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Research Article

The involvement of the renin-angiotensin system in the regulation of cell proliferation in the rat endometrium

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Abstract. Oestrogens are known to enhance angiotensin biosynthesis by increasing the elaboration of its precursor, angiotensinogen. On the other hand, we found that inhibition of angiotensin-converting enzyme (ACE) suppressed the proliferative response of the rat anterior pituitary gland to oestrogens. To answer the question whether the angiotensin system is involved in the control of the cell proliferation of the uterine epithelium, the effects of an ACE inhibitor, enalapril maleate, and of angiotensins II and IV, alone or together with losartan, an antagonist of angiotensin receptor type 1 (AT1), on endometrial epithelial cell proliferation have been studied. The experiments were performed on ovariectomized female Wistar rats. In the first experiment the animals were injected with a single dose of oestradiol benzoate or received an injection of solvent only. Half of the oestrogen-treated rats were injected additionally with enalapril maleate (EN, twice daily). The incorporation of bromodeoxyuridine (BrDU) into endometrial cell nuclei was used as an index of cell proliferation. It was found that oestradiol alone dramatically increased the BrDU labelling index (LI) of endometrial cell nuclei, and this effect was partially blocked by the simultaneous treatment with EN. In the second experiment, the animals were injected intraperitoneally with angiotensin II (AII), angiotensin IV (AIV) or saline, alone or together with losartan. It was found that AIV induced an increase in the LI in uterine epithelium, and this effect was not blocked by the simultaneous treatment with losartan. The increase in LI in uterine epithelium was also observed in the rats treated with AII and with losartan. These findings suggest an involvement of angiotensin IV in the control of uterine epithelium cell proliferation.

Key words. Angiotensin II; angiotensin IV; enalapril; losartan; cell proliferation; rat endometrium.

Angiotensins are members of a family of peptides that derive from a common precursor molecule, angiotensinogen. The specific protease renin cleaves angiotensinogen to the essentially inactive decapeptide angiotensin I (AI). AI is converted by the angiotensin converting enzyme (ACE) into a potent vasoconstrictor octapeptide angiotensin II (AII). Angiotensin II can be cleaved into shorter peptides, including a hexapeptide fragment 3–8 called angiotensin IV (AIV). Initially, AIV was considered to be devoid of physiological relevance, but its functional implications have been recently suggested [1, 2]. At least three subtypes of angiotensin receptors were characterized and classified as AT1, AT2 and AT4 [3, 4]. AT1 receptors preferentially bind AII and can be blocked by losartan. These receptors are connected with activation of phospholipase C, leading to increased phosphoinositide breakdown and subsequent intracellular calcium release. They are also responsible for inhibition of adenylate cyclase by AII. The majority of the known physiological effects of AII are thought to be

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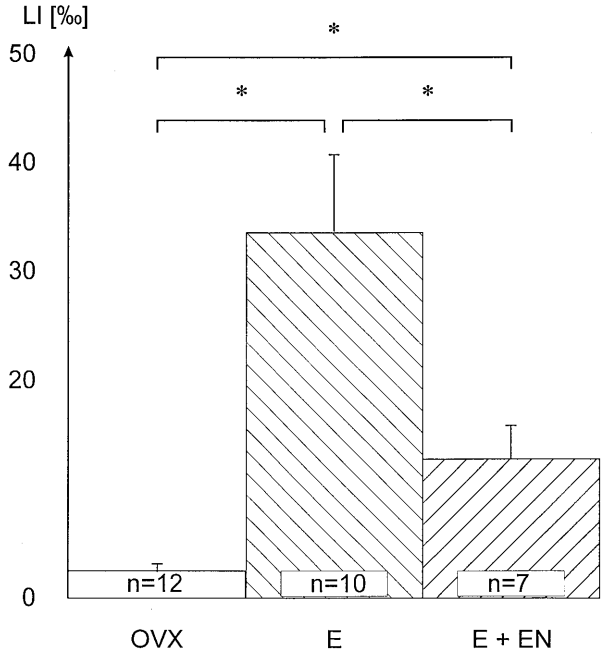


Figure 1. BrDU LI in the endometrial epithelial cells of the rats ovariectomized and treated with solvent (OVX), oestradiol benzoate (E) or with oestradiol benzoate and enalapril (E + EN). Bars represent means ± SEM, and numbers in bars indicate the number of animals in each group. $*P < 0.05$.

