JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2005, 56, 4, 571–585
www.jpp.krakow.pl

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# ANGIOTENSIN II VIA $AT_1$ RECEPTOR ACCELERATES ARTERIAL THROMBOSIS IN RENOVASCULAR HYPERTENSIVE RATS

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Although there are some *in vitro* evidence that angiotensin II (Ang II) may promote thrombosis, there is still no data concerning effect of Ang II on arterial thrombus formation. In the present study we have investigated the influence of Ang II on electrically induced arterial thrombosis in a common carotid artery of renovascular hypertensive rats. Furthermore, we examined if Ang II effect is mediated via $AT_1$ receptor. We measured some coagulation and fibrinolytic parameters at the same time. Since platelets play crucial role in the initiation of arterial thrombosis their contribution in the mode of Ang II action was also determined. Intravenous infusion of Ang II caused significant increase in arterial thrombus weight, which was reversed by losartan, selective $AT_1$ receptor antagonist. The prothrombotic effect of Ang II was accompanied by increase in haemostatic and decrease in fibrinolytic potential of rat plasma. While number of data has clearly demonstrated that Ang II can augment human platelets aggregation, at least in rats, platelets were not involved in the mechanism of Ang II action. Our study shows that Ang II via $AT_1$ receptor accelerates arterial thrombosis in renovascular hypertensive rat, therefore may be considered as a risk factor of myocardial infarction or stroke.

Key words: *angiotensin II, hypertensive rat, arterial thrombosis*

## INTRODUCTION

There are mounting evidences for a link between the activation of renin - angiotensin system (RAS) and cardiovascular-related complications, such as unstable angina, myocardial infarction (1) and stroke (2). The main pathological process responsible for those urgent clinical events is acute intravascular arterial

thrombosis. Numerous clinical trials, like CONSENSUS (3), SAVE (4), SOLVED (5), HOPE (6), ELITE (7) and ELITE II (8) support the hypothesis that drugs interfering with RAS, such as angiotensin-converting enzyme inhibitors (ACE-Is) and $AT_1$ receptor antagonists ($AT_1$-As) reduce the primary and secondary risk of arterial thrombosis. It has become obvious that the clinical benefit of those drugs is far greater than can be explained by mere reduction of blood pressure alone. Although we have previously demonstrated that ACE-Is (captopril) and $AT_1$-As (losartan) exert strong antithrombotic effect, in nitric oxide (NO) and prostacyclin (PGI2) - dependent mechanism as well as by antagonising TXA2/PGH2 receptors (9-13), the final answer to the question what is a key to the beneficial activity of the ACE-Is and $AT_1$-As is still evolving.

The best known and simultaneously the most active peptide of RAS is Ang II. The role of this octapeptide in the regulation of arterial blood pressure, electrolyte balance and plasma volume homeostasis is well established. Recent studies, however, have shown that Ang II is endowed also with nonhemodynamic effects such as prothrombotic activity. There are a few *in vitro* and *ex vivo* studies indicating that Ang II influences fibrinolysis (14-17), coagulation (14, 18, 19) and platelets activation (19-23), which can promote thrombosis. Moreover, we have recently published results indicating that Ang II may promote venous thrombosis (24). In present study we made attempt to examine if Ang II is also involved in the arterial thrombosis development, thus may be consider as a risk factor of cardiovascular events such as myocardial infarction and stroke. Therefore our aim was to determine the influence of Ang II on arterial thrombosis *in vivo* and to estimate the mechanism of its action.

## MATERIALS AND METHODS

### *Animals*

Male Wistar rats (350-450g) were used in the study. The animals were housed in a room with a 12-h light/dark cycle, in group cages as appropriate, were given tap water and fed a standard rat chow. 24 hours before the experiments the animals were deprived of food but had free access to water.

Procedures involving the animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research (25).

### *Chemicals and drugs*

Ang II (Sigma Chemical Co., USA), losartan (Dup 753, DuPont Merck Pharmaceutical Co., USA), collagen type I (Collagen, Chronolog, USA), Tris buffer [Tris(hydroxymethyl)-aminomethane hydrochloride (Sigma Chemical Co., USA) and Tris(hydroxymethyl)-aminomethane (Merck, Germany)], thrombin (Trombina 400, Biomed, Poland), t-PA (Actylise, Boehringer Ingelheim, Germany), pentobarbital (Vetbutal, Biovet, Poland), 3.13% trisodium citrate, natrium chloride (Gliwice, Poland) were used in the study.

