ANGIOTENSINS II AND IV STIMULATE THE RAT ADRENOCORTICAL CELL PROLIFERATION ACTING VIA DIFFERENT RECEPTORS

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Objective. The effects of angiotensins II (AngII) and IV (Ang IV,3-8 fragment of angiotensin II) on the adrenocortical cell proliferation have been investigated in the rat.

Methods. The male adult Wistar rats were injected subcutaneously with saline, captopril or captopril together with either Ang II or Ang IV. A part of animals received additionally losartan – an antagonist of AT1 subtype of angiotensin receptors. Bromodeoxyuridine (BrDU) incorporation into cell nuclei was used as the index of cell proliferation.

Results. It was found that both Ang II and Ang IV increased the BrDU labeling in the adrenal cortex of captopril-pretreated rats. This effect involved mainly the zona glomerulosa cells.The proliferogenic effect of Ang II was blocked by AT1 receptor antagonist losartan. In contrast, losartan did not block the effect of Ang IV.

Conclusion. Both Ang II and Ang IV stimulate the adrenocortical cell proliferation in the rat, but they act via different receptors – AT1 in the case of Ang II and non-AT1 (probably AT4) in the case of Ang IV.

Key words: Adrenal cortex – Cell proliferation – Angiotensin II – Angiotensin IV

It is well known that angiotensin II (AngII) is a major physiological stimulator of aldosterone secretion. Moreover, as early as in the seventies it was shown that AngII exerts a stimulating effect on adrenocortical cell proliferation (GILL et al. 1977). This observation was confirmed in several further studies (SZKUDLINSKI and LEWINSKI 1989; PAWLIKOWSKI et al. 1990; MC EWAN ET AL. 1996; MAZZOCCHI et al. 1997). Recently, another peptide, corresponding to 3-8 fragment of AngII, called angiotensin IV (AngIV) has been suggested to have a physiological role (for review see WRIGHT et al. 1995). AngIV can be formed by the enzymatic cleavage of AngII via the intermediary step of angiotensin III (fragment 2-8 of AngII). A specific subtype of the angiotensin receptor, called AT4, which binds preferetially AngIV, has been characterized and localized in different tissues including the bovine adrenal cortex (JARVIS et al. 1992; SWANSON et al. 1992; SDINIAAR et al. 1994). However, AngIV can act also via AT1 receptors as it was demonstrated in the case of the pressor response to this peptide the intracerebroventricular administration (WRIGHT et al. 1996). In our laboratory we have found that AngIV enhanced the tritiated thymidine incorporation into rat pituitary lactotrophs (PAWLIKOWSKI and KUNERT-RADEK 1997). We have also found a stimulatory effect of AngIV on cell proliferation in the uterine endometrium in the rat (PAWLIKOWSKI et al. 1999). Both effects were not inhibited by AT1 receptor antagonist, losartan. A question arises whether AngIV can also exert a proliferogenic action on the adrenal cortex. To our knowledge, no observations have been done on the AngIV effects on either adrenocortical function or

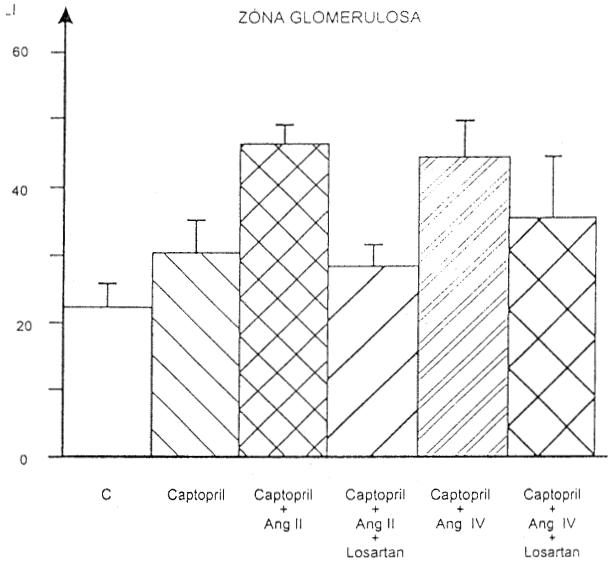

Fig 1
The number of bromodeoxyuridine (BrDU)-labeled nuclei in zona glomerulosa (mean ? SEM) per equatorial section of the adrenal gland (LI). C- controls; ANGII – angiotensin II; ANGIV – angiotensin IV.

