prevent hypersecretion of this hormone. Other investigators have also proposed a possible function

of the angiotensin system in the hypothalamus to limit the magnitude of PRL secretion (Saavedra 1992; Steele 1992).

It has been shown the colocalization of receptor mRNA, Ang II binding sites, Ang II immunoreactivity nerve terminals and Ang II receptors expression in the preoptic-hypothalamic area (Lenkei et al, 1997), Ang II binding sites (Gomes et al, 2006) and AT_1 receptors (Moreno and Franci,2005) in the MPOA. There are consistent evidences about the regulation of brain Ang II receptors by estrogen and progesterone as well the interaction of this regulation with brain areas related with the control of gonadotropin and PRL secretion. Arcuate nucleus from cycling rats on the estrus day or estrogen-primed ovariectomized rats treated with progesterone presented an increased expression of AT_1 receptors (Seltzer et al, 1993). AT_1 receptors as well the expression of their mRNA are induced in the arcuate nucleus DA neurons of ovariectomized rats treated with estrogen and progesterone (Johren etal, 1997). Our group showed that Ang II receptors in the locus coeruleus, median preoptic nucleus and subfornical organ (Donadio etal, 2005) and ARC (Donadio et al, 2006) are upregulated in ovariectomized rats by treatment with estrogen and progesterone.

Is there interaction between actions of Ang II and DA into the MPOA on PRL secretion? The microinjection of sulpiride or Ang II in the MPOA reduced PRL secretion in unprimed ovariectomized rats. However, the effect of sulpiride was observed earlier and persisted up to 60 minutes (figure 2.4), while the effect of Ang II was observed at 60 minutes (figure 2.1). Plasma PRL showed a similar profile in the group receiving the sulpiride / Ang II combination and the group receiving sulpiride / saline (figure 2.4). Thus, the effect of Ang II seems somehow to be masked by sulpiride. On the other hand, SCH (figure 2.3) and haloperidol (figure 2.2) blocked the effect of Ang II. Therefore, basal PRL secretion can be maintained in part by the stimulatory action of DA through D_2 receptors in

unprimed ovariectomized rats, while the Ang II inhibitory action seems to depend on the action of DA mediated by D_1 receptors, since SCH did block the effect of Ang II.

The inhibitory action of Ang II on PRL secretion in estrogen-primed ovariectomized rats was blocked by haloperidol, which acts through D_1 and D_2 receptors, and was partly reduced by sulpiride (D_2 receptor antagonist) and SCH (D_1 receptor antagonist). Thus, the blockade of both receptors did impede the Ang II inhibitory effect, while the blockade of either type (D_1 or D_2) partly did reduced the effect of Ang II. A previous study (Durhan et al. 1998) showed that simultaneous activation or inhibition of D_1 and D_2 receptors did block the actions on TIDA neurons mediated by these receptors.

Since PRL levels close to 40 ng/ml (at -20 minutes) decreased to near 25 ng/ml under the effect of sulpiride / saline or of the sulpiride / Ang II combination in estrogen primed ovariectomized rats (figure 3.4), it seems that the effect of sulpiride masks the effect of Ang II also in this case, as it was observed for unprimed ovariectomized rats.

Electrical stimulation of the MPOA decreased the magnitude of PRL surges in cycling rats during proestrous and estrous afternoon, in lactanting rats during sucling as well in pregnant rats during day and night. These inhibitory mechanisms may be in the MPOA itself or another region whose projections continue through the MPOA 9Wierma and Kastelijn, 1990). On the other hand, basal plasma levels of PRL decreased after microinjections of kainic acid in preoptic-anterior hypothalamic area (POA/AHA). The authors discovered that kainic acid caused extensive damage of medial region but not periventricular region of this area (Day et al, 1982). Suties of deafferentation of this ame area showed that POA neurons integrate some inhibitory tonic mechanism of basal PRL secretion (Jakubowisky et al, 1988).