### *Induction of renovascular hypertension (Two-kidney one-clip renovascular hypertension)*

Rats, weighing 160-180 g, were anesthetized with pentobarbital (40mg/kg, i.p.). Two-kidney one-clip (2K-1C) renovascular hypertension was induced by a partial, standardized clipping of the left renal artery (26). After 6 weeks the systolic blood pressure (SBP) was measured using the "tail cuff" method (Student Oscillograph, Harvard Rat Tail Blood Pressure Monitor, UK) in conscious rats (27). Each value was the average of three consecutive readings. Only hypertensive rats (SBP higher than 140 mmHg) were used in the experiments. Sham operated rats (SO) served as a control to 2K-1C hypertensive rats. They underwent the same surgical intervention apart from clipping of the renal artery.

### *Angiotensin and losartan administration*

Ang II in doses of 100, 200 and 400 pmol/kg/min or 0,9% NaCl (VEH) were administered by i.v. continuous infusion 5 min before the induction of arterial thrombosis and were continued for 110 minutes, to the end of the experiment. To investigate the involvement of $AT_1$ receptors in the prothrombotic action of Ang II, losartan (Los), selective $AT_1$ receptor antagonist, was administered into left femoral vein in dose of 30 mg/kg 5 minutes before Ang II (400 pmol/kg/min) or VEH infusion (*Fig. 1*).

### *Induction of arterial thrombosis*

Male renovascular hypertensive Wistar rats were anesthetized by intraperitoneal injection of pentobarbital (40 mg/kg) and then fixed in the supine position on a operation table. The right femoral vein was cannulated to administer drug. We induced arterial thrombosis by electrical stimulation of the common carotid artery according to the method described by Schumacher et al. (28). A segment of the left common carotid artery, about 15 mm long, was exposed and

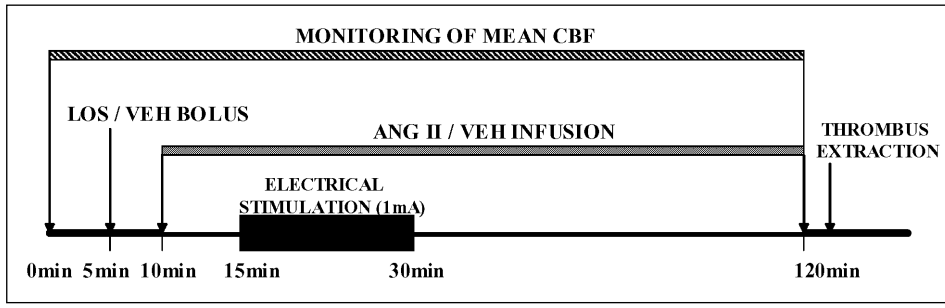


*Figure 1:* The experimental protocol. Angiotensin II (ANG II) in doses of 100, 200 and 400 pmol/kg/min or 0,9% NaCl (VEH) in a volume of 2 ml/kg/h were administered by i.v. continuous infusion 5 min before the electrical induction of arterial thrombosis and were continued for 110 minutes, to the end of the experiment. Bolus injection of losartan (LOS) in dose of 30 mg/kg or VEH was administered into left femoral vein 5 minutes before ANG II (400 pmol/kg/min) or VEH infusion. The arterial thrombosis was induced by electrical stimulation (1mA) for 15 minutes. 90 min after electrical stimulation the formed arterial thrombus was completely removed, air-dried in 37°C and weighed 24 hours after the end of experiment. The mean carotid blood flow (CBF) was continuously monitored from the beginning to the end of experiment (120 minutes).

cautiously dissected free of surrounding tissue. A pace of parafilm (5mm x 20mm) was placed under the exposed vessel to provide electrical isolation. Two electrodes were used. The anode, the stainless steel L-shaped wire, was inserted under the artery and connected to a circuit with a constant current generator. The cathode was a subcutaneous metal needle attached to the hindlimb. The stimulation (1mA) took 15 min. A Doppler flow probe was placed in contact with the carotid artery downstream from the site of electrical stimulation and connected to the flowmeter (Hugo Sachs Elektronik, Germany). The degree of thrombotic occlusion was expressed by the decrease in carotid blood flow (CBF) which was continuously monitored before, during and after electrical stimulation. Final CBF was the CBF measured at the end of experiment in 120 min. The time (min) from the end of the electrical stimulation to the point at which the CBF decreased to zero was defined as the occlusion time. 90 min after stimulation the segment of the common carotid artery with the formed thrombus was dissected opened lengthwise and the thrombus was completely removed, air-dried in 37°C and weighed 24 hours after the end of experiment.

