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ORIGINAL ARTICLE

The effects of angiotensin peptides and angiotensin receptor antagonists on the cell growth and angiogenic activity of GH3 lactosomatotroph cells in vitro

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Abstract The local renin–angiotensin system (RAS) is present in the pituitary gland, and inhibitory effects of angiotensins on the lactosomatotroph (GH3) cell growth have been revealed. The aim of this study was to examine the influence of various angiotensin peptides and angiotensin AT1, AT2, and AT4 receptors antagonists on the cell proliferation, viability, and VEGF secretion in pituitary lactosomatotroph GH3 cell culture in order to identify receptors involved in antiproliferative effects of angiotensins on GH3 tumor cells. Cell viability and proliferation using Mosmann method and BrdU incorporation during DNA synthesis, and VEGF secretion using ELISA assay were estimated. The inhibitory effects of ang II, ang IV, and ang 5–8 on the cell viability and BrdU incorporation in GH3 culture were not abolished by AT1, AT2, and AT4 receptors antagonists. Ang II, as well as ang III and ang IV at lower concentrations stimulated the secretion of VEGF in GH3 cell culture. The secretion of VEGF was inhibited by ang III and ang IV at higher concentrations. AT1 and AT2 receptors antagonists prevented the proangiogenic effects of ang II. Ang II, ang IV, and ang 5–8 decrease the cell number and proliferation in GH3 cell culture independently of the AT1, AT2, and AT4 receptors. These peptides affect also secretion of VEGF in culture examined. Both the AT1 and AT2 receptors appear to mediate the proangiogenic effects of ang II.

Keywords Pituitary tumor · GH3 cell line · Angiotensin · Angiotensin receptor antagonist · Cell proliferation · VEGF

Introduction

Angiotensin II (ang II), the main peptide of renin–angiotensin system (RAS) is converted into smaller peptides angiotensin III (ang III) and angiotensin IV (ang IV) by aminopeptidase A and aminopeptidase N, respectively [1]. All these angiotensin peptides possess biological activity and as the components of the local tissue RASs, are involved in the regulation of cellular proliferation, differentiation, and survival [2–5]. On the contrary, the ang IV derivatives, ang 4–8 and ang 5–8 are known to be biologically inactive. Besides well-documented growth-promoting effects of angiotensins in various tissues [6–11], ang II, ang III, and ang IV have been demonstrated to possess antiproliferative activities. Ang II has been found to inhibit bFGF-induced proliferation of the bovine adrenal fasciculata cells (BAC) and the rat coronary endothelial cells (CECs) [12, 13]. This peptide decreased also the cellular proliferation in primary culture of the rat adrenal glomerulosa cells [14, 15]. Moreover, ang II, ang III, and ang IV inhibited the growth of the DU-145 androgen independent human prostate cancer cell line in vitro [16].

Recent studies have also revealed an association of the RAS with angiogenesis [17]. The ang II-stimulated blood vessel growth has been previously demonstrated in some

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experimental models [18–20]. Moreover, in some tissues, ang II was capable to induce the expression of key pro-angiogenic cytokine, vascular endothelial growth factor (VEGF), to up-regulate the VEGF receptor expression or to potentiate the VEGF-induced endothelial progenitor cells proliferation and tube formation [21–24]. Ang IV is known to increase DNA and protein synthesis in mammalian vascular endothelial cells [25, 26].

Most of the well-described effects of ang II and ang III are mediated by the AT1 and AT2 receptors [1, 6], whereas ang IV possesses its specific receptor subtype called AT4 [27, 28]. The AT4 site exhibits a high tissue distribution, especially in the central nervous system, blood vessels, heart, kidney, prostate, and adrenals [27]. This receptor subtype is suggested to mediate memory acquisition and retrieval, and to participate in the regulation of cerebral and renal blood flow [6].

