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ORIGINAL ARTICLE

Steroid modulation of angiotensin II action in the rat anterior pituitary gland

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Abstract

OBJECTIVES: The present study was designed to test whether various steroid hormones modulate differently angiotensin II (AngII) action in the anterior pituitary in males and females.

MATERIAL AND METHODS: Adult female and male rats were treated with one of the following substances: oil (control), pregnenolone sulfate (PREG-S), 17β-estradiol benzoate (E2,) progesterone (P), or dehydroepiandrosterone sulfate (DHEA-S), given in intraperitoneal injections for five days in dose of 50 μg per animal per day. Because AngII is known to act in the anterior pituitary through the phosphatidiloinositol breakdown, thus increasing the level of inositol-1,4,5-trisphosphate (IP3), the IP3 concentration was determined 24 hours after the injection in the anterior pituitary homogenate exposed to AngII.

RESULTS: In control animals (without steroids) AngII stimulated concentration of IP_3 stronger in females than in males. E_2 and DHEA-S enhanced AngII effects in both males and females. PREG-S increased AngII-induced IP_3 concentration in females, but not males. Progesterone raised AngII effect on IP_3 concentration in males, only when high concentrations of peptide were used.

CONCLUSION: These results indicate that pituitary sensitivity to AngII stimulation is modulated by steroid hormones and is related to the gender of the animal.

Abbreviations

AngII – angiotensin II
RAS – renin-angiotensin system
DHEA – dehydroepiandrosterone
DHEA-S – dehydroepiandrosterone sulfate
IP_3 – inositol-1,4,5-trisphosphate
ACE – angiotensin converting enzyme
PREG-S – pregnenolone sulfate
E2 – 17-β-estradiol benzoate
P – progesterone

Introduction

Angiotensin II (AngII) is an octapeptide generated by the RAS and involved in the regulation of the mechanisms relevant to body fluid. The presence of AngII receptors in the anterior pituitary and the hypothalamus suggest that AngII may be also an important neuroendocrine modulator [1,2]. Renin, angiotensinogen and enzymes necessary for the generation of AngII have been also identified in the both pituitary and the brain [1,3]. In the anterior pituitary AngII has been shown to bind to AT_1 receptors [4,5], which are coupled to the activation of a phosphoinositide-specific phospholipase C [6,7]. The activation of phospholipase C leads to hydrolysis of the membrane phosphoinositides into inositol phosphates (among others, to inositol-1,4,5-trisphosphate, IP_3) and diacyloglycerol [6,8]. AT_1 receptor subtypes are found in the anterior pituitary of mature and immature animals of both sexes [2,9,10]. The gender and hormonal status is important for function of the local RAS systems. For example, it is observed that i.e. hypertension occurs mainly in men. Women after menopause, abandoned from the protective influence of estrogens, more often develop higher blood pressure. In the anterior pituitary estrogen treatment has been shown to decrease AT_1 binding sites as well as AT_1 receptor mRNA level [11]. The administration of estrogens reduces circulating levels of AngII in ovx rats, and significantly decreases the concentration of angiotensin converting enzyme (ACE) [12].

Testosterone was reported to influence the AngII action and angiotensinogen mRNA level in the kidney [13] and anterior pituitary [14]. Administration of progesterone caused the various effects on AngII receptors density and regulation in various tissues. In rat uterus progesterone given for a shorter period of time (two days) did not change AngII receptors in uterus. However after longer time (over seven days), a significant decrease was observed [15]. In human trophoblast progesterone downregulated AngII receptor density [16]. But in general, the influence of the steroid hormones on AngII action was investigated in the anterior pituitary mainly in one gender, and their action was not compare between sexes. So far mainly estrogen and progesterone were subject of particular interest; little is known about the influence of other steroids. Some of them belong to the family of neurosteroids, hormones which can be synthesized and act within the brain tissue. This group of hormones, including among others pregnenolone, dehydroepiandrosterone and their sulfates can modulate neurotransmitters and neuropeptides expression, action of G proteins, or activity of enzymes involved in neurochemical metabolism [17,18]. These hormones play also important role during brain development and physiological responses involved in sexual maturity.

The purpose of this study was to compare the changes in angiotensin II effects on IP_3 turnover after the in vivo treatment with pregnenolone sulfate (PREG-S) and dehydroepiandrosterone sulfate (DHEA-S), 17-β-estradiol benzoate (E_2) and progesterone (P) in male and female rats. We have evaluated the effects of the examinated steroids on inositol-1,4,5-trisphosphate concentration in the anterior pituitary.

