

Angiotensin II AT₁ Receptor Antagonists Inhibit Platelet Adhesion and Aggregation by Nitric Oxide Release

Leszek Kalinowski, Tomasz Matys, Ewa Chabielska, Włodzimierz Buczko, Tadeusz Malinski

Abstract—This study investigated the process of nitric oxide (NO) release from platelets after stimulation with different angiotensin II type 1 (AT₁)-receptor antagonists and its effect on platelet adhesion and aggregation. Angiotensin II AT₁-receptor antagonist-stimulated NO release in platelets was compared with that in human umbilical vein endothelial cells by using a highly sensitive porphyrinic microsensor. In vitro and ex vivo effects of angiotensin II AT₁-receptor antagonists on platelet adhesion to collagen and thromboxane A₂ analog U46619-induced aggregation were evaluated. Losartan, EXP3174, and valsartan alone caused NO release from platelets and endothelial cells in a dose-dependent manner in the range of 0.01 to 100 μmol/L, which was attenuated by NO synthase inhibitor N^G-nitro-L-arginine methyl ester. The angiotensin II AT₁-receptor antagonists had more than 70% greater potency in NO release in platelets than in endothelial cells. The degree of inhibition of platelet adhesion (collagen-stimulated) and aggregation (U46619-stimulated) elicited by losartan, EXP3174, and valsartan, either in vitro or ex vivo, closely correlated with the NO levels produced by each of these drugs alone. The inhibiting effects of angiotensin II AT₁-receptor antagonists on collagen-stimulated adhesion and U46619-stimulated aggregation of platelets were significantly reduced by pretreatment with N^G-nitro-L-arginine methyl ester. Neither the AT₂ receptor antagonist PD123319, the cyclooxygenase synthase inhibitor indomethacin, nor the selective thromboxane A₂/prostaglandin H₂ receptor antagonist SQ29,548 had any effect on angiotensin II AT₁-receptor antagonist-stimulated NO release in platelets and endothelial cells. The presented studies clearly indicate a crucial role of NO in the arterial antithrombotic effects of angiotensin II AT₁-receptor antagonists. (*Hypertension*. 2002;40:521-527.)

Key Words: platelets ■ nitric oxide ■ endothelium ■ angiotensin II ■ angiotensin antagonist

Platelets play an important role in arterial thrombosis and the onset of acute myocardial infarction after atherosclerotic plaque rupture. Inhibition of platelet aggregation has become a critical step in preventing thrombotic events that are associated with stroke, heart attack, and peripheral arterial thrombosis.¹ Thrombosis is a multicellular event in which other cells, such as endothelial cells, are involved in the regulation of platelet reactivity. During the past several years, clear evidence has emerged that a concerted action of nitric oxide (NO) generated by either endothelial or platelet NO synthases regulates platelet activation, causing inhibition of adhesion and aggregation.^{2,3} Recently developed nonpeptide angiotensin II (Ang II) AT₁-receptor antagonists (AT₁-As) make up a new generation of antihypertensive agents that also modulate hemostasis,^{4,5} and apparently this effect is not solely a result of Ang II-receptor blockage. Ang II induces an early phase of platelet activation⁶ and increases secretion of plasminogen activator inhibitor type I from vascular endothelial cells,⁷ whereas AT₁-As inhibit the vasoconstrictor and platelet aggregation effects induced by thromboxane A₂ (TXA₂)/prostaglandin H₂ (PGH₂) agonists acting at the TXA₂/

PGH₂ receptors.⁸⁻¹⁰ In addition, it has been demonstrated that NO, through activating phosphorylation of TXA₂/PGH₂ receptors, may inhibit the TXA₂-dependent intraplatelet pathway.¹¹ Because NO is a potent inhibitor of platelet adhesion and aggregation, it cannot be excluded that AT₁-As could inhibit platelet function through an endothelial/platelet NO-associated mechanism. In this regard, it has been reported that administration of NO synthesis inhibitors impairs the blood pressure-lowering action induced by losartan in rats in both short-term¹² and longer treatments.^{13,14} The results from the in vitro studies performed directly on isolated vessels are less consistent regarding the action of AT₁-As, which could involve enhanced NO release from endothelial cells. Although the preincubation of rat aortic rings with losartan reduced the contractile response to TXA₂ analog (U46619), and this action was reversed by N^G-nitro-L-arginine methyl ester (L-NAME),¹⁵ no influence of NOS inhibition on the cessation of U46619 effect by losartan in human gastroepiploic artery and saphenous vein,¹⁶ as well as irbesartan in canine coronary arteries,⁹ was observed. The limitation of these studies is that the release of NO from endothelium

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could be suggested on the basis of the comparison of vessel relaxation. Meanwhile, the direct effect of AT₁-As on vascular smooth muscle cells might mask the endothelium-dependent action of a compound.