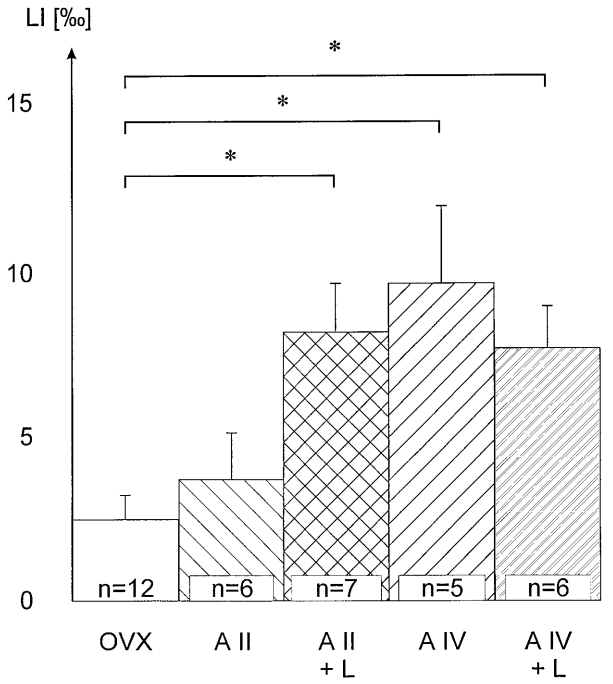


Figure 2. The effects of AII, AIV and L on BrDU LI in the endometrium of the ovariectomized rats. Bars represent means ± SEM, and numbers in bars indicate the number of animals in each group. $*P < 0.05$.

mediated by AT1 receptors. AT2 receptors do not bind losartan, but can be blocked by other antagonists, including the compound PD 123177. Stimulation of AT2 receptors results in activation of protein tyrosine phosphatases and inhibition of guanylate cyclase [5]. The recently characterized AT4 receptors bind AIV with high affinity, but they do not bind selective antagonists for AT1 (losartan) or AT2 (PD 123177) receptors. Their transduction mechanisms remain unclear [6].

It is well known that oestrogens indirectly enhance the synthesis of angiotensins by increasing production of their precursor molecule, angiotensinogen [7, 8]. In our earlier studies we showed that ACE inhibitors, enalapril and enalaprilate, suppressed the oestrogen-induced hyperplasia of the anterior pituitary gland [9, 10]. Since AII was found to stimulate the proliferation of pituitary lactotrophs [11–14], the data quoted above suggested an involvement of the renin-angiotensin system in oestrogen-induced growth of the anterior pituitary gland. A question has arisen whether angiotensins could be also involved in the control of cell growth of other oestrogen target organs such as the uterus. The existence of the local renin-angiotensin system in the human endometrium has been suggested [13]. It is well known that oestrogens induce endometrial proliferation, whereas oestrogen withdrawal results in its atrophy. The present paper reports the suppression of oestradiol-induced cell proliferation under the influence of an ACE inhibitor, enalapril maleate, and on stimulation of cell proliferation by angiotensin IV in the endometrium of ovariectomized rats.

