Alpha- but not beta-adrenergic receptors mediate the effect of angiotensin II in the medial preoptic area on gonadotropin and prolactin secretion

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Abstract

The aim of this work was to study the participation of α - and β -adrenergic receptors on the effect of microinjection of angiotensin II (A II) into the medial preoptic area (MPOA) on the secretion of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL) in estrogen-primed ovariectomized (OVX) rats. Microinjection of A II into the MPOA increased plasma LH. This effect was reduced by previous microinjection of an α - (phentolamine), but not a β - (propranolol) adrenergic blocker into the MPOA. Plasma PRL decreased after microinjection of A II into the MPOA, but this effect was unchanged by phentolamine or propranolol. There was no change in plasma FSH in any condition. These results indicate that A II in the MPOA stimulates LH secretion and inhibits PRL secretion in estrogen-primed OVX rats. The effect on secretion of LH, but not of PRL, is mediated by α -adrenergic receptors in the MPOA.

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Introduction

The distribution of angiotensin II (A II)-AT₁ receptors overlaps with that of luteinizing hormone-releasing hormone (LHRH) in some brain areas, including the medial preoptic area (MPOA) and median eminence (1), and the number of A II receptors in the preoptic area and the pituitary gland is reduced during estrus or by chronic estrogen treatment (2, 3). The intracerebroventricular (i.c.v.) microinjection of A II increases plasma luteinizing hormone (LH) and decreases plasma prolactin (PRL) in normal female rats or estrogen-primed ovariectomized (OVX) rats (4). LH surges during proestrus and ovulation are blocked by i.c.v. microinjection of the A II receptor blocker, saralasin, or the A II synthesis inhibitor, enalapril (5, 6). In OVX rats, the i.c.v. microinjection of specific antiserum against A II decreases the plasma LH and LHRH content in the median eminence and MPOA, but increases plasma PRL (7–9). A II may have a role in the release of LH and PRL that is modulated by estrogen (5, 10).

In contrast, norepinephrine has a stimulatory action on LH secretion (11). Furthermore, there is an interaction between A II and catecholamines in certain brain structures (3), among them the MPOA, where A II facilitates the release of norepinephrine and dopamine (12).

The aim of this work was to evaluate if, in the MPOA, there is an interaction between A II, estrogen and

adrenergic receptors in the control of gonadotropin and PRL secretion.

Material and methods

Adult female Wistar rats weighing 180–200 g were ovariectomized and housed under conditions of controlled temperature (23–25°) and light (lights on from 0700 h to 1900 h) with free access to water and food. Two weeks later, a stainless guide cannula was placed stereotaxically into the MPOA, using the following coordinates: 0.8 mm lateral to the sagittal line; 2.2 mm anterior to the bregma; 8.0 mm ventral to the dorsum of the skull. The cannula was attached to the skull with dental cement and two metal screws. About 1 week after this surgery and 24 h before the experiment, a systatic catheter was introduced into the external jugular vein. The animals received a prophylactic injection of antibiotic (Veterinary Pentabiotic, Fontoura-Whyte). For anaesthesia, 2.5% tribromethanol (Aldrich; 1 ml/100 g body weight, i.p.) was used. For 3 days before the experiment, the animals received an s.c. injection of estradiol benzoate (Schering; 25 μ g/day) in corn oil.

Experimental procedure

Each experiment began between 0900 h and 1000 h. Heparinized blood samples (0.8 ml) were collected from

the jugular catheter 20 min before (basal), immediately before (zero time) and 10, 20, 30 and 60 min after the microinjection of A II (Sigma; 100 pmol) or saline (NaCl 0.15 mol/l) into the MPOA. Ten minutes after the first blood sample (that is, 10 min before the microinjection of A II or saline), 1 μ l saline, phentolamine (Sigma; 100 pmol) or propranolol (Sigma; 100 pmol) was injected into the MPOA. Saline was injected through the jugular cannula to replace the volume of blood collected. The plasma was removed after refrigerated centrifugation and frozen for later measurement of LH, follicle-stimulating hormone (FSH) and PRL. The brains were removed, fixed in 10% formalin and stained with Nissl in order to determine the site of microinjection. Only animals having a cannula inserted into the MPOA were used in this work.

Radioimmunoassay

Plasma LH, FSH and PRL were measured by RIA using kits supplied by the National Institute of Diabetes, Digestive and Kidney Disease (NIDDK). All results were expressed in terms of the NIH-RP₂ reference standards. The smallest detectable doses were 0.04 ng/ml for LH, 0.35 ng/ml for FSH and 0.28 ng/ml for PRL. The intra-assay coefficients of variation were 3, 6 and 4% for LH, FSH and PRL respectively.

Statistics

The significance of differences between groups and times were tested by ANOVA and Newman-Keuls test for multiple comparisons.

Results

The microinjection of saline, phentolamine or propranolol into the MPOA did not produce a change in plasma concentrations of LH (Fig. 1A) or PRL (Table 1). However, microinjection of A II increased plasma LH (Fig. 1B) and decreased plasma PRL (Table 1). The increase in plasma LH was significant at 10, 20, 30 and 60 min (Fig. 1B) and the decrease in PRL release was significant at 20, 30 and 60 min (Table 1).