Our preliminary data suggested that the antiplatelet action of losartan might also involve a modification of intraplatelet NO synthesis.¹⁷ The aim of this study therefore was to assess a role of NO stimulated by AT₁-As on inhibition of platelet adhesion and aggregation. We have also compared the NO-stimulating potency of the AT₁-As in platelets with that in endothelial cells. We used a porphyrinic microsensor for direct electrochemical measurements of biologically active NO¹⁸ (diffusible NO), which is particularly suitable for NO quantification, with a high sensitivity in both platelets¹⁹ and cultured endothelial cells.²⁰

Materials and Methods

Direct Measurements of NO Released From Platelets and Endothelial Cells

Nitric oxide sensor was prepared according to the procedures published previously.¹⁸ Platelet-rich plasma was obtained from blood samples directly drawn from the heart of the rats.¹⁷ The washed platelets were finally suspended in a calcium-free Tyrode-HEPES buffer to ascertain that NO was not generated by activation of the aggregating process in the presence of extracellular Ca²⁺.¹⁹ The platelets suspended in the buffer (3×10^8 /mL) were incubated in an aggregometer with a NO sensor (working electrode) placed in the platelet suspension. Before the experiments with endothelial cells, human umbilical vein endothelial cells (HUVECs) were trypsinized as for the passage procedure and next rinsed twice with Tyrode-HEPES buffer with 1.8 mmol/L CaCl₂. NO detection studies were performed in endothelial cell suspensions (3×10^8 /mL) in the same incubation conditions as for a platelet suspension.

Before NO determination, either platelets or endothelial cells were pretreated for 30 minutes with different agents to investigate the potential mechanism of the action of the AT₁-As (Losartan, EXP3174, valsartan). To determine whether AT₁-As mediates NO release through interaction with TXA₂/PGH₂ receptors or activation of the prostaglandins production, the selective TXA₂/PGH₂ receptors antagonist SQ29,548 (1.0 μmol/L) and the cyclooxygenase inhibitor indomethacin (10 μmol/L) were chosen to pretreat platelets and endothelial cells. In addition, to investigate the effect of Ang II AT₂-receptor blockade on NO response to the AT₁-As, both the platelets and the culture cells were pretreated with the AT₂-receptor antagonist, PD123319 (10 μmol/L). To ascertain whether NO release after administration of the AT₁-As is specified for endothelial NO synthase, both platelets and endothelial cells were pretreated with L-NAME (100 μmol/L). After pretreatment, the AT₁-As (0.01 to 100 μmol/L) were added, and the release of NO was measured for 3 minutes. In some experiments, AT₁-As were replaced with 1 μmol/L *D*-myo-inositol-(1,4,5)-triphosphate (IP₃) for demonstration of maximal NO release by platelets under these experimental conditions (in the absence of extracellular Ca²⁺). To record the maximum of NO release from endothelial cells, the AT₁-As were replaced with 1 μmol/L calcium ionophore (CaI) A23187.

Platelet Adhesion and Aggregation

Platelet adhesion to fibrillar collagen and platelet aggregation induced by thromboxane A₂ analog U46619 were carried out according to previously described methods.¹⁷ In *in vitro* studies, AT₁-As (0.1 to 10 μmol/L) were added to the washed platelets samples and preincubated for 5 minutes. At the end of preincubation, collagen (50 μg/mL) for adhesion or U46619 (5 μmol/L) for aggregation was added and the platelets were further incubated for 15 minutes. In *ex vivo* studies, AT₁-As were injected intraperitoneally 1 hour before the blood sampling in doses of 3, 10, or 30 mg/kg. In some