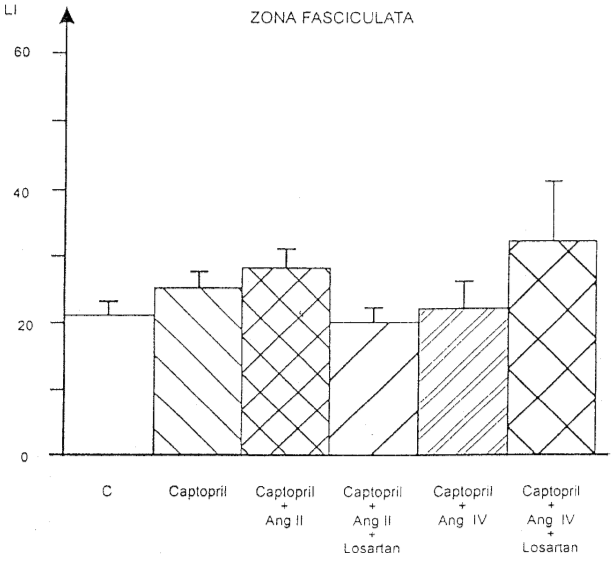

Fig 2
The number of BrDU – labeled nuclei in zona fasciculata/ equatorial section of the adrenal gland. Abbreviations as in Fig 1

growth. In the present paper we compared the effects of both angiotensin peptides AngII and AngIV on the adrenocortical cell proliferation in the rat.

Materials and Methods

The experiment was carried out in 42 adult male Wistar rats (from the Central Animalery of the Medical Faculty, Medical University of Lodz) weghing initially 290±10 g each. The animal were divided into six groups receiving 4 injections in the interval of 12 hrs of the following substances: Group I (controls) – 0.9 % NaCl, intraperitoneally (i.p.); Group II – Captopril (Jelfa SA, Poland) 20 mg/kg of body weight (b.w.), subcutaneously (s.c.); Group III – Captopril as above + Angiotensin II (AngII, Sigma), 50 µg/kg of b.w. i.p.; Group IV – Captopril as above + Angiotensin IV (AngIV, ICN Pharmaceuticals,inc.), 50 µg/kg of b.w. i.p.; Group V – Captopril as above + AngII as above + Losartan (LOS, Merck, Sharp and Dohme, USA), 10 mg/kg of b.w. i.p.; Group VI – Captopril as above + AngIV as above + LOS as above.

Twelve hours after the last injection the animals were sacrificed. Ninety minutes before that all the animals received a single i.p.injection of bromodeoxyuridine (BrDU, Sigma, 50mg/kg of body weight). The adrenals were collected and fixed in Bouin's fixative. The tissues were embedded in paraffin wax and immunostained using the Amersham cell proliferation kit. The The cell proliferation was assessed according to procedure described by Michat and Nouet (1975). The serial sections of each gland were estimated, the largest (equatorial) being chosen for counting. The number of BrDU-immunopositive cell nuclei were counted per equatorial section of each gland, and per each adrenocortical zone separately (zona glomerulosa, fasciculata and reticularis). The numerical data were statistically analyzed using the one-way analysis of variance test.

Results

The results were given in Fig 1-4. As can be seen there, the administration of captopril alone had no effect on the number of BrDU-immunopositive cells in the adrenal cortex. In contrast, the joint treatment with captopril plus AngII resulted in the statistically significant increase in the number of BrDU-labeled nuclei as estimated for either the all adrenocortical

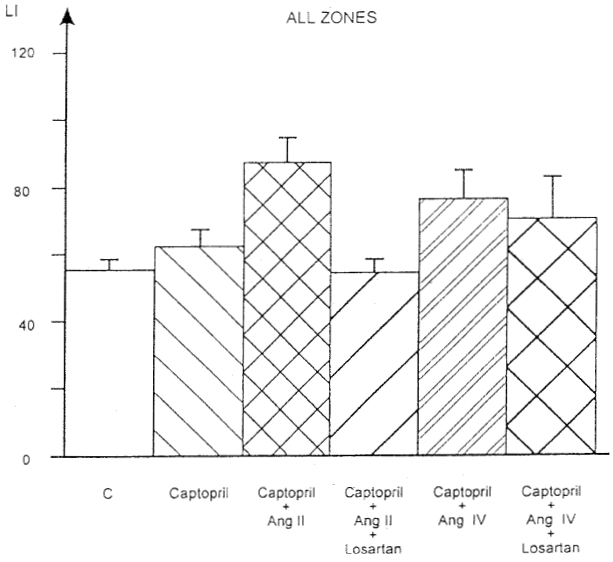

Fig 3
The number of BrDU -labeled nuclei in zona reticularis/ equatorial secrion of the adrenal gland. Abbreviations as in Fig 1

