

ORIGINAL PAPERS

Adv Clin Exp Med 2005, 14, 3, 451–458
ISSN 1230-025X

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Modulatory Effect of Redox Agents and Antihypertensive Drugs: Captopril and Enalapril on Angiotensin II Induced Signal Transduction in Human Endothelium

Modulatorowy wpływ czynników redoks oraz leków przeciwnadciśnieniowych: kaptoprylu i enalaprylu na sygnalizację wewnątrzkomórkową indukowaną przez angiotensynę II w ludzkim śródbłonku

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Abstract

Background. Angiotensin II, acting through its AT₁ receptor, has been shown to trigger signaling pathway mediated by superoxide anion and hydrogen peroxide that induce mitogen activated protein kinases.

Objectives. The aim of this study was to investigate the effect of hydrogen peroxide, diamide, captopril, enalapril and calcimycin (calcium ionophore: A 23187) on angiotensin II induced extracellular signal-regulated kinase 2 (ERK2) activation as well as on protein tyrosine kinases and phosphatases activity.

Material and Methods. The experiments were conducted on human vascular endothelial cell line (HVEC) that was divided into samples incubated with the studied agents at specific time intervals, and after the lysis they were analyzed using Western Blot (with antibodies against phosphotyrosine and phospho-ERK1/2) and enzymatic assays (detecting tyrosine phosphatases and kinases activities).

Results. Angiotensin II, acting on human vascular endothelial cells through AT₁ receptor, was shown to induce phosphorylation of ERK2 that was abolished by calcimycin and captopril, but not enalapril. In contrast to enalapril, captopril was found to increase protein tyrosine phosphatases activity. Oxidants such as hydrogen peroxide and diamide showed inhibitory effect on protein tyrosine phosphatases activity.

Conclusions. Analysis of the obtained results allows for the notion that captopril (an antihypertensive drug being an ACE inhibitor) may possibly interfere with angiotensin II signaling pathway based on protein tyrosine phosphorylation in human vascular endothelial cells. Since calcimycin is a calcium ionophore, its abolishing of ERK2 activation suggests that ERK2 phosphorylation induced by angiotensin II is calcium dependent. Oxidants such as hydrogen peroxide may show modulatory effect on angiotensin II signaling (*Adv Clin Exp Med* 2005, 14, 3, 451–458).

Key words: A 23187, captopril, enalapril, ERK2, hydrogen peroxide.

Streszczenie

Wprowadzenie. Angiotensyna II, działając przez aktywację receptora AT₁, uruchamia ścieżkę sygnalizacji wewnątrzkomórkowej, w której uczestniczą anion ponadtlenkowy i nadtlenek wodoru mające zdolność do indukcji kinaz MAP.

Cel pracy. Zbadanie wpływu nadtlenu wodoru, diamidu, kaptoprylu, enalaprylu i jonoforu wapnia (A 23187) na proces aktywacji kinazy ERK2 za pośrednictwem angiotensyny II, jak również na aktywność białkowych kinaz i fosfatyz tyrozynowych.

Materiał i metody. W eksperymentach zastosowano ludzką linię komórek śródbłonka naczyniowego (HVEC), którą inkubowano z badanymi związkami w określonych odstępach czasu, a następnie poddawano lizie i analizowano przy użyciu techniki western blotting (stosując przeciwciała przeciwko fosfotyrozynie i ufosforylowanej postaci białka ERK1/2) oraz badano aktywność fosfatyz i kinaz tyrozynowych (z wykorzystaniem biotynylowanych peptydów).

Wyniki. Zaobserwowano, że angiotensyna II, działając na ludzkie komórki śródbłonna za pośrednictwem receptora AT_1 , indukuje fosforylację białka ERK2, która była znoszona przez A 23187 i kaptopril, ale nie enalapril. W przeciwieństwie do enalaprilu, kaptopril wpływał aktywnie na białkowe fosfatazy tyrozynowe. Utleniacze, takie jak nadtlenek wodoru i diamid, wpływały hamująco na białkowe fosfatazy tyrozynowe.