The intrinsic RAS and its components including peptides, enzymes, and receptors have been identified in all types of the hormone-secreting anterior pituitary cells, with the highest expression within the lactotropes [29, 30]. Ang II is known to stimulate the secretion of anterior pituitary hormones, including prolactin (PRL), adrenocorticotropic hormone, growth hormone, and luteinizing hormone [31, 32]. Although an involvement of only AT1 receptor subtype in Ang II-induced PRL release has been confirmed by Moreau et al. [34], the later study of Pawlikowski et al. [33] revealed, that also specific antagonist of AT2 receptor PD123319 is effective in inhibiting PRL secretion in rats with estrogen-induced hiperprolactinemia. In contrast to the well-established role of RAS in the regulation of anterior pituitary hormones' release, the influence of angiotensin peptides on the cellular growth within the pituitary gland is poorly defined. There are reports on stimulatory effects of ang II and ang IV on the proliferation of lactotropes isolated from the estrogen-induced rat pituitary tumor [33, 35]. Furthermore, angiotensin-converting enzyme inhibitor, enalapril, prevented the pituitary proliferative response to a single injection of estrogens in rat and decreased the density of lactotroph cells in the diethylstilbestrol (DES)-induced rat anterior pituitary tumors [33, 36]. Nevertheless, our recent studies revealed that ang II, ang IV, and surprisingly, ang 5–8 inhibited cell proliferation and viability in the lactosomatotroph (GH3) cell culture [37]. For our best knowledge, these data were the first one, indicating the inhibitory influences of angiotensin peptides on the growth of anterior pituitary cells and suggesting the biological activity of ang IV-degradation products. Until now, we have not identified the receptors involved in the antiproliferative effects of angiotensins. For this reason, in this study we examined the influence of angiotensin receptor antagonists on the proliferation of angiotensin-treated GH3 cells. Moreover, taking into consideration the proved role of RAS in the angiogenic aspect of the pathogenesis of solid tumors [38–40], the secretion of VEGF in this model was also studied. In our study, we used the GH3 cell line. The GH3 cells represent lactosomatotrophs, expressing both PRL and growth hormone within the same cell [41]. It is known that lactotropes have been defined to be the major pituitary target cells of angiotensin [42]. GH3 cells, although differing in some points from non-tumoral pituitary lactotropes, may present a suitable model to study the angiotensin effects on anterior pituitary gland.

Materials and methods

Materials

The chemicals used in present experiments were obtained from the following sources: angiotensin II (ang II, Sigma), angiotensin III (ang III, Bachem), angiotensin IV (ang IV, Bachem), angiotensin 5–8 (ang 5–8, Bachem), Losartan (Merck), PD123319 (Sigma), Divalinal-ang IV (Pacific Northwest Biotechnology), Nutrient Mixture F-10 [Ham] (Sigma), Sodium bicarbonate, $NaHCO_3$ (Sigma), Penicillin/Streptomycin Solution (Sigma), Fetal Bovine Serum, FBS (Sigma), Horse Serum (Sigma), Trypsin–EDTA (Sigma).

Cell culture

The rat lactosomatotroph tumor cell line GH3 was obtained from ATCC, LGC Promochem. Continuous GH3 cell culture was maintained in culture flasks (Nunc Eas Y Flask 25 cm^2, NUNC). All the cells were grown in Ham's F-10 medium supplemented with 1.2 g/l Sodium Bicarbonate, 100 U/ml Penicillin and 100 μg/ml Streptomycin solution, 15 % heat-inactivated horse serum and 2.5 % heat-inactivated fetal bovine serum at 37 °C and in a humidified atmosphere of 95 % air and 5 % CO_2. Every 7 days, the cells were harvested after 2-min incubation at 37 °C in the presence of trypsin–EDTA (0.05 or 0.02 %, respectively) in Hanks balanced solution. The cells were washed twice in complete F-10 medium and after the last centrifugation seeded at 1×10^5 cells in 5 ml of fresh medium.

Proliferation assays

We have previously demonstrated that ang II and ang IV in concentrations of 10^{-8} M and ang 5–8 in concentration of 10^{-10} M exerted the maximal inhibitory effect on the viability and proliferation of GH3 cells [37]. For this reason, we have chosen the concentrations mentioned for the studies on simultaneous actions of these angiotensins and

the angiotensin receptors antagonists in this experimental model.

