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Angiotensin II as a factor modulating protein tyrosine kinase activity in two breast cancer lines — MCF-7 and MDA-MB-231

Angiotensyna II jako czynnik modulujący aktywność kinaz tyrozynowych w komórkach raka gruczołu sutkowego — MCF-7 i MDA-MB-231

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Abstract

Introduction: Angiotensin II (AngII), a peptide that regulates the water-electrolytic balance and blood pressure, is also known to influence cell proliferation. It can either induce cell growth, when binding to angiotensin type-I receptor, or trigger growth inhibition via angiotensin type-II receptor. AngII stimulates proliferation of some normal and tumour cell lines, e.g. pituitary, adrenal glands and breast cancer.
Material and methods: The aim of this study was to evaluate possible AngII effect on the growth of two breast cancer cell lines — hormone-dependent MCF-7 and hormone-independent MDA-MB-231. We measured tyrosine kinase activity as a potential proliferation marker. We also estimated the influence of 17β-oestradiolon AngII-induced changes.
Results: In the MDA-MB-231 line, AngII radically slowed the activity of tyrosine kinases and 17β-oestradiol only at a concentration of 10^{-6} M, while it enhanced the effect of angiotensin II at a concentration of 10^{-9} M. In MCF-7, Ang II had a strong inhibitory effect in the presence of oestradiol (10^{-6} M). Oestradiol alone decreased the activity of examined enzymes in both cell lines. AngII receptor type 1 was found in both studied lines, but type 2 only in MDA-MB-231.
Conclusions: Our results show that AngII can modulate tyrosine kinase activity in breast tumour cell lines.
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Key words: *angiotensin, breast cancer, MDA-MB-231, MCF-7, 17β-oestradiol, PTKs*

Streszczenie

Wstęp: Angiotensyna II (AngII), peptyd systemu renina-angiotensyna biorący udział w regulacji równowagi wodno-elektrolitowej oraz ciśnienia krwi, jest także związkiem wpływającym na proliferację i wzrost komórek. Efekt działania tego peptydu uzależniony jest od typu receptora. Angiotensyna II po związaniu z receptorem AT1 stymuluje proliferację i wzrost komórek natomiast poprzez aktywację receptora AT2 — hamuje wzrost komórek oraz pobudza proces apoptozy. Proproliferacyjne działanie angiotensyny II zaobserwowano zarówno w komórkach tkanek zdrowych, jak i w komórkach nowotworowych między innymi komórkach przysadki i nadnerczy, ale także raka piersi czy hiperplazji mięśni naczyń krwionośnych.
Materiał i metody: Celem pracy było zbadanie wpływu AngII na aktywność białkowych kinaz tyrozynowych dwóch linii nowotworowych gruczołu piersiowego: hormono-zależnej linii MCF-7 i hormono-niezależnej MDA-MB-231. Aktywność kinaz tyrozynowych jest przyjętym markerem proliferacji. Oszacowano także wpływ 17β-estradiolu na zmiany w aktywności kinaz tyrozynowych wywołane angiotensyną II.
Wyniki: AngII radykalnie hamowała aktywności kinaz tyrozynowych w linii MDA-MB-231, natomiast 17β-estradiol tylko w stężeniu 10^{-6} M pogłębiał efekt działania angiotensyny II w stężeniu 10^{-9} M. W linii hormono-zależnej MCF-7 Ang II miała silny hamujący wpływ na aktywność badanych enzymów w obecności estradiolu 10^{-6} M. Sam estradiol obniżał aktywność badanych enzymów w obu liniach komórkowych. Obecność receptora angiotensynowego typu 1 została potwierdzona w obydwu badanych liniach raka piersi, natomiast ekspresję receptora typu 2 odnotowano jedynie w komórkach MDA-MB-231.
Wnioski: Otrzymane wyniki wskazują, że AngII może modulować aktywność kinaz tyrozynowych w badanych komórkach raka gruczołu sutkowego. **(Endokrynol Pol 2011; 62 (2): 151–157)**

Słowa kluczowe: *angiotensyna, rak piersi, MDA-MB-231, MCF-7, 17β-estradiol, PTKs*

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Introduction

In classical terms, the central renin-angiotensin system (RAS) works as a co-ordinated cascade, which mainly controls the cardiovascular, renal and adrenal functions. Angiotensin II (AngII) is its principal effector hormone. AngII can either induce cell growth and proliferation by binding to the angiotensin type-1 receptor (AT1R), or can cause an opposite effect: growth inhibition and stimulation of apoptosis via the angiotensin type-2 receptor (AT2R) [1].