Materials and methods

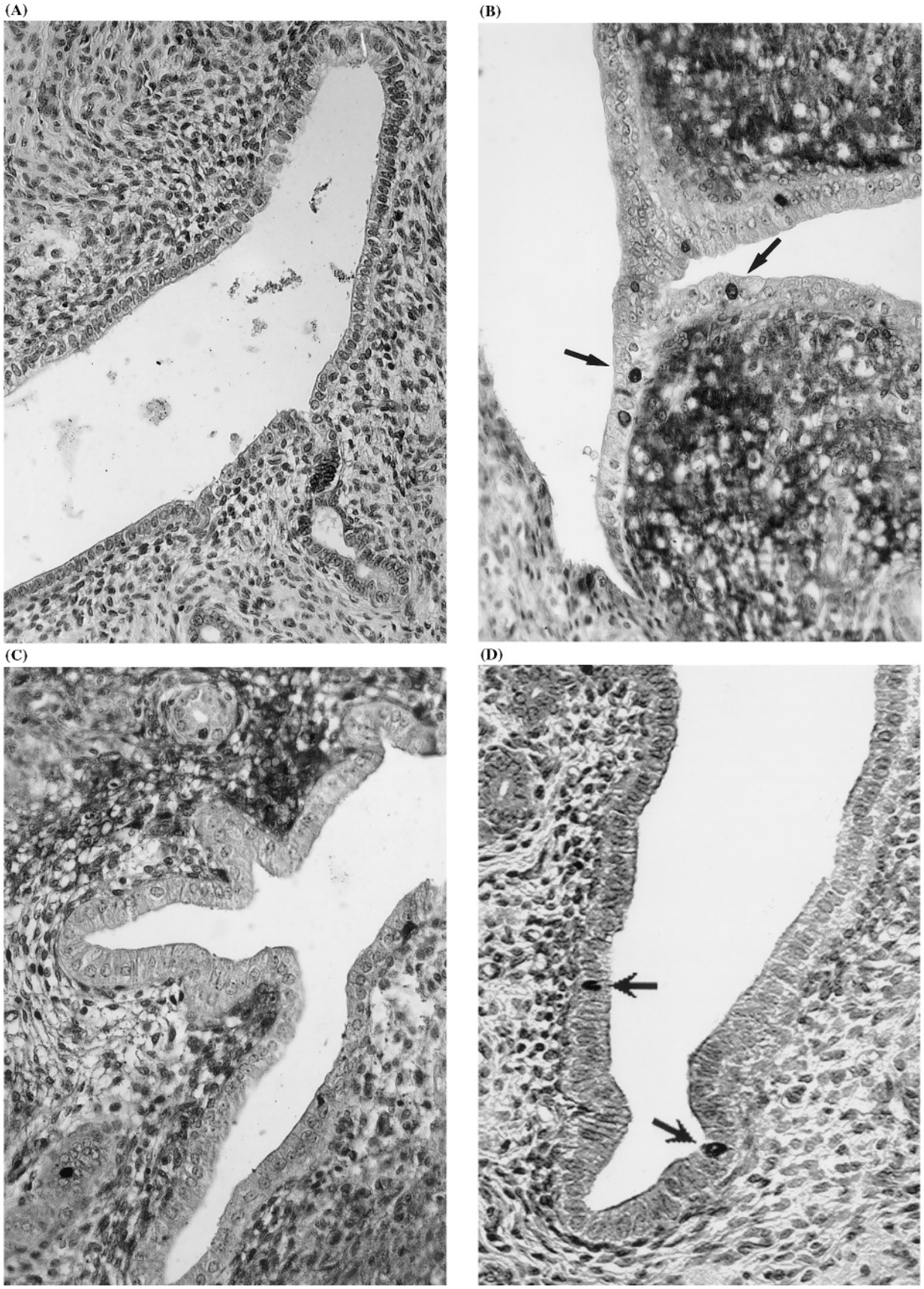
Two independent experiments were performed. The studies were carried out on female Wistar rats. The animals, weighing ~250 g each, were ovariectomized (OVX) and 10 days later treated as follows:

In the first experiment group OVX received a single subcutaneous (s.c.) injection of olive oil (0.25 ml) and 12 h later a single intraperitoneal (i.p.) injection of saline (0.5 ml);

group E was s.c.-injected with 250 μg of oestradiol benzoate (E; Oestradiolum benzoicum, Polfa, Poland) and 12 h later received an i.p. injection of saline; group E + EN was s.c.-injected with 250 μg of oestradiol benzoate and received two i.p. injections (at 12 h intervals) of enalapril maleate at a dose of 25 mg/kg body weight (b.w.)

In the second experiment, animals were divided into five groups treated as follows:

group OVX was i.p.-injected with saline, once daily, for 2 days; group AII received i.p. injections of angiotensin


(A)
(B)
(C)
(D)

II (AII, Sigma) at a dose of 50 μg/kg b.w., once daily, for 2 days; group AII + L received AII injections as above and i.p. injections of losartan (Merck, Rahway, NJ, USA) at a dose of 10 mg/kg b.w., twice daily, for 2 days; group AIV received two i.p. injections of angiotensin IV (AIV, Sigma), once daily, for 2 days; group AIV + L received i.p. injections of AIV and of losartan as described above.