The dual participation of neurons in the preoptic area to control PRL secretion is a puzzle with some few known pieces. Furthermore, there are not specific studies about dopamine receptors in this brain area correlated with PRL secretion control, estrogen levels or angiotensin II action in rat. Thus, the present work represents some contribution for the restricted literature of this subject.

Taken together, our results raise some conclusions regarding the integration of mechanisms for the control of PRL secretion: I- the estrogen stimulatory action is mediated in part by the stimulatory action of DA through D_2 receptors and so, the sulpiride should reduce part of the estrogen effect on PRL secretion (figure 3); II- the inhibitory action of Ang II depends on estrogen and it is mediated in part by the inhibitory action of DA through D_1 receptors and in other part by inhibition of the stimulatory action of DA through D_2 receptors and so, the haloperidol should inhibit the effect of Ang II by blockade of both receptors; III- plasma PRL induced by estrogen is mediated by Ang II and DA actions in the MPOA of manner that very high levels of plasma PRL should be prevented by the inhibitory action of Ang II, while very low levels should be impeded by the stimulatory action of DA through D_2 receptors.

REFERENCES

ARAI Y, YAMANOUCHI K: Possible role of anterior input to the medial basal hypothalamus (MBH) in regulating prolactin release during pseudopregnancy in the rat. *Brain Res* **83:** 51-58, 1975.

BEN-JONATHAN N, HNASKO R: Dopamine as a Prolactin (PRL) Inhibitor. *Endocr Rev* **22:** 724-76, 2001.

BJÖRHLUND A, LINDVALL O, NOBIN A: Evidence of an incerto-hypothalamic dopamine neurons system in the rat. *Brain Res* **89:** 29-42, 1975.

BUNNEMANN B, FUXE K, GANTEN D: Extrarenal renin systems: The Brain. In: *The renin - angiotensin system.* JIS ROBERTSON and MG NICHOLLS (eds), Mosby, London, 1993, chapter 41.

CARRER HF, TALEISNICK S: Induction and maintenance of pseudopregnancy after interruption of preoptic hypothalamic connections. *Endocrinology* **86:** 231-236, 1970.

CONRAD LCA, PFAFF DW: Axonal projections of medial preoptic area and anterior hypothalamic neurons. *Science* **190:** 112-114, 1975.

CROWLEY WR, O' DONOHUE TL, JACOBOWITZ M: Changes in CA content in discrete brain nuclei during the estrous cycle of the rat. *Brain Res* **147:** 315-326, 1978.

DAY TA, BLESSING W, WILLOUGHBY JO: Noradrenergic and dopaminergic projections to the medial preoptic area of the rat. A combined horseradish peroxidase / catecholamine fluorescence study. *Brain Res* **193:** 543-548, 1980.

DAY TA, OLIVER JR, MANADUE MF, DAVIES B, WILLOUGHBY JO: Stimulatory role for medial preoptic / anterior hypothalamic area neurones in growth hormone and prolactin secretion. A kainic acid study. *Brain Res* **238:** 55-63, 1982.

DEMAREST KT, RIEGLE GD, MOORE KE: Long-term treatment with estradiol induces reversible alterations in tuberoinfundibular dopaminergic neurons: a decreased responsiveness to prolactin. *Neuroendocrinology* **39:** 193-200, 1984.

DÖCKE F, ROHDE W, OELSSNER W, SCHLEUSSNER E, GUTENSCHWAGER I, DÖRNER G: Influence of the medial preoptic dopaminergic activity on the efficiency of the negative estrogen feedback in the prepubertal and cyclic female rats. *Neuroendocrinology* **46:** 445-452, 1987.

DONADIO MVF, GOMES CM, SAGAE SC, FRANCI CR, ANSELMO-FRANCI JA, LUCION AB, SANVITTO GL: Angiotensin II receptors are upregulated by estradiol and progesterone in the locus coeruleus, median preoptic nucleus and subfornical organ of ovariectomized rats. *Brain Res* **1065:** 47-52, 2005.