After removal of formed thrombus blood samples were taken from the heart and drawn into 3.13% trisodium citrate in a volume ratio of 9:1. Platelet aggregation was assessed in whole blood directly after experiment. The rest blood samples were centrifuged at 2000 x g in 4°C for 20 min and plasma was deep-frozen (- 70°C) in aliquots of 1 ml until further assays could be preformed.

## *Platelet aggregation*

Platelet aggregation was studied in *ex vivo* and *in vitro* conditions. Whole blood platelet aggregation was monitored by measuring electrical impedance in Chronolog aggregometer (Chrono-log Corp., USA) according to the method described by Cardinal et al. (29). Blood samples were drawn into 3.13% trisodium citrate in a volume ratio 9:1. Collagen (5 μg/ml) was added after 15 minutes of incubation at 37°C with 0.9% NaCl. Then changes in resistance were registered for 6 minutes. The maximal extension of the aggregation curve at $6^{th}$ minute was expressed in Ohms (Ω). In addition in *in vitro* study blood samples were mixed with Ang II ($10^{-7}$ M) or VEH solutions before incubation.

## *Overall plasma potentials*

We used previously described method (30-31), modified and adapted to use on laboratory animals (32). Briefly, two fibrin curves, changing during clot formation, were made by the registration of optical density at $\lambda$ = 405 nm using microplate reader (Dynex Tech., USA). To determine Overall Haemostatic Potential (OHP), $CaCl_2$, thrombin and t-PA were added to the Tris buffer (66 mM Tris and 130 mM NaCl, pH 7,4) and mixed with plasma sample. To determine Overall Coagulation Potential (OCP) fibrin time curve was created without adding t-PA. Values of optical density were recorded in 1 minute intervals for 30 minutes. Based on the principal of integrals, the area under the curve, illustrating OHP and OCP, was expressed by summation of the optical density values. OCP reflects mainly coagulant plasma activity, while OHP determines haemostatic plasma activity. The difference between these two curves equals Overall Fibrinolysis Potential (OFP) in plasma calculated as: [(OCP-OHP)/OCP] x 100%.

## *Statistical analysis*

The data are shown as mean ± SEM. In calculating the thrombus weight, the lack of the thrombus was regarded as 0 mg. To test whether the mean of a variable differs between two groups

two-tail, Mann-Whitney test was used consistently throughout the study. Correlations were analyzed using a Pearson test. The p values less than 0.05 were considered significant.

## RESULTS

### *The effect of angiotensin II and losartan on carotid blood flow (CBF), occlusion time and incidence of occlusion.*

The CBF monitored during whole experiment is shown on *Figure 2A, B* and *Table 1*. The initial CBF was comparable in all studied groups. Only in the group of animals given losartan alone and followed with Ang II infusion we observed a significant reduction of CBF just after their administration. The arterial thrombus formation induced by electrical stimulation led to gradual reduction of CBF in all studied groups. We found significant difference between initial and final CBF in groups treated with Ang II 100 pmol/kg/min or losartan alone when compare to control. Neither occlusion time, defined as the time from the end of the electrical stimulation to the point at which the mean CBF decreased to zero, nor frequency

*Table 1:* CBF - Carotid Blood Flow monitored from the beginning of the experiment (Initial CBF) to the end (Final CBF); ΔCBF - the difference between Initial Carotid Blood Flow value and at particular minute. Positive values represent increases and negative values decreases in comparison to Initial Carotid Blood Flow; * p<0.05; ** p<0.01; *** p<0.001 vs VEH, # p<0.05; ## p<0.01; ### p<0.001 vs Ang II (400 pmol/kg/min); ^ p<0.05; ^^ p<0.01 vs Los (30 mg/kg). Groups consisted of 4 to 24 animals. Data are expressed as mean ± SEM.

