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Nitric oxide modulates angiotensin II-induced endothelial vasoconstrictor prostanoid release

Susana Jerez *, Maria Peral de Bruno, Alfredo Coviello

Departamento de Bioingeniería, Instituto Superior de Investigaciones Biológicas (INSIBIO), CONICET, Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Tucumán, Argentina

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Abstract

This study investigated the modulation of angiotensin II-induced endothelial prostanoid release in rabbit aortic rings. Two cumulative dose response curves with 90-min washing interval were performed. Incubation with L-N^G-nitroarginine methyl ester (L-NAME) 10^{-4} M increased angiotensin II maximal contractile response (E_{max}). This effect was reversed by indomethacin 10^{-5} M, diphenyliodinum 10^{-5} M, Tempol 10^{-5} M or ascorbic acid 10^{-4} M in both cumulative dose response curves and by SQ 29548 10^{-6} M in the second cumulative dose response curve. When segments were treated with tetraethylamonium 10^{-3} M but not with glibenclamide 10^{-5} M during the washing period, L-NAME recovered its ability to enhance the E_{max} in arteries incubated with SQ 29548. Conclusions: nitric oxide modulates angiotensin II-induced endothelial release of cyclooxygenase-dependent eicosanoids, one of which acts through thromboxane A_2/prostaglandin H_2 receptors and would decrease K_{Ca} channel activity. An increase in free radical production may account for the enhancement of such prostanoid release. Furthermore, it was found that in the present conditions, the release of the hyperpolarizing factor would improve in order to maintain the vascular tone.

Keywords: Angiotensin II; Eicosanoid; Nitric oxide; Cyclooxygenase; Cytochrome P_{450} epoxygenase; TXA_2/PGH_2 receptor

1. Introduction

It is now well documented that angiotensin II stimulates endothelial synthesis and release of vasodilators such as nitric oxide (NO) (Zhang et al., 1994), epoxyeicosatrienoic acids (Arima et al., 1997), prostacyclin (PGI_2) and vasoconstrictors such as endothelin (Chen et al., 1995), lipoxygenase-derived eicosanoids (Lin et al., 1994), superoxide anions (Touyz, 2004), endoperoxides, thromboxane A_2 (TXA_2) (Keen et al., 1997) and cytochrome P_{450} omega hidroxylase metabolites (Alonso-Galicia et al., 2002). Under physiological conditions, relaxing factors appear to dominate. In contrast, in hypertensive and atherosclerotic arteries the release of endothelium-derived relaxing factors and/or the responsiveness of vascular smooth muscle cells to the relaxing factors is reduced, while that of endothelium-derived contracting factors (EDCF) is increased. This imbalance of endothelium-derived relaxing and contracting factors may be important in the pathogenesis of hypertension and its cardiovascular complications (Luscher, 1990).

NO production is continuous, imparting a constant vasodilatory effect and helping maintain resting vascular tone and normal blood pressure (Moncada et al., 1991). Furthermore, development of hypertension has been documented in animals with long-term blockade of NO synthesis or knock-out of the nitric oxide synthase (NOS) (Huang et al., 1995).

Although the exact chemical identity of endothelium-derived hyperpolarizing factor (EDHF) has not yet been elucidated, a number of studies suggest that cytochrome P_{450} derived metabolite may be responsible for hyperpolarization

* Corresponding author. España 3706-4000, Tucumán, Argentina. Tel.: +54 381 4364120; fax: +54 381 4364120.
E-mail address: sjerez@herrera.unt.edu.ar (S. Jerez).

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of the vascular smooth muscle observed in the presence of inhibitors of cyclooxygenase (COX) and NOS (Hecker, 2000). In the coronary system, NO inhibits the activity of the cytochrome P_{450} (CYP)-like (EDHF) synthase. A decrease of bioavailability of NO, as demonstrated in various states associated with endothelial dysfunction, alleviates this intrinsic inhibition so that the activity of the EDHF synthase and the production of vasodilating epoxyeicosatrienoic acids are increased. As a consequence of this interaction, vascular responsiveness is thought to be at least partially maintained despite the apparent loss of NO (Bauersachs et al., 1996).

In the aorta of the spontaneously hypertensive rat (SHR), endothelium-dependent contractions to acetylcholine involve reactive oxygen species that activate the COX-1 pathway with the production of endoperoxide(s), which stimulate thromboxane receptors on the aortic vascular smooth muscle (Yang et al., 2002). Both endothelial cells and smooth muscle cells express COX-1 and thromboxane receptors. Lin et al. (1994) and Dellipizzi et al. (1997) reported that treatment with a thromboxane A_2/prostaglandin H_2 (TXA_2/PGH_2) receptor blocker lowers the blood pressure of rats with established angiotensin II and salt induced hypertension as well as those in the early stage of the aortic coarctaction-hypertension.

Furthermore, it has been demonstrated that both basal and stimulated bioavailability of NO within the vascular wall are attenuated by an elevation in the superoxide anion production (Rubanyi and Vanhoutte, 1986; Jerez et al., 2001a).