DONADIO MVF, GOMES CM, SAGAE SC, FRANCI CR, ANSELMO-FRANCI JA, LUCION AB, SANVITTO GL: Estradiol and progesterone modulation of angiotensin II receptors in the arcuate nucleus of ovariectomized and lactating rats. *Brain Res* **1083:** 103-109, 2006.

DORNELLES RCM, FRANCI CR: Action of AT_1 subtype angiotensin II receptors of the medial preoptic area on gonadotropins and prolactin release. *Neuropeptides* **32:** 51-55, 1998a.

DORNELLES RCM, FRANCI CR: Alpha- but not beta- adrenergic receptors mediate the effect of angiotensin II in the medial preoptic area on gonadotropins and prolactin secretion. *Eur J Endocrinol* **138:** 583-586, 1998b.

DURHAM RA, JOHNSON JD, EATON MJ, MOORE KE, LOOKINGLAND KJ: Opposing roles for dopamine D_1 and D_2 receptors in the regulation of hypothalamic tuberoinfundibular dopamine neurons. *Eur J Pharmacol* **355:**141-147, 1998.

FRANCI CR, ANSELMO-FRANCI JA, MCCANN SM: The role of endogenous atrial natriuretic peptide in resting and stress- induced release of corticotropin, prolactin, growth hormone and thyroid-stimulating hormone. *Proc Natl Acad Sci, USA* **89:** 11391-11395, 1992.

FRANCI CR, ANSELMO-FRANCI JA, MCCANN SM: The hypothalamic angiotensinergic neurons play a physiologically significant inhibitory role to suppress plasma prolactin, growth hormone and TSH, but not ACTH by central action in ovariectomized rats. *Peptides* **18:** 971-976, 1997.

FREEMAN ME, KANYICSKA B, LERANT A, NAGY G: Prolactin: Structure, function, and regulation of secretion. *Physiol Rev* **80:** 1523-1631, 2000.

GOMES CM, DONADIO MVF, FRANSKOVIAK I, ANSELMO-FRANCI JA, FRANCI CR, LUCION AB, SANVITTO GL: Neonatal handling reduces angiotensin II receptor density in the medial preoptic area and paraventricular nucleus and locus coeruleus of female rats. *Brain Res* **1067:** 177-180, 2006.

GRONAN RJ, YORK DH: Effects of angiotensin II and acetylcholine on neurons in the medial preoptic area. *Brain Res* **154:** 172–177, 1978.

HIEMKE C, FROHNE D, BRUDER D, GHRAF R: Effect of oestradiol benzoate and progesterone on luteinizing hormone release and catecholamine turnover in the preoptic-hypothalamic brain area of ovariectomized rats. *J Endocrinol* **97:** 437-445, 1983.

JAKUBOWSKI M, DOW RC, FINK G: Preoptic-hypothalamic pathways controlling nocturnal prolactin surges, pseudopregnancy, and estrous cyclicity in the rat. *Neuroendocrinology* **47:** 13-19, 1988.

JÖHREN O, SANVITTO GL, EGIDY G, SAAVEDRA JM: Angiotensin II AT$_{1A}$ receptor mRNA expression is induced by estrogen-progesterone in dopaminergic neuron of the female rat arcuate nucleus. *J Neurosci* **17:** 8283-8392, 1997.

KAWAKAMI M, KIMURA F, KONNO T: Possible role of the medial basal prechiasmatic area in the release of LH and prolactin in rats. *Endocrinol Jap* **20:** 335-344, 1973.

KAWAKAMI M: Electrophysiological evidences for possible participation of periventricular neurons in anterior pituitary regulation. *Brain Res* **101:** 79-94, 1976.

KRULICH L, QUIJADA M, ILLNER P: Localization of prolactin inhibiting factor (PIF), prolactin releasing factor (PRF), growth hormone – RF (GRF) and GIF activies in the hypothalamus of the rat. *Proceedings 53rd Meeting of Endocrine Society*, 83, 1971.