Cell viability

The cells were subjected to the trypsinization process and than suspended at 4×10^5/ml in complete F-10 medium. 50 µl aliquots of cell suspension (2×10^4 cells) were placed in the wells of plastic 96-well culture plates (96 Cell Culture Cluster Dish, Costar; Nunclon, Microwell Plates, NUNC). After 12 h period of preincubation (5 % CO_2, 37 °C, 95 % humidity) the tested substances were added to the appropriate wells: 10^{-8} M ang II or 10^{-8} M ang IV alone or with losartan at final concentrations of 10^{-8} or 10^{-7} M, losartan at the above final concentrations; 10^{-8} M ang II or 10^{-8} M ang IV alone or with PD123319 at final concentrations of 10^{-8} or 10^{-7} M, PD123319 at the above final concentrations; 10^{-8} M ang II or 10^{-8} M ang IV or 10^{-10} M ang 5–8 alone or with divalinal at final concentrations of 10^{-8} or 10^{-7} M, divalinal at the above final concentrations. The appropriate volume of the culture medium was added to the wells of control group ($n = 8$) and to the wells with one tested substance, to the final volume 200 µl in each well. After 72 h of incubation (5 % CO_2, 37 °C, 95 % humidity), the cell viability was estimated using the modified Mosmann method, following the procedure recommended by the producer of the kit (EZ4Y, Easy for You, The 4th Generation Non Radioactive Cell Proliferation & Cytotoxity Assay, Biomedica Gruppe, Austria, Belco Biomedica Poland). The optical density (OD) of each sample was measured by a microplate reader at 450 nm.

BrdU incorporation

After trypsinization process the cells were suspended at 3×10^5/ml in complete F-10 medium. 50 µl aliquots of cell suspension (1.5×10^4 cells) were placed in the wells of plastic 96-well culture plates (96 Cell Culture Cluster Dish, Costar; Nunclon, Microwell Plates, NUNC). After 12 h period of preincubation (5 % CO_2, 37 °C, 95 % humidity) the tested substances were added to the appropriate wells: 10^{-8} M ang II or 10^{-8} M ang IV alone or with losartan at final concentrations of 10^{-8} or 10^{-7} M, losartan at the above final concentrations; 10^{-8} M ang II or 10^{-8} M ang IV alone or with PD123319 at final concentrations of 10^{-8} or 10^{-7} M, PD123319 at the above final concentrations; 10^{-8} M ang II or 10^{-8} M ang IV or 10^{-10} M ang 5–8 alone or with divalinal at final concentrations of 10^{-8} or 10^{-7} M, divalinal at the above final concentrations. The appropriate volume of the culture medium was added to the wells of control group ($n = 8$) and to the wells with one tested substance, to the final volume 100 µl in each well. The cells were cultured in the presence of the tested substances for 72 h (5 % CO_2, 37 °C, 95 % humidity). After 68 h of incubation 10 µl BrdU labeling solution (final concentration: 10 µM BrdU) was added to each well, and the cells were reincubated for additional 4 h, necessary for pyrimidine analog BrdU to incorporate into DNA of proliferating cells. Then, cell proliferation was measured following the procedure recommended by the producer of the kit (Cell Proliferation ELISA, BrdU [colorimetric], colorimetric immunoassay for the quantification of cell proliferation, based on the measurement of BrdU incorporation during DNA synthesis, an alternative to the [^{3}H]-thymidine incorporation assay). The OD of each sample was measured by a microplate reader at 450 nm.

VEGF measurement

The cells were subjected to the trypsinization process, suspended at 10^6/ml in complete F-10 medium, and 100 µl aliquots of cell suspension (10^5 cells) were placed in the wells of cell culture plates (24 Cell Culture Cluster Dish, Nuclon Multidishes, Nunc). All the plated cells were preincubated 12 h (5 % CO_2, 37 °C, 95 % humidity), and after this preincubation period treated with the following substances: ang II, ang III, or ang IV at final concentrations of 10^{-12}, 10^{-10}, 10^{-8}, or 10^{-6} M; 10^{-10} M ang II + losartan at final concentrations of 10^{-8} or 10^{-7} M; 10^{-10} M ang II + PD123319 at final concentrations of 10^{-8} or 10^{-7} M, losartan at final concentrations of 10^{-8} or 10^{-7} M; PD123319 at final concentrations of 10^{-8} or 10^{-7} M. The same volume of fresh serum-free culture medium was added to the control wells. All the cells were treated with the substances for 24 h. The supernatant was than collected from the wells, and the secreted VEGF isoforms, among them dominating mouse/rat VEGF164, were measured in terms of pg/ml using specific ELISA assay kits for mouse/rat VEGF (Mouse VEGF Immunoassay, Quantikine M, R&D System, USA).