All these previously mentioned data suggest a complicate interaction between the different factors released from the endothelium by angiotensin II. The aim of the present investigation is to study the modulation of the prostanoid release induced by angiotensin II in experimental conditions of endothelial dysfunction by inhibition of the NOS activity.

2. Materials and methods

2.1. Rabbit aortic ring preparation

Experiments were performed on isolated rabbit thoracic aorta from male Flanders hybrid rabbits (1.5–2.5 kg) obtained from a slaughterhouse. The thoracic aorta was carefully dissected and all adherent fat and connective tissue were removed. Five-millimeter wide rings were cut and mounted in a 10 ml organ bath containing Krebs solution of the following composition (mM): NaCl 128, KCl 4.7, $NaHCO_3$ 14.4, NaH_2PO_4 1.2, Na_2-EDTA 0.1, $CaCl_2$ 2.5, glucose 11.1, pH 7.2. Krebs solution was kept at 37 °C and aerated with 95% O_2 and 5% CO_2.

Isometric contractions were measured by using force-displacement transducers and recorded under an initial tension of 2 *g*, which was found to be the optimal tension for KCl-induced contraction (100 mM). All preparations were allowed to equilibrate for 90 min and washed with Krebs solution at 15 min intervals. The endothelium was kept intact in some rings, but in other groups the endothelium was removed by rubbing the luminal surface. Acetylcholine was used to test the endothelium integrity. The rings were stimulated with noradrenaline 5.10^{-6} M and when the maximal contraction was achieved, acetylcholine 10^{-6} M was added to establish its relaxing effect.

2.2. Experimental protocols

Unrubbed and rubbed aortic rings were exposed to increasing doses of angiotensin II (10^{-10} to 10^{-6} M) separated by a 90 min interval to construct two cumulative dose response curves. In order to evaluate the effect of NOS-inhibition, unrubbed arteries were treated with L-N^G-nitroarginine methyl ester (L-NAME 10^{-4} M) or D-N^G-nitroarginine methyl ester (D-NAME 10^{-4} M, the inactive isomer). After the first stimulation, the arteries were washed every 15 min during the 90 min interval and the drugs were added to the bath at each change of bath. To study the role of prostaglandins in the increase of the maximal contractile response (E_{max}) observed in arteries treated with L-NAME, unrubbed and rubbed arteries were treated with indomethacin 10^{-5} M (COX inhibitor) in absence and presence (only in unrubbed arteries) of L-NAME. To establish whether the prostanoid released by angiotensin II in L-NAME-treated arteries contracts the vascular smooth muscle via interaction with TXA_2/PGH_2 receptors, a blocker of such receptors (SQ 29548 10^{-6} M) was used in a similar protocol as that performed with indomethacin in unrubbed arteries. An inhibitor of cytochrome P_{450} epoxygenase (miconazol 10^{-6} M) was used in order to evaluate the possible role of cytochrome P_{450} epoxygenase — metabolites in the loss of affinity observed in arteries treated with L-NAME plus indomethacin. Unrubbed arteries were bathed in miconazol either in absence or in presence of L-NAME plus indomethacin. The role of O_2^- on the COX activation was investigated with the NADPH inhibitor, diphenyliodinum 10^{-5} M, the membrane-permeable superoxide dismutase mimetic (Tempol 10^{-5} M) and ascorbic acid 10^{-4} M. These drugs were added to the bath in absence or in presence of L-NAME 10^{-4} M. All the drugs used in the protocols previously mentioned were added to the bath 30 min before the first cumulative dose response curve and maintained all along the experiment. In order to check the hypothesis that the endoperoxide released by angiotensin II during NOS-inhibition would decrease K channel activity, the effects of blockade of Ca^{2+}-activated K^+ channels (K_{Ca}) with tetraethylamonium (TEA) 10^{-3} M and ATP-activated K^+ channels (K_{ATP}) with glibenclamide 10^{-5} M were determined. Taking into account that the aim of the protocol designed was to evaluate the effect of the prostanoid at a medium term, these drugs were added to

the bath after the first cumulative dose response curve. Control arteries and those treated with L-NAME plus SQ 29548 were incubated with TEA and glibenclamide during the 90-min washing period. The second stimulation was performed in absence of the K^+ channel blockers.

The results are expressed as milligrams of isometric contraction.

2.3. Statistical analysis

Data are presented as mean values ± S.E.M. The pEC_{50} (negative log of molar concentration of angiotensin II inducing 50% of the maximal contraction) and the maximal contractile response (E_{max}) were calculated using a curve-fitting analysis program. Student's t test for paired data was used in order to compare pEC_{50} values or maximal response between first and second cumulative dose response curves. $P<0.05$ was considered statistically significant (two-tail test). The significance of the differences between and within the groups was examined with an analysis of variance (ANOVA) for repeated measures followed by a Duncan's test.

3. Results

3.1. Effects of L-NAME

The contractile response to angiotensin II (10^{-10} to $2.5.10^{-6}$ M) was dose dependent. Unrubbed and rubbed arteries showed no significant differences in E_{max} between them or between first and second cumulative dose response curves. However, there was a significant shift to the right of the second exposure to angiotensin II (Table 1).