Wnioski. Analiza powyższych wyników pozwala przypuszczać, że w ludzkich komórkach śródbłonna kaptopril może modyfikować drogę sygnalizacyjną indukowaną przez angiotensynę II z udziałem kinaz i fosfataz tyrozynowych. Zniesienie aktywacji ERK2 przez A 23187, będący jonoforem wapnia, wydaje się świadczyć, że proces ten jest zależny od wapnia. Utleniacze, takie jak nadtlenek wodoru, mogą modulować proces przekazywania sygnałów przez angiotensynę II (*Adv Clin Exp Med* 2005, 14, 3, 451–458).

Słowa kluczowe: A 23187, kaptopril, enalapril, ERK2, nadtlenek wodoru.

Angiotensin II (Ang II) is a hormone that increases blood pressure through the promotion of vasoconstriction and retention of sodium and water in the kidneys. It exerts its effects through multiple pathways; it stimulates the secretion of aldosterone, vasopressin, catecholamines as well as proliferation of vascular smooth muscles. Almost all of these effects are mediated by abundantly occurring AT_1 receptors whose stimulation in vascular smooth muscle cells leads to phospholipase C activation and finally to calcium mobilization which initiates muscle contraction. Besides this well known signaling pathway, Ang II has been implied to induce other signal transduction routes. One of them is connected with the generation of superoxide and hydrogen peroxide which in turn induce various kinases. Both in vascular smooth muscle cells and endothelial cells Ang II has been shown to activate NAD(P)H oxidase that is involved in superoxide production [1]. Superoxide is then converted to hydrogen peroxide by superoxide dismutase. Subsequently these two oxidants serve to mediate downstream signaling events such as activation of p38MAPK, JnK, ERK as well as gene transcription [2, 3].

Ang II receptors of the second type, AT_2 , are expressed in low levels in limited organs in adults [3]. Although their role is not fully elucidated, from the recent reports it seems that stimulation of AT_2 leads to opposite effects as compared to AT_1 ; they induce vasodilation and inhibit proliferation, probably acting through the promotion of nitric oxide production [3, 4].

Most of the latest experiments investigating Ang II signaling based on protein tyrosine phosphorylation pathways, as well as the involvement of redox agents, have been conducted on vascular smooth muscle cells. Thus, here it was decided to study these processes in human vascular endothelial cell (HVEC) line. The purpose was to establish whether Ang II induces ERK1/2 signaling pathway or this pathway could be mediated by calcium ions, and modified by redox agents: H_2O_2 , diamide and glutathione.

Angiotensin converting enzyme (ACE) inhibitors, captopril and enalapril, are used in treatment of hypertension. Their antihypertensive properties result from inhibiting ACE activity. Moreover, captopril shows protective qualities in some diseases, which probably derive from its antioxidative character [5–7]. Reducing properties of captopril are likely to result from its sulfhydryl group that is absent in enalapril molecule. Therefore, in this study it was resolved to compare the two inhibitors; to investigate their effect on protein tyrosine kinases (PTKs) and phosphatases (PTPs) activities as well as protein tyrosine phosphorylation pattern in Ang II stimulated HVECs.

Material and Methods

Human vascular endothelial cell (HVEC) line was from the Department of Histology at Wrocław Medical University. Endothelial cells were grown in DMEM (Dulbecco) supplemented with 5% FBS (foetal bovine serum) at 37°C under humidified atmosphere of 95% air and 5% CO_2 . Medium was changed every 48 h. Enalapril ([S]-1-[N-(1-[Ethoxycarbonyl]-3-phenylpropyl)-L-alanyl]-L-proline maleate), captopril ([2S]-N-[3-Mercapto-2-methyl-propionyl]-L-proline), an ionophore calcimycin (A 23187), genistein, nitrocellulose membrane were purchased from Sigma Chemical Company (USA), materials for electrophoresis were from Roth (Germany). Tyrosine Kinase Assay Kit 153 4505 and Tyrosine Phosphatase Assay Kit 153 413 were from Boehringer-Mannheim GmbH (Germany). Mouse monoclonal anti-phosphotyrosine antibodies were from Chemicon (USA), rabbit polyclonal anti-mouse IgG antibodies conjugated with horseradish peroxidase were from Amersham (Great Britain), rabbit polyclonal anti-ERK1/2 antibodies raised against synthetic phosphorylated peptide analogous to 196–209 fragment of active form of p44 MAPK (ERK1) (DHTGFLTEY(P)VATRW) were from Genosphere Biotechnologies (France), goat polyclonal anti-rabbit IgG antibodies conjugated with

