

Actions of Angiotensin II and Dopamine in the Medial Preoptic Area on Prolactin Secretion

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Summary

Dopamine (DA) is known as a primary regulator of prolactin secretion (PRL) and 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). Our study of these interactions has shown that 1) estrogen-induced PRL secretion is mediated by Ang II and 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 D₂ receptors, 2) the inhibitory action of Ang II depends on estrogen and is mediated in part by inhibitory action of DA through D₁ receptors and in other part by inhibition of stimulatory action of DA through D₂ receptors.

Key words

Prolactin • Medial preoptic area • Angiotensin II • Dopaminergic antagonists • Estrogen

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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 others.

The rostral group of neurons in A₁₄ nucleus, a periventricular structure, is responsible for 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 transection 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 deafferentation 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 to exhibit PRF activity *in vitro* (Kulich *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 (Bunemann *et al.* 1993) and AT₁ 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 AT₁ 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: a) if the Ang II action in the MPOA on PRL secretion would be mediated by D₁ and/or D₂ receptors as well, and b) if the presence or absence of estrogen would modify this putative interaction.

Methods

Animals

Adult female Wistar rats weighing 180-200 g were kept in a light- and temperature-controlled environment (lights on from 7:00 to 19:00 h, 22 ± 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/100 g 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 h, 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 min, 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 min, 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 (D₁/D₂ receptor antagonist, RBI, USA), sulpiride (D₂ receptor antagonist, RBI) or SCH 23390 (D₁ 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 min (NaCl, haloperidol, sulpiride or SCH) and 20 min (NaCl or Ang II) after basal bleeding (-20 min). The doses used for microinjections of drugs were based on literary data: 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

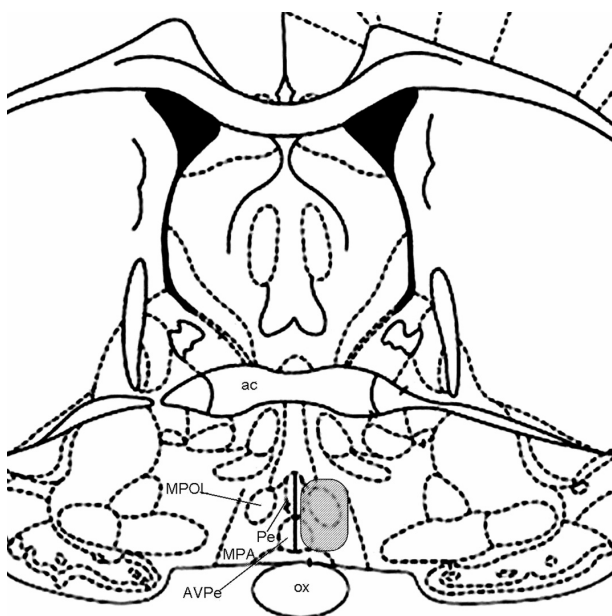


Fig. 1. The gray area represents the site of cannulas tips localization for microinjections in the preoptic area: ac – anterior commissure; ox – optic chiasm; MPA – medial preoptic area; MPOL –medial preoptic area, lateral part; Pe – periventricular nucleus; AVPe – anteroventral periventricular nucleus.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

(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 (Fig. 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 \pm standard error.