LENKEI Z, PALKOVITS M, CORVOL P, LLORENS-CORTES C: Expression of angiotensin type-1 (AT$_1$) and type-2 (AT$_2$) receptor mRNA in the adult rat brain: a functional neuroanatomical review. *Front Neuroendocrinol* **18:** 383-439, 1997.

LIND RW, SWANSON LW, GANTEN D: Organization of angiotensin II immunoreactive cells and fibers in the rat central nervous system. *Neuroendocrinology* **40:** 2-24, 1985.

LINDVALL O, BJÖRHLUND A, SKAGERBERG G: Selective histochemical demonstration of dopamine terminal systems in rat di- and telencephalon: new evidence for dopaminergic innervation of hypothalamic neurosecretory nuclei. *Brain Res* **306:** 19-30, 1984.

MATUSZEWICH L, LORRAIN DS, HULL EM: Dopamine release in the medial preoptic area of female rats in response to hormonal manipulation and sexual activity. *Behav Neurosci* **114:** 772- 782, 2000.

MAURER RA, GORSKI J: Effects of estradiol-17β and pimozine on prolactin synthesis in male and female rats. Endocrinology **101:** 76-84, 1977.

MORENO AS, FRANCI CR: Angiotensin II receptors and estrogen receptors in neurons on preoptic área of female rats. 35th Annual Meeting, Society for Neurosciences, Wahsington-DC, Abstract: **521.12**, 2005.

MORUTTO CL, PHILLIPS GD: Isolation rearing enhances the locomotor stimulant properties of intra-perifornical sulpiride, but impairs the acquisition of a conditioned place preference. *Psychopharmacology* **133:** 224-232, 1997

MOSES J, LOUCKS JA, WATSON HL, MATUSZEVICH L, HULL EM: Dopaminergic drugs in the medial preoptic area and nucleus accumbens: effects on motor activity, sexual motivation, and sexual performance. *Pharmacol Biochem Behav* **51:** 681-686, 1995

MYERS LS, STEELE MK: The brain renin-angiotensin system and the regulation of prolactin secretion in female rats: Influence of ovarian hormones. *J Neuroendocrinol* **1:** 299-303, 1989.

MYERS LS, STEELE MK: The brain renin-angiotensin system and prolactin secretion in the male rat. *Endocrinology* **129:** 1744-1748, 1991.

PAN J-T, GALA RR: Central nervous system regions involved in the estrogen-induced afternoon prolactin surge. II. Implantation studies. *Endocrinology* **117:** 388-395, 1985.

PAXINOS G, WATSON C: The rat brain in stereotaxic coordinates. Academic Press, San Diego,Compact 3rd Edition,1997.

PHILLIPS MI, SHEN L, RICHARDS EM, RAIZADA MK: Immunohistochemical mapping of angiotensin AT_1 receptors in the brain. *Regul Pept* **44:** 95-107, 1993.

PILOTTE NJ, BURT DR, BARRACLOUGH CA: Ovarian steroids modulate the release of dopamine into hypophysial portal blood and the density of anterior pituitary (3H) spiropene-binding sites in ovariectomized rats. *Endocrinology* **114:** 2306-2311, 1984.

QUADRI F, BADOER E, STADLER T, UNGER T: Angiotensin II induced noradrenaline release from the anterior hypothalamus in consious rats: a brain microdialysis study. *Brain Res* **563:** 137-141, 1991.

RAYOMOND V, BEAULIEU M, LABRIE F, BOISSIER J: Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. *Science* **200:** 1175 - 1178, 1978.

RETTORI V, MILENKOVIC L, RIEDEL M, MCCANN SM: The role of neuropeptide Y (NPY) in the control of gonadotropins and prolactin release in rat. *Gynecol Endocrinol* **4:** 169-179, 1990.