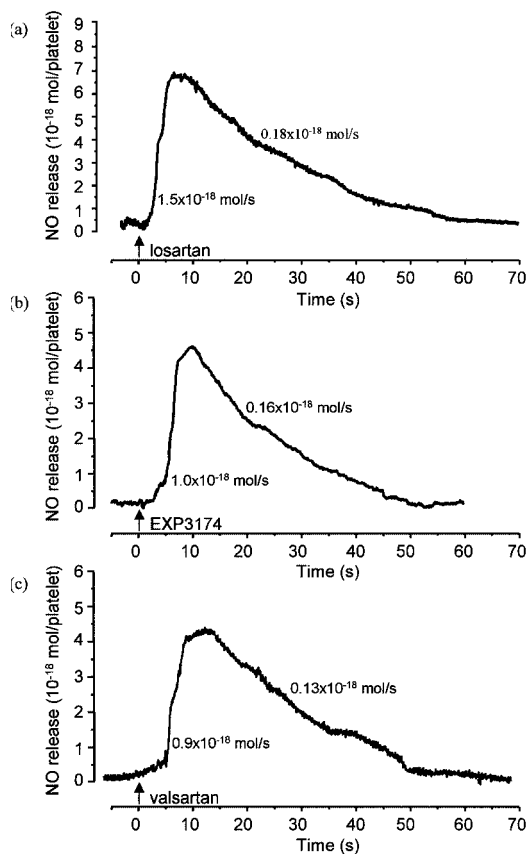


Figure 1. Representative recordings of NO release from platelets. NO release was measured after addition of 10 μmol/L of losartan (a), EXP3174 (b), and valsartan (c).

experiments, platelet NO synthase was inhibited with L-NAME, added to the sample in a concentration of 100 μmol/L (*in vitro* studies), or injected intraperitoneally in a dose of 10 mg/kg (*ex vivo* studies).

An expanded Methods section can be found in an online supplement available at <http://www.hypertensionaha.org>.

Results

Nitric Oxide Release From Blood Platelets and Cultured Endothelial Cells

Figure 1 shows representative amperometric curves for NO release by platelets stimulated with losartan, EXP3174, and valsartan. The pattern of NO release was similar for all tested antagonists. The rates of concentration increase ranged between 0.9 and 1.5×10^{-18} mol/s per platelet, and the maximum NO concentration was reached after about 5 seconds. After 5 seconds of a semiplateau, a slow decrease in NO concentration with a rate-range from 0.13×10^{-18} to 0.18×10^{-18} mol/s per platelet was observed. The kinetics of NO release was appreciably slower for all tested inhibitors in comparison with a receptor-independent NO agonist, ie, IP₃, during entire courses of NO release (Figure 2). Immediately after addition of IP₃ (1 μmol/L), NO was released with the rate of 7.3×10^{-18} mol/s per platelet. After reaching the sharp peak of NO concentration after about 2.8 seconds after stimulation, a rapid decrease of NO concentration with a rate of 1.6×10^{-18} mol/s per platelet was observed.

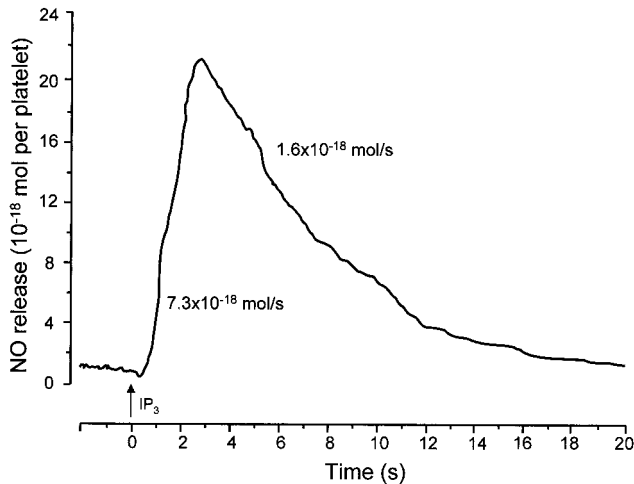


Figure 2. Representative recording of NO release from platelets after stimulation with 1 $\mu\text{mol/L}$ IP₃.

In the absence of adhesion/aggregating agents, the release of NO from platelet-rich plasma (PRP) was not detected. The addition of AT₁-As alone resulted in a concentration-dependent release of NO; a linear response for all 3 AT₁-As at the concentration range from 0.01 to 100 $\mu\text{mol/L}$ was observed. A maximum NO release response was constant at concentrations higher than 10 $\mu\text{mol/L}$ for each tested AT₁-A. There were no significant differences in the concentration-response relationship for EXP3174 and valsartan, although the NO response after addition of losartan were about 2 times higher and shifted to the left compared with the former 2 antagonists ($P < 0.01$) (Figure 3). The maximum NO release (plateau) amounted to $8.8 \pm 0.6 \times 10^{-18}$, $6.2 \pm 0.5 \times 10^{-18}$, and $5.7 \pm 0.5 \times 10^{-18}$ moles per platelet for losartan, EXP3174, and valsartan, respectively.