horseradish peroxidase were from Amersham (Great Britain). All the other chemicals were purchased from Sigma Chemical Company (USA).

Preparation of Cell Lysates, Immunoprecipitation and Western Blotting

Stimulated and non-stimulated human vascular endothelial cells (HVECs) were prepared by washing cells 2× with phosphate-buffered saline (PBS), followed by scraping, sonicating into 150 µl of lysis buffer (5 mM Tris-HCl, 50 mM NaCl, 0.1% SDS and 1% Triton X-100, 1 mM dithiothreitol, 0.1 mg phenylmethylsulphonyl fluoride, 1 µg aprotinin and 2 µg leupeptin, pH 7.6). Lysates were cleared by centrifugation at 7,500 g for 15 minutes, and the protein concentration of the cleared lysates was determined by the BioRad protein assay. Then lysates were subjected to immunoprecipitation with mouse anti-phosphotyrosine polyclonal antibody and A/G-Sepharose. Immunoprecipitated proteins were separated by SDS polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose by electroblotting and probed with anti-phosphotyrosine or anti-ERK1/2 antibodies, and with anti-mouse or anti-goat IgG antibodies conjugated with horseradish peroxidase.

Enzymatic Assay

Activity of phosphotyrosine phosphatases and tyrosine kinases was estimated with specific methods using biotinylated peptides. The procedure was due to the protocol of Phosphotyrosine Phosphatase and Tyrosine Kinase Assay Kit (Boehringer-Mannheim GmbH).

Statistical Analysis

All the measurements were done in triplicate and the data are expressed as the mean. The level of significance was assessed with Student's t-test. Differences were considered significant at $p < 0.05$.

Results

To assess the influence of redox agents on protein tyrosine phosphatases (PTPs) activity and compare it with ACE inhibitors effect, HVEC line was divided into six samples. They were incubated for 5, 10 and 40 minutes in PBS buffer contain-

ing 1 mM of different compounds; the oxidizing agents: diamide and H_2O_2 , the reducing agent: glutathione, and ACE inhibitors: captopril and enalapril. Subsequently, the cells were cleared by centrifugation at 2000 g, lysed, and PTPs activity was measured. PTPs activity assessed in non-stimulated HVEC line was treated as a control. The obtained data shown in Figure 1 indicate that the oxidizing agents: H_2O_2 and diamide were capable of lowering PTPs activity in endothelial cells. On the other hand, the reducing agents: glutathione and captopril apparently increased PTPs activity. The other ACE inhibitor – enalapril did not affect PTPs activity. In comparison with glutathione, captopril caused significantly greater increase of PTPs activity.

To assess the influence of the redox agents and ACE inhibitors, captopril and enalapril on protein tyrosine kinases (PTKs) activity in human endothelial cells, the same experimental scheme was used as previously, but instead of PTPs, PTKs activity was measured. The results shown in Figure 2 indicate that neither ACE inhibitors nor glutathione influenced PTKs activity in human endothelium. The minor effect was observed only in the case of the oxidizing agents: diamide and H_2O_2 , which slightly elevated PTKs activity shortly after stimulation, but after longer incubation with the agents, PTKs activity returned to the control level.