Statistical evaluation

Data concerning the cell viability and proliferation are given as the absolute values of the OD, and represents 8–10 different measurements, run in duplicate, ±SD. In the case of VEGF secretion, data express VEGF concentration in the culture medium. The normality of distribution of the results was examined by the Student test. Comparisons of individual groups were evaluated by analysis of variance (ANOVA). Differences were considered significant if $p < 0.05$.

Results

Cell viability and proliferation

Angiotensin peptides decrease the total cell number and cellular proliferation in the lactosomatotroph GH3 cell culture.

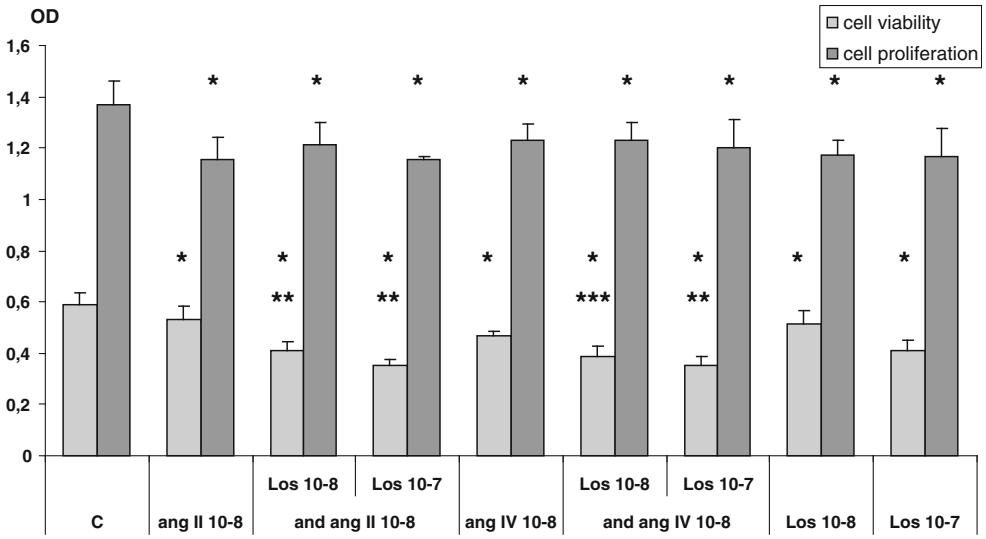

Fig. 1 The influence of AT1 receptor antagonist losartan (Los) at concentrations of 10^{-8} and 10^{-7} M on angiotensin II (ang II)- and angiotensin IV (ang IV)-induced decrease of the cellular viability and proliferation in the lactosomatotroph GH3 cell culture. X axis—absolute values of the OD, C—control (unstimulated cells), X ± SEM; *$p < 0.05$ vs C, **$p < 0.05$ vs ang II, ***$p < 0.05$ vs ang IV

We have previously demonstrated that angiotensin peptides, including ang II, ang IV and ang 5–8 inhibited the cellular proliferation and viability in GH3 cell culture [37]. In our present study, we confirmed the results mentioned above (data not shown).

The influences of AT1, AT2 and AT4 receptors antagonists on the effects of some angiotensin peptides and on the basal GH3 cell viability and proliferation.

The influence of AT1 receptor antagonist losartan on the angiotensins growth-regulatory effects were examined with respect to ang II and ang IV. We did not study the effects of combined treatment with losartan and ang 5–8, presuming the insignificant affinity of ang IV derivatives for the AT1 receptor. This presumption results from the data, that N-terminal residue of ang II is important for the AT1 receptor binding and duration of action. Since lacking *Arg-Asp*-terminus ang IV is known to be very weak agonist of the AT1 receptor, we would expect extremely low binding affinity of ang 5–8 for the AT1 receptor subtype. Our study demonstrated, that AT1 receptor blocker did not prevent, but even strengthened, the decrease of total number of the GH3 cells in ang II- and ang IV-treated groups (Fig. 1). Moreover, losartan abolished neither the inhibitory effects of ang II nor the effects of ang IV on the BrdU incorporation in GH3 cell culture (Fig. 1). Similarly to AT1 antagonist, the effects of AT2-blocker were examined with respect to ang II and ang IV. As it can be seen in Fig. 2, PD123319 did not abolish the inhibitory effects of both peptides on cell viability and cellular proliferation. As for the AT4 receptor antagonist, we assessed its influences on the effects of three angiotensin peptides, including ang II, ang IV, and ang 5–8. Analogically to losartan and PD123319, divalinal prevented none of the inhibitory effects of peptides that have been examined (Fig. 3). The incubation of the GH3 cells with losartan or divalinal alone in both concentrations tested, or with PD123319 alone at concentration 10^{-7} M resulted in the decrease of the basal GH3 cell number and proliferation (Figs. 1, 2, 3).

