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Effect of 17-β-Estradiol and Progesterone on Angiotensin II-Induced Changes in Inositol-1,4,5-Trisphosphate Content and Protein Kinase C Activity in Anterior Pituitary

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Angiotensin II (AngII) is known to act in the anterior pituitary through phosphatidiloinositol breakdown, increasing the level of inositol-1,4,5-trisphosphate (IP_3) and diacyloglycerol (DAG), a potential activator of protein kinase C (PKC). We examined the effect of estradiol and progesterone treatment *in vivo* on IP_3 levels and activity of PKC under the influence of AngII. Three groups of intact female rats received *in vivo* injections of 17-β-estradiol, progesterone, and oil (control) for five days, and then the *in vitro* effect of AngII was examined using homogenate of the anterior pituitary. AngII increased either the IP_3 concentration or the synapsin I phosphorylation catalyzed by PKC. Estradiol enhanced the basal (without AngII) IP_3 level and PKC activity induced by AngII. Progesterone did not change the basal and AngII-induced IP_3 concentrations. On the other hand, it decreased the basal PKC activity and blocked the effect of AngII. Our data suggest that ovarian steroids can modulate the effect of AngII on the anterior pituitary gland.

Key Words: **angiotensin II; inositol 1,4,5-trisphosphate; pituitary; estradiol; progesterone.**

Angiotensin II (AngII), an octapeptide of the renin–angiotensin family, is mostly involved in regulating the fluid balance and the arterial pressure, but can also mediate release of anterior pituitary hormones such as prolactin, luteinizing hormone, and adrenocorticotropic hormone (1). It is likely that the effects of AngII in the pituitary gland could be modulated by sex steroids. Angiotensin II release from the brain (2) and the number of AngII receptors in the anterior pituitary (3) change during the estrus cycle. Estrogens can also modulate AngII action in the pituitary in ovariectomized rats (4). In the anterior pituitary AngII has been shown to bind to AT_1 receptors (5), which are coupled to the activation of a phosphoinositide-specific phospholipase C (4). The activation of phospholipase C hydrolyzes membrane phosphoinositides into inositol phosphates (among others, inositol-1,4,5-trisphosphate, IP_3) and *sn*-1,2-diacyloglycerol (DAG) (6). The release of IP_3 mobilizes calcium from intracellular stores, and increases on DAG, in concert with cellular calcium, activates protein kinase C (PKC) (7). The calcium ions and protein kinase C have a major role in signal transduction, processes of cell proliferation and hormone release also in the anterior pituitary (8). The purpose of this study was to investigate changes in angiotensin II action after the treatment with two ovarian steroids, 17-β-estradiol and progesterone, *in vivo.* We have evaluated the effects of both steroids on inositol-1,4,5-trisphosphate concentration and protein kinase C activity in the anterior pituitary. Synapsin I, the phosphoprotein, evoked the neurotransmitter release and found to be a good brain substrate for various protein kinases, including PKC (9).

MATERIALS AND METHODS

Randomly chosen cycling female, Wistar rats weighing 180–220 g were used. The rats had been kept in light- and temperature-controlled rooms with tap water and food available *ad lib.* The rats had been divided into three groups. The first group had been injected intraperitoneally with estradiol benzoate (15 mg/g of body weight) for 5 days and the second one with progesterone, at the same dose and for the same time. The third control group was injected with similar volume of oil. After the decapitation, the pituitary gland was collected, the posterior lobe was removed, and the anterior lobe was weighed and homogenized at 0–4°C in medium containing aprotinine as protease inhibitor. Protein content in the samples was estimated according to the method of Ohnishi and Barr (10), with bovine serum albumin as a standard. The dose-dependent effects of Ang II were investigated *in vitro* after 15 min incubation with peptide concentration from 10^{-11} to 10^{-5} M. The time-dependent effects were studied using incubation times of 3, 5, 15, and 30 min, with 10^{-7} M of angiotensin II. The homogenates incubated without angiotensin II served as control. The IP_3 concentration was measured by using