To assess the redox agents modulatory effect on the phosphorylation processes induced by Ang II, HVEC line was divided into 5 samples and incubated for 40 minutes in PBS buffer containing 100 nM Ang II in the presence of losartan (250 nM), genistein (0.05 mg/ml), sodium vanadate (0.01 mM), H_2O_2 (1 mM) or diamide (1 mM). Subsequently, phosphotyrosine proteins were detected by Western blotting using anti-phosphotyrosine antibodies. Losartan – AT_1 receptor antagonist – was used to check whether Ang II acts through this receptor type. As shown in Figure 3, the cells incubated with losartan (lane 1) failed to show any bands of phosphorylated proteins within the range of 40–80 kDa, similarly as the cells with genistein (PTKs inhibitor) (lane 2). Since losartan application abolished phosphorylation of some proteins, it could be assumed that Ang II induced phosphorylation processes in the studied endothelial cells by the stimulation of AT_1 receptors.

As it can be seen in Figure 3, in the cells incubated with Ang II and hydrogen peroxide (lane 4) or diamide (lane 5), an additional band of phosphorylated protein appeared in the range of 60–80 kDa, in comparison to the cells incubated only with Ang II and sodium vanadate (PTPs inhibitor) (lane 3).

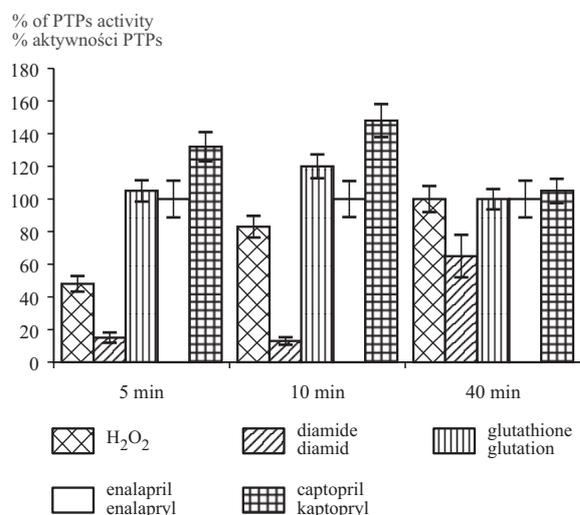


Fig. 1. Effect of the redox agents and ACE inhibitors on PTPs activity in HVECs, after different incubation periods, shown as per cent of the control activity (100% = 0.15 nmol/min/mg protein)

Ryc. 1. Wpływ czynników redoks oraz inhibitorów ACE na aktywność białkowych fosfataz tyrozynowych (PTPs) w ludzkich komórkach śródbłonna po różnym okresie inkubacji, przedstawione jako procent aktywności kontroli (100% = 0.15 nmol/min/mg białka)

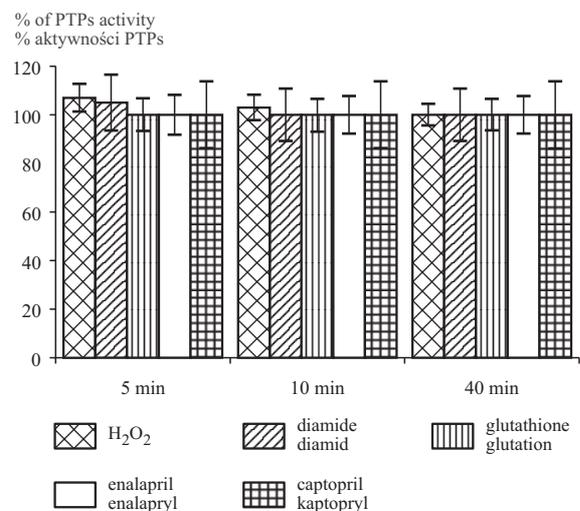


Fig. 2. Effect of the redox agents and ACE inhibitors on PTKs activity in HVECs, after different incubation periods, shown as per cent of the control activity (100% = 0.05 nmol/min/mg protein)