The release of NO in response to IP₃ represents the maximum amount of NO that can be released by platelets via mobilization of intracellular Ca²⁺. This maximum release of NO ($21.4 \pm 0.9 \times 10^{-18}$ moles per platelet) achieved in the

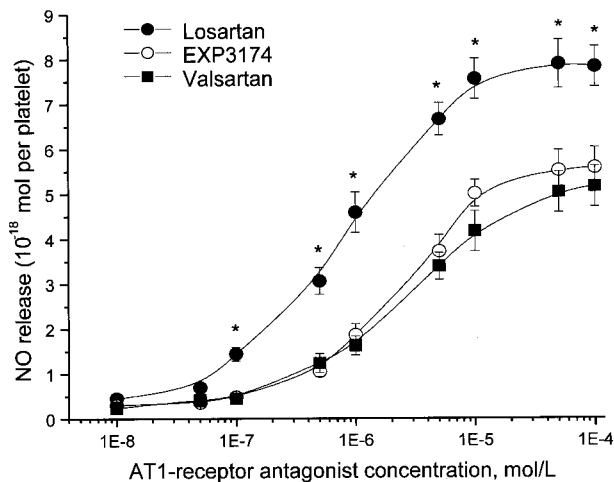


Figure 3. NO release from platelets in dose-dependent response to the AT₁ receptor antagonists: losartan, EXP3174, and valsartan ($n = 6$). * $P < 0.01$ versus EXP3174 or valsartan.

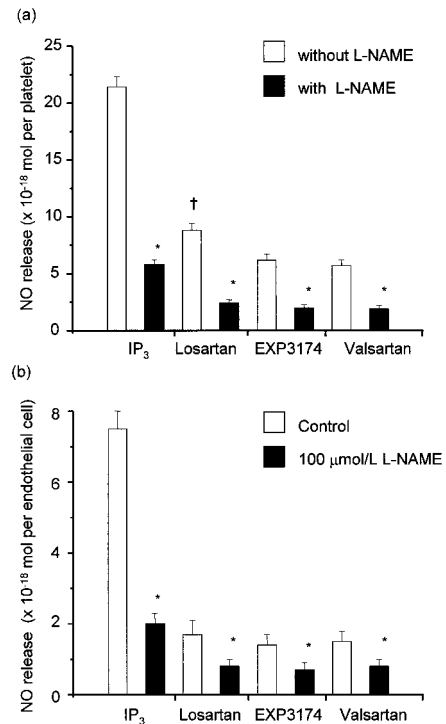


Figure 4. NO release stimulated by 5 $\mu\text{mol/L}$ AT₁ receptor antagonists in platelets (a) and endothelial cells (b) in the presence or absence of 100 $\mu\text{mol/L}$ L-NAME. After pretreatment for 30 minutes with the eNOS inhibitor, NO release was measured for 3 minutes in the platelets or endothelial cells suspensions ($3 \times 10^9/\text{mL}$ each). IP₃- and Cal-stimulated NO release represent the maximum NO release that can be achieved under the experimental conditions in platelets and endothelial cells, respectively ($n = 6$). * $P < 0.001$ versus without L-NAME.

presence of IP₃ was about 2.5 to 3 times higher than those in the presence of the AT₁-As (Figure 4a). The maximal NO release in endothelial cells ($7.5 \pm 0.5 \times 10^{-18}$ moles per endothelial cell), recorded after addition of CaI, was about 3 times lower than the maximal NO release after addition of IP₃ in platelets (Figures 4a and 4b). The potency in releasing NO after addition of AT₁-As was 30% to 50% higher in platelets than in endothelial cells. The potency was calculated as a ratio of NO concentration stimulated by AT₁-As to the maximal concentration stimulated by IP₃ and CaI in platelets and endothelial cells, respectively. As expected, the presence of the NO synthesis inhibitor (100 $\mu\text{mol/L}$ L-NAME) decreased by about 70% the NO release stimulated with either IP₃ in platelets or CaI in endothelial cells. Also, to the same extent, L-NAME inhibited NO release after stimulation with AT₁-As alone in both platelets and endothelial cells. Although the maximum NO release in endothelial cells after stimulation with losartan was higher than after stimulation with either EXP3174 or valsartan, in contrast to platelets the difference did not reach statistical significance. NO release stimulated with losartan, EXP3174, and valsartan alone was not altered by the pretreatment of platelets or endothelial cells with indomethacin (10 $\mu\text{mol/L}$) in either the maximal or the half-maximal response. Similarly, neither PD123319 (10 $\mu\text{mol/L}$) nor SQ29,548 (1.0 $\mu\text{mol/L}$) was able to modify AT₁-As-stimulated NO release in both platelets and endothelial cells (the data for losartan are shown in Figure 5).