SAAVEDRA JM: Brain and pituitary angiotensin. *Endocr Rev* **13:** 329-380, 1992.

SAMSON WK, BIANCHI R, MOGG R: Evidence for dopaminergic mechanism for the prolactin inhibitory effect of the atrial natriuretic peptide factor. *Neuroendocrinology* **47:** 268-271, 1988.

SAMSON WK, LUMPKIN MD, MCCANN SM: Evidence for physiological role for oxytocin in the control of prolactin. *Endocrinology* **119:** 554-561, 1986.

SCHALLY AV, REDDING TW, ARIMURA A, DUPONT A, LINTHICUM GL: Isolation of gamma-amino butyric acid from pig hypothalami and demonstration of its prolactin release inhibiting (PIF) activity in vivo and in vitro. *Endocrinology* **100:** 681-691, 1977.

SELTZER A, TSUTSUMI K, SHIGEMATSU K, SAAVEDRA JM: Reproductive hormones modulate angiotensin AT_1 receptors in the arcuate nucleus of the female rat. *Endocrinology* **133:** 939-941, 1993.

SILVEIRA NA, FRANCI CR: Antisense mRNA for NPY-Y_1 receptors in the medial preoptic area increases prolactin secretion. *Braz J Med Biol Res* **32:** 1161-1165, 1999.

STEELE MK: The role of brain angiotensin II luteinizing hormone and prolactin release. *Trends Endocrinol Metabol* **3:** 295-301, 1992.

STEELE MK, NEGRO-VILAR A, MCCANN SM: Effect of angiotensin II on in vivo and in vitro release of anterior pituitary hormones in the female rat. *Endocrinology* **109:** 893-899, 1981.

SUMNERS C, PHILLIPS MI: Central injection of angiotensin II alters catecholamine activity in the brain. *Am J Physiol* **244:** R257-263, 1983.

TINDAL JS, KNAGGS GS: Pathways in the forebrain of the rat concerned with the release of prolactina. *Brain Res* **119:** 211-221, 1972.

TOHYAMA M, TAKATSUJI K: Atlas of neuroactives substances and their receptors in the rat, Oxford University Press, Oxford,1998.

VICIAN L, SHUPNIK MA, GORSKI J: Effects of oestrogen on primary ovine pituitary cell cultures: stimulation of prolactin secretion, synthesis and preprolactin messenger ribonucleic acid activity. *Endocrinology* **104:** 736-743, 1979.

WUTTKE W, MANSKY T, STOCK KW, SANDMANN R: Modulatory actions of estradiol on CA and GABA turnover and effects on serum PRL and LH. In: *Steroid regulation of the brain*. K Fuxe, JA Gustafsson and L Wetterberg (eds), Perganon Press, New York, 1981, pp 135-146.

WEISS F, ETTENBRG A: Comparison of cycling induced by unilateral intrastriatal microinjections of haloperidol, clozapine and CCK-8 in rats. *Pharmacol Biochem Behav* **24:** 983-989, 1986.

LEGENDS

Figure 1- The hatched area represents the site of cannulas tips localization for microinjections in the preoptic area: ac-anterior commissure; ox- optic chiasm; MPA-medial preoptic area; MPOL-medial preoptic area, lateral part; Pe- periventricular nucleus; AVPe- anteroventral periventricular nucleus. (Adapted from Paxinos and Watson, 1997; bregma -0.26mm / interaural 8.74 mm).

Figure 2- Plasma PRL after following microinjections in the medial preoptic area (MPOA) of ovariectomized rats: NaCl +NaCl (O) and NaCl+ Ang II (●), panel 2.1; haloperidol +NaCl (O) and haloperidol + Ang II (●), panel 2.2; SCH +NaCl (O) and SCH + Ang II (●),panel 2.3; sulperide +NaCl (O) and sulperide + Ang II (●), panel 2.4; $^{*}P<0.05$ vs NaCl + NaCl; $^{\#}P<0.05$ vs NaCl + Ang II. The number of animals in the groups was 10 to 14.