Ryc. 2. Wpływ czynników redoks oraz inhibitorów ACE na aktywność białkowych kinaz tyrozynowych (PTKs) w ludzkich komórkach śródbłonna, po różnym okresie inkubacji, przedstawione jako procent aktywności kontroli (100% = 0.05 nmol/min/mg białka)

To assess whether Ca²⁺ ions are necessary for PTKs activation in HVECs stimulated with Ang II, a Ca²⁺ ionophore (A 23187), calcimycin was used. HVEC line was divided into two samples. The first sample was incubated for 5, 10 and

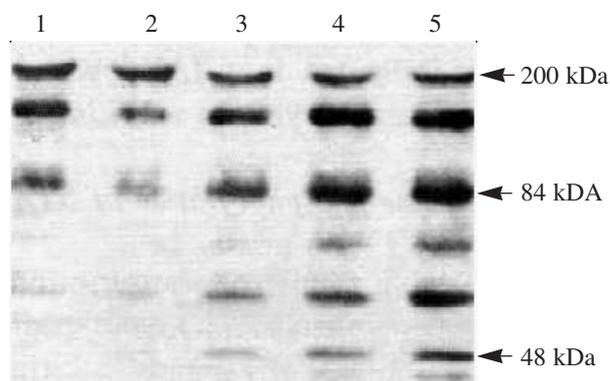


Fig. 3. Effect of the redox agents and ACE inhibitors on protein tyrosine phosphorylation pattern in HVECs incubated with Ang II and: losartan (1), genistein (2), sodium vanadate (3), H₂O₂ (4), diamide (5)

Ryc. 3. Wpływ czynników redoks oraz inhibitorów ACE na profil fosforylacji tyrozyn białkowych w ludzkich komórkach śródbłonna inkubowanych z angiotensyną II oraz: losartanem (1), genisteiną (2), wanadanem sodu (3), H₂O₂ (4), diamidem (5)

40 minutes in PBS buffer containing 100 nM Ca²⁺, 5 mM EGTA and 100 nM Ang II. The second sample was incubated for 5, 10 and 40 minutes in the same solution, containing additionally 10 μM calcimycin. Subsequently, after the lysis of the cells, PTKs activity was measured and compared with the control (PTKs activity in non-stimulated HVECs). Whereas a significant increase in PTKs activity was observed in the cells incubated only with Ang II (with the greatest, two-fold increase after 40-minute incubation), the cells containing additionally calcimycin demonstrated PTKs activity at the control level (Fig. 4). From these results it could be concluded that calcimycin abolished PTKs activity increase stimulated by Ang II. Then it was decided to compare a modulatory effect of calcimycin, ACE inhibitors and H₂O₂ on the process of protein tyrosine phosphorylation in HVECs stimulated with Ang II. Thus HVEC line was divided into seven samples and incubated for 40 minutes in PBS buffer containing 100 nM Ang II in the presence of 1 mM captopril (sample 1), 1 mM enalapril (sample 2), 1 mM H₂O₂ (sample 3), 10 μM calcimycin (sample 4), 10 μM calcimycin + 1 mM captopril (sample 5), 10 μM calcimycin + 1 mM enalapril (sample 6) and 10 μM calcimycin + 1 mM H₂O₂ (sample 7). HVECs incubated for 40 minutes only with 100 nM Ang II were treated as a reference. Subsequently the cells underwent the procedure described in the Material and Methods section and were probed with anti-phosphotyrosine and anti-ERK1/2 antibodies. The obtained phosphorylation patterns are shown in Figures 5 and 6. Since using anti-ERK1/2 antibodies the