Materials and methods

Adult male and randomly chosen cycling female Wistar rats weighing 180–220 g were used in the experiment. The rats had been kept in light- and temperature-controlled rooms with tap water and food available *ad lib*. Every examined group (8 animals per group) received for a five days intraperitoneally injections of one of the following steroid hormone: 1) 17β-estradiol benzoate (E_2), 2) progesterone (P), 3) pregnenolone sulfate (PREG-S), 4) dehydroepiandrosterone sulfate (DHEA-S) in a dose of 50 μg per animal per day, the dose allowing to abolish the cycling activity of the ovaries. The control group was injected with the same volume of oil. Twenty four hours after the last injection the rats were sacrificed. After the decapitation, the pituitary gland was collected, the posterior lobe was removed, the anterior lobe was weighed and homogenized at 0–4 °C in medium containing aprotinine as proteases inhibitor. Protein content in the samples was estimated according to the method of Ohnishi and Barr [19], with bovine serum albumin as a standard. The time-dependent effects of Ang II were studied using incubation times of 3, 5, 10 and 30 min, with 10^{-7}M of peptide. The time of incubation – 10 minutes was chosen for dose-dependent experiments, based on the results of time-dependent studies. The dose-dependent effects were investigated *in vitro* with AngII concentration from 10^{-11}M to 10^{-5}M. The homogenates incubated without angiotensin II served as control. IP_3 concentration was measured using assay kits, obtained from Amersham International plc., according to the procedure provided by the manufacturer. The data were calculated as mean of values ± SD ($p < 0.02$).

The comparison between means was performed using ANOVA test followed by Newman-Keul's test and regression and correlation coefficient analysis.

Results

In control animals (female and male rats receiving oil instead the steroid) AngII alone stimulated IP_3 concentration, but in females in much higher degree. Estrogen treatment stimulated AngII-induced IP_3 concentration in both females and males, however in males after the treatment with AngII in high doses (10^{-7}

and 10^{-5}M) only slight effect in comparison to controls was observed (Fig. 1B). DHEA-S also stimulated the effect of AngII on IP_3 concentration in both males and females, and the degree of stimulation was similar in both groups of donor animals (Fig. 1A). Progesterone enhanced AngII effect in males, it was observed only when high doses of AngII (10^{-7} and 10^{-5}M) was applied (Fig. 1D). PREG-S strongly increase AngII-induced IP_3 concentration only in females rats, in males there was no difference to the control animals (Fig. 1C).

Discussion

In the present study we have examined the role of two classical sex hormones, 17β-estradiol and progesterone and of other two steroid hormones, DHEA-S and PREG-S, which can act within the brain as neurosteroids. Although the influence of steroid hormones on AngII action within the anterior pituitary is a focus of interest, the previous experiments are usually conducted on classical sex hormones, i.e. estrogens and testosterone, and concerned only one gender. Our results indicate that E_2, progesterone, DHEA-S and pregnenolone sulfate can modulate AngII action in the female and male rat anterior pituitary. However, it seems that depending on the gender of animals this modulation may have different directions and degree. It is known that the rat RAS system, (also in the anterior pituitary), is sensitive to either female or male sex hormones [11, 16, 20–22]. In our experiment AngII itself stimulated much stronger IP_3 concentration in females than in males. This finding might be explained by the lower binding to AngII receptors in the male anterior pituitary [5]. The hormonal milieu plays an important role in setting the sensitivity to exogenous steroid hormones. Moreover, the brain is able to metabolize the steroid hormones [23]. All the steroid hormones used in the present study can be synthesized within the brain. Two of them, DHEA-S and pregnenolone sulfate, may act not only as hormones but can be also the precursors of other steroids.

PREG-S can also serve as a prohormone and the potential source of DHEA, progesterone, or estradiol [17]. However, it seems unlikely that the effect of