In both the experiments, 12 h after the last injection all the animals received i.p. bromodeoxyuridine (BrDU; Sigma), 50 mg/kg b.w. Ninety minutes later the animals were sacrificed by decapitation, and uteri were collected and fixed in Bouin's fixative. The tissues were embedded in paraffin wax, and paraffin sections were immunostained using the Amersham cell proliferation kit to detect the incorporated BrDU [15]. The labelling indices (LI), expressed as a number of BrDU-immunopositive nuclei per 1000 randomly scored cells of endometrial epithelium, were estimated in microscopic preparations. The numerical data were evaluated statistically by means of the Mann-Whitney U test. Differences were considered significant if $P < 0.05$.

Results

The results obtained from both OVX groups were very similar; thus we have joined them in a single control group. As expected, the BrDU LI was low in the endometrium of ovariectomized rats (2.2 ± 0.6‰, mean ± SEM), (figs 1, 3A) and raised dramatically after the administration of oestradiol benzoate (33.5 ± 6.3‰), (figs 1 and 3B). Simultaneous treatment with enalapril maleate resulted in significant suppression, but not in total inhibition, of this oestrogen-induced effect (12.1 ± 3.9‰), (figs 1 and 3C).

Administration of AII alone did not significantly change BrDU incorporation into endometrial epithelial cell nuclei (3.0 ± 1.1‰), (fig. 2). However, the joint administration of AII and losartan produced a significant increase in the BrDU LI (7.7 ± 1.8‰). The increases in BrDU labelling were also observed in groups treated with AIV, either alone (9.6 ± 3.4‰) (fig. 3D), or together with losartan (7.7 ± 1.2‰) (fig. 2).

Discussion

The finding that an ACE inhibitor, enalapril maleate, counteracted oestrogen-induced endometrial cell proliferation suggests that involvement of the renin-angiotensin system in the growth-promoting action of oestrogens is not limited to the anterior pituitary gland, but is a more generalized phenomenon. It is worth recalling here that angiotensin II exerts a stimulatory effect on the proliferation of several cell types including adrenocortical cells [16], vascular cells [17, 18], cardiac fibroblasts [19], spleen lymphocytes [20] and intestinal epithelial cells [21]. However, as reported above, treatment with AII alone failed to stimulate endometrial cell proliferation in ovariectomized rats, at least under the conditions of our experiment. On the other hand, the stimulatory effect of AIV was demonstrated. This effect was less pronounced in comparison with the action of oestrogens. Although our data suggest that oestrogen action may be partially mediated by AIV, it certainly involves other mechanisms, for example generation of insulin-like growth factor 1 [22].

This observation is compatible with the recent data that AIV stimulated DNA and RNA synthesis in cultured rabbit cardiac fibroblasts [23]. A recent observation from our laboratory indicated the stimulatory effect of AIV on thymidine incorporation into rat lactotrophs in vitro [24]. The AT4 receptor, which binds AIV with high affinity, but not AII, is structurally similar to growth factor and cytokine receptors [1]. All these findings taken together with the observation presented above on AIV stimulation of rat endometrial proliferation strongly support the hypothesis of involvement of AIV in the control of cell growth. AT1, AT2 and AT4 sites have been found in the rat uterus [4, 6]. Inhibition of ACE by enalapril results not only in decreased synthesis of AII but also (indirectly) in decreased synthesis of AIV, as a consequence of the inhibition of the entire angiotensin cascade. Thus, the finding that enalapril attenuated oestrogen-induced endometrial cell proliferation corroborates the hypothesis of AIV involvement in endometrial cell growth. It remains unclear why combined treatment with AII and losartan resulted in stimulation of cell proliferation in contrast to administration of AII alone. One possible explanation is that losartan favours its conversion into further active peptides including AIV by inhibiting AII binding to AT1 sites, but direct proof is lacking. It cannot be ruled out that losartan may have an intrinsic activity on cell proliferation. However, such a possibility seems unlikely since losartan alone, in contrast to AII and AIV, did not alter [^{3}H]-thymidine incorporation into lactotrophs in vitro [24]. In conclusion, our data suggest involvement of the angiotensin cascade, and especially of angiotensin IV, in the control of uterine growth.

Figure 3. Identification of BrDU-labelled nuclei (arrows) in the rat endometrium. (*A*) in ovariectomized (OVX) rats; (*B*) in OVX animals treated with oestradiol benzoate; (*C*) in OVX rats treated with oestradiol benzoate and then with enalapril maleate; (*D*) in rats treated only with angiotensin IV. Magnification × 400.

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