Microinjection of phentolamine (an α -adrenergic antagonist) into the MPOA blocked the release of LH elicited by the microinjection of A II (Fig. 1B), but propranolol (a β -adrenergic antagonist) did not block this release (Fig. 1B). The areas under the curves of Fig. 1B were 270 ± 13 in the NaCl $\pm$ AII group and 191 ± 14 in the phentolamine + AII group ($P < 0.05$); that for the propranolol $\pm$ AII group was 306 ± 18 (not statistically different from the value in the NaCl $\pm$ AII group).

Microinjection of phentolamine or propranolol into the MPOA did not block the decrease in plasma PRL in response to the microinjection of A II (Table 1).

There was no change in plasma FSH in any experimental condition (data not shown).

Figure 1 Effect of previous microinjection (–10 min time) of saline, phentolamine or propranolol on plasma LH in estrogen-primed OVX rats submitted to microinjection of saline (A) or A II (B) into the MPOA at time zero. Values are means $\pm$ S.E.M.; numbers in parentheses are the number of animals in the group. * $P < 0.05$ compared with control values at –20 min time; + $P < 0.05$ compared with saline group at the same time.

Discussion

LH secretion was stimulated by microinjection of A II into the MPOA. A preceding microinjection of α - but not of β -adrenergic antagonists reduced this response.

The regions of the preoptic area and anterior hypothalamus of rat are responsive to A II, which can stimulate LH release. A II excites a large proportion of neurons in this region, increasing the neuronal discharge frequency (13) and facilitating the release of norepinephrine and dopamine (12) in the MPOA. Norepinephrine is released in a pulsatile pattern and stimulates LHRH secretion (11).

A stimulatory action of A II on LH release has been shown in OVX steroid-primed and proestrus rats and in hemipituitaries from OVX rats incubated *in vitro*. This stimulatory action is dose dependent (5, 10). During proestrus and ovulation, i.c.v. microinjection of saralasin or enalapril inhibits the LH surge (5, 6). The i.c.v. microinjection of antiserum against A II decreases plasma LH, indicating a central role for endogenous AII (7). Our results have confirmed the effect of A II and demonstrated an interaction between A II and

Table 1 Effects of previous microinjection (–10 min time) of saline, phentolamine or propranolol on plasma PRL in estrogen-primed OVX rats submitted to microinjection of saline or A II into the MPOA at time zero. Values are means $\pm$ S.E.M.; numbers in parentheses are number of animals in the group.

Microinjection	Time (min)					
	–20	0	10	20	30	60
NaCl + NaCl (n = 13–17)	30.3 $\pm$ 3.9	31.3 $\pm$ 3.3	30.3 $\pm$ 3	31.5 $\pm$ 3.2	28.5 $\pm$ 3	29.9 $\pm$ 3.8
Phentolamine + NaCl (n = 10–13)	31.1 $\pm$ 3.6	32.1 $\pm$ 3	32.1 $\pm$ 3.7	27.7 $\pm$ 4.1	30.4 $\pm$ 4.4	26.7 $\pm$ 4.4
Propranolol + NaCl (n = 7–8)	31.3 $\pm$ 6.8	35.5 $\pm$ 6	33.3 $\pm$ 6.3	29.1 $\pm$ 6.5	35.9 $\pm$ 6.3	24.3 $\pm$ 4.9
NaCl + A II (n = 19–24)	28.3 $\pm$ 3.1	28.8 $\pm$ 4	19.6 $\pm$ 2.7*	16.7 $\pm$ 2.5*	16.3 $\pm$ 2.1*	16.3 $\pm$ 2.6*
Phentolamine + A II (n = 10–18)	28.5 $\pm$ 3.2	26.5 $\pm$ 3.1	15.3 $\pm$ 2.6*	11.1 $\pm$ 1.9*	10.7 $\pm$ 1.8*	11.1 $\pm$ 1.8*
Propranolol + A II (n = 10–15)	27.4 $\pm$ 5.3	26.6 $\pm$ 5.1	14 $\pm$ 3.2*	13.6 $\pm$ 3.6*	10.8 $\pm$ 1.6*	15.3 $\pm$ 3.2*

* $P < 0.05$ compared with control values at –20 min time.

norepinephrine. This interaction must include LHRH neurons, as i.c.v. microinjection of antiserum against A II decreased the LHRH content of the median eminence and MPOA (8). Furthermore, the involvement of norepinephrine in these events in the MPOA was shown to occur through α -adrenergic receptors, but not β -adrenergic receptors.

Microinjection of A II into the MPOA decreased plasma PRL. It has been shown previously that A II may differ in its action on PRL secretion at the level of brain or the pituitary (14). *In vivo* and *in vitro* studies have revealed evidence for a stimulatory action of A II at the pituitary level (4, 15). Another study showed that i.v. injection of A II did not alter PRL secretion (10), whereas i.c.v. microinjection of A II inhibited PRL secretion and this effect was blocked by dopamine receptors antagonists (10). The i.c.v. microinjection of antiserum against A II has also been shown to increase PRL secretion in OVX rats (9). Our present results have demonstrated that the inhibition of PRL secretion by A II injected to the MPOA is not affected by α - or β -adrenergic antagonists. Other authors have demonstrated an interaction between A II and central dopaminergic neurons, as A II increased the content of dopamine in the arcuate nucleus and the release of dopamine from the hypothalamus. The peptide may affect the activity of the dopaminergic tuberoinfundibular system (10). It can be hypothesized that dopamine released in the primary plexus of the median eminence reaches the anterior pituitary through portal vessels, inhibiting the release of PRL.

In conclusion, our results show that A II injected into the MPOA has opposite effects on the release of LH and PRL, stimulating the former and inhibiting the latter.

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