| | VEH | Ang 100 | Ang 200 | Ang 400 | Ang400+Los | Los |
|---|---|---|---|---|---|---|
| Initial CBF (ml/min) | 6.7±0.5 | 7.3±0.3 | 5.7±0.5 | 6.3±0.3 | 6,2±0.5 | 6.9±0.5 |
| ΔCBF8min (ml/min) | 0.4±0.3 | -0.4±0.3 | 1,0±0.5 | 0.8±0.3 | -1.9±0.3***### | -1.9±0.3***### |
| ΔCBF13min (ml/min) | 0.07±0.4 | -0.2±0.8 | 0.4±0.6 | 0.2±0.2 | -1.8±0.6## ^ | -1.5±0.2** |
| ΔCBF20min (ml/min) | 0.3±0.4 | 0.6±0.5 | 0.1±1.3 | 0.07±0.2 | -2.6±0.5**### | -2.0±0.3*** |
| ΔCBF25min (ml/min) | 0.2±0.4 | -0.15±0.6 | 0.1±1.3 | -0.15±0.3 | -2,4±0.6**## | -1.9±0.3*** |
| ΔCBF30min (ml/min) | 0.05±0.4 | -0.05±0.7 | 0.2±1.4 | -0.03±0.6 | -2,5±0.7**# | -1.7±0.3** |
| ΔCBF40min (ml/min) | -1.9±0.7 | -2.5±0.6 | -1.3±1.6 | -2.5±0.4 | -2.6±0.7 | -3.3±0.7 |
| ΔCBF60min (ml/min) | -4.3±0.5 | -6.3±0.7 | -2.8±1.9 | -4.6±0.7 | -3,3±0.7^^ | -6.2±0.6 |
| ΔFinal CBF (ml/min) | -3.7±0.7 | -6.3±0.8* | -4,1±1.6 | -4.9±0.8 | -3,5±0.9^^ | -6.5±0.7* |

