Angiotensins II and IV stimulate the rat anterior pituitary cell proliferation independently of the AT1 receptor subtype

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Abstract

OBJECTIVES. The purpose of this study was to investigate the effect of angiotensin II (AII) and angiotensin IV (AIV, 3–8 fragment of AII) on the cell proliferation in the anterior pituitary of rat *in vivo*.
MATERIALS AND METHODS. The female adult Wistar rats, ovariectomized 10 days before experiment were injected intraperitoneally with saline, AII, AIV and AII or AIV together with losartan – the specific AT1 receptor subtype antagonist. Bromodeoxyuridine (BrDU) incorporation into anterior pituitary cell nuclei was used as the index of cell proliferation.
RESULTS. We observed the increased BrDU-labeling index (LI) in the anterior pituitary of rat treated with either AII or AIV. The proliferogenic effect of neither AII nor AIV was reversed by AT1 specific antagonist losartan.
CONCLUSIONS. AII and AIV stimulate the rat anterior pituitary cell proliferation *in vivo*. This action of both angiotensin peptides is connected with activation of receptor different from AT1 subtype.

Introduction

The renin-angiotensin system (RAS) consists of the biologically active angiotensin peptides and enzymes necessary to its degradation and formation from inactive precursors. [1]. Angiotensin II (AII), the best known active peptide of the RAS, is generated from angiotensin I by angiotensin converting enzyme (ACE). AII is known to be degraded at its N-terminal position by aminopeptidase A (APA) leading to angiotensin III (AIII). AIII is subsequently cleaved by aminopeptidase N (APN), resulting in the formation of angiotensin IV (AIV), the 3–8 fragment of AII [2]. Three subtypes of angiotensin receptors, AT1, AT2 and AT4, have already been described [3]. AT1 receptor appears to be responsible for mediating most classic angiotensin effects. AT2 receptor subtype seems to mediate antiproliferative effects but its role in the vascular growth has also been postulated [4]. AT1 subtype preferentially binds AII, but possesses also affinity for AIII. In turn, AIII has affinity for AT2 receptor and to the lesser extent for AT1 receptor. Losartan and PD 123319 are the specific antagonists of AT1 and AT2

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respectively. Whereas AII and AIII share the same receptors, AIV possesses its own receptor called AT4 [5].

Next to the systemic RAS, the local, independent systems have already been described in many tissues including brain, pituitary, adrenal, ovaries and testis [6]. In the anterior pituitary gland the presence of renin, AII and ACE has been demonstrated in gonadotropes. AII exerts some influence on the secretion of hypothalamic and anterior pituitary hormones [7]. At the level of pituitary AII has been shown to stimulate prolactin (PRL) and adrenocorticotropin (ACTH) secretion through the direct interaction with AT1 receptor [6, 8, 9]. The AT1B subtype of this receptor is expressed on the anterior pituitary cells, predominantly on lactotropes (80%) and to a lesser extent (20%) on corticotropes [10]. The effects of AIII and AIV (the AII derivatives) upon the anterior pituitary function remain unclear.

The recent *in vitro* study has indicated that both AII and AIV enhanced proliferation of cells isolated from the rat pituitary tumors and from the human prolactinomas [11, 12]. The RAS has also been suggested to mediate the estrogen-stimulated hyperprolactinemia and pituitary hyperplasia [13]. Specific AT1 receptor antagonist Losartan failed to reverse stimulatory action of AII and AIV upon anterior pituitary cell proliferation [14]. However, the examination of angiotensin effect should not be limited to the *in vitro* study, as modifying action of various endogenous factors is eliminated. These factors may influence both the specific receptor subtype expression as well as postreceptor events. For this reason, in order to investigate the *in vivo* effect of AII, AIV and Losartan on the cell proliferation in the anterior pituitary, the present study has been undertaken.

Materials and methods

The studies were carried out on female Wistar rats weighting 250 g, ovariectomized 10 days before experiment. The animals were divided into five groups, receiving the injections of the following substances: Group I (controls) – 0.9% NaCl, intraperitoneally (i.p.); Group II – Angiotensin II (AII, Sigma), 50 μg\kg of body weight (b.w.), i.p.; Group III – AII as above + Losartan (LOS, Merck USA, specific AT1 receptor antagonist), 10 mg/kg of b.w., i.p.; Group IV – Angiotensin IV (AIV, Sigma, 3–8 AII fragment), 50 μg\kg of b.w., i.p.; Group V – AIV as above + LOS as above. AII and AIV were injected 2 times in 24 hours intervals and LOS 4 times in 12 hours intervals. Twelve hours after the last injection the animals were sacrificed. Ninety minutes before that, the animals had received a single i.p. injection of bromodeoxyuridine (BrDU, Sigma). All procedures were approved by The Local Animal Use and Care Committee (No. Ł/BD/126). The pituitaries were collected and fixed in Bouin's fixative. The tissues were embedded in paraffin wax and paraffin section were immunostained for BrDU using the Amersham cell proliferation kit. The labeling index (LI), expressed as a number of BrDU-immunopositive nuclei per 1000 randomly scored pituitary cells was estimated in microscopic preparations. Data were evaluated using the Mann-Whitney test. P values less than 0.05 were considered statistically significant.

Results

The results are presented in Fig. 1. The treatment with either AII or AIV significantly increased BrDU-labeling index in the rat anterior pituitary (2.75+/–0.4 for AII and 2.1+/–0.95 for AIV vs 0.6+/–0.25 in the control group). The treatment with losartan reversed the effect of neither AII nor AIV. The BrDU-labeling indices in AII+LOS and AIV+LOS treated group were 2.2+/–0.85 and 1.6 +/–0.5 respectively.