It has recently been shown that many structures have local renin-angiotensin systems. All components of RAS, including angiotensinogen, prorenin, ACE and two main angiotensin receptors (AT1R and AT2R) have been found both in normal cells and in different types of tumour cells, including breast tumour cells [2]. These local systems could be involved in the pathogenesis of cancer and might play an important role in controlling cell growth [2, 3]. The proliferogenic action of AngII is not limited to the circulatory system: various studies have demonstrated this peptide can modulate proliferation of adrenocortical, endometrial and anterior pituitary cells as well [4–6]. AngII also appears to be a potentially important growth promoter, since in a variety of cell types it activates phosphatidylinositol turnover, causing a rise in cytosolic Ca^{2+}, a process linked to mitogenesis [7]. In the oestrogen-dependent breast cancer cell line MCF-7, AngII is responsible for induction of cell growth, probably via AT1R receptors. It is presumed that the two main second messengers for AT1R are triphosphoinositol (IP3) and diacylglycerol, both of which activate protein kinase C (PKC) [1]. The proproliferative effects of AngII, after binding to AT1R, could be connected to activation of PKC. Another intracellular mechanism by which angiotensin peptides can control cell growth is modulation of protein tyrosine kinases (PTKs). Tyrosine kinases are coupled to the receptors of several growth factors and are involved in the transduction of growth inducing signals. AngII, acting via classical AT1R receptors, may modulate cell growth [8, 9] and tyrosine kinase activity [10–12] in various normal and cancer tissues, such as vascular muscle cells, a normal and tumoral pituitary gland and a hormone–independent prostate cancer. There is also evidence that AngII-induced biological effects may depend on the concentration and presence of gonadal steroids [13].

The aim of this study was to examine the effect of AngII on tyrosine kinase activity in oestrogen-dependent and oestrogen-independent breast cancer cells and the influence of 17β-oestradiol on AngII-induced changes. Additionally, we checked if classical AngII receptors are present in these cell lines. The modulatory effect of AngII on PTKs activity in two cell lines of breast tumours, hormone-independent MDA-MB-231 and hormone-dependent MCF-7, is reported for the first time in the present study.

Material and methods

The radioactive g-32P — ATP was purchased from Isotopes Center (Hungary).

Cell culture

Breast cancer-derived cell lines, purchased from the American Type Culture Collection (ATCC), were kindly donated by Prof. Andrzej Bednarek from the Department of Molecular Carcinogenesis, Medical University of Lodz, Poland. MCF-7 cells were cultured in DMEM supplemented with 10% foetal bovine serum; MDA-MB-231 cells were maintained in McCoy's medium. The cell lines were cultured under standard conditions (5% CO_2 at 37°C). The media and other cell culture reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Phosphorylation assay