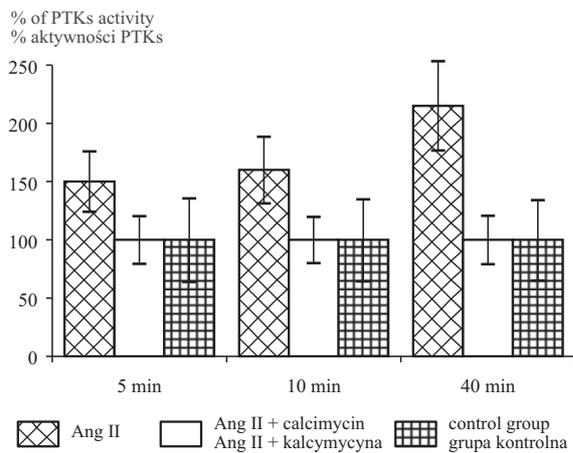


Fig. 4. Effect of calcimycin on PTKs activity in HVECs stimulated with Ang II, after different incubation periods, shown as per cent of the control activity (100% = 0.05 nmol/min/mg protein)

Ryc. 4. Wpływ A 23187 na aktywność białkowych kinaz tyrozynowych w ludzkich komórkach śródbłonna stymulowanych angiotensyną II po różnym okresie inkubacji, przedstawione jako procent aktywności kontroli (100% = 0,05 nmol/min/mg białka)

band of molecular mass of about 42 kDa was detected (depicted in Figures 5 B and 6 B), it can be regarded as ERK2 protein (molecular mass of ERK1 is 44 kDa). In comparison with control (endothelial cells stimulated only with Ang II; lane 4 in Figure 5), addition of captopril prevented phosphorylation of proteins in the range of 40–50 kDa, including ERK2 protein (lane 1 in Figure 5 A and B). In the second lane, where enalapril influence was shown, there was a weak ERK2 band visible (Fig. 5 B). Similar phosphorylation patterns for captopril and enalapril were observed after calcimycin treatment (lanes 3 and 4 in Figure 6). Comparing phosphorylation pattern between the cells incubated with Ang II with and without calcimycin it could be noticed that the ionophore addition caused the disappearance of phosphorylated ERK2 protein (lanes 1 and 2 in Figure 6 B).

Discussion

It is known that Ang II is engaged in tyrosine phosphorylation and dephosphorylation processes, triggering mitogen activated protein kinases (MAPKs) pathway. Most experiments trying to elucidate the mechanism of signal transduction of this type have been conducted on vascular smooth muscle cells (VSMCs). It has been shown that in VSMCs Ang II, through its AT₁ receptor is able to induce extracellular signal-regulated kinase (ERK1/2), c-Jun amino terminal kinase (JNK) and

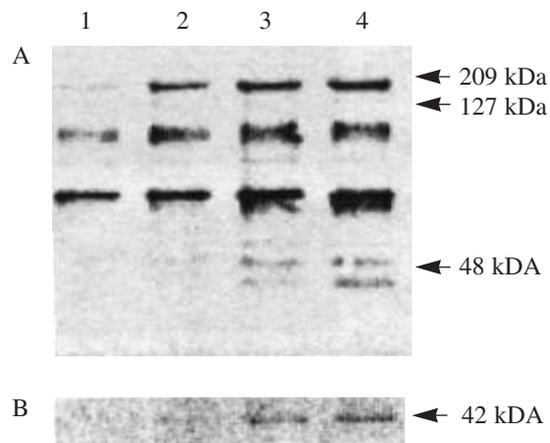


Fig. 5. Effect of ACE inhibitors and H₂O₂ on protein tyrosine phosphorylation pattern (A) and ERK2 phosphorylation (B) in HVECs incubated with Ang II + captopril (1), Ang II + enalapril (2), Ang II + H₂O₂ (3), and Ang II (4)

Ryc. 5. Wpływ inhibitorów ACE i H₂O₂ na profil fosforylacji tyrozyn białek (A) i fosforylacji ERK2 (B) w ludzkich komórkach śródbłonna inkubowanych z angiotensyną II i kaptoprylem (1), angiotensyną II i enalaprylem (2), angiotensyną II i H₂O₂ (3) oraz angiotensyną II (4)