Discussion

The result of present study indicates that AII and its 3–8 derivative AIV stimulate cell proliferation in the rat anterior pituitary *in vivo*. Our results are consistent with the results of the previous *in vitro* study. We have found that both AII and AIV enhanced the tritiated thymidine incorporation into cells isolated from pituitary of estrogenized rats [11, 14]. Moreover,

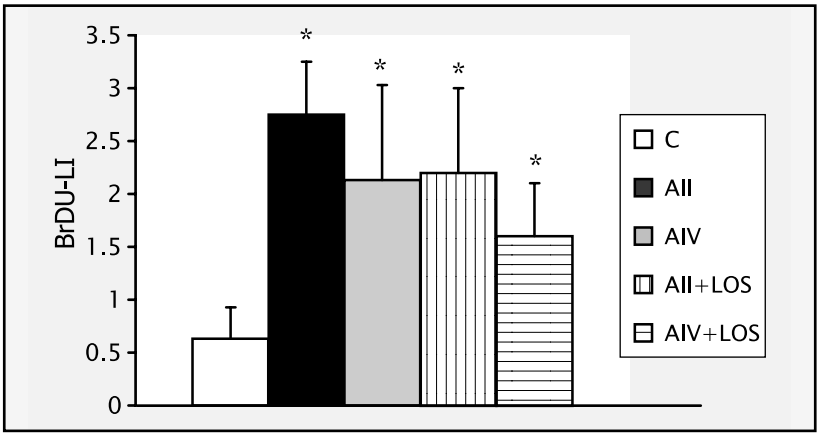

Fig. 1. Bromodeoxyuridine-labeling indices (BrDU-LI) in the rat anterior pituitary; C – control, AII – angiotensin II, AIV – angiotensin IV, LOS – losartan.
*p<0.05 *vs* control.

the regulatory action of AII upon cell proliferation has been observed in the other tissues including adrenal cortex [15], vascular smooth muscle cell [16, 17], intestinal cell [18], prostatic epithelial cell [19] and pancreas [20]. In another experiment we have proved, that chronic treatment with ACE inhibitor enalapril caused an inhibition of lactotropes proliferation in rats with DES-induced pituitary tumors. This phenomenon may suggest the possible mediatory role of AII in estrogen-induced pituitary hyperplasia [13]. The regulatory action of AIV upon cell proliferation has already been proved. AIV was shown to exert a proliferogenic effect on rat adrenocortical cell [15], prostatic epithelial cell [19], and uterine endometrium cell [21]. Moreover, our recent study has indicated that AII and AIV stimulate the activity of tyrosine kinases in estrogen-induced rat pituitary tumors [22].

The involvement of particular receptor subtypes in the effect of angiotensins on cell proliferation may differ depending on the tissue and physiological state. In zona glomerulosa of the rat adrenal cortex losartan – the specific AT1 antagonist – reversed the proliferogenic effect of AII. In contrast, losartan did not affect the AIV action, suggesting the involvement of angiotensin receptor different from AT1 subtype. [15]. In the rat prostate losartan only partially blocked proliferation-stimulating action of AII and, in the same time, enhanced the effect of AIV. It may suggest that in prostate, additionally to AT1, another angiotensin receptor may participate in this AII activity, whereas the action of AIV is completely independent of AT1 receptor [19]. In the glomerular mesangial cells the influence of angiotensin peptides upon cell proliferation and apoptosis was connected with activation of AT2 receptor subtype [23]. The balance between the pro- and antiproliferative effects might have been dependent on various modulators acting in the paracrine manner.

In our present study losartan failed to block the proliferogenic effect of AIV in the anterior pituitary. Moreover, losartan did not reverse the effect of AII either, unlike the influence of this AT1 receptor antagonist on the AII-stimulated cell proliferation in the adrenal cortex and the prostate. This finding corroborates with the results of earlier *in vitro* experiment and indicates that angiotensin effect on the anterior pituitary cell proliferation is not connected with activation of AT1 receptor [14]. The similar results of both *in vitro* and *in vivo* studies eliminate the formerly mentioned problem of potential endogenous factors, modulating at the level of receptor and/or postreceptor events the angiotensin peptides activity. The question still remains to the mechanism of action of AII and its derivative in the anterior pituitary. It is possible that AII does not influence pituitary cell proliferation itself but indirectly, after previous conversion into AIV. The specific AT4 receptor may be implicated in this process. This mechanism is all the more probable taking into consideration the result of our recent study. In our laboratory it was found that inhibition of APA and APN by EC33 and PC18 markedly diminished the stimulatory effect of AII on the tyrosine kinases activity in estrogen-induced rat pituitary tumors [22]. The presence of AIV receptor AT4 has already been reported in a variety of tissues including the guinea pig pituitary gland (JW. Wright – personal communication) and its properties are consistent with those of a growth factor and cytokine receptor [5, 24]. Recently, the AT4 receptor was recognized as insulin-regulated aminopeptidase [25]. Moreover, AT4 site is suggested to regulate the processes of cellular growth in the other tissues. It appears for example to affect neurite outgrowth and angiogenesis [4]. However, the determination of the potential role of AT4 receptor in the proliferogenic action of AII and AIV on the anterior pituitary needs further studies.

In summary, both AII and AIV stimulate the proliferation of cells in the anterior pituitary *in vivo*. The effect of neither AII nor AIV is connected with interaction to AT1 receptor. The hypothesis arises that AII stimulates pituitary cell proliferation after previous conversion to AIV, which subsequently binds to its specific receptor (possibly AT4).

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