Figure 3- Plasma PRL after following microinjections in the medial preoptic area (MPOA) of estrogen-primed ovariectomized rats: NaCl +NaCl (O) and NaCl+ Ang II (●), panel 3.1; haloperidol +NaCl (O) and haloperidol + Ang II (●), panel 3.2; SCH +NaCl (O) and SCH + Ang II (●), panel 3.3; sulperide +NaCl (O) and sulperide + Ang II (●), panel 3.4; $^{*}P<0.05$ vs NaCl + NaCl; $^{\#}P<0.05$ vs NaCl + Ang II. The number of animals in the groups was 10 to 14.
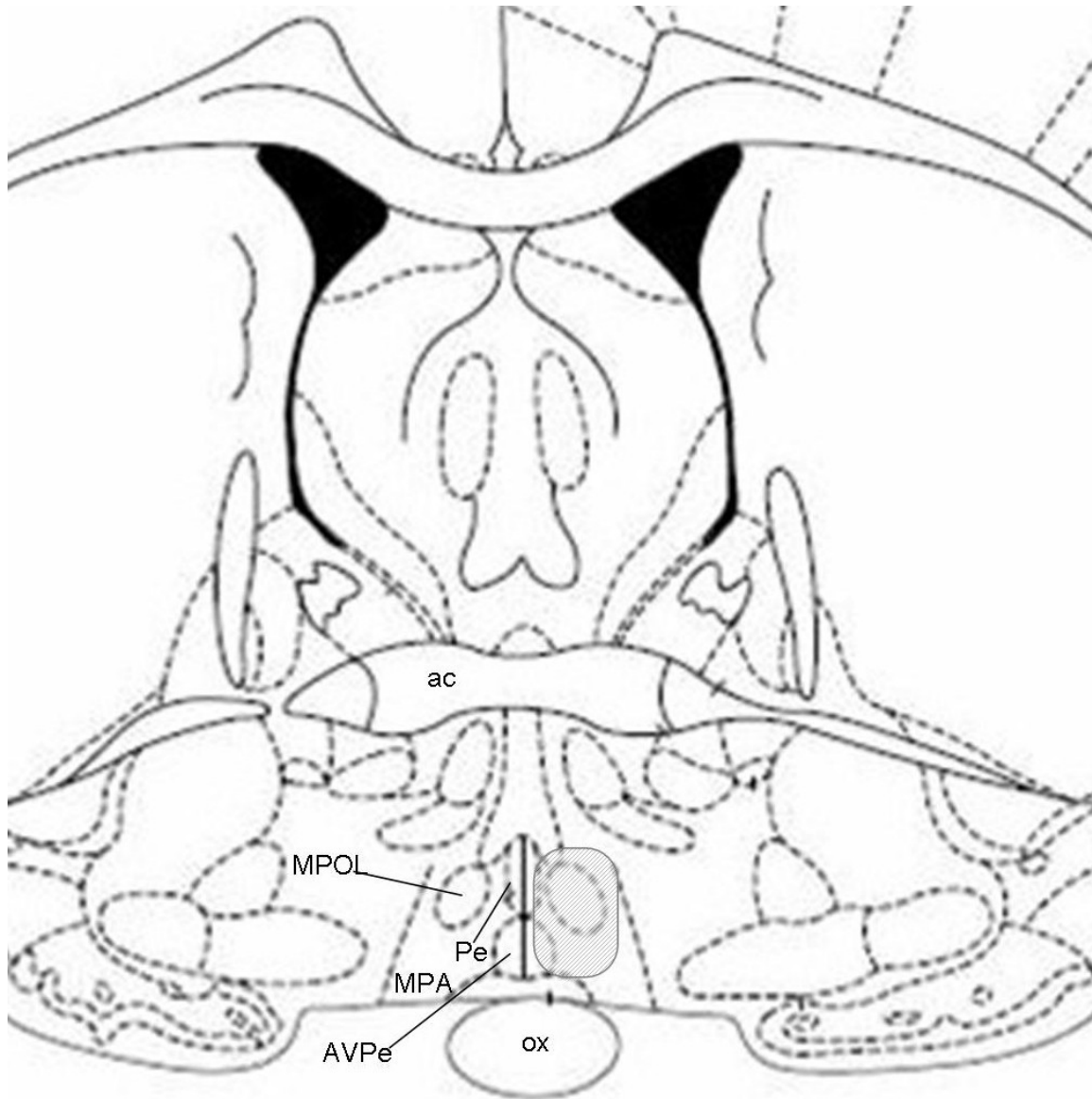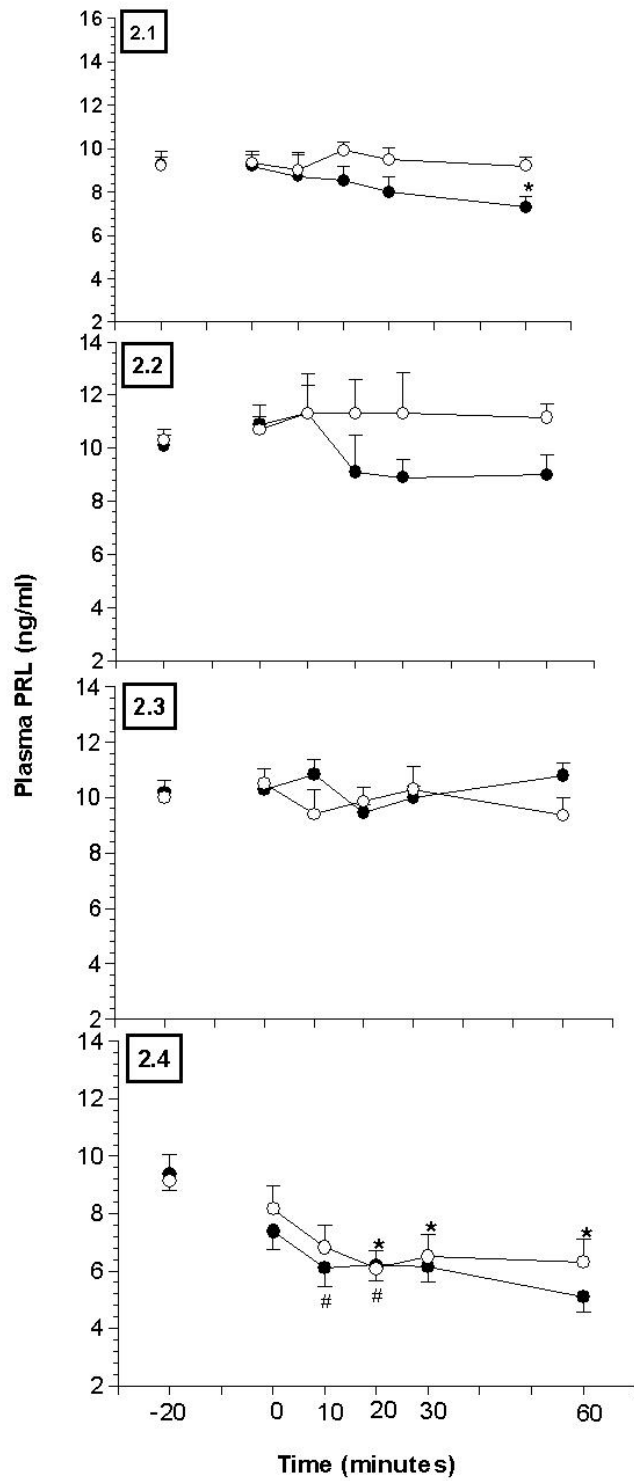
Figura 1 – ms878

Figura 2 – ms878

Figura 3 – ms878