300,000 cells per sample were sonicated in medium containing 0.32 M sacharose, 0.05 mM EDTA, 10 mM TRIS/HCl pH 7.4, 0.05 mM PMSF and aprotinin 25 KIU/ml at 4°C. The samples were preincubated for 15 min at 37°C with angiotensin II alone at several concentrations (10^{-11} M–10^{-9} M), and with 17β-oestradiol at concentrations of 10^{-5} M and 10^{-6} M, added to the mixture separately or together with AngII. Then 0.1% TRITON-X –100 was added to all samples for 15 min (0°C). The reaction medium contained: 20 mM TRIS/HCl pH 7.4; 20 mM $MgCl_2$; 1 mM $MnCl_2$; 1 mM EGTA; 0.5 mM EDTA; 0.1 M DDT, 1 mM ouabain and 1 mM sodium orthovanadate [14]. The phosphorylation was initiated by adding g-[^{32}P] – ATP (200 mM) to the mixture containing the preincubated cells and an artificial specific substrate for tyrosine kinases — polyGlu,Tyr in a ratio of 4:1. The control group did not contain the substrate. Incubation was carried out for 7 min at 30°C and then it was stopped by adding a dissociation buffer with 5 mM ATP and 0.25 mM EDTA. The reaction mixture was spotted onto Whatman 3MM paper and washed three times with cold 10% TCA with 5% sodium pyrophosphate. The radioactivity of ^{32}P incorporated into the substrate was counted as Cerenkov radiation in liquid scintillation analyser. The activity of protein tyrosine kinase was defined as the amount of pmoles of ^{32}P incorporated into polyGlu,Tyr per mg of protein during 1 min. The results were compared to the basal activity of protein tyrosine kinase obtained from samples which did not contain any of the studied compounds. The degree of ^{32}P incorporation by PTKs into the specific substrate in the control group (basal activi-

ty) was assumed to be 100% and averaged to approximately 300 pmoles/mg/min.

Source of RNA

The research was carried out on two human breast cancer cell lines: MCF-7 and MDA-MB-231 (2×10^6 cells per RNA isolation). RNA was extracted using Trizol® reagent (Invitrogen™, Carlsbad, CA, USA) according to the manufacturer's guidelines. Two independent extractions from each type of cell were performed in this study. The concentrations of the resulting RNA samples were determined by spectrophotometric measurements (Beckman DU-650).

cDNA synthesis

Synthesis of cDNA was performed from 10 μg of total RNA in a total volume of 70 μl using ImProm-II™ reverse transcriptase (Promega, Madison, WI, USA). Next, cDNA samples were diluted with sterile deionised water to a total volume of 140 μl. Volumes of 2.5 μl (corresponding to 0.18 μg of total RNA) were used for PCR.

PCR technique

Reactions were performed according to the manufacturer's instructions. The 25 ml volume of reaction mixture contained 2.5 ml of cDNA (non-diluted or 10-fold diluted for AT1R-specific, AT2R-specific and b actin-specific PCR, respectively), primers (0.5 mM each), and 2 mM $MgCl_2$. A typical protocol included 3 min of initial denaturation followed by 45 cycles of 94°C denaturation for 40 min, annealing at 55/60/65°C (depending on the PCR product) for 40 min and extension at 72° for 40 min. The sequences of primers and annealing temperatures are presented in Table I. The sequences of primers and the length of PCR product for AT1R and AT2R were designed using AMPLIFY 3.1.4 For MacOS X program. The primers for β-actin were as described by Robert et al. [15]. All reactions were performed in duplicate. The PCR products were confirmed by molecular weight determination under UV light after electrophoresis in 10% polyacrylamide gel. As a first step, all cDNA preparations were used as templates for PCR with b-actin-specific primers to evaluate whether the constitutively expressed gene could be amplified from the preparations. AT1R-specific RT-PCR experiment was accompanied by an identical reaction prepared with total RNA isolated from DU-145, a hormone-independent prostate cancer cell line, where the presence of AT1R expression has previously been documented [16].

Statistical evaluation

All data is expressed as means ± SD. Statistical significance was assessed by analysis of variance (One-Way ANOVA) for repeated measurements followed by the LSD (Least Significant Difference) post-hoc test to determine the extent of the differences with the use of the StatGraphics Plus 6.0. A significance level of 5% was chosen ($p < 0.05$).