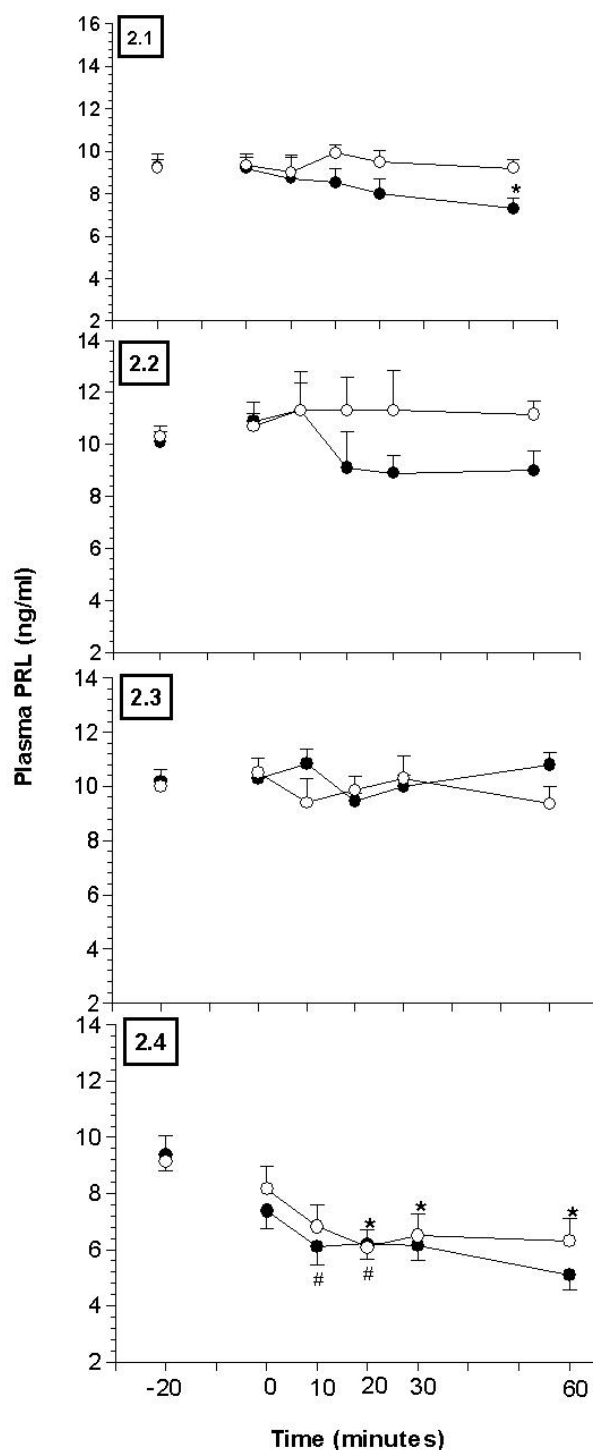


Fig. 2. Plasma PRL after following microinjections in the medial preoptic area (MPOA) of ovariectomized rats: NaCl + NaCl (O) and NaCl + Ang II (●), panel 2.1; haloperidol + NaCl (O) and haloperidol + Ang II (●), panel 2.2; SCH + NaCl (O) and SCH + Ang II (●), panel 2.3; sulpiride + NaCl (O) and sulpiride + 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.

Results

The basal plasma PRL at -20 min was around 10 ng/ml in the several unprimed ovariectomized groups