The incubation of segments with L-NAME 10^{-4} M induced an increase in the E_{max} of the first cumulative dose response curve ($P<0.05$, Table 1). However, no differences were found in pEC_{50} values (Table 1). In addition, L-NAME increased E_{max} of the second cumulative dose response curve. Despite this enhancement on the vasoconstrictor activity, a shift to the right of the second exposure to angiotensin II was observed ($P<0.05$, Table 1). The magnitude of the rightward shift was similar to that of the control arteries ($P>0.05$, ANOVA and Duncan's test).

3.2. Effects of indomethacin

The incubation of the unrubbed or rubbed aortic rings with indomethacin 10^{-5} M did not modify either the contractile response or the affinity of the first and the second cumulative dose response curves (Table 1). However, when unrubbed arteries were treated with indomethacin plus L-NAME, indomethacin was capable of blocking the increase in the E_{max} induced by L-NAME. In such conditions, a significant shift to the right in the first cumulative dose response curve with respect to control arteries and those treated either with L-NAME or indomethacin was observed ($P<0.05$, ANOVA and Duncan's test, Table 1). The rightward shift of the second cumulative dose response was similar in arteries treated with indomethacin, L-NAME or indomethacin plus L-NAME ($P>0.05$, ANOVA and Duncan's test).

3.3. Effects of SQ 29548

Treatment with SQ 29548 10^{-6} M modified neither the contractile response nor the affinity of first and second cumulative dose response curves (Table 1). However, when the segments were incubated with SQ 29548 plus L-NAME,

Table 1
pEC_{50} and maximal contractile response (E_{max}) to angiotensin II in rabbit aortic rings with and without (E−) endothelium

	First curve		Second curve	
	E_{max} (mg)	pEC_{50}	E_{max} (mg)	pEC_{50}
Control	3703±818	8.27±0.12	3696±696	7.94±0.19 [a]
Control (E−)	3162±511	8.30±0.12	3635±593	7.90±0.17 [a]
L-NAME	6086±1044 [b]	8.20±0.10	7005±1122 [a]	7.64±0.21 [a]
Indomethacin	3474±821	8.26±0.03	3400±818	7.81±0.18 [a]
Indomethacin (E−)	3743±962	8.36±0.07	3.630±1013	8.01±0.09 [a]
L-NAME+Indom.	3764±821	7.86±0.20 [d]	3582±918	7.51±0.20 [a]
SQ 29548	3703±893	8.36±0.05	3857±1070	8.16±0.08 [a]
L-NAME+SQ 29548	5339±1003 [c]	8.53±0.04	5025±1052	8.23±0.09 [a]
Miconazol	3718±951	8.35±0.03	4450±940 [a]	8.06±0.09 [a]
L-NAME+Miconazol+Indomethacin	4652±1119	8.48±0.12	4771±1360	8.09±0.13 [a]

Values are expressed as means ± S.E.M. of 8 experiments.

[a] $P<0.05$ indicates differences statistically significant between the first and the second cumulative dose response curves (paired t test).

[b] $P<0.01$ indicates differences in E_{max} of the first cumulative dose response curve between arteries treated with L-NAME and control arteries or those incubated with L-NAME plus indomethacin or L-NAME plus miconazol plus indomethacin (ANOVA and Duncan´s test).

[c] $P<0.05$ indicates differences in E_{max} of the first cumulative dose response curve between control or SQ 29548 treated arteries and those treated with L-NAME plus SQ 29548 (ANOVA and Duncan's test).

[d] $P<0.01$ indicates differences in pEC_{50} of segments incubated with L-NAME plus indomethacin and arteries treated with L-NAME, indomethacin or L-NAME plus indomethacin plus miconazol (ANOVA and Duncan's test).

SQ 29548 was capable of inhibiting the second but not the first cumulative dose response-increase in the E_{max} to angiotensin II induced by treatment with L-NAME (Table 1). In addition, a shift to the left with respect to arteries treated with L-NAME or SQ 29548 was observed ($P<0.05$, ANOVA and Duncan's test, Table 1).

3.4. Effects of miconazol

Treatment with miconazol 10^{-6} M had no effects on contractile response or affinity of the first cumulative dose response curve with respect to control (Table 1). However, miconazol increased E_{max} of the second cumulative dose response curve (Table 1). In spite of the increase in the contractile activity miconazol induced a shift to the right of the cumulative dose response curve to the same magnitude as the control arteries ($P>0.05$, ANOVA and Duncan's test). In turn, the addition of miconazol on arteries incubated with L-NAME plus indomethacin improved angiotensin II affinity of the first and the second cumulative dose response curves with respect to arteries treated with L-NAME plus indomethacin (Table 1).

3.5. Effects of diphenyliodinum, Tempol and ascorbic acid

The maximal contractile response to angiotensin II in aortic rings was decreased by treatment with diphenyliodinum and remained unmodified by Tempol or ascorbic acid either in the first or the second cumulative dose response curves (Table 2). In turn, the addition of diphenyliodinum, Tempol or ascorbic acid in segments incubated with L-NAME eliminated the increase on E_{max} induced by L-NAME both in the first and the second cumulative dose response curves. A leftward shift of the first cumulative dose response curve to angiotensin II was observed in segments treated with diphenyliodinum, Tempol and ascorbic acid with or without L-NAME with respect to control arteries (Table 2). Furthermore, the rightward shift of the second cumulative dose response curve that had been observed in control arteries disappeared in arteries treated with diphenyliodinum and Tempol.