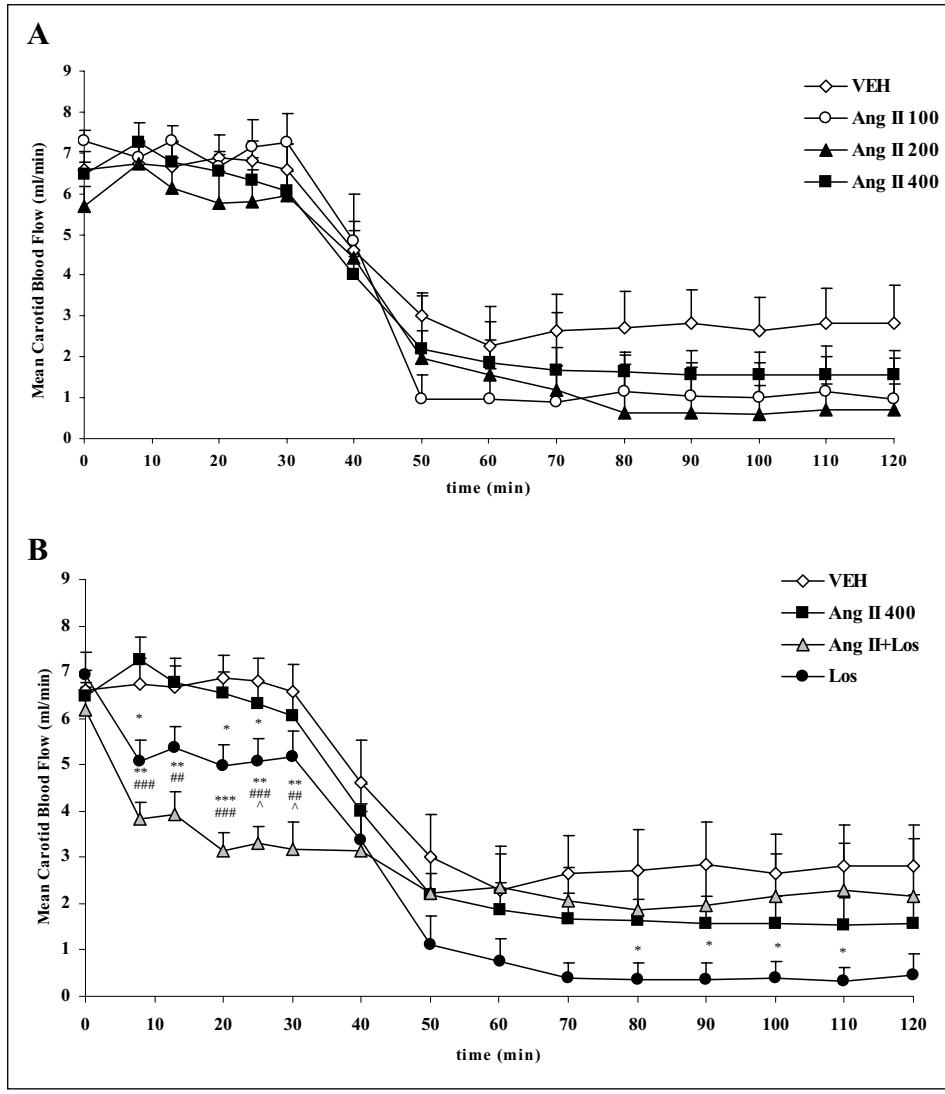


*Figure 2:* The diagram represent the mean carotid blood flow (CBF) monitored during whole experiment in hypertensive rats treated with: 0,9% NaCl (VEH), Ang II (100, 200, 400 pmol/kg/min) **(A)**; 0,9% NaCl (VEH), Los - selective $AT_1$ receptor antagonist (30mg/kg) followed by infusion of 0,9% NaCl (VEH), Los followed by infusion of Ang II (400 pmol/kg/min), Ang II (400 pmol/kg/min) **(B)**; * p<0,05; ** p<0,01, *** p<0.001 vs VEH; ## p<0,01; ### p<0,001 vs Ang II (400 pmol/kg/min); ^ p<0,05 vs Los. Groups consisted of 4 to 24 animals. Data are expressed as mean ± SEM.

of occlusion was accompanied by a marked statistical difference among all groups (data not shown).

*The effect of angiotensin II and losartan on thrombus formation*

As shown in *Figure 3* the infusion of Ang II caused significant increase of arterial thrombus weight (1.5 ± 0.1 and 1.4 ± 0.2 mg, for doses of 200 and 400 pmol/kg/min respectively vs 0.8 ± 0.1 mg in VEH treated group; $p<0.01$). Additional administration of Los (30mg/kg) in group infused with Ang II (400 pmol/kg/min) reversed effect of Ang II (0.4 ± 0.1 mg vs 1.4 ± 0.2 mg in Ang II 400 pmol/kg/min; $p<0.001$). In these animals the mean thrombus weight was significantly reduced in comparison to control group (VEH) (0.4 ± 0.1 mg vs 0.8 ± 0.1 mg; $p<0.05$). Arterial thrombus weight slightly, but not significantly, decreased in group of animals pretreated with Los alone (30mg/kg) [0.7 ± 0.1 mg vs 0.8 ± 0.1 mg; not significant (ns)].

*The effect of angiotensin II on plasma overall haemostasis potentials*

*Table 2* presents influence of Ang II on OCP, OHP and OFP. Infusion of Ang II at three doses used was accompanied by a marked increase in OHP comparing

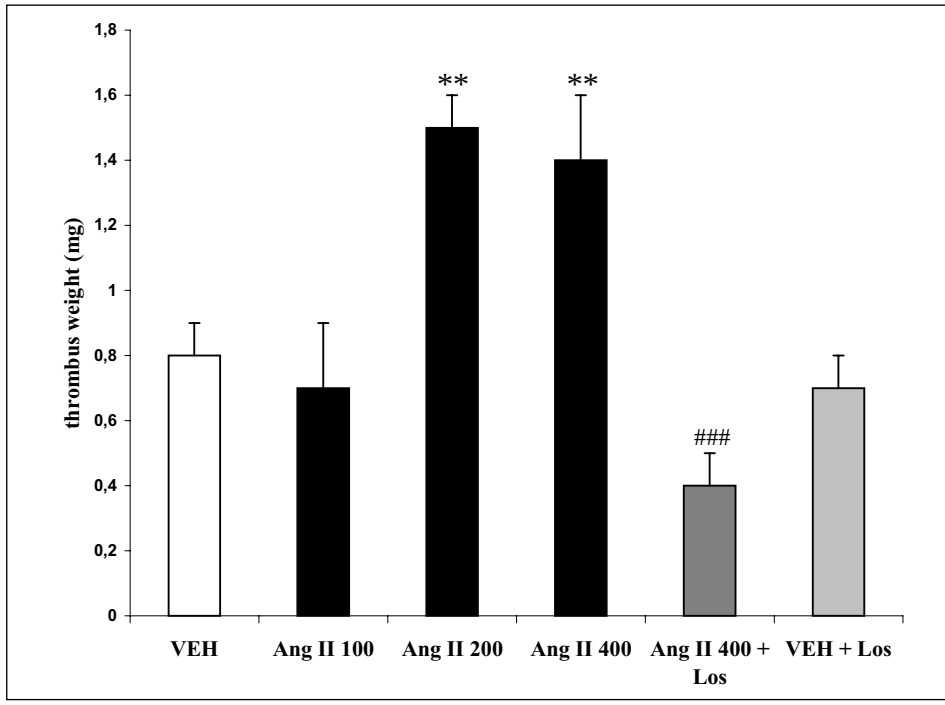


*Figure 3:* The columns represent the thrombus weight in hypertensive rats treated with: 0,9% NaCl (VEH), Ang II (100, 200, 400 pmol/kg/min), Los followed by infusion of Ang II (400 pmol/kg/min), Los (30mg/kg) followed by infusion of 0,9% NaCl (VEH); * $p<0.05$, ** $p<0.01$ vs VEH, ### $p<0.001$ vs Ang II (400 pmol/kg/min). Groups consisted of 4 to 24 animals. Data are expressed as mean ± SEM.