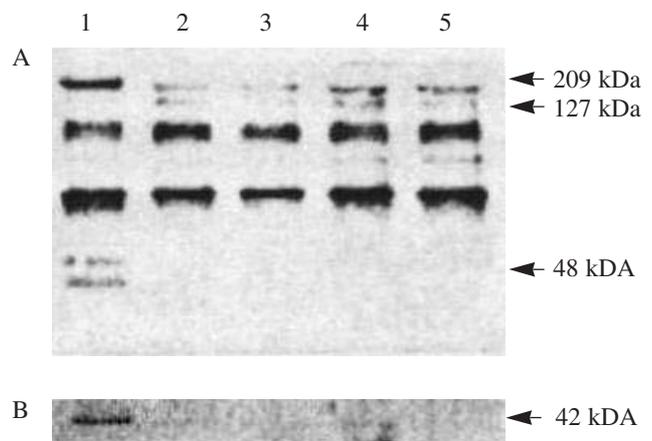


Fig. 6. Effect of calcimycin, ACE inhibitors and H₂O₂ on protein tyrosine phosphorylation pattern (A) and ERK2 phosphorylation (B) in HVECs incubated with Ang II (1), Ang II + calcimycin (2), Ang II + calcimycin + captopril (3), Ang II + calcimycin + enalapril (4), and Ang II + calcimycin + H₂O₂ (5)

Ryc. 6. Wpływ A 23187, inhibitorów ACE i H₂O₂ na profil fosforylacji tyrozyn białek (A) i fosforylacji ERK2 (B) w ludzkich komórkach śródbłonna inkubowanych z angiotensyną II (1), angiotensyną II i A 23187 (2), angiotensyną II, A 23187 i kaptoprylem (3), angiotensyną II, A 23187 i enalaprylem (4) oraz angiotensyną II, A 23187 i H₂O₂ (5)

p38 MAP kinase [8, 9]. In this study Ang II effect on ERK1/2 stimulation in human vascular endothelial cells (HVECs) was investigated. It was shown that in HVECs Ang II induced ERK2 phos-

phorylation after 40-minute incubation. Fischer et al. [10] and Xie et al. [11] have found out that Ang II induced ERK1/2 phosphorylation in cardiac microvascular endothelial cells, however Fischer et al. [10] observed ERK2 phosphorylation after 5 minutes and its quenching within 20 minutes. Shorter phosphorylation period could be a result of different type of cell line (Fischer et al. [10] conducted experiments on rat, not human cells). In VSMCs Ang II has been shown to activate ERK1/2 from about 2 to 30 minutes [8, 12, 13]. Whereas Hamaguchi et al. [12] and Touyz et al. [13] observed maximal phosphorylation after 5 minutes of Ang II stimulation, Viedt et al. [8] obtained phosphorylation peak after 15 minutes. Thus, it seems to depend on experimental conditions.

Investigations conducted on VSMCs indicate that Ang II-induced ERK1/2 activation is dependent on calcium signaling, since it was blocked by inhibition of PLC, calmodulin or intracellular calcium mobilization [14]. Haendeler et al. [15] have demonstrated that binding of Ang II to AT₁ activates ERK1/2 via two pathways; the major pathway stimulates Ras independently of cytoplasmic Ca²⁺, whereas the minor pathway activates Rap1 and is dependent on cytoplasmic Ca²⁺.

Therefore, Ca²⁺ ionophore, calcimycin was used to establish the potential role of these ions in Ang II stimulated HVECs. The experimental conditions were adjusted so that the introduction of calcimycin would abolish the potential Ca²⁺ augmentation upon Ang II induction. Calcimycin builds in both plasma and intracellular membranes mediating the passive transport of Ca²⁺ across them down existing Ca²⁺ gradients. Since the incubation buffer was supplemented with 100 nM Ca²⁺ (comparable to the cytosolic Ca²⁺ concentration) and 5 mM EGTA, it was assumed that addition of calcimycin would result in Ca²⁺ depletion from intracellular sequestration sites followed by its efflux from the cell.