VEGF secretion

The results of the quantitative analysis and the statistical evaluation of these results are presented in Figs. 4 and 5. The incubation of the GH3 cells with ang II at final concentrations of 10^{-12}, 10^{-10}, 10^{-8}, and 10^{-6} M, ang III at concentrations of 10^{-12} and 10^{-10} M, or ang IV at concentration of 10^{-8} M led to a significant increase in VEGF secretion, whereas exposure of cells to 10^{-6} M concentration of ang III or ang IV resulted in the important inhibition of cytokine release (Fig. 4). The stimulatory effect of ang II at concentration of 10^{-10} M on the VEGF secretion in GH3 cell culture was abolished by losartan or PD123319 in both concentrations tested. Incubation of lactosomatotroph cells with losartan (10^{-8} or 10^{-7} M) or PD123319 (10^{-7} M) alone was followed by the decrease in VEGF concentration in culture medium (Fig. 5).

Discussion

The stimulatory effects of some angiotensin peptides on the growth of lactotroph cells have been demonstrated in

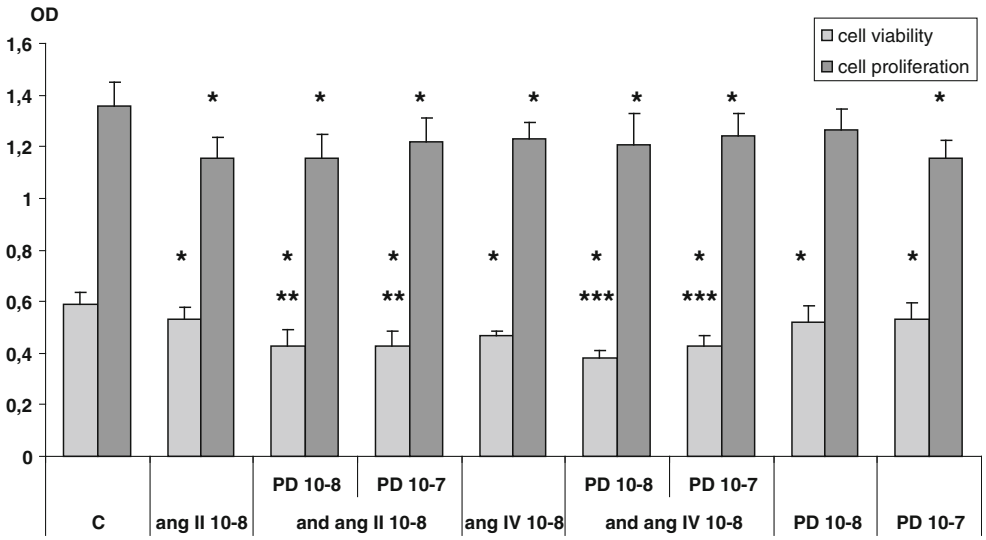

Fig. 2 The influence of AT2 receptor antagonist PD123319 (PD) at concentrations of 10^{-8} and 10^{-7} M on angiotensin II (ang II)- and angiotensin IV (ang IV)-induced decrease of the cellular viability and proliferation in the lactosomatotroph GH3 cell culture. X axis—absolute values of the OD, C—control (unstimulated cells), X ± SEM; *$p < 0.05$ vs C, **$p < 0.05$ vs ang II, ***$p < 0.05$ vs ang IV