*Table 2:* OCP - Overall Coagulation Potential, OHP - Overall Haemostatic Potential, OFP - Overall Fibrinolytic Potential. ***p<0,001 vs VEH. Groups consisted of 4 to 24 animals. Data are expressed as mean ± SEM.

| | OCP % | OHP % | OFP % |
|---|---|---|---|
| VEH | 58,3±3,4 | 16,7±1,2 | 75,2±2,5 |
| Ang 100 | 65,0±5,4 | 29,8±2,3*** | 52,7±0,9*** |
| Ang 200 | 58,0±3,4 | 26,8±1,2*** | 55,7±1,7*** |
| Ang 400 | 70,2±6,1 | 30,4±2,9*** | 55,2±0,5*** |

to VEH treated rats. Moreover, administration of Ang II led to significant reduction of OFP. There was a linear correlation between OHP and OFP changes and thrombus weight (*Fig. 4 A* and *B*).

*The effect of angiotensin II on platelet aggregation.*

Ang II failed to influence collagen induced platelet aggregation both in *in vitro* and in *ex vivo* study (data not shown).

## DISCUSSION

Acute arterial thrombosis leading to myocardial infarction or ischemic stroke is the most common reason of death or disability of human being in developing and developed countries (33). Searching the particular risk factors of arterial thrombosis is needed and legitimate. There are some studies indicating that one of the RAS peptide, Ang II may contribute to the thrombosis development, by inhibiting fibrinolysis or stimulating coagulation and platelets activation (14-23). Interestingly, we have just found that Ang II may enhance venous thrombus formation *in vivo* (24). We observed that the prothrombotic effect of Ang II partially depended on enhanced leukocytes adhesion to endothelial cells accompanied by accelerated fibrin formation and increased plasma level of plasminogen activator inhibitor type 1 PAI-1. However, it is intriguing, if Ang II could also affect arterial thrombus formation and if platelets are involved in this process.

Since hypertension is one of the most important risk factors of arterial thrombosis and its clinical events such as acute coronary syndrome and ischemic stroke, this study was performed on renovascular hypertensive rats. We used two - kidney, one - clip model (2K-1C) to induce hypertension. This model of renovascular hypertension is mediated by the increased activities of both systemic and tissues (heart, aorta, lung and kidney) RAS (34-35) and is recommended for the evaluation of chemicals affecting RAS such as ACE-K, $AT_1$-As or RAS peptides (36-38).

To investigate the influence of Ang II on arterial thrombosis we used the model of experimental arterial thrombosis induced by electrical stimulation (28).