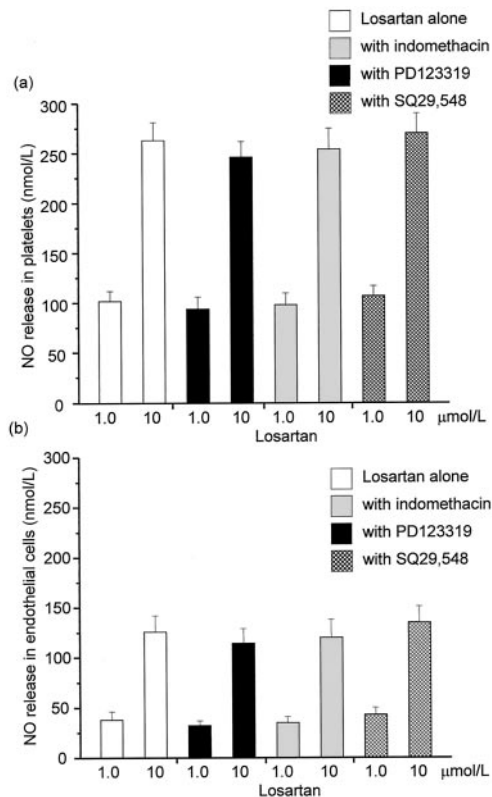


Figure 5. Pretreatment with indomethacin (10 $\mu\text{mol/L}$), PD123319 (10 $\mu\text{mol/L}$), or SQ29,548 (1.0 $\mu\text{mol/L}$) did not affect NO release stimulated with losartan (1.0 and 10 $\mu\text{mol/L}$) in platelets (a) and endothelial cells (b). After pretreatment for 30 minutes with the tested agents, NO release was measured for 3 minutes after addition of losartan in the platelet or endothelial cell suspensions ($3 \times 10^8/\text{mL}$ each; $n=6$).

In Vitro Platelet Adhesion and Aggregation

When the platelets were incubated with collagen, $41.2 \pm 0.7\%$ of the platelets adhered. Addition of AT_1 -As to the sample markedly inhibited the number of adhering platelets in a concentration-dependent manner (Figure 6a). The most pronounced effect was exerted by losartan (10 $\mu\text{mol/L}$), which decreased the index of adhesion to $16.8 \pm 1.2\%$ ($P < 0.001$). The lowest concentration of losartan, which showed significant antiadhesive effect, was 0.1 $\mu\text{mol/L}$, whereas equal concentrations of EXP3174 and valsartan were ineffective. Moreover, in the whole range of concentrations used, losartan decreased the index of adhesion to a greater extent than corresponding concentrations of EXP3174 or valsartan ($P < 0.05$).

The mean value of maximal U46619-induced platelet aggregation in the control group equalled $65.3 \pm 1.4\%$. AT_1 -As addition to PRP significantly decreased this parameter in a concentration-dependent manner (Figure 6b). Similar to the adhesion studies, the most pronounced inhibition of platelet aggregation was obtained with losartan, which was effective in the whole range of concentrations used, with the maximal reduction being $37.2 \pm 3.0\%$ by the highest concentration ($P < 0.001$). The actions of EXP3174 and valsartan were less pronounced than that of losartan ($P < 0.05$), starting from a concentration of 1 $\mu\text{mol/L}$.

Preincubation of platelets with AT_1 -As alone did not affect platelet adhesion (without collagen) and aggregation (without U46619).

Ex Vivo Platelet Adhesion and Aggregation

Pretreatment of the animals with AT_1 -As before the blood sampling resulted in a dose-dependent inhibition of platelet adhesion to collagen (Figure 6c), with the maximal reduction of the index of adhesion from $42.0 \pm 0.9\%$ to $22.1 \pm 1.6\%$. This reduction was observed for a 30 mg/kg dose of losartan ($P < 0.01$). A marked decrease in this parameter was already achieved using the lowest dose of losartan, whereas EXP3174 and valsartan were effective only in higher doses (10 and 30 mg/kg), and their effects were significantly weaker ($P < 0.05$).

Administration of AT_1 -As also caused a significant inhibition of ex vivo platelet aggregation U46619 (Figure 6d). Again, losartan proved to be the most effective, reducing platelet aggregation from $67.2 \pm 2.1\%$ to $32.2 \pm 3.0\%$ in the highest dose ($P < 0.001$). The lowest dose of losartan and EXP3174 inhibited aggregation to a similar extent, whereas an equal dose of valsartan was ineffective. However, with a dosage increment, the action of losartan was more pronounced than that of the remaining AT_1 -As ($P < 0.05$).