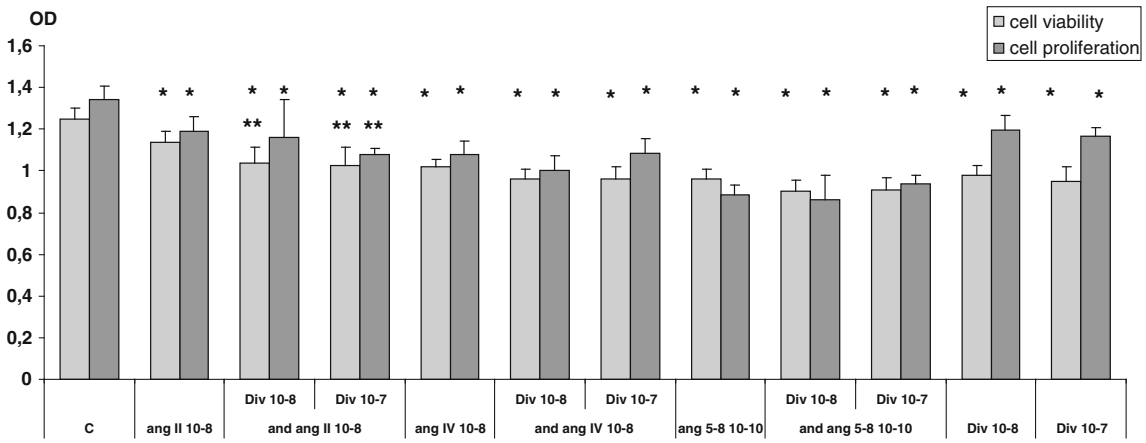

Fig. 3 The influence of AT4 receptor antagonist divalinal (Div) at concentrations of 10^{-8} and 10^{-7} M on angiotensin II (ang II)-, angiotensin IV (ang IV)- and angiotensin 5–8 (ang 5–8)-induced decrease of the cellular viability and proliferation in the lactosomatotroph GH3 cell culture. X axis—absolute values of the OD, C—control (unstimulated cells), X ± SEM; *$p < 0.05$ vs C, **$p < 0.05$ vs ang II

earlier studies [33, 35, 43–45]. Surprisingly, our recent experiments performed on the lactosomatotroph GH3 cell model have revealed, that ang II, ang III, and ang IV, as well as their derivatives ang 4–8 and ang 5–8, were able to inhibit the cellular proliferation and viability [37]. Nevertheless, the influence of the antagonists of main angiotensin receptors, AT1, AT2, and AT4, on the angiotensin-dependent antiproliferative effects has not been examined as yet. A question arises why the angiotensin peptides could inhibit proliferation of GH3 cells.

In contrast to the earlier reports on AT1- or AT2-linked antiproliferative actions of ang II in bovine adrenocortical cell (BAC) culture or in primary culture of the rat adrenal glomerulosa cells [12, 14] the growth-inhibitory effects of angiotensin peptides in GH3 cell culture appear to be independent of the AT1, AT2, or even AT4 receptors, since we demonstrated in this study that losartan, PD123319 or divalinal did not prevent the inhibition of total cell viability and proliferation in the angiotensins-treated groups. The lack of the functional AT1 receptors can be ruled out because in this study we also revealed AT1-dependent stimulation of VEGF release (see below). The possible involvement of ang 1–7 receptor was not tested in our study but should be taken into consideration. It is worth to recall that the main agonist of this receptor, ang 1–7 was shown to inhibit cell proliferation of lung cancer cells [46].