Table 3
pEC_{50} and maximal contractile response (E_{max}) to angiotensin II in rabbit aortic rings with endothelium

	First curve		Second curve	
	E_{max} (mg)	pEC_{50}	E_{max} (mg)	pEC_{50}
Control	3142±671	8.29±0.09	3179±586	7.98±0.14 [a]
TEA	2380±256	8.28±0.10	2541±391	8.08±0.09 [a]
L-NAME+SQ 29548+TEA	2684±465	8.37±0.10	3160±574 [a]	8.02±0.14 [a]
Glibenclamide	2529±502	8.45±0.06	2754±687	8.38±0.11
L-NAME+SQ 29548+ Glibenclamide	2919±507	8.51±0.12	3382±669	8.23±0.15 [a]

Control arteries and those treated with L-NAME plus SQ 29548 were incubated with TEA or glibenclamide during the 90-min washing period, that means these drugs were added to the bath after the first cumulative dose response curve to angiotensin II and the second stimulation was performed in absence of the K^+ channel blockers. Values are expressed as means±S.E.M. of 9 experiments.

[a] $P<0.05$ indicates statistically significant differences between the first and the second cumulative dose response curves (paired t test).

3.6. Effect of K^+ channel blockers

There were no differences on E_{max} or pEC_{50} either in the first or the second cumulative dose response curves between arteries incubated with TEA and glibenclamide during the 90-min washing period with respect to control (Table 3). However, when arteries were treated with TEA but not with glibenclamide, L-NAME recovered its ability to enhance E_{max} in the second cumulative dose response curve in arteries incubated with SQ 29548. On the other hand, glibenclamide but not TEA was capable of preventing the shift to the right of the second cumulative dose response curve (Table 3).

Table 2
pEC_{50} and maximal contractile response (E_{max}) to angiotensin II in rabbit aortic rings with endothelium

	First curve		Second curve	
	E_{max} (mg)	pEC_{50}	E_{max} (mg)	pEC_{50}
Control	4011±988	8.29±0.10 [c]	4435±724	7.99±0.14 [a]
Diphenyliodinum	3134±763 [b]	8.61±0.10	3190±660	8.40±0.05
L-NAME+Diphenyliodinum	2889±724 [b]	8.60±0.08	2810±564	8.46±0.06
Tempol	4218±804	8.59±0.07	4703±883	8.54±0.06
L-NAME+Tempol	3924±760	8.67±0.03	4542±883	8.62±0.09
Ascorbic acid	4329±752	8.79±0.15	4611±751	8.19±0.17 [a]
L-NAME+Ascorbic acid	4929±781	8.53±0.10	5349±720	8.32±0.13

Values are expressed as means±S.E.M. of 8 experiments.

[a] $P<0.05$ indicates statistically significant differences between the first and the second cumulative dose response curves (paired t test).

[b] $P<0.05$ indicates differences in E_{max} of the first cumulative dose response curve between arteries treated with diphenyliodinum or L-NAME plus diphenyliodinum and control arteries (ANOVA and Duncan's test).

[c] $P<0.01$ indicates differences in pEC_{50} between control arteries and those incubated either with diphenyliodinum or Tempol or ascorbic acid in absence or the presence of L-NAME.

4. Discussion

Results obtained in the present study showed that inhibition of NO production improves the maximal contractile response to angiotensin II at short (first cumulative dose response curve) and at medium term (second cumulative dose response curve). This is in agreement with Zhang et al. (1994). The increase in the intrinsic activity may be due to the fact that no counteracting action of NO was observed and the equilibrium was displaced by the release of vasoconstrictors.