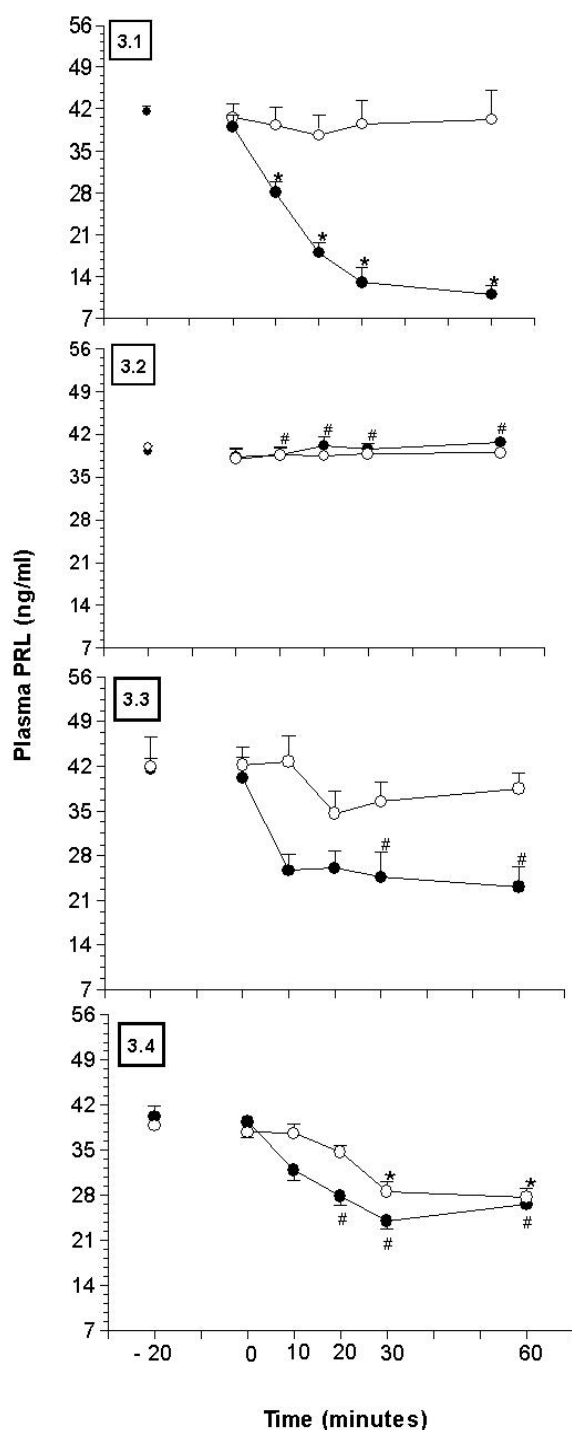


Fig. 3. Plasma PRL after following microinjections in the medial preoptic area (MPOA) of estrogen-primed ovariectomized rats: NaCl + NaCl (O) and NaCl + Ang II (●), panel 3.1; haloperidol + NaCl (O) and haloperidol + Ang II (●), panel 3.2; SCH + NaCl (O) and SCH + Ang II (●), panel 3.3; sulpiride + NaCl (O) and sulpiride + 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.

(Fig. 2) and 40 ng/ml in the several estrogen-primed ovariectomized groups (Fig. 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, Fig. 3.4) and unprimed ($F = 5.216$ for critical level of 2.331, Fig. 2.4) ovariectomized rats while microinjections of saline (Fig. 2.1 and 3.1), haloperidol (a D_1/D_2 receptor antagonist, Fig. 2.2 and 3.2) or SCH (D_1 receptor antagonist, Fig. 2.3 and 3.3) in the MPOA did not change plasma PRL. The decrease of plasma PRL was significant at 30 and 60 min in estrogen-primed (Fig. 3.4) and at 20, 30 and 60 min in unprimed ovariectomized rats (Fig. 2.4) that received sulpiride microinjection when compared with the control groups that received just microinjections of saline.

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 min in estrogen-primed (Fig. 3.1) and at 60 min in unprimed ovariectomized rats (Fig. 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 (Fig. 2.2) or SCH (Fig. 2.3) with Ang II 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, Fig. 2.4). This decrease was significant at 10 and 20 min in the group subjected to sulpiride microinjection combined with Ang II when compared with the group subjected to the microinjection of NaCl combined with Ang II (Fig. 2.1)

The combined microinjections of haloperidol with Ang II (Fig. 3.2) in the MPOA in estrogen-primed ovariectomized rats did not change plasma PRL. However, there was a change of plasma PRL in the group that received combined microinjections of sulpiride with Ang II ($F = 97.744$ for critical level of 2.315, Fig. 3.4) or SCH with Ang II ($F = 115.412$ for critical level of 2.315, Fig. 3.3). Plasma PRL at 20, 30 and 60 min in the group submitted to the microinjection of sulpiride combined with Ang II (Fig. 3.4), at 30 and 60 min in the group, which had received a microinjection of SCH combined

with Ang II (Fig. 3.3) or at 10, 20, 30 and 60 min in the group received a microinjection of haloperidol combined with Ang II (Fig. 3.2) was significantly higher than in the group submitted to the microinjection of NaCl combined with Ang II (Fig. 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), and c) altered sensitivity of the pituitary to the regulating factors of PRL secretion (Raymond *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 (Fig. 3) or unprimed (Fig. 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 diestrus and proestrus than during estrus and metestrus. On the other hand, estrogen significantly reduces the DA turnover in the MPOA of ovariectomized rats (Hiemke *et al.* 1983). It has been suggested that the estrogen can block the DA neurotransmission in the MPOA at the postsynaptic level (Döcke *et al.* 1987).

How could the DA activity in the preoptic area influence the TIDA neurons that control the pituitary PRL secretion? PRL release involves two pathways, one originating from midbrain (ascending) and the other one from prefrontal cortex (descending), both projecting to the lateral and medial preoptic area. Then, the PRL release common pathway projects from the preoptic area caudally until the anterior hypothalamic area (Tindal and Knaggs 1972), which projects monosynaptically to the arcuate region (Kawakami and Sakuma 1976). Such a pathway would be well located 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 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 (Tohyama and Takatsuji 1998).