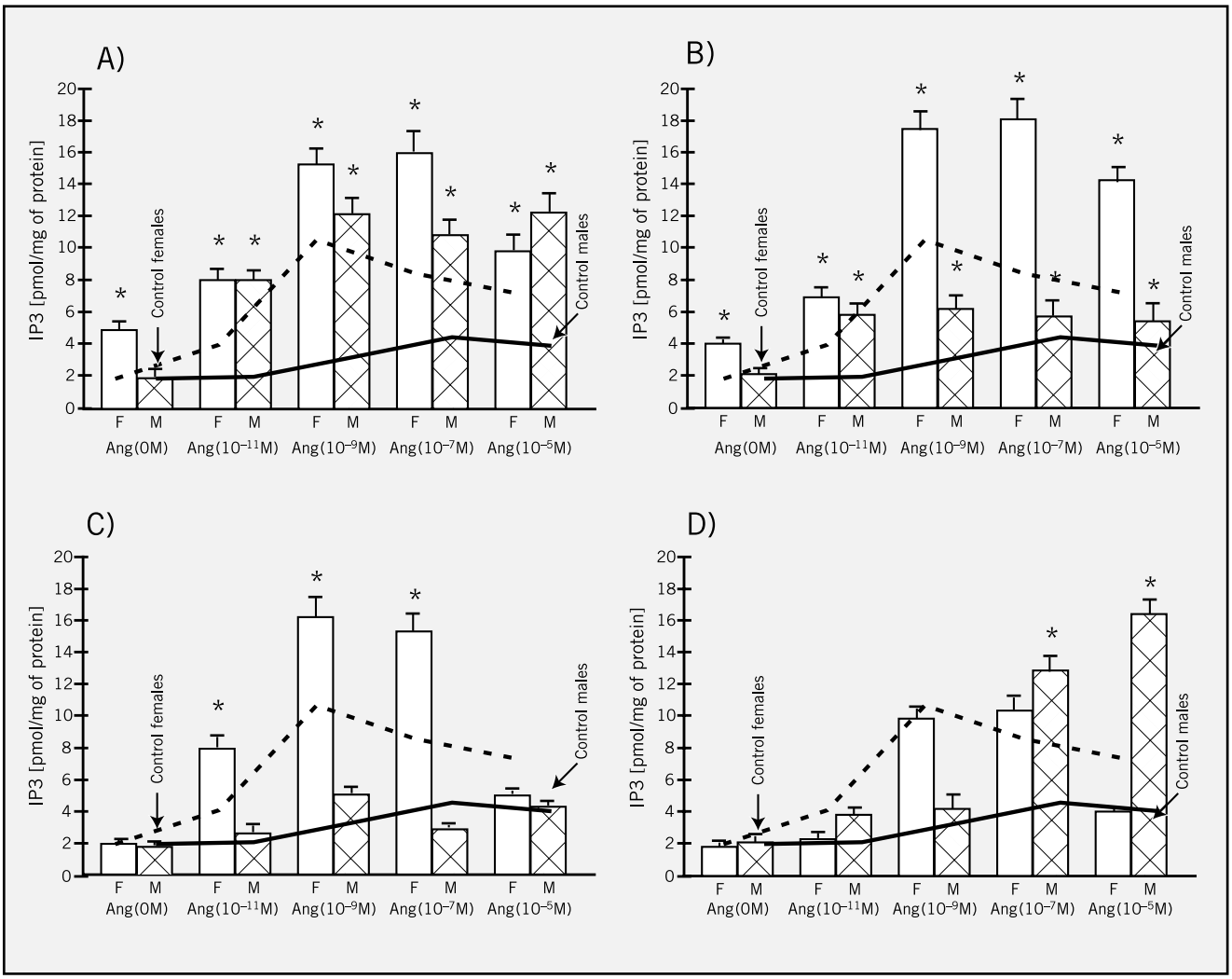

Fig. 1. The changes in IP_3 concentration in anterior pituitary of the rats previously treated with dehydroepiandrosterone sulfate (A), estradiol (B), pregnenolone sulfate (C) and progesterone (D) in dose 50 μg per animal after incubation with various doses of angiotensin II. Time of incubation – 10 min. The asterisk means the statistical significance to the control values, $p<0.02$. Control animals (dotted line – females; solid line – males) did not receive hormone treatment.

PREG-S observed in this study occurred *via* transformation into the above mentioned steroids. It is known that only a small percent of pregnenolone sulfate can be converted to the metabolites [24–26]. In addition, some neurosteroids have different mode of action from their metabolites [27–29]. In fact, in females PREG-S acts similarly to DHEA and E_2 in the presence of AngII, although PREG-S does not stimulates basal (without AngII) level of IP_3 as E_2 does. On the other hand, in males PREG-S does not stimulate either basal or AngII-induced IP_3 concentration whereas E_2 and DHEA-S both enhances the angiotensin action. Steroid hormones can be metabolized not only within the brain but also in the peripheral glands, i.e. in ovaries and testes. It is possible that the ovaries are the main site of conversion of PREG-S to its metabolites. The testes does not posses so powerful and specialized enzyme machinery. According to our earlier results, ovariectomy does not change PREG-S modulation of AngII function in the anterior pituitary [30]. It is also known that the brain is the main place of enzymatic conversion of rather early products of cholesterol cleavage such as PREG-S and DHEA-S. Estradiol and other C_{18} and C_{19} steroids are mainly transported to the brain with blood, and crosses the blood-brain barrier. The endogenous C_{18} and C_{19} steroids present in the brain rather originate from the periphery then are synthesized in situ, so their concentration in the brain strongly depends on gender and hormonal status of animal [28]. Another investigated steroid, progesterone, which belongs to the group C_{21} steroids, also shows the differences related to the gender of animals in modulation of AngII action, exerting its effect only in males. Among the examinated steroids, E_2 and DHEA-S act in a similar way in both sexes. Both hormones increase the AngII-stimulated IP_3 release. In females, E_2 stimulates basal (without AngII) level of IP_3 in the anterior pituitary stronger than in males. After adding AngII to the pituitary homogenates, the values of IP_3 were lifted in all concentrations of the used peptide, but the original shape of the curve was kept. In males, although the basal stimulation of IP_3 was lower, the AngII-induced IP_3 generation is comparable to that in females. DHEA acts also very similarly in both sexes. It slightly enhances the basal levels of IP_3 and then strongly enhances AngII-induced IP_3 release. The degree of stimulation was the same in both genders. The similarity of the dose-response curve as well as almost the same degree of stimulation after using E_2 and DHEA-S may suggest that DHEA-S may act *via* transformation to estrogens. This observation is in agreement with our previous studies showing that ovariectomy completely abolished the effect of E and DHEA-S, but not of PREG-S [30].