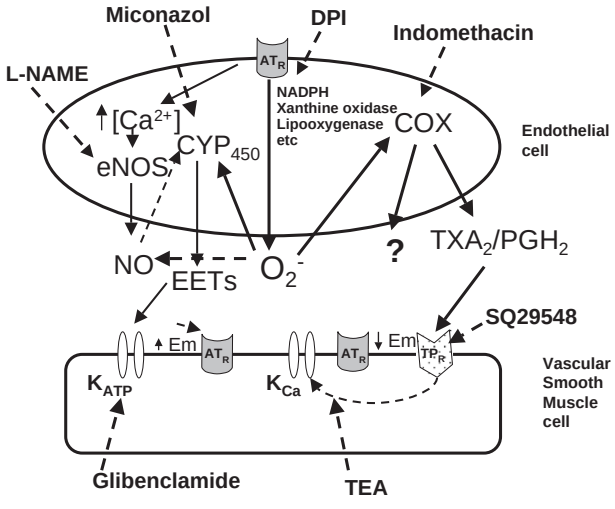
In the present work no effect of indomethacin in the contractile response to angiotensin II was found either in rubbed or unrubbed arteries, which is in agreement with Gruetter et al. (1988). That means there is no basal release of COX products in the normal aorta, except in the case of pathological circumstances such as hypertension (Dellipizzi et al., 1997). In that sense, it has been reported that treatment with indomethacin lowers blood pressure in rats with aortic coarctation-induced hypertension and rats with two-kidney-one clip hypertension, models of angiotensin-dependent hypertension in which the production of TXA_2 and prostaglandins by vascular structures is increased (Nasjletti, 1998). Furthermore, previous studies have demonstrated impaired vascular relaxation of mesenteric resistance arteries of SHR because of increased production of a COX-1-dependent endothelium-derived contracting factor (Dai et al., 1992). On the other hand, development of hypertension has been documented in animals with blockade of NO synthesis or knock-out of the NOS gene (Raij, 2001). Taking into account these data from the literature, the present work has checked the hypothesis that the endothelial release of vasoconstrictor prostanoids may account for the increase on the angiotensin II intrinsic activity observed during the NOS-inhibition. It has been found that indomethacin blocked the increase on the maximal contractile response induced by L-NAME. These data as well as those previously mentioned about the lack of effect of indomethacin on rubbed arteries, would mean that angiotensin II releases from the endothelium a vasoconstrictor prostanoid, which may be partially responsible for the intrinsic activity improvement that had been observed in the presence of L-NAME. In agreement with these findings, a number of in vivo and in vitro studies have demonstrated that the vascular response to angiotensin II is associated with synthesis of constrictor prostanoids such as prostaglandins and TXA_2 in vascular and renal tissues (Mistry and Nasjletti, 1988; Lin and Nasjletti, 1991). TXA_2 and PGH_2 stimulate contraction of the vascular smooth muscle via interaction with a common receptor (Mais et al., 1985). Of the products of the COX pathway, endoperoxides (in particular PGH_2) are likely candidates as EDCF in the SHR aorta. This conclusion is supported by the observations that inhibitors of COX-1 and thromboxane receptor antagonists eliminated the endothelium-dependent contraction while inhibitors of thromboxane synthase failed to do so (Lin et al., 1994). In addition, the aortas from SHR without endothelium showed a higher sensitivity to exogenous PGH_2 than those of normotensive control rats (Ge et al., 1995). Relevant to this point, Zerrouk et al. (1998) reported that after blockade of thromboxane receptors with SQ 29548, the angiotensin II-induced contractions were significantly reduced in SHR aorta with endothelium. Furthermore, treatment with a TXA_2/PGH_2 receptor blocker lowers the blood pressure of rats with established angiotensin II and salt induced hypertension as well as those in the early stage of aortic coarctaction-hypertension (Nasjletti, 1998). Taking these data into account, the present study intends to demonstrate that the prostanoid released during the NOS-inhibition interacts with a TXA_2/PGH_2 receptor. Treatment with SQ 29548 eliminated the enhancement in the maximal contractile response observed at medium (second cumulative dose response curve) but not at short term (first cumulative dose response curve) in arteries treated with L-NAME. This result would indicate that there is more than one cyclooxygenase-dependent eicosanoid released by angiotensin II in absence of NO production. Both of them would be released together, but one of them accounts for the increase in the intrinsic activity at short term and the other, which acts throughTXA_2/PGH_2 receptors, accounts for the increase in the contractile response at medium term. Crane and Garland (2004) showed that an early consequence of TXA_2 receptor stimulation is a reduction in the arterial hyperpolarization and relaxation attributed to EDHF and this effect appears to reflect a loss of K_{Ca} activity. According to these data, it has been verified the hypothesis that the prostanoid released by angiotensin II during the NOS-inhibition that acts through TXA_2/PGH_2 receptors may reduce K_{Ca} activity. In such sense, it was found that TEA but not glibenclamide was capable of reversing the inhibition of the maximal contractile response induced by SQ 29548 in arteries treated with L-NAME. This result would mean that the action mechanism of the prostanoid released by angiotensin II during the NOS-inhibition would involve a loss of K_{Ca} activity. The appearance of this effect would occur on the second cumulative dose response curve (at medium term) because during the 90-min washing period the diminished activity of K_{ca} channels induces Ca^{2+} influx increase and vascular reactivity improvement. Nevertheless, further studies will be necessary to establish the subtype of the K_{Ca} channels involved.

Griendling and Ushio-Fukai (2000) reported that angiotensin II stimulates a NADPH oxidase to produce superoxide ($\bullet O_2^-$) and hydrogen peroxide (H_2O_2), both of which may act on intracellular growth-related proteins and enzymes to mediate the final physiological response. In physiological conditions in aqueous solutions at a neutral pH, $\bullet O_2^-$ is transformed into H_2O_2. However, when produced in excess, a significant amount of O_2^- reacts with NO, which results in the formation of the highly aggressive reactive nitrogen species $ONOO^-$. Therefore, in addition to the loss of important NO-mediated signaling events, oxidative destruction of NO may have direct cytotoxic action as well (Mihm et al., 2003). That