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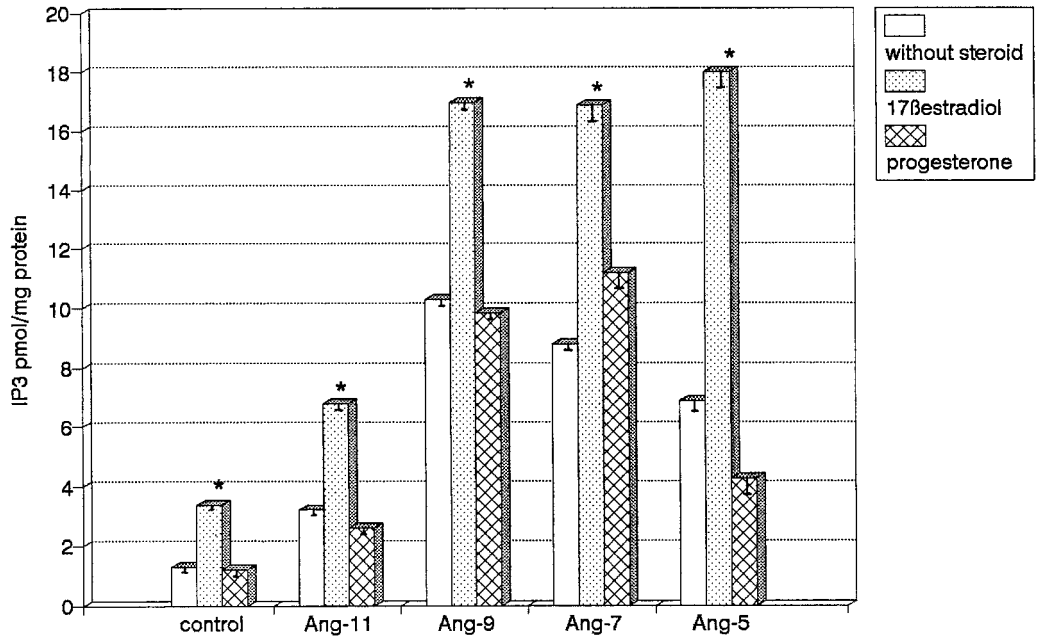

FIG. 1. The changes in IP_3 concentration in anterior pituitary of rats previously treated with 17-β-estradiol and progesterone after incubation with various doses of angiotensin II. White bars, rats previously treated with oil; dotted bars, rats treated with estradiol; cross-hatched bars, rats treated with progesterone. The asterisk means the statistical significance compared to the control values (without angiotensin) $P < 0.01$.

assay kits, obtained from Amersham International plc according to the procedure provided by producer. The standard phosphorylation assay medium contained 50 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mM EGTA, 1 mM $CaCl_2$ 1 mM PMA and 1 mM vanadyl sulfate as phosphatase inhibitor. Medium also contained 1 μM phorbol 12-myristate 13-acetate (PMA) which is a specific activator of the protein kinase C. The reaction was started by adding [γ^{32}P]ATP to the reaction mixture, in the presence of endogenous synapsin I in the homogenate as a substrate. After incubation for 2 min at 37°C the reaction was stopped by the addition of dissociation buffer containing 50 mM Tris–HCl, pH 6.8, 20% (v/v) glycerol, 0.1% β-mercaptoethanol, 6% SDS and 0.002% bromophenol blue and heating at 100°C for 2 min. The solubilized proteins were resolved on 10% SDA–polyacrylamide gel as described by Laemmli (11). Simultaneously, the pattern proteins with molecular weights of 24–100 kDa were separated. After electrophoresis, the gels were silver stained (12) and dried. The radioactivity of the band was detected by autoradiography. Protein phosphorylation was quantified from densitometric scans of autoradiograms by measuring peak areas and comparing to the control values. Synapsin I was identified by limited proteolysis (proteases from *Staphylococcus aureus* V8) (13), and using monoclonal antibody (Calbiochem). The data were calculated as mean values ± SD ($P < 0.01$). The comparison between means was performed using ANOVA followed by Neuman–Keul's test and regression and correlation coefficient analysis. The experiment was repeated in triplicate with similar results.