In this work calcimycin abolished induced by Ang II increase in protein tyrosine kinases (PTKs) activity, as well as prevented tyrosine phosphorylation of proteins in the range of 40–50 kDa, including ERK2. Thus, it could be concluded that in HVECs Ang II triggers the pathway in which Ca²⁺ dependent ERK2 phosphorylation may take place.

There is much evidence that redox agents could modulate Ang II induced ERK1/2 signaling pathway [2, 16]. On one hand, it is an excessive formation of reactive oxygen species (ROSS) in pathology that can disturb signaling pathways, leading to hypertension and atherosclerosis [17]. On the other hand, superoxide and hydrogen peroxide (H₂O₂) are thought to be important signaling

molecules in Ang II induced pathways in vascular cells [1, 2]. Therefore, it was decided to investigate the effect of H₂O₂ on phosphorylation and dephosphorylation processes activated by Ang II in HVECs. H₂O₂ was shown to decrease PTPs activity by 50% after 5-minute incubation, whereas the longer incubation resulted in abolishing of this effect (after 40 minutes PTPs activity regained its control level). Inhibitory effect of H₂O₂ on PTPs has also been shown by Koshio et al. [18] and Denu et al. [19]. Regaining of 100% of PTPs activity after longer incubation with H₂O₂ could suggest reversible type of inhibition. Reversible inactivation of PTPs by H₂O₂ has been proposed by Denu et al. [19] who found out that the catalytic cysteine thiolate of PTPs was the selective oxidation target yielding sulfenic acid intermediate (SOH). In the presence of -SH group donors (such as glutathione) this intermediate is completely reducible.

Stimulation of HVECs with Ang II and H₂O₂ resulted in much stronger phosphorylation pattern than stimulation with Ang II and sodium vanadate. Since sodium vanadate is PTPs inhibitor, it can be concluded that H₂O₂ is able to shift phospho-/dephosphorylation balance towards phosphorylation not only by inhibiting PTPs activity but also by enhancing phosphorylation processes. As the direct effect of H₂O₂ on PTKs activity was minor (only 7% of activity increase after 5-minute incubation) it might be suggested that an indirect mechanism is involved here. Stimulation of PTKs activity and inhibition of PTPs activity by H₂O₂ in endothelial cells have also been observed by Natarajan et al. [20]. These observations are in agreement with the results obtained by Hsu et al. [16] who have found out that ERK phosphorylation induced by AngII was inhibited by antioxidants.

Clinical observations suggest that captopril shows protective effects in some diseases, such as myocardial ischemia – reperfusion injury. These effects have not been noticed in the case of enalapril. Andreoli [5] has shown that captopril, in contrast to enalapril, protects endothelial cells from free radicals. Therefore, it was resolved to compare the influence of the two ACE inhibitors on endothelial cells stimulated with Ang II, and verify the hypothesis that reducing properties of captopril might account for the possible differences. The obtained results showed that in contrast to enalapril, captopril enhanced PTPs activity by nearly 50% after 10-minute incubation. However, comparison with glutathione effect on PTPs activity, that was much weaker (20% activation after 10 minutes), suggests that this is not only the -SH group that is involved in the mechanism increasing PTPs activity. Since both glutathione and captopril

contain the sulfhydryl group, and in the experiment equimolar concentration of them was used, some other properties of captopril must have been partially responsible for the observed effect. Addition of captopril to Ang II stimulated HVECs prevented phosphorylation of the proteins in the range of 40–50 kDa, including ERK2 protein, what could result from this inhibitor's effect on

PTPs activity increase (since it did not show any effect on PTKs activity). Thus, it can be suggested that captopril interferes with Ang II signaling pathway based on ERK2 activation in HVECs. The probable mechanism might be through inducing excessive PTPs activation, what could be partly ascribed to the sulfhydryl group's reducing properties.

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Received: 16.07.2004

Revised: 10.09.2004

Accepted: 5.10.2004

Praca wpłynęła do Redakcji: 16.07.2004 r.

Po recenzji: 10.09.2004 r.

Zaakceptowano do druku: 5.10.2004 r.