Effect of NO Synthase Inhibition on the Antiplatelet Action of AT_1 Receptor Antagonists

In the next step of the studies, the influence of NO synthase inhibition on the antiplatelet effect of AT_1 -As was examined. In the following experiments, concentrations of 1 $\mu\text{mol/L}$ (in vitro) and doses of 10 mg/kg (ex vivo) of AT_1 -As were used, together with the NO synthase inhibitor, L-NAME (100 $\mu\text{mol/L}$ and 10 mg/kg, respectively). L-NAME given alone changed neither collagen-stimulated adhesion nor U46619-stimulated aggregation of platelets. However, NO synthase inhibition markedly attenuated the antiadhesive ($P < 0.01$) and antiaggregative effects of losartan ($P < 0.05$) in vitro and completely inhibited those of EXP3174 and valsartan ($P < 0.05$), as shown in Figures 7a and 7b, respectively. Similarly, coadministration of L-NAME with losartan partially reversed its inhibitory effect on ex vivo platelet adhesion ($P < 0.05$) and aggregation ($P < 0.05$; Figures 7c and 7d), whereas it completely inhibited that observed in the case of EXP3174 and valsartan ($P < 0.05$, $P < 0.01$).

Discussion

This study reports for the first time that AT_1 -As may not only counteract the pathologic actions of Ang II on the evolution of arterial hypertension but also may have an additional and separate action in preventing platelet adhesion and aggregation through a NO-dependent mechanism. Using a porphyrinic microsensor, we have directly shown that AT_1 -As are potent in releasing NO in a concentration-dependent manner in both platelets and endothelial cells. From an analytical point of view, detection of NO in biological fluids is a challenging problem. The short biological half-life of NO, in the range of 100 ms to 2 s, results in the action of the NO produced being most likely confined to the vicinity of its production site. The most currently available methods for the detection of NO are based on the measurements of biologi-

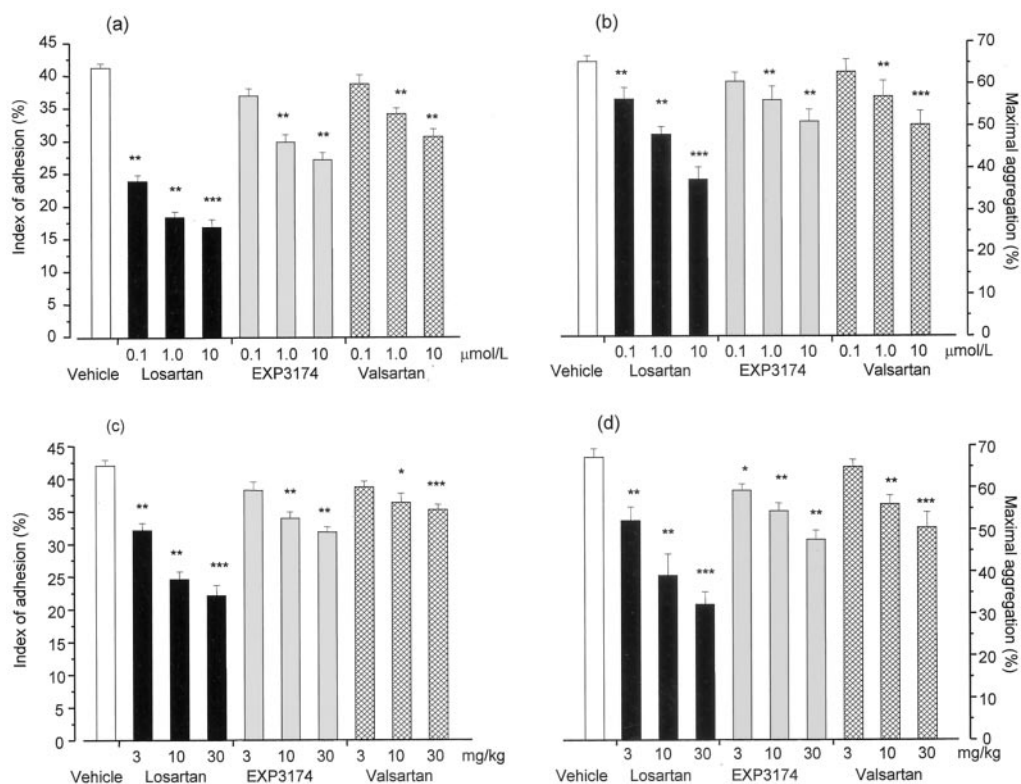


Figure 6. Dose-dependent effect of losartan, EXP3174, and valsartan on collagen-stimulated adhesion and U46619-stimulated aggregation of rat platelets in in vitro (a and b) and ex vivo (c and d) experiments (n=4 to 6). **P*<0.05, ***P*<0.01, ****P*<0.001 versus vehicle.

cally inactive products of NO (nitrite) or bioassays that rely on secondary effects of NO (eg, vasorelaxation after inhibition of NO synthesis or endothelium damage). The extremely small size of the porphyrinic sensor tip and the proximity of the sensor to the site of NO formation offers the advantage of allowing measurements to be made in situ and in real time.¹⁸ The detection limit of a porphyrinic microsensor for NO is about 10-fold higher than that of the oxyhemoglobin technique, another known direct NO-detecting method, which is

devoid of the above attributes of the electrochemical method for NO measurements in biological fluids.