Table I. *Oligonucleotides used for PCR*

Tabela 1. *Sekwencje starterowe zastosowane w łańcuchowej reakcji polimerazy*

Gene primers (5'> 3')	Annealing temperature (°C)	PCR product size (bp)
Angiotensin II receptor type 1 (AT1R)		
AGC CGG CCC TCG GCG GGA CGTG TCA TTT TGA TCA CCT GGG TCG AAT TTG TTG C	65	126/235
Angiotensin II receptor type 2 (AT2R)		
TTC TGC AGC CTG AAT TTT GAA GGA GTG TG	60	147
AGT AGT GGC AAG GGT GGA GTT GCC CTT C		
β-actin		
CGTGACATTAAGGAGA AGCTGTGC	55	374
CTCAGGAGGAGCAATG ATCTTGAT		

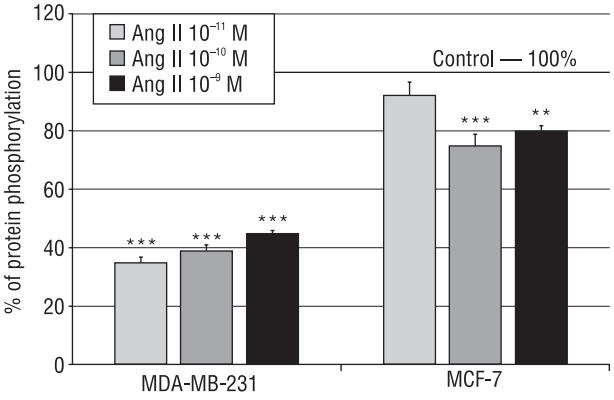

Figure 1. *The effect of angiotensin II on protein tyrosine kinase activity in MDA-MB-231 and MCF-7 cell lines. X ± SD; *p < 0.05 **p < 0.01, ***p < 0.001 vs. C*

Rycina 1. *Wpływ angiotensyny II na aktywność białkowych kinaz tyrozynowych w komórkach linii MDA-MB-231 i MCF-7. X ± SD; *p < 0,05, **p < 0,01, ***p < 0,001 v. C*

Results

The basic activities of PTKs did not differ between the studied cell lines. The concentration-dependent effects of AngII on tyrosine kinase activity in MCF-7 and MDA-MB-231 are presented in Figure 1. As can be seen, the

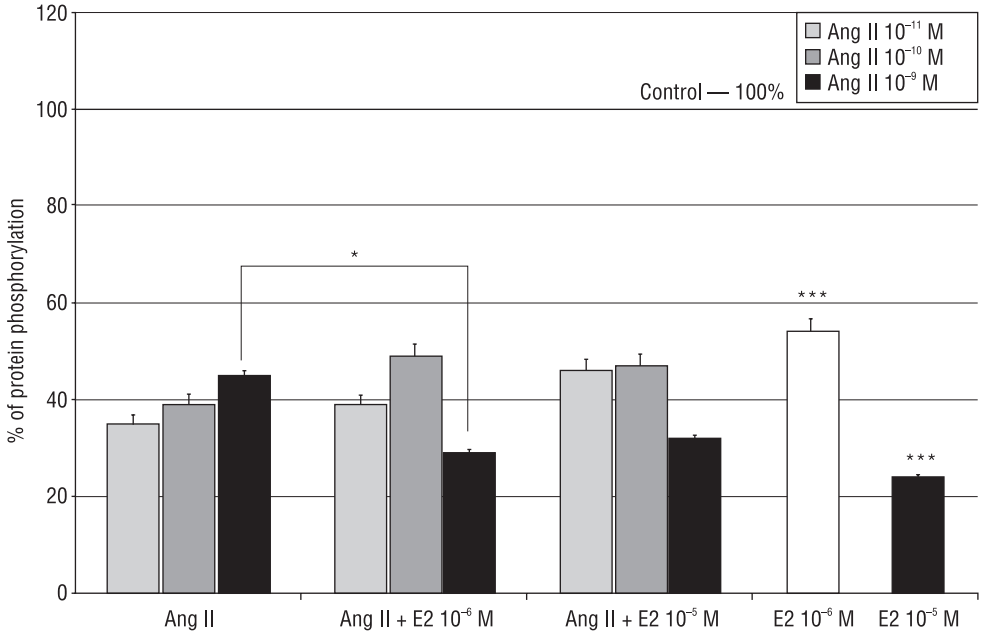

Figure 2. *The effect of angiotensin II on protein tyrosine kinase activity in the hormone-independent cell line MDA-MB-231 in the presence of 17β-oestradiol. X ± SD; *p < 0.05, **p < 0.01, ***p < 0.001*