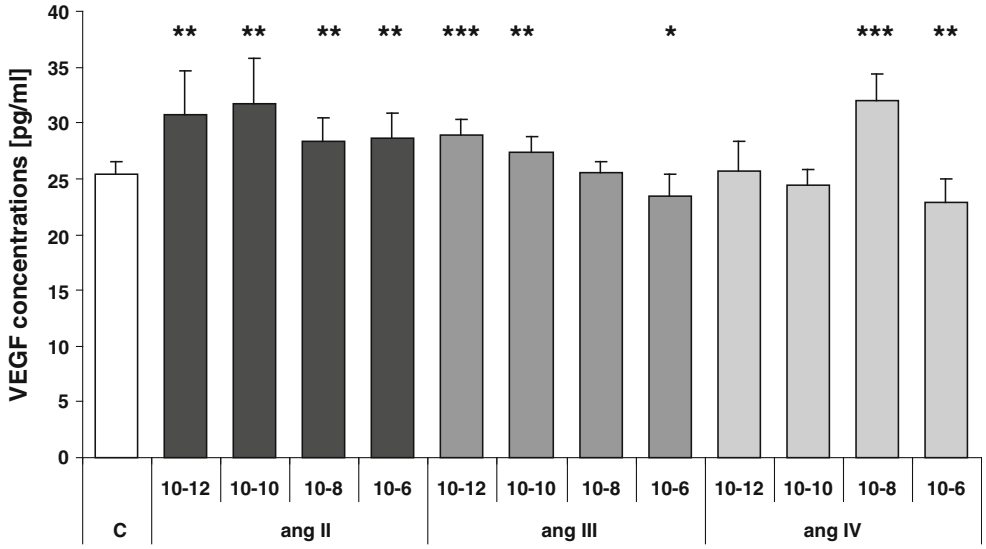

Fig. 4 The influence of 24-h treatment with angiotensin II (ang II), angiotensin III (ang III) and angiotensin IV (ang IV) on the secretion of VEGF in the GH3 cell culture. C—control (unstimulated cells), X ± SEM; *$p < 0.05$ vs C, **$p < 0.01$ vs C, ***$p < 0.001$ vs C

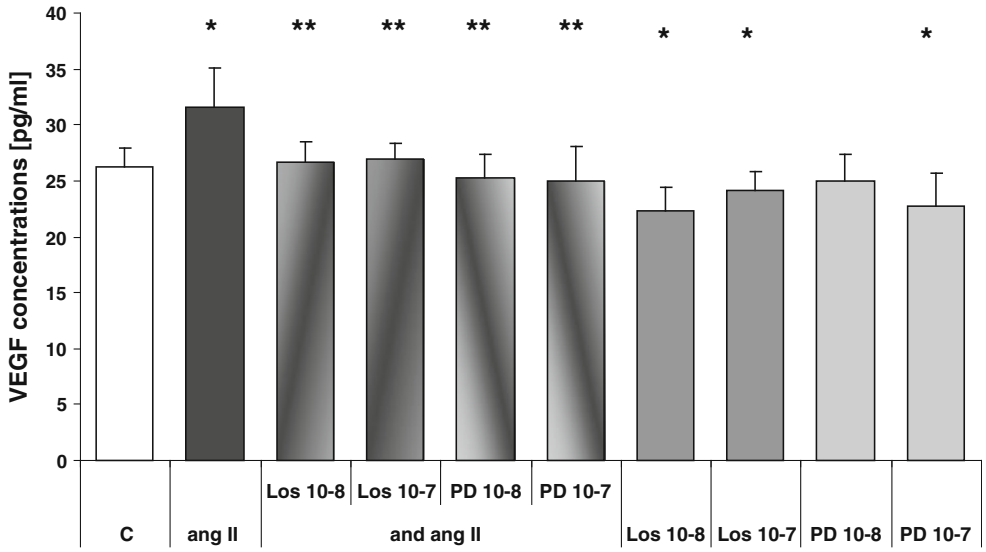

Fig. 5 The influence of treatment with angiotensin II (ang II, 10^{-10} M), AT1 receptor antagonist losartan (Los), AT2 receptor antagonist PD123319 (PD) and of the combined treatment with angiotensin II and losartan or angiotensin II and PD123319 on VEGF secretion in the GH3 cell culture. C—control (unstimulated cells), X ± SEM, *$p < 0.05$ vs C (control), **$p < 0.05$ vs ang II

Interestingly, we observed that AT1, AT2, or AT4 receptor antagonists alone exerted the inhibitory influences on basal proliferation of the GH3 cells, and lowered the number of viable GH3 cells in culture. We hypothesize that this unexpected phenomenon may result from the inhibition of the autocrine and paracrine proliferative actions exerted by locally generated endogenous angiotensin peptides. Such an explanation seems relevant, as the components of RAS, including the precursors, enzymes and bioactive angiotensin peptides, as well as the AT1, AT2, and AT4 receptor subtypes, have been identified within the cells of lactosomatotroph GH3 line [6, 29, 30, 47]. However, a question arises why the angiotensin peptides could both promote and inhibit proliferation of GH3 cells, and why antiproliferative actions of exogenous angiotensin peptides overcame their probable AT1-, AT2-, or AT4-dependent proliferogenic effects in our experimental model. Stoll et al. [13] demonstrated the ability of ang II to exert a dual control of cell proliferation in cultured CECs. In this experiment, ang II alone did not influence proliferation of quiescent CECs, as growth-promoting actions of the peptide mediated by the AT1 receptor subtype offset