The AT₁-As-stimulated NO release occurred rapidly. It was short-lived and inhibited by L-NAME, thereby indicating that NO release was due to activation of platelet Ca²⁺-dependent constitutive NO synthase (eNOS). This concept can be confirmed by our finding that IP₃, by mobilizing intraplatelet Ca²⁺, activated NO release even more rapidly than AT₁-As, and that it was also inhibited by L-NAME. The

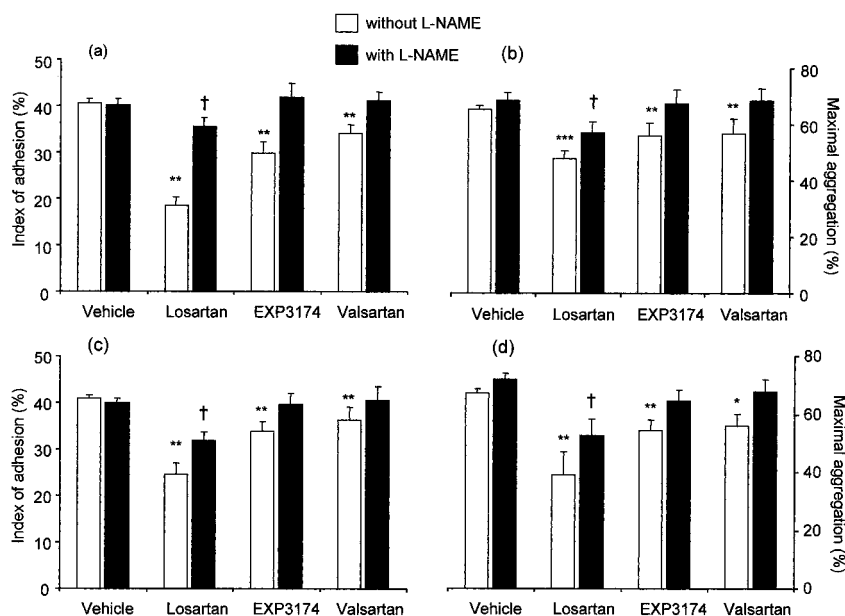


Figure 7. Effect of NO synthase inhibitor L-NAME on the antiplatelet action of losartan, EXP3174, and valsartan. In in vitro experiments on platelet adhesion (a) and aggregation (b) 1 μmol/L AT₁-receptor antagonists and 100 μmol/L L-NAME were used; in ex vivo studies on platelet adhesion (c) and aggregation (d), 10 mg/kg of L-NAME was administered with 10 mg/kg of antagonists (n=5 to 6). **P*<0.05, ***P*<0.01, ****P*<0.001 versus vehicle; †*P*<0.05 versus L-NAME. Drug versus drug+L-NAME differences are not indicated.

pretreatment of platelets with L-NAME also prevented the antiadhesive and antiaggregative effect of AT₁-As. There was a good correlation between the degree of eNOS stimulation (NO release) and the magnitude of inhibition of adhesion and aggregation of platelets by AT₁-As, both being revealed at micromolar concentrations. These data confirm that NO formation in platelets is associated with the antiadhesive and antiaggregative effect of AT₁-As. It is interesting that L-NAME given alone neither in vitro nor ex vivo changed platelet adhesion and aggregation. It has been found that the stimulation of platelets by aggregating agents results in activation of eNOS.^{19,21} However, the amounts of NO produced by platelets in this response were small; therefore, the ability of the platelet NOS to regulate aggregation may not be revealed with strong aggregating agents.²