is why NO may be considered a $\bullet O_2^-$ scavenger. Yang et al. (2002) demonstrated that free radicals activate COX-1, most likely to produce endoperoxides. Considering these data from the literature, it has been hypothesized that in conditions of NOS-inhibition there is an increase of free radicals that would activate COX to improve the synthesis of vasoconstrictor prostanoids. In order to check this hypothesis, the following antioxidants were used: an inhibitor of flavin containing enzymes including NADPH oxidase (diphenyliodinum), a superoxide mimetic (Tempol), and ascorbic acid, whose antioxidant properties were demonstrated in previous works (MacKenzie and Martin, 1998; Adeagbo et al., 2003). Diphenyliodinum treatment reduced the angiotensin II contractile response in control arteries. According to this finding, Pagano et al. (1995) reported that diphenyliodinum caused a 78% reduction in basal superoxide levels in rabbit aorta, and Souza et al. (2001) found that superoxide generated in the vessel wall by an NADPH-dependent oxidase modulates the vascular contractile tone. However, Tempol and ascorbic acid did not modify the angiotensin II contractile response per se. This is in agreement with Schuijt et al. (2003) who found that diphenyliodinum unlike Tempol reduced the angiotensin II constriction in human coronary arteries. That would mean NADPH oxidase products might be involved in the improvement of the contractile response to angiotensin II in physiological conditions. However, there are other potential sources of vascular O_2^- production, including xanthine oxidase, lipoxygenase, etc. (Cai and Harrison, 2000). Moreover, diphenyliodinum, Tempol and ascorbic acid were capable of blocking the L-NAME-induced increase in the angiotensin II-intrinsic activity at short (first cumulative dose response curve) and at medium term (second cumulative dose response curve). Therefore, the free radicals release improvement during the NOS-inhibition accounts for the increase of vasoconstrictor prostanoid production.

On the other hand, a previous work (Jerez et al., 2001a,b) demonstrated that in rabbit aorta there are two mechanisms involved in the development of angiotensin II-tachyphylaxis, one of them involves endothelium influence and the other, which is endothelium independent, occurs at smooth muscle level. The endothelium-dependent tachyphylaxis is related to the angiotensin II intrinsic contractile property and the endothelium-independent tachyphylaxis is related to its loss of affinity. Further studies (Jerez et al., 2004) demonstrated no differences in the maximal contractile responses between the first and the second cumulative dose response curves to angiotensin II in control arteries when the recovery time after the first cumulative dose response curve was longer. This result suggests the disappearance of the endothelium-dependent desensitisation. However, there was a shift to the right of the second cumulative dose response curve to angiotensin II. It would mean that the endothelium-independent tachyphylaxis is not reversible after a 90 min recovery time.

The incubation of unrubbed arteries with L-NAME did not modify angiotensin II affinity in the first cumulative dose response curve, but induced a shift to the right of the second cumulative dose response curve in the same magnitude as the controls. In addition, when indomethacin but not SQ 29548 was added to L-NAME treated arteries, a significant shift to the right in first and second cumulative dose response curves was found. In a previous paper (Jerez et al., 2004), it was pointed out that endothelium dependent hyperpolarization might modify angiotensin II affinity. That view is in agreement with Oriowo et al. (1991) who suggested the presence of a factor(s) within the receptor microenvironment capable of modulating affinity and hence tissue sensitivity. As it was stated in the Introduction, although the exact chemical identity of EDHF has not yet been elucidated, there is much evidence in favour of EDHF being a cytochrome P_{450}-derived arachidonic acid metabolite. 5,6-, 8,9-, 11,12- and 14,15-Epoxyeicosatrienoic acids are produced by endothelium intact coronary, renal, carotic and mesenteric arteries and, when applied to endothelium-denuded artery segments, are capable of producing both hyperpolarization and relaxation (Campbell et al., 1996; Quilley et al., 1997). Taking these data into account, in the present study it has been hypothesised that the epoxyeicosatrienoic acids production would be increased when NOS and COX are inhibited simultaneously and this fact would account for the desensitisation observed. The results obtained showed that inhibition of cytochrome P_{450} epoxygenase with miconazol prevented the shift to the right observed in arteries treated with indomethacin plus L-NAME without modification of the contractile response. It would mean that, in such experimental conditions, the hyperpolarization induced by epoxyeicosa-



Scheme 1. Representation of the nitric oxide (NO) modulator role in the angiotensin II-induced release of vasoconstrictor prostanoids. AT_R: angiotensin II receptor; COX: cyclooxygenase; CYP_{450}: cytochrome P_{450} epoxygenase; DPI: diphenyliodinum; EETs: epoxyeicosatrienoic acids; Em: membrane potential; eNOS: endothelial nitric oxide synthase; L-NAME: L-N^G-nitroarginine methyl ester; K_{Ca} calcium-activated potassium channels; KATP: ATP-activated potassium channels; TEA: tetraethylamonium; TP: thromboxane A_2/prostaglandin H_2 receptor; TXA_2/PGH_2: thromboxane $_2$/prostaglandin H_2.

trienoic acids is more effective on the affinity than on the intrinsic activity. That would be the reason why a modification of the affinity but not of the maximal contractile response was observed. The fact that glibenclamide was able to avoid the loss of affinity on the second cumulative dose response curve to angiotensin II supports this view. This result is in agreement with previous findings (Jerez et al., 2001a, 2004). In addition, Bauersachs et al. (1996) demonstrated that NO inhibits the activity of the cytochrome P_{450}-like endothelium-derived hyperpolarizing factor (EDHF) synthase in the coronary system and Udosen et al. (2003) reported that NO directly and reversibly inhibits epoxygenase dependent dilation of rat renal microvessels.