Rycina 2. *Wpływ angiotensyny II na aktywność białkowych kinaz tyrozynowych w hormono-niezależnej linii komórkowej MDA-MB-231 w obecności 17ß-estradiolu. X ± SD; *p < 0,05, **p < 0,01, ***p < 0,001*

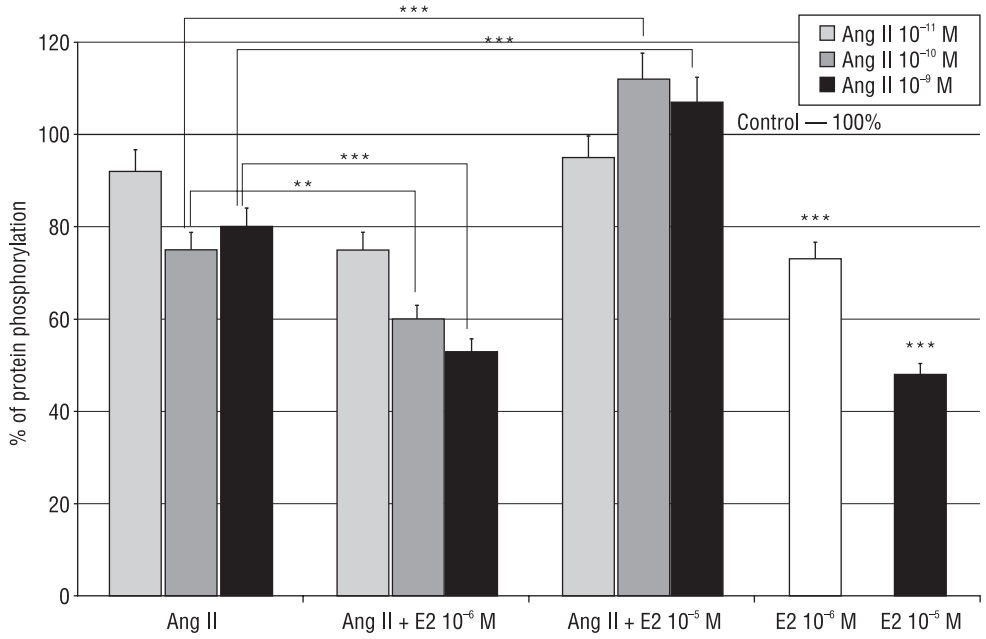

Figure 3. *The effect of angiotensin II on protein tyrosine kinase activity in the hormone-dependent cell line MCF-7 in the presence of 17β-oestradiol. X ± SD; *p < 0.05, **p < 0.01, ***p < 0.001*

Rycina 3. *Wpływ angiotensyny II na aktywność białkowych kinaz tyrozynowych w hormono-zależnej linii komórkowej MCF-7 w obecności 17β-estradiolu. X ± SD; *p < 0,05, **p < 0,01, ***p < 0,001*

activity of the studied enzymes in the oestrogen-independent cell line MDA-MB-231 was strongly reduced by the peptide.

In MDA-MB-231, the presence of 17-ßoestradiol (E_2) only at a concentration of 10^{-6} M changed significantly the AngII (10^{-9} M)-induced effect on PTKs activity (Fig. 2).