RESULTS

Both of examined steroids caused different effects even on basal, (i.e., nonstimulated by angiotensin), concentration of IP_3. In the homogenates of anterior pituitary estradiol caused the increase of basal IP_3 content (Fig. 1), whereas progesterone did not alter the basal IP_3 level. After adding the AngII to the homogenates of rats not treated with any steroid, we have observed a significant increase of IP_3 in comparison to the control (Fig. 1), also after using doses as low as 10^{-11} M. The maximum stimulation occurred at 10^{-9} M of AngII ($P < 0.01$). Angiotensin II added to the homogenates of estradiol-treated rats caused much greater elevation of IP_3 level, statistically significant compared to the rats injected with oil. However, when we use the tissue from progesterone-treated rats. AngII elevated the levels of IP_3 in the same degree as in olive oil-treated rats. The increase of IP_3 concentration was relatively short, maximum 20 min, either in estradiol-treated animals or in animals injected with oil (data not shown). The protein kinase C-induced phosphorylation of synapsin I is dependent on various steroids (see Fig. 2). Angiotensin and estradiol alone increase the PKC activity; nevertheless, the stimulation caused by estradiol was greater. AngII and estradiol together raise the PKC-stimulated phosphorylation to a greater degree than when used alone. Progesterone alone decreased PKC activity and reduced the effect of AngII.

DISCUSSION

Previous studies concerning the influence of ovarian steroids on angiotensin II action have been suggested that endogenous estrogen *in vivo* reduced the number

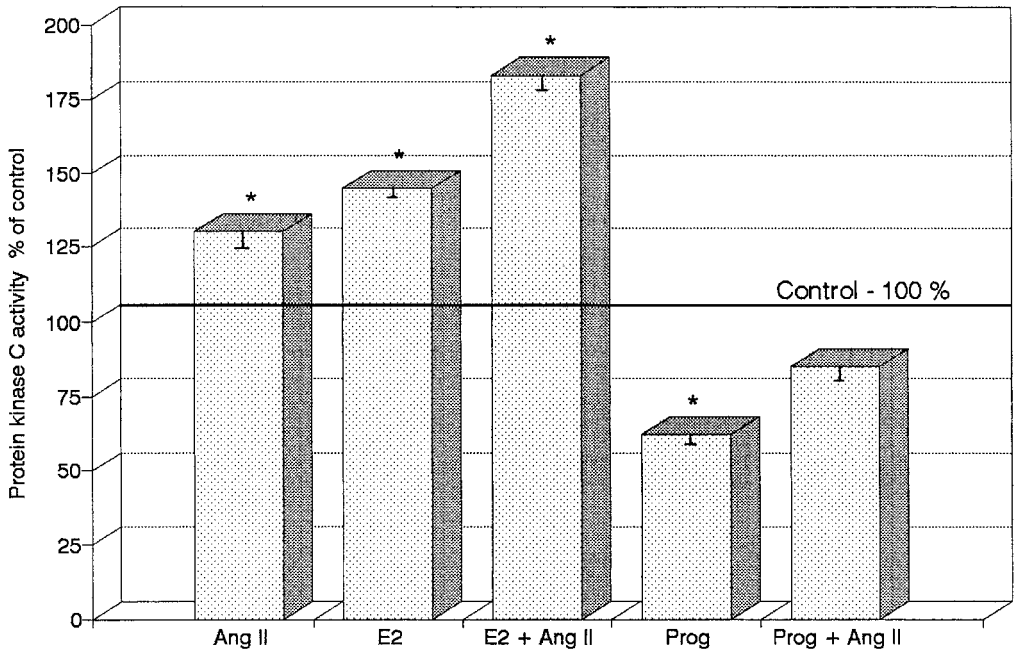

FIG. 2. The effects of angiotensin II alone and with steroids on phosphorylation of synapsin I catalyzed by PKC. Control values with regard to group received neither steroids nor AngII. The results are expressed as a percentage of control activity = 100%. The asterisk means the statistical significance compared to the control values (without angiotensin) $P < 0.01$.