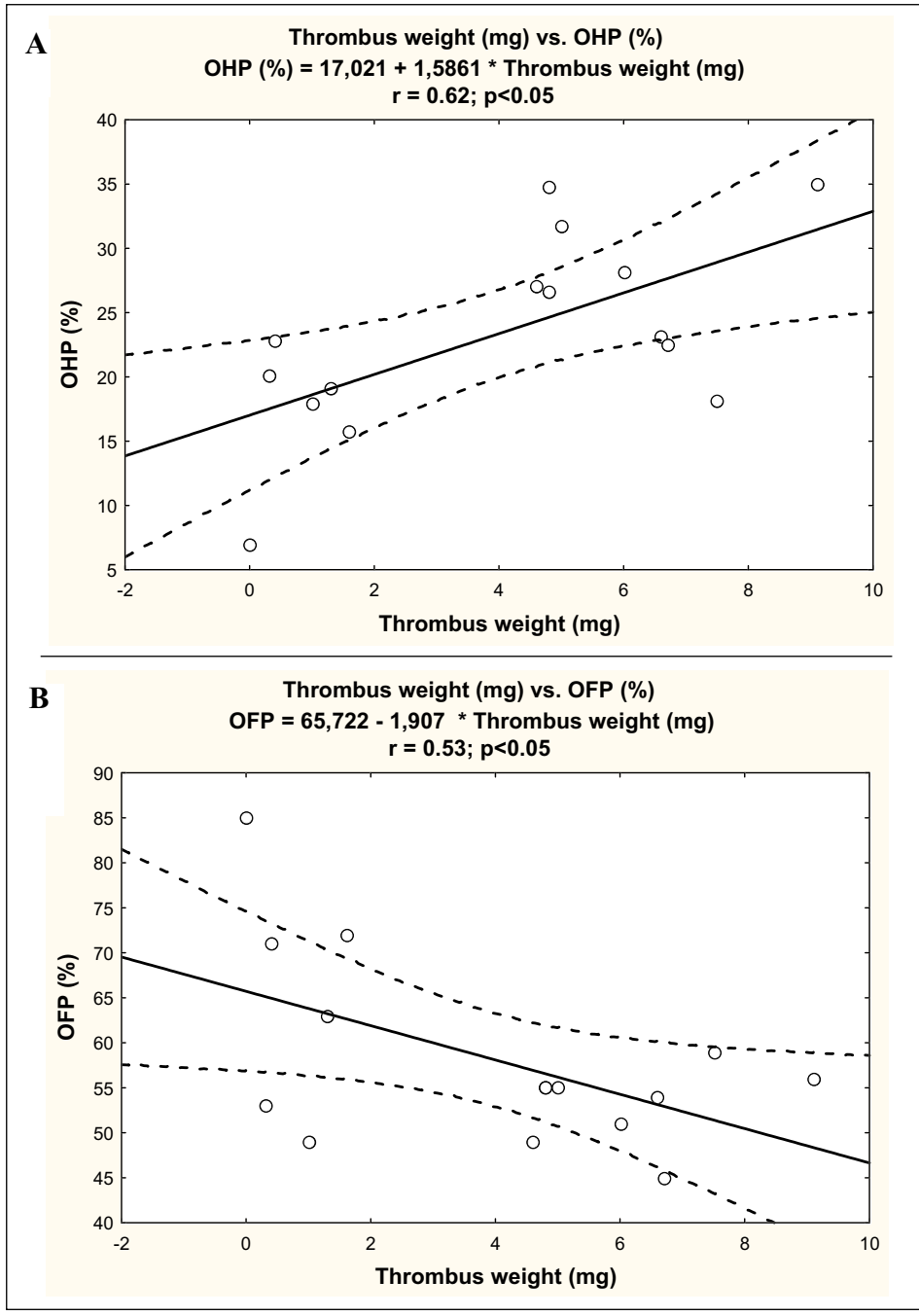


*Figure 4:* Correlation between OHP - Overall Haemostatic Potential **(A)**, OFP - Overall Fibrinolytic Potential **(B)** and thrombus weight in hypertensive rats treated with Ang II (100, 200, 400 pmol/kg/min)

Although thrombus formation was initiated by electrical stimulation producing arterial injury that is unrelated to clinical situation, thrombus morphology, suggests that growth of rich in platelets, intravascular thrombotic material in response to electrolytic injury is physiologically relevant (39). Nevertheless, this method was found to be successful to investigate compounds influencing arterial thrombosis (28, 40-43). In this experimental model the arterial thrombus formation in rat's common carotid artery was monitored by measuring the CBF during whole experiment. The marked reduction in CBF was recorded 10 minutes after electrical stimulation in all groups. We expected that Ang II, a potential prothrombotic factor, will shorten occlusion time, but we did not observe any significant change (data not shown). We previously found that infusion of Ang II at this range of doses significantly increases blood pressure and slightly CBF (24). Therefore, Ang II could make the vessel total occlusion difficult and mask the final result. However, we found slightly greater reduction of final CBF in group infused with Ang II (*Table 1*). This effect was especially seen in animals treated with the lowest dose of Ang II, exerting the weakest hypertensive activity, which confirms our earlier hypothesis. Despite similar time of occlusion of carotid artery in all studied groups the weight of formed arterial thrombus was significantly greater in animals treated with Ang II comparing with control group (*Fig. 3*). Therefore, we provide clear evidence that Ang II enhances arterial thrombosis development *in vivo*.

In purpose to investigate the mechanism by which Ang II promotes arterial thrombosis we used losartan to block $AT_1$ receptor that is considered to mediate the majority of Ang II effects contributing to the thrombosis development such as enhancing expression of tissue factor (TF) and PAI-1 or platelet activation (14, 23). Interestingly, just after administration of losartan alone and followed with Ang II infusion we observed a significant reduction of CBF. This finding may be related to the haemodynamic response. In our previous studies losartan given alone as well as simultaneous administration of Ang II and losartan exerted significant hypotensive effect (44). As expected losartan, a nonpeptide angiotensin II $AT_1$ receptor antagonist (45), successfully inhibited prothrombotic activity of Ang II. Our results demonstrate that Ang II enhances arterial thrombosis development in hypertensive rats mainly via $AT_1$ receptor. Moreover simultaneous administration of Ang II and losartan leads to far greater reduction of thrombus weight than by mere blocking of $AT_1$ receptor. Based on the patterns of *in vitro* expression, cell-based studies, and transgenic/knockout models, angiotensin receptor type 2 ($AT_2$) appear to modulate tissue development and repair and to counterbalance the effects of the $AT_1$ receptor (46). Moreover, we have previously demonstrated that Ang II paradoxically decreases blood pressure after blocking $AT_1$ receptor (44). There are also some findings describing the involvement of $AT_2$ receptor stimulation in the antithrombotic action of losartan in renal hypertensive rats (47). Thus, the role of angiotensin receptor type 2 ($AT_2$) should be taken into consideration.