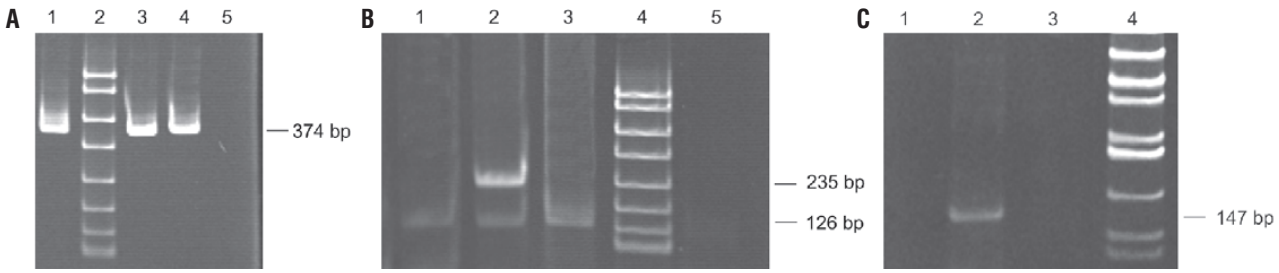

Figure 4. *Electrophoregram of PCR products of angiotensin II receptors, AT1R and AT2R, in both studied cell lines (MDA-MB-231 and MCF-7).* ***A.*** *β-actin-specific PCR products: lane 1, HL60 (positive control; PCR product — 374 bp); lane 2, DNA size markers; lane 3, MDA-MB-231 (PCR product — 374 bp); lane 4, MCF-7 (PCR product — 374 bp); lane 5, H_2O (negative control).* ***B.*** *AT1R-specific PCR products: lane 1, DU145 (positive control; PCR product — 126 bp); lane 2, MCF-7 (PCR products — 126 bp and 235 bp); lane 3, MDA-MB-231 (PCR product — 126 bp); lane 4, DNA size markers; lane 5, H_2O (negative control).* ***C.*** *AT2R-specific PCR products: lane 1, H_2O (negative control); lane 2, MDA-MB-231 (PCR product — 147 bp); lane 3, MCF-7 (lack of PCR product — 147 bp); lane 4, DNA size markers*

Rycina 4. *Elektroforegram przedstawiający produkty PCR dla receptorów angiotensyny II (AT1R i AT2R) w obydwu badanych liniach (MDA-MB-231 i MCF-7).* ***A.*** *Produkty PCR dla β-aktyny: kanał 1, HL60 (kontrola pozytywna; produkt PCR — 374 pz); kanał 2: wzorce; kanał 3: MDA-MB-231 (produkt PCR — 374 pz); kanał 4: MCF-7 (produkt PCR — 374 pz), kanał 5: H_2O (kontrola negatywna).* ***B.*** *Produkty PCR dla AT1R: kanał 1, DU145 (kontrola pozytywna; produkt PCR — 126 pz); kanał 2: MCF-7 (produkt PCR — 126 pz i 235 pz), kanał 3: MDA-MB-231 (produkt PCR — 126 pz); kanał 4: wzorce; kanał 5: H_2O (kontrola negatywna).* ***C.*** *Produkty PCR dla AT2R: kanał 1: H_2O (kontrola negatywna), kanał 2: MDA-MB-231 (produkt PCR — 147 pz), kanał 3: MCF-7 (brak produktu PCR — 147 pz), kanał 5: wzorce*

In MCF-7, the oestrogen-dependent cancer cell line, E_2 at the concentration of 10^{-5} M reversed the effect of AngII (10^{-9} M and 10^{-10} M) (Fig. 3). However, preincubation with E_2 at the concentration of 10^{-6} M enhanced AngII-induced changes in PTKs activity (AngII 10^{-9}, 10^{-10} M) (Fig. 3). Preincubation with 17-βoestradiol alone lowered PTKs activity in both studied cell lines and the inhibition was much stronger at the concentration of 10^{-5} M than at 10^{-6} M (Figs. 2 and 3).

Figure 4 illustrates the presence of mRNA for classical AngII receptors, AT1R and AT2R, in both studied cell lines. In order to examine both cell lines for the expression of the type 1 and 2 angiotensin II receptors, we performed receptor-specific RT-PCR using total RNA isolated from MCF-7 and MDA-MB-231 cells obtained from β-actin-specific PCR product of 374 bp as shown in Fig. 4A. AT1R-specific PCR ga-ve positive results for MCF-7-, MDA-MB-231- and DU145-derived cDNA. As shown in Fig. 4B, AT1R--specific PCR products of 126 bp were observed in both types of cDNA preparations, and an additional product of 235 bp was detected only in MCF-7-derived cDNA.

AT2R-specific primers were applied in the search for the expression of angiotensin II receptor type 2 in both cell lines. Independently of applied PCR conditions, AT2R-specific PCR product of the expected length of 147 bp was observed only for MDA-MB-231 cDNA, whereas MCF-7-derived cDNA did not exhibit AT2R expression at a level sufficient for PCR detection (Fig. 4C).