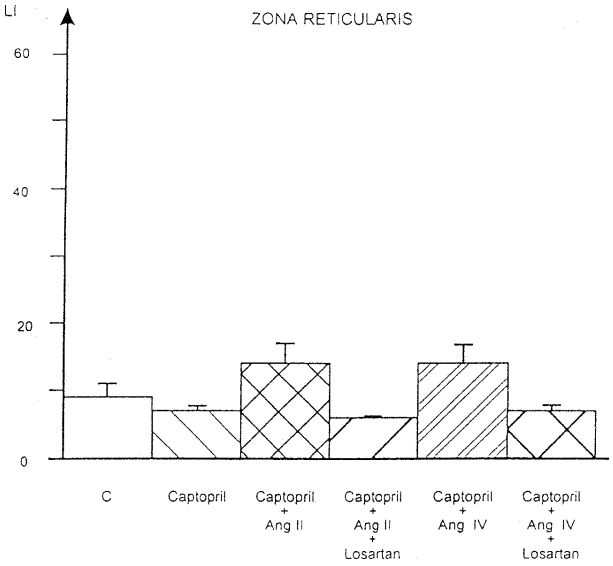

Fig 4
The number of BrDU – labeled nuclei in all zones of the adrenal cortex/equatorial section of the adrenal gland. Abbreviations as in Fig 1

zones or for zona glomerulosa (P<0.05 vs either controls or captopril group). The effect is totally blocked by the simultaneous administration of LOS. AngII did not significantly influence the BrDU labelling in zona fasciculata and zona reticularis; however, a tendency towards higher number of BrDU – labeled nuclei in zona reticularis could be observed. The effect of the joint administration of captopril + AngIV was very similar to that of captopril + AngII: namely, the increase of the BrDU labelling in zona glomerulosa (P<0.05 vs controls) and the slight non-significant increase in zona reticularis was observed. However, in this case, the simultaneous administration of LOS did not block the proliferogenic response.

Discussion

The observation that the administration of AngII to the captopril-pretreated rats increases the adrenocortical cell proliferation and that this effect involves mainly or even exclusively zona glomerulosa corroborate with the earlier data of McEwan et al. (1996; 1999) and Mazzocchi et al (1997). The tendency towards the higher number of BrDU-immunopositive cells in zona reticularis of AngII-treated animals, albeit not significant, also corroborates with the earlier finding of McEwan et al. (1996). Our data confirm also the finding of the quoted authors, that AngII stimulate the proliferation of zona glomerulosa acting via AT1 type of angiotensin receptors. Our novel observation is that AngIV, a 3-8 fragment of AII, exerts also a proliferogenic activity on zona glomerulosa. Moreover, in contrast to AngII, the action of AngIV is not blocked by a selective antagonist of AT1 receptors. It means that AngIV acts on adrenocortical cell prolifertion via other receptor than AT1. The involvement of AT2 receptors is unlikely because this receptor subtype mediates rather the antiproliferative and proapoptotic effects (de Gasparo and Siragy 1999). It seems to concern the AT2 receptors in the rat adrenal gland, since PD 123319, AT2 receptor antagonist was found to increase DNA synthesis in the rat adrenal cortex (Mazzocchi et al. 1997). Although we do not present a direct proof that AngIV produces its proliferogenic effect via AT4 receptors, such a presumption seems to be the most probable. The bovine adrenal cortex is a tissue which contains the very high concentration of AT4 receptors (Wright et al. 1995). The presence of a non-AT1/ non-AT-2 receptors in the murine adrenal cortex was

also suggested by NARUSE et al (1998). The further studies using the specific antagonists of AT4 receptors are needed to prove our presumption. The lack ot the effect of captopril (the inhibitor of angiotensin-convering enzyme) when given alone does not exclude the role of the endogenous angiotensins in the control of the adrenocortical cell growth. Firstly, the action of captopril may be limited to the states of the enhanced activity of the renin-angiotensin system. Secondly, the effects of angiotensins on the adrenocortical cell growth may be opposite in dependence of the receptors involved, as it has been shown for Ang II acting via AT1 and AT2 receptors. It is also possible that the use of the other ACE inhibitor which more effectively decreases the tissue concentrations of Ang II might affect the adrenocortical cell\proliferation. Interestingly, MCEWAN et al. (1996) also did not observe the effect of captopril on the adrenocortical proliferation in spite of the marked proliferogenic effect of Ang II.

Acknowledgements

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