Furthermore, it has been observed that diphenyliodinum, Tempol and ascorbic acid improved the angiotensin II affinity both in control and L-NAME treated arteries. This finding would indicate that free radicals, which are increased during angiotensin II stimulation, would act in increasing the release of epoxyeicosatrienoic acids in order to counterbalance their effects (Scheme 1).

In conclusion, evidence has been provided that angiotensin II stimulates endothelium release of COX-dependent vasoconstrictor prostanoids during NOS inhibition. In such experimental conditions the increase in free radical production may be responsible for the COX-dependent activation pathway towards the vasoconstrictor eicosanoid synthesis. One of the released prostanoids acts through TXA/PGH_2 receptors and its action mechanism would imply the decrease of the K_{ca} channels activity, effect which is evident at medium term. Furthermore, it was found that in the present conditions, the release of the hyperpolarizing factor would improve in order to maintain the vascular tone.

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References

Adeagbo, A.S., Joshua, I.G., Falkner, C., Matheson, P.J., 2003. Tempol, an antioxidant, restores endothelium-derived hyperpolarizing factor-mediated vasodilation during hypertension. Eur. J. Pharmacol. 481, 91–100.

Alonso-Galicia, M., Maier, K.G., Greene, A.S., Cowley Jr., A.W., Roman, R.J., 2002. Role of 20-hydroxyeicosatetraenoic acid in the renal and vasoconstrictor actions of angiotensin II. Am. J. Physiol. 283, R60–R68.

Arima, S., Endo, Y., Yaoita, H., Omata, K., Ogawa, S., Tsunoda, K., Abe, M., Takeuchi, K., Abe, K., Ito, S., 1997. Possible role of P_{450} metabolite of arachidonic acid in vasodilator mechanisms of angiotensin II type receptor in the isolated microperfused rabbit afferent arteriole. J. Clin. Invest. 100, 2816–2823.

Bauersachs, J., Popp, R., Hecker, M., Sauer, E., Fleming, I., Busse, R., 1996. Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor. Circulation 94, 3341–3347.

Cai, H., Harrison, D.G., 2000. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ. Res. 87, 840–844.

Campbell, W.B., Gebremedhin, D., Pratt, P.F., Harder, D.R., 1996. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ. Res. 78, 415–423.

Chen, L., McNeill, J.R., Wilson, T.W., Gopalakrishnan, V., 1995. Heterogeneity in vascular smooth muscle responsiveness to angiotensin II: role of endothelin. Hypertension 26, 83–88.

Crane, G.J., Garland, C.J., 2004. Thromboxane receptor stimulation associated with loss of SKCa activity and reduced EDHF responses in the rat isolated mesenteric artery. Br. J. Pharmacol. 142, 43–50.

Dai, F.X., Skopec, J., Diederich, A., Diederich, D., 1992. Prostaglandin H2 and thromboxane A2 are contractile factors in intrarenal arteries of spontaneously hypertensive rats. Hypertension 19, 795–798.

Dellipizzi, A., Pucci, M.L., Mosny, A.Y., Deseyn, K., Nasjletti, A., 1997. Contribution of constrictor prostanoids to the calcium-dependent basal tone in the aorta from rats with aortic coarctation-induced hypertension: relationship to nitric oxide. J. Pharmacol. Exp. Ther. 283, 75–81.

Ge, T., Hughes, H., Junquero, D.C., Wu, K.K., Vanhoutte, P.M., Boulanger, C.M., 1995. Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H synthase-1 and hypersensitivity to prostaglandin H2 in the SHR aorta. Circ. Res. 76, 1003–1010.

Griendling, K.K., Ushio-Fukai, M., 2000. Reactive oxygen species as mediators of angiotensin II signaling. Regul. Pept. 28, 21–27.

Gruetter, C.A., Ryan, E.T., Lenke, S.M., Bailly, D.A., Fox, M.K., Schoepp, D.D., 1988. Endothelium-dependent modulation of angiotensin II-induced contraction in blood vessels. Eur. J. Pharmacol. 146, 85–95.

Hecker, M., 2000. Endothelium-derived hyperpolarizing factor—fact or fiction? News Physiol. Sci. 15, 1–5.

Huang, P.L., Huang, Z., Mashimo, H., Block, J.D., Moskowitz, M.A., Bevan, J.A., Fishman, M.C., 1995. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 377, 239–242.

Jerez, S., Peral de Bruno, M., Coviello, A., 2001a. Efecto de antagonistas de los receptores de angiotensin II y α_2-adrenérgicos sobre la liberación de NO inducida por angiotensin II. Medicina 61, 431–436.