Discussion

Breast cancer is the most commonly diagnosed cancer among women in Western countries. The renin-angiotensin system plays an important role in water and electrolyte homeostasis and its main effector (AngII) has been recently attributed with angiogenic and growth factor actions in a breast tissue. AngII originated from its precursor by angiotensin I-converting enzyme (ACE) has been recently shown to mediate growth of breast cancer cell lines via ligand-induced activation of the angiotensin II Type I receptor [17]. Another effect of AngII in breast cancer is the reduction of apoptosis. In the MCF-7 cell line, AngII suppressed adriamycin-induced apoptosis via AT1 receptor and protein kinase cascade [18]. Activation of tyrosine kinases (PTKs) and phospholipase C (PKC) is a part of the second messenger dependent response after AngII has been bound to AT1 receptor.

Our data shows for the first time that AngII inhibits PTKs activity in breast cancer cell lines. It is worth underlining that such an effect was clearly evident in the oestrogen-independent cell line MDA-MB-231 (all results were highly significant: $p < 0.001$). On the other hand, in the MCF-7 cell line, AngII alone did not change significantly the basal PTKs activity (AngII at concentrations of 10^{-11}M) or had a weak inhibitory effect (AngII at concentrations of 10^{-9} and10^{-10}M). Generally, the results were surprising, because AngII is known to stimulate cell proliferation (including breast cancer cells), thus we in fact expected an increase in PTKs activity.

The technique used-provides global information on the level of activation of a large pool of non-receptor tyrosine kinases, e.g. Src, Jak, Fak, Pyk2, and Abl. All of them are involved in such processes in cells as: proliferation, growth, differentiation, and apoptosis. It is possible that some of these kinases can be inhibited by AngII but another can be activated. In most cases, PTKs activity may represent a potentially useful proliferation marker. Our earlier study showed that phosphorylation assay can give results analogous to those derived from MTT assay. In both methods, we observed similar effects of angiotensin on a hormone-dependent prostate cancer cell line (LNCaP) [12].

It is worth noting that the proproliferative action of peptides from the family of angiotensins is a result of the activation exclusively of the receptor AT1R. The activation of the receptor AT2R leads rather to the stimulation of apoptosis. Additionally, AT1R and AT2R are capable of forming heterodimers, where through AT2R abolishes functions of the receptor AT1R and is considered to be a his antagonist [19].

This could be the reason why in the cellular line MDA-MB-231, in which we detected expressions of both types of receptor, the activity of tyrosine kinases was significantly decreased. In the line MCF-7, in which we did not observe the expression of the receptor AT2A, the effect of AngII was significantly weaker but so also was the inhibition of the activity of examined enzymes. Moreover, we detected two AT1R-specific PCR products in MCF-7. This suggests that the expression of two AT1R-specific mRNA variants might be a characteristic feature of that cell line and the additional variant could be responsible for this inhibitory effect of AngII. It might be connected to the invasiveness of the cancer. In advanced breast cancer cells, where regulatory pathways initiated by AngII are not needed, expression of AT1R on the cell membrane disappears. However De Paepe et al. suggested that specific overexpression of AT1R receptor on the cell membrane can occur in cells of hyperplastic lesions with and without atypia [20].

The most active oestrogen, 17β-oestradiol, is involved in tumour cell proliferation and breast cancer development. It has been reported that enzymes which synthesise oestradiol by both aromatase and sulfatase pathways, (especially 17β-hydroxysteroid dehydrogenases) are very important in the development of breast cancer, including MCF-7 and MDA-MB-231 cell lines [21].

The effect of oestradiol on tyrosine kinase activity in the presence of AngII has not been studied. Nevertheless, it has been reported that this steroid hormone can affect the activities of other physiological compounds involved in breast cancer growth. For example, it has been found that 17β-oestradiol can modify the expression of adiponectin receptor and adiponectin action on cell growth in MCF-7 and MDA-MB-231 cells [22]. Moreover, oestradiol can modulate the biological properties of local RAS, too. For example, Pawlikowski et al. demonstrated significant differences between the AngII effects on the adrenocortical cell proliferation in ovariectomised and intact rats [23]. Previous publications also indicated that sensitivity of tissues to angiotensins is modulated by the steroid hormone [13, 24].