Our results suggest a tonically active stimulation of PRL secretion by DA acting on 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 not rule out a presynaptic D_2 stimulatory autoreceptor regulation of endogenous DA release. In this case, the blockade of these receptors could induce an increase in DA endogenous that could act exclusively at postsynaptic 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 postsynaptic 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 has also 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 (Fig. 3.1) in estrogen-primed ovariectomized rats (from close to 40 ng/ml at -20 min to near 10 ng/ml at 60 min). This lower level was similar to that found in unprimed ovariectomized rats at -20 min (Fig. 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 AT₁ receptor antagonist (Dornelles and Franci 1998a), but it was not altered 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).

There is 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), moreover Ang II binding sites (Gomes *et al.* 2006) and AT₁ receptors (Moreno and Franci 2005) colocalized in the MPOA. There is consistent evidence 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 AT₁ receptors (Seltzer *et al.* 1993). AT₁ receptors as well the expression of their mRNA are induced in the arcuate nucleus DA neurons of ovariectomized rats treated with estrogen and progesterone (Jöhren *et al.* 1997). Our group showed that Ang II receptors in the locus coeruleus, median preoptic nucleus and subfornical organ (Donadio *et al.* 2005) and ARC (Donadio *et al.* 2006) are upregulated in ovariectomized rats by treatment with estrogen and progesterone.

Is there any interaction between actions of Ang II and DA in 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 min (Fig. 2.4), while the effect of Ang II was observed at 60 min (Fig. 2.1). Plasma PRL showed a similar profile in the group receiving the sulpiride/Ang II combination and the group receiving sulpiride/saline (Fig. 2.4). Thus, the effect of Ang II seems somehow to be masked by sulpiride. On the other hand, SCH (Fig. 2.3) and haloperidol (Fig. 2.2) blocked the effect of Ang II. Therefore, basal PRL secretion can be maintained in part by the stimulatory action of DA through D₂ receptors in unprimed ovariectomized rats, while the Ang

II inhibitory action seems to depend on the action of DA mediated by D₁ 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 D₁ and D₂ receptors, and was partly reduced by sulpiride (D₂ receptor antagonist) and SCH (D₁ receptor antagonist). Thus, the blockade of both receptors did impede the Ang II inhibitory effect, while the blockade of either receptor type (D₁ or D₂) partly reduced the effect of Ang II. A previous study (Durham *et al.* 1998) showed that simultaneous activation or inhibition of D₁ and D₂ receptors blocked the actions on TIDA neurons mediated by these receptors.

Since PRL levels close to 40 ng/ml (at -20 min) decreased to near 25 ng/ml under the effect of sulpiride/saline or of the sulpiride/Ang II combination in estrogen primed ovariectomized rats (Fig. 3.4), it seems that the effect of sulpiride masks the effect of Ang II also in this case, as 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 lactating rats during suckling 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 (Wiersma 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). Studies of deafferentation of this area showed that POA neurons integrate some inhibitory tonic mechanism of basal PRL secretion (Jakubowski *et al.* 1988).

The dual participation of neurons in the preoptic area to control PRL secretion is a puzzle with only few known pieces. Furthermore, there are no specific studies about the relationship between dopamine receptors in this brain area and PRL secretion control, estrogen levels or angiotensin II action in rat. Thus, the present work represents some contribution to the limited literature on this subject.

Taken together, our results raise some conclusions regarding the integration of mechanisms for the control of PRL secretion: 1) the estrogen stimulatory action is mediated in part by the stimulatory action of DA through D₂ receptors and thus, the sulpiride should reduce

part of the estrogen effect on PRL secretion (Fig. 3), 2) the inhibitory action of Ang II depends on estrogen and it is mediated in part by the inhibitory action of DA through D₁ receptors and in other part by inhibition of the stimulatory action of DA through D₂ receptors and so, the haloperidol should inhibit the effect of Ang II by blockade of both receptors, 3) plasma PRL induced by estrogen is mediated by Ang II and DA actions in the MPOA so 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 D₂ receptors.

Conflict of Interest

There is no conflict of interest.

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