the antiproliferative effects exerted via the AT2 receptor. However, in our study the involvement of AT2 was excluded since AT2 receptor blocker failed to counteract the antiproliferative action of angiotensin peptides. On the other hand, ang II, ang III, and ang IV can exert indirect antiproliferative actions, determined by the previous conversion into the smallest peptides, ang 4–8 or ang 5–8. In such a case, the balance between growth-promotion and anti-proliferation would be determined by the ang IV degradation rate, and we would expect the shift into antimitogenic effects if the activity of ang IV-degrading aminopeptidases were high. This explanation is probable in context of our previous study on the same experimental model. We demonstrated, that the inhibitor of aminopeptidases, amastatin, completely abolished the inhibitory effect of ang IV on the viability of GH3 cells [37]. Nevertheless, identification of the mechanisms, connected with the growth-inhibitory effects of angiotensin peptides in the GH3 cell culture needs further studies.

Apart from the influences on cellular proliferation and viability, our results indicate the involvement of angiotensin peptides in the regulation of angiogenic activity of the GH3 cells. We revealed, that ang II, ang III, and ang IV, at the concentrations of 10^{-12}–10^{-8} M were able to stimulate VEGF release in the GH3 cells culture. There are several reports on VEGF-dependent proangiogenic activities of angiotensins. Ang II was found to stimulate in vitro VEGF secretion in human vascular smooth muscle cells, human mesangial cells, rat heart ECs and bovine retinal microcapillary pericytes [21–23, 48]. Ang IV was shown to enhance the proliferation of the lung endothelial cells [25]. Nevertheless, independently of the proangiogenic effects of peptides, we noticed that treatment of the GH3 cells with ang III or ang IV at the concentration of 10^{-6} M led to a significant decrease in VEGF levels in the culture medium. The stimulatory or inhibitory effects on VEGF release possibly reflect biphasic action of angiotensin peptides on angiogenesis. The similar phenomenon was previously reported with respect to estrogens [49]. The unclear role of angiotensin system in angiogenesis is supported by the findings of Benndorf et al. [50] who observed that AT2-inhibited VEGF-induced migration and tube formation of human endothelial cells and Fujiyama et al. [51] who revealed that AT1 and AT2 differentially regulated angiopoietin-2 and VEGF expression and angiogenesis.

The proangiogenic effect of ang II in lactosomatoproph GH3 cell culture is mediated by the AT1 and AT2 receptors. As we reported above, specific AT1 receptor antagonist losartan or AT2 receptor antagonist PD123319 abrogated the stimulatory action of exogenous peptide on VEGF secretion by GH3 cells. Moreover, similarly to the inhibitory influences on basal cell proliferation, AT1 and AT2 receptor antagonists decreased basal VEGF secretion in the culture. The latter phenomenon possibly reflects the interference in proangiogenic activity of endogenous angiotensins and strengthens the hypothesis of the AT1- or AT2-dependent VEGF stimulation by angiotensins acting at low concentrations. This is noteworthy, that our results are consistent with the results of previous in vivo study. Pawlikowski et al. [52] have found, that estrogen-dependent increase of vessel area in the anterior pituitary of DES-treated rats was blocked by losartan, and, to a lesser degree, by PD123319. Moreover, both the AT1 and AT2 receptors have been found to participate in vascular growth-promoting effects in other tissues [24, 53–55]. Interestingly, non-tumoral and tumoral human pituitary cells express VEGF receptors and are not only a source of VEGF, but its target as well [56]. Recently, it was shown that a somatostatin analog suppressed in vitro the viability of human pituitary tumoral cells via inhibition of VEGF secretion [57]. Thus, VEGF is not only the angiogenic factor but a growth and/or survival factor for pituitary cells. However, in the case of GH3 cells, in spite of AT1- and AT2-dependent VEGF secretion enhancement, the angiotensin peptides failed to stimulate the cell growth and viability.

Summing up, we revealed the ability of various angiotensin peptides to affect the proliferation and angiogenic activity of the lactosomatotroph GH3 cells. The AT1 and AT2 receptors mediate mitogenic and proangiogenic effects of angiotensins, whereas antiproliferative and antiangiogenic actions are determined by the other, unidentified receptors, and appear to involve higher concentrations of peptides. Since the intrinsic RAS has been found in cells of the PRL-secreting adenoma, our results strongly support the hypothesis of an involvement of locally generated angiotensin peptides in the regulation of lactotroph tumor growth.

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