In both studied cell lines, E_2 alone strongly reduced PTKs activity. The term — 'oestrogen-dependent line' usually means that cells show the expression of the classical receptor for the hormone and effects are the result of the genomic way of oestrogen action. But in hormone-sensitive cells, receptors for steroid hormones are present not only in the nucleus or cytosol but also in the cell membrane. Steroid hormone action using membrane receptors is a fast way of signal transmission and is called non-genomic [25]. Oestradiol may bind not only to its own specific membrane receptor, but also to various G-protein-coupled receptors similar to AT1R and AT2R [26]. For instance, in oestrogen-receptor negative breast cancer cells, oestradiol can modify cell functions via AT1R [27].

We can assume that the oestrogen-induced effects on PTKs activity observed by us were in fact non-genomic rather than genomic, bearing in mind the short time (15 min) of incubation with oestradiol and the fact that steroid can modify AngII — receptor interaction. In our work preincubation of hormone-independent MDA-MB-231 cell line with oestradiol (10^{-6} M) in fact did not change the inhibitory effect of AngII on PTKs activity (AngII at concentrations of 10^{-10} and10^{-11}M) and slightly enhanced the inhibitory effect only of AngII at a concentration of 10^{-9} M.

Although E_2 alone (at a concentration of 10^{-5} M) strongly reduced PTKs activity in hormone-dependent MCF-7 cell line, such high concentration of steroid reversed the inhibitory effect of AngII. A ten times lower concentration of E_2 in the presence of AngII decreased PTKs activity much more strongly than AngII alone. The simplest explanation for this discrepancy is that the observed effects probably depend on the type of AngII receptors present in the studied cancer cell lines.

In MCF-7, we detected two AT1R-specific PCR products, but none corresponding to AT2R. Specific AT1R and AT2R products were detected in MDA-MB-231. The absence of AT2R might be responsible for the slight inhibitory effect of AngII alone in MCF-7 cells. In MDA--MB-231cell line, in which we detected AT2R, binding of AngII to that receptor may result in a decrease in PTKs activity and may inhibit proliferation.

On the other hand, the mechanism of inhibition of PTKs activity by AngII may be more complicated. The

RAS system, including the density of AngII receptors, is sex-hormone sensitive. Thus, we cannot exclude the influence of steroid hormones or steroid hormone receptors on AngII action in the cells. Our earlier study revealed that AngII decreased in PTKs activity in the hormone-independent prostate cancer cell line DU-145 (similarly to the effect reported for MDA-MB-231 in the present work), although we did not detect the presence of AT2R in DU-145 [16]. In a hormone-dependent prostate cancer line (LNCaP) AngII did not change PTKs activity, similar to AngII action in MCF-7 [12].

We cannot also exclude the possibility of downregulation of AT1R mRNA expression in hormone-dependent cell lines, but additional studies are needed to investigate the influence of steroid hormones on the expression of AT1 and AT2 receptor in hormone-dependent and hormone-independent cell lines.

It is known that long exposure to oestrogens downregulates AT1R receptor expression [28] and that oestradiol influences ACE1, ACE2, AT1R and AT2R in kidneys, lungs and hearts and that this effect is tissue-dependent [29, 30]. The upregulation of specific AT1R and AT2R expression might well be an important step in the pathogenesis of hyperplasia and breast cancer. The drugs used in the treatment of hypertension (such as AngII receptor blockers and inhibitors of enzymes involved in angiotensin metabolism) can reduce tumour size but they are not sufficiently effective. New and more effective compounds are still needed which could be used in breast tumour therapy and act via RAS. One of them could be a shorter fragment of anti-AT1R antibody [31].

Conclusions

The enzymes involved in intracellular signalling associated with angiotensin receptor activation, including tyrosine kinases, could be the next target for anti-cancer drugs.

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