Jerez, S., Peral de Bruno, M., Coviello, A., 2001b. Endothelium-dependent desensitization to angiotensin II in rabbit aorta: the mechanisms involved. Can. J. Pharmacol. 79, 481–489.

Jerez, S., Peral de Bruno, M., Coviello, A., 2004. Cross-talk between angiotensin II and alpha 1 adrenergic receptors in rabbit aorta: role of endothelium. J. Cardiovasc. Pharmacol. 43, 402–409.

Keen, H.L., Brands, M.W., Smith, M.J., Shek, E.W., Hall, J.E., 1997. Thromboxane is required for full expression of angiotensin hypertension in rats. Hypertension 29, 310–314.

Lin, L., Nasjletti, A., 1991. Role of endothelium-derived prostanoid in angiotensin-induced vasoconstriction. Hypertension 18, 158–164.

Lin, L., Balazy, M., Pagano, P.J., Nasjletti, A., 1994. Expression of prostaglandin H2-mediated mechanism of vascular contraction in hypertensive rats. Circ. Res. 74, 197–205.

Luscher, T.F., 1990. Imbalance of endothelium-derived relaxing and contracting factors: a new concept in hypertension? Am. J. Hypertens. 3, 317–330.

MacKenzie, A., Martin, W., 1998. Loss of endothelium-derived nitric oxide in rabbit aorta by oxidant stress: restoration by superoxide dismutase mimetics. Br. J. Pharmacol. 124, 719–728.

Mais, D.E., Saussy, D.L., Chaikouni, A., Kochel, P.J., Knapp, D.R., Hamanaka, N., Halushka, P.V., 1985. Pharmacologic characterization of human and canine thromboxane A_2/prostaglandin H_2 receptors in platelets and blood vessels: evidence for different receptors. J. Pharmacol. Exp. Ther. 233, 418–424.

Mihm, M.J., Wattanapitayakul, S.K., Piao, S.F., Hoyt, D.G., Bauer, J.A., 2003. Effects of angiotensin II on vascular endothelial cells: formation of receptor-mediated reactive nitrogen species. Biochem. Pharmacol. 65, 1189–1197.

Mistry, M., Nasjletti, A., 1988. Role of pressor prostanoids in rats with AII-salt-induced hypertension. Hypertension 11, 758–762.

Moncada, S., Palmer, R.M., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Nasjletti, A., 1998. The role of eicosanoids in angiotensin-dependent hypertension. Hypertension 31, 194–200.

Oriowo, M.A., Bevan, R.D., Bevan, J.A., 1991. Variable receptor affinity and tissue sensitivity. Blood Vessels 28, 115–121.

Pagano, P.J., Ito, Y., Tornheim, K., Gallop, P.M., Tauber, A.I., Cohen, R.A., 1995. An NADPH oxidase superoxide-generating system in the rabbit aorta. Am. J. Physiol. 268, H2274–H2280.

Quilley, J., Fulton, D., McGiff, J.C., 1997. Hyperpolarizing factors. Biochem. Pharmacol. 54, 1059–1070.

Raij, L., 2001. Hypertension and cardiovascular risk factors. Role of the angiotensin II–nitric oxide interaction. Hypertension 37, 767–773.

Rubanyi, G.M., Vanhoutte, P.M., 1986. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. Am. J. Physiol. 250, 822–827.

Schuijt, M.P., Tom, B., de Vries, R., Saxena, P.R., Sluiter, W., van Kats, J.P., Danser, A.H., 2003. Superoxide does not mediate the acute vasoconstrictor effects of angiotensin II: a study in human and porcine arteries. J. Hypertens. 21, 2335–2344.

Souza, H.P., Laurindo, F.R., Ziegelstein, R.C., Berlowitz, C.O., Zweier, J.L., 2001. Vascular NAD(P)H oxidase is distinct from the phagocytic enzyme and modulates vascular reactivity control. Am. J. Physiol. 280, H658–H667.

Touyz, R.M., 2004. Reactive oxygen species and angiotensin II signaling in vascular cells—implications in cardiovascular disease. Braz. J. Med. Biol. Res. 37, 1263–1273.

Udosen, I.T., Jiang, H., Hercule, H.C., Oyekan, A.O., 2003. Nitric-oxide epoxygenase interactions and arachidonate-induced dilation of rat renal microvessels. Am. J. Physiol. 285, H2054–H2063.

Yang, D., Félétou, M., Boulanger, C.M., Wu, H.F., Levens, N., Zhang, J. N., Vanhoutte, P.M., 2002. Oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in aortas from spontaneously hypertensive rats. Br. J. Pharmacol. 136, 104–110.

Zerrouk, A., Auguet, M., Chabrier, P.E., 1998. Augmented endothelium-dependent contraction to angiotensin II in the SHR aorta: role of an inducible cyclooxygenase metabolite. J. Cardiovasc. Pharmacol. 31, 525–533.

Zhang, J., Van Meel, J.C.A., Pfaffendorf, M., Zhang, J.S., Van Zwieten, P. A., 1994. Endothelium-dependent, nitric oxide-mediated inhibition of angiotensin II-induced contractions in rabbit aorta. Eur. J. Pharmacol. 262, 247–253.