Comparing the effects of PREG-S and P to the effects of its putative metabolites, (among others DHEA-S and E_2) in both genders we can assume that the observed effects are rather due to the specific action of PREG-S or P. It is also possible that depending on the presence of different type of gonads, PREG-S and P can be differently metabolized, forming the compounds characteristic for the respective genders. The ovaries and testes differ in type and activity of enzymes converting steroids. There is also a possibly that PREG-S can modulate AngII action only in the presence of highly enough concentrations of exogenous estrogens, as it was shown above. In females, PREG-S and E_2 seems to act in a synergistic way, whereas such an effect is not present in males. In males, progesterone and estradiol have dissimilar influence on AngII-induced generation of second messenger (IP_3). Progesterone seems to modulate the angiotensin action only in the high (10^{-5}, 10^{-7}M) concentration of the peptide. On the contrary, estradiol influenced AngII-induced IP_3 after using lower (10^{-9}, 10^{-11}M) concentration. It is well known, that sex differences in the gonadal steroid hormone milieu especially during the puberty, can exert the important effect on the function hypothalamo-pituitary-gonadal axis [10, 31]. The influence of some steroid hormones on AngII receptors were extensively studied. Both typical pituitary AngII receptor subtypes AT_{1A} and AT_{1B} in various tissues may be regulated by estrogens [3, 9, 12, 32]. Although both types have almost similar properties, the expressions of their mRNAs are differentially modulated. In the anterior pituitary, estrogen treatment suppressed AT_{1B} but not AT_{1A} mRNA levels [3]. The anterior pituitary express predominantly AT_{1B} type, but AT_{1A} is also present. Another difference is the affinity to the ligand, for AT_{1B} subtype is proposed bell-shape curve for AngII binding [1, 5]. In our study in females the shape of dose-response relationship of IP_3 values after AngII treatment was typical as proposed for AT_{1B} receptor. The stimulation by steroid hormones changed the degree of IP_3 concentration but did not change the shape of the dose-response relationship. In males the shape of IP_3 response curve to AngII is respective for AT_{1A} subtype. Both subtypes of AT_1 receptor interacts with the same second messenger cascade, so the effects of their activation should be the same. Due to the different hormonal regulation, it is possible the exposure for 5 days to the exogenous steroid hormones might cause the different effect on receptor subtype density in different sexes.

The physiological role of neurosteroids in the brain and in the anterior pituitary remains still unclear. The steroid hormones action has been described as having mainly the genomic effects [27], but recently it was also well established that neurosteroids may act also in a non-genomic way, as the agonists or antagonists of some inhibitory neurotransmitters, such as $GABA_A$ or glycine or as modulators of the ion currents and second messenger systems. Thus, the receptors belonging to the family of G-proteins coupled receptors like AT_1 might be potential target for neurosteroid modulation. Within the brain PREG-S, 17β-estradiol and progesterone was shown to modulate the various neuronal receptors as well as ion current channels and G-protein systems in a rapid way [17, 18, 33]. Ovarian steroids were shown to modulate lactotrophs responsiveness to various stimuli, as well as affecting the G-protein systems in this type of cells [20, 33]. In our experiment the hormones was administered *in vivo*, and it raises the question whether the observed effects occur directly in

the anterior pituitary or indirectly through the brain. Although the time between the hormone injection and the sacrification of the animals was long enough for the induction of the genomic effects of PREG-S, it seems that its effect is rather non-genomic. Because we cannot exclude any of the ways of action, the additional studies including in vitro investigations are required to elucidate this problem, as well as physiological role of this modulation.

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