Arterial thrombosis is a dynamic and a very complex process in which participate multiple factors such as: endothelium, platelets, plasma coagulation and fibrinolytic system. The mechanism by which Ang II can promote arterial thrombosis seems to be equally complicated. There is a variety of factors which can be modified by Ang II.

Ang II, activating $AT_1$ receptor, increases TF mRNA expression in cultured rat aortic endothelial cells without influence on tissue factor pathway inhibitor (TFPI) (14). This action of Ang II impairs physiological balance between TF, the initiator of the extrinsic pathway of the coagulation cascade, and its inhibitor. In *in vivo* study Ang II activates coagulation cascade with increases in plasma levels of thrombin-antithrombin complexes and prothrombin fragments F1 + 2 (19). In accordance to these observations, we found using newly described method (31) adapted by us to apply on laboratory animals (32), increased clot formation in rats infused with Ang II, expressed by raise of OHP (*Table 2*).

An important defense against intravascular thrombosis is fibrinolytic system (48). The interaction of Ang II with the fibrinolytic system has been previously documented. Local administration of Ang II into cultured rat and bovine aortic endothelial cells increased PAI-1 antigen and mRNA expression (49). The same results were observed in smooth muscle cells obtained from human umbilical and internal mammary arteries (50). In our present *in vivo* study we observed that Ang II caused significant decrease of OFP (*Table 2*). This finding can be explained by our recently published results showing that infusion of Ang II enhances PAI-1 synthesis in renovascular hypertensive rats developing venous thrombosis (24). According to the literature influence of Ang II on PAI-1 level in human are unclear. Ridker et al. (17) found Ang II - dependent rapid increase in circulating PAI-1 levels in both normotensive and hypertensive patients. Labinjoh C et al. did not confirm these results (51). Nevertheless, inhibition of fibrinolytic system could be responsible for the prothrombotic activity of Ang II observed in our study. The significant, linear correlation between OFP and thrombus weight (*Fig. 4B*) only confirms this conclusion.

There is number of data supporting the concept that Ang II augments platelets activation. Ang II enhances collagen, adenosine diphosphate (ADP), epinephrine, thrombin and the thromboxane A2 (TXA2) analogue U44069 - induced platelet activation (20,22,52,53). Incubation of human platelets with Ang II significantly increased concentration of $Ca^{2+}$ and pH in platelets that may be associated with increased aggregatory response (52). Ang II, added in platelet rich plasma, prepared from healthy subjects, caused a significant platelet shape change (PSC) which is an early phase of platelet activation that precedes platelet aggregation. Moreover, Ang II significantly enhanced sub-maximal PSC induced by ADP and serotonin (23). Also *in vivo* studies demonstrated enhanced human platelets activation by Ang II. Infusion of Ang II in normotensive volunteers caused significant increase of platelet activation markers such as beta-thromboglobulin and P-selectin (19). In our study Ang II failed to influence collagen induced

platelet aggregation. The prothrombotic action of Ang II, at least in rat, seems not to be platelet dependent, probably because the presence of $AT_1$ receptors on the surface of rats' thrombocytes was not confirmed.

In conclusion, recently we have demonstrated that Ang II enhances venous thrombus formation (24). Our present study documents that Ang II also exerts prothrombotic activity in experimental model of arterial thrombosis, probably due to the fibrinolysis inhibition. This is direct evidence pointing to Ang II as a prothrombotic agent and therefore the risk factor of acute coronary syndrome or ischemic stroke. Similarly to our previous findings, Ang II action was reversed by the $AT_1$ - receptor antagonist, confirming the role of $AT_1$ receptor in the prothrombotic effect of this peptide.

*Acknowledgments:* This study was supported by Grant No. 3-26581F from Medical University of Białystok, Poland.

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Received: July 28, 2005
Accepted: November 4, 2005

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