The potency of the AT₁-As to stimulate NO release was about twice as low in endothelial cells than in platelets. The peak value of NO concentration for losartan, the most potent NOS activator among the tested drugs, equalled ≈40% in platelets and ≈20% in endothelial cells of those revealed in the presence of receptor-independent agonists (IP₃ or CaI). These results could suggest that, in vivo, the amounts of NO involved in platelet antiadhesive and antiaggregative action of AT₁-As may be supplemented by NO release from endothelium stimulated by the drugs. In this regard, it has been demonstrated that the amounts of NO available for regulation of platelet function are considerably boosted by the synthesis and release of this mediator from vascular endothelium. Platelet adhesion and aggregation in vitro induced by a variety of agonists was inhibited by NO release from fresh and cultured endothelial cells.² Also, stimulation of NO release in vivo by cholinergic stimuli resulted in inhibition of platelet aggregation induced by some aggregating agents.²² Activation of endothelium-derived NO release may also contribute to the non-Ang II-related actions of AT₁-As in their blood pressure-reducing effects. It has been shown that, 48 hours after administration of a single dose of losartan, blood pressure was still reduced in the presence of normal responses to Ang I and Ang II in SHR.¹² Likewise, the involvement of endothelium-derived NO has been suggested in the mechanism of the blood pressure-lowering action of losartan in rats in long-term treatment.^{13,14} The chronic treatment of stroke-prone SHR with losartan increased aortic cyclic GMP content. Maeso et al²³ reported that losartan reduced constrictor responses to phenylephrine in blood vessels of SHR by stimulating production of NO, and this effect might be partially mediated through blockage of Ang II T₂-type receptors by the AT₁-As. In our study, addition of an AT₂-receptor antagonist, PD123319, to either platelets or endothelial cells, failed to modify the AT₁-As-stimulated NO release. Although the presence of AT₂ receptors in platelets remains to be established, the addition of PD123319 did not affect in vitro the inhibitory effect of AT₁-As on platelet adhesion and aggregation (data not shown). Additionally, the blocking action of AT₁-As on endogenous Ang II found in platelets and endothelial cells leading to NO release appears to be excluded in our experimental conditions. Degradation of Ang II during preparation of the platelet and endothelial cell

suspensions might be expected despite the prior occupancy of Ang II receptors.

Recent reports indicate that several AT₁-As interact with TXA₂/PGH₂ receptors in human platelets and inhibit the TXA₂ analog U46619-induced platelet aggregation along with canine^{8,9} and human artery contraction.¹⁶ It was also important to evaluate any endogenous participation of prostanoids in AT₁-As-stimulated NO release, because losartan has been shown to increase the dose-dependent release of PGI₂ in vascular smooth muscle cells.²⁴ However, our data presented here demonstrated that neither SQ29,548, a selective TXA₂/PGH₂-receptor antagonist, nor indomethacin, an inhibitor of cyclooxygenase synthase, affected AT₁-As-stimulated NO release in platelets and endothelial cells. It is in agreement with our previous in vitro studies on platelets showing that indomethacin did not inhibit antiadhesive and antiaggregative effects of AT₁-As.¹⁷

Following the studies in our laboratory with a variety of potent eNOS agonists, the kinetics of NO release recorded after stimulation with AT₁-As resembled the patterns of NO release obtained for the receptor-dependent eNOS agonist (eg, acetylcholine) rather than for the typical receptor-independent eNOS agonists (CaI and IP₃).^{20,25,26} Among the tested AT₁-As, losartan had a greater potency to release NO and inhibit platelet adhesion and aggregation than EXP3174 and valsartan. All these drugs have some similarities in their chemical structure. Both losartan and EXP3174 have a benzylimidazole moiety, with EXP3174 differing from losartan only by having a carboxylic radical in place of a hydroxylic radical. Similar to EXP3174, valsartan also contains a carboxylic radical, which could explain similar ability to stimulate NO release and to inhibit platelet adhesion and aggregation. On the other hand, it has been shown¹⁰ that the inhibitory effect of losartan on platelet aggregation is not shared by the AT₁ receptor antagonist candesartan, suggesting that these actions are not general for all AT₁-As, but specific for the structure of certain AT₁-As. Further studies beyond the scope of this research are needed to determine the exact mechanism of release of NO by different AT₁-As, with respect to other proposed mechanisms of action of these drugs.

Similar NO-dependent antiadhesive and antiaggregative effects were obtained, both when AT₁-As were added to platelets and when administered to the rats before the blood sampling. In rats, the concentration of losartan was estimated to reach approximately 250 μmol/L after a 10 mg/kg IV infusion.²⁷ In humans, the blood concentration of losartan was ≈1 μmol/L after an oral therapeutic dose of losartan.²⁸ Hence, NO release stimulated with AT₁-As may be considered clinically relevant and contributes to the thrombosis prevention and blood-pressure reduction.

Perspectives

The results of this study provide direct evidence that platelet antiadhesive and antiaggregative properties of losartan, its metabolite EXP3174, and valsartan are linked to activation of NO release. In addition, the tested drugs reveal the ability to release NO directly, acting on both resting platelets and, with the lesser potency, culture endothelial cells. These findings

suggest that AT₁-As, not associated with AT₂ or TXA₂/PGH₂ receptors with direct action to stimulate NO production in platelet and endothelial cells, may have additional therapeutic benefits in the treatment of arterial thrombosis. The observed effects of NO stimulation with AT₁-As in both platelet and endothelial cells are of special interest in the setting of arterial hypertension, renal protection, and antiproatherogenic actions, all of which are associated with vasoconstriction and platelet aggregation.²⁹

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