of AngII receptors in anterior pituitary, especially in proestrus, when endogenous estrogen levels are high (14). The administration of exogenous estrogens to ovariectomized or to intact rats also caused decrease number of AngII receptors (15, 16). It was also shown that in ovariectomized rats treated with estrogen the angiotensin II-induced level of IP_3 was lower than that in animals treated with vehicle (4). However, in our earlier study performed on male rats, estradiol benzoate was found to enhance the AngII-induced rise of IP_3 level in the anterior pituitary (17). We have also shown that estradiol, but not progesterone, enhanced these effects. The results of the present experiment corroborate with our earlier finding. Our study demonstrates that angiotensin II increases the IP_3 concentration and protein kinase C activity in the anterior pituitary gland. Although the homogenate used in our study contained the mixed population of the pituitary cells, it seems that the observed changes concern the lactotrophs which are known to be the main target of angiotensin II. It was shown that the estrogen treatment did not decrease the AngII-induced prolactin release (14), and even increased the PRL synthesis in spite of reduced number of AT_1 receptors (15, 18). The results above indicate that receptor down-regulation did not change the target cells responsiveness (14); they also corroborate with our results. Although progesterone did not influence the basal and AngII-induced IP_3 levels, we have found that this hormone decreased the basal PKC activity. It also blocked the angiotensin-induced activation of PKC. This novel finding indicates that progesterone may antagonize, at least in part, the action of AngII within the anterior pituitary. This assumption corroborates with the recent findings of Kalenga *et al.* (19) that progesterone down-regulates the AT_1 receptors in human placenta and of Johren *et al.* (20) who found the decrease in the number of AT_{1B} receptors in the anterior pituitary of estradiol-primed ovariectomized rats treated with progesterone. The mechanism by which the ovarian steroids modulate the IP_3 generation or PKC activity remains unclear. They may involve the genomic actions, but there is also a possibility of direct action on cell membranes. Estradiol and progesterone were shown to act in the brain as neuroactive steroids; their membrane receptors were found in the brain (21). Both steroids can bind to $GABA_A$ and NMDA receptors (progesterone also to $\alpha_1$adrenergic receptors (22)) and thus could change the neurotransmitter release (23). Bression *et al.* suggest that there is evidence for an estradiol binding to the pituitary cells membrane (24), and also we could not exclude the direct involvement of estradiol but not progesterone in membrane phospholipids breakdown and thus also regulation of protein kinase C activity. Angiotensin II has been shown to activate the membrane-bound protein kinase C activity in smooth muscle (25), intestinal epithelium (26), and adrenal cortex (27) but to date not in the anterior pituitary. The phospholipid-dependent protein kinase C is assumed to have a major role in signal transduction and probably in cell proliferation. Estrogens can induce pituitary prolactin cell hyperplasia and induce

pituitary PRL-secreting tumors (28) and there is a possibility that AngII is involved in this process (29–31). The increase in secretion of normal lactotrophs is associated with high levels of PKC activity, and estrogens have also been found to increase PKC activity in normal and hyperplastic pituitaries (32, 33). Moreover, estrogens can mediate, similar to AngII, the LH release through facilitated responses to GnRH and GnRH secretion, probably via PKC (34). On the other hand, the role of progesterone in modulating the hypothalamus–pituitary axis is not yet clear. In the brain, progesterone caused the attenuation of phosphoinositol hydrolysis, via α-adrenergic stimulation, but this effect occurred only in the presence of estrogens (22).

In conclusion, we suggest that ovarian steroids modulate angiotensin II action in the pituitary. Estradiol enhances the effect of AngII on phosphatidylinositol hydrolysis and protein kinase C activity and, in this way, can modulate cell proliferation and hormone secretion. Progesterone, in turn, may alternate the effect of AngII on PKC activity.

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