Cross Talk between Angiotensin II and Alpha 1 Adrenergic Receptors in Rabbit Aorta: Role of Endothelium

Susana Jerez, PhD, Peral de Bruno María, PhD, and Coviello Alfredo, PhD

Abstract: Interaction between the renin-angiotensin system and the sympathetic nervous system has been proposed to be like a physiological regulation mechanism. The present work was designed to study the cross talk between angiotensin II and adrenergic receptors on the smooth muscle contractile response and the endothelium influence in this phenomenon. Homologous and endothelium independent desensitization of angiotensin II-contractile response was observed. Treatment with noradrenaline between two cumulative doses response curves (CDRC) to angiotensin II caused a rightward shift of the second CDRC in unrubbed arteries and increased the maximal response in rubbed arteries. Prazosin blocked these effects. No homologous desensitization of noradrenaline contractile response was found. Treatment with angiotensin II between two CDRC to noradrenaline caused a loss of affinity in the second CDRC in unrubbed arteries. Losartan was able to avoid this phenomenon. Maximal response was enhanced both in arteries with and without endothelium treated or not with angiotensin II. Results demonstrate homologous and endothelium-independent desensitization of the contractile response to angiotensin II but not to noradrenaline. In addition, heterologous and endothelium-dependent desensitization induced by noradrenaline and angiotensin II on the contractile response to each other was found. Furthermore, results provided the first evidence that there is an endothelium-dependent cross talk between α_1-adrenergic and angiotensin II receptors in smooth muscle of rabbit aorta.

Keys Words: adrenergic receptors, angiotensin II, cross talk, endothelium

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Several experimental and clinical studies have clearly demonstrated physiologically important interactions between the renin-angiotensin system and the sympathetic nervous system. Experimental evidence suggests that these 2 systems have an effect on each other both at the level of the peripheral vasculature and at the central nervous system level.[1] Angiotensin II (Ang II) produces vasoconstriction not only through the interaction with AT_1 receptors in the vascular smooth muscle but also through its ability to modulate sympathetic neural function.[2] It is known that Ang II facilitates neurotransmitter release from the presynaptic nerves terminals, which can cause vasoconstriction and myocardial damage.[3,4] Furthermore, important interactions between AT_1 receptors and α_1-adrenergic receptors (α_1-ARs) also exist. It has been shown that released noradrenaline (NA) negatively regulates Ang II receptors in cultured brain neurons[5] and in vascular tissue through its interactions with α_1-Ars.[6] In neonatal rat cardiac myocytes Ang II selectively down-regulates α_{1a}-AR subtype mRNA and its corresponding receptors.[7] However, recently there has been increasing evidence that the cross talk between AT_1 and α_1-ARs is present only under physiologic conditions because it is lacking in neurons of spontaneously hypertensive rat brain[8] and aortic rings of cardiomyophatic hamsters.[9]

Numerous reports have shown that the exposure to elevated catecholamines or Ang II results in homologous desensitization of both adrenergic or AT_1-mediated vascular smooth muscle contraction in the rat or rabbit aorta.[10–12] This desensitization mediated by G-protein coupled receptors may result from changes in receptors, G proteins, carriers, or the interaction among these component systems.[13,14] Furthermore, it has been reported that desensitization of NA receptor function and homologous short-term desensitization to Ang II may be attributed to G protein uncoupling.[15,16]

On the other hand, it has also been shown[17] that the control of vascular tone is the function of 2 regulatory systems: the sympathetic nervous system and the endothelium. NA can affect responses of the endothelium and endothelium-derived factors can alter responses of the NA. Moreover, Ang II stimulates endothelial synthesis of vasodilators such as NO[18–20] and prostaglandins[21] and vasoconstrictors such as endothelin[22] and lipoxygenase-derived eicosanoids.[23,24] However, the endothelium influence on the interaction between AT_1 and α_1-ARs has not been studied. In a previous work we have found that the

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

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Reprints: Susana Jerez, PhD, España 3706, 4000, Tucumán, Argentina (e-mail: sjerez@herrera.unt.edu.ar).

endothelium was able to enhance the Ang II-desensitization in rabbit aortic rings.[25]

The present study was designed to study (i) the cross talk between Ang II and adrenergic receptors in the smooth muscle contractile response and (ii) the influence of the endothelium in this phenomenon.

METHODS

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 cleaned of adherent fat and connective tissue. 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 were recorded under an initial tension of 2 g, which had been found to be the optimal tension for KCl-induced contraction (100 mM). All preparations were allowed to equilibrate for 90 minutes and were washed with Krebs solution at 15-minute 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 whether the endothelium had been removed. The rings were stimulated with NA 5.10^{-6} M and when the maximal contraction was achieved, acetylcholine 10^{-6} M was added to establish its relaxing effect.

Experimental Protocol

Aortic rings (rubbed or unrubbed) were exposed to increasing doses of Ang II (10^{-10} to $2.5\ 10^{-6}$ M) at 90-minute intervals to construct 2 cumulative dose-response curves (CDRC I and CDRC II). Because many pharmacologists have indicated that the contractile response to Ang II is impaired as compared CDRC to multiple single doses, the maximal contractile response of the tissue was carefully reached every time. This means that intrinsic activity would not be blunted.

To evaluate Ang II-NA cross talk and the endothelium influence in this phenomenon, arteries with or without endothelium were treated with one CDRC to NA following the first CDRC to Ang II. Rings were rinsed and a 30-minute recovery period was allowed before the next exposure to Ang II (Fig. 1). To study the role of α_1-ARs in the heterologous desensitization to Ang II the α_1-ARs antagonist, prazosin 10^{-6} M was added to the bath 30 minutes before CDRC I in arteries with and without endothelium. Prazosin was removed by washing before the CDRC to NA. Furthermore, to establish the influence of agonists-exposition time in the cross talk, 2 stimulations with single doses of NA 5.10^{-7} M were allowed in arteries with and without endothelium. In matched experiments 1 CDRC to Ang II between the 2 stimulations with NA 5.10^{-7} M was performed.

In a similar protocol, 2 CDRC to NA (10^{-8} to $2.5\ 10^{-5}$ M) at 90-minute intervals were constructed both in rubbed and unrubbed arteries. The other group was treated with 1 CDRC to Ang II following the first CDRC to NA. Rings were rinsed and a 30-minute recovery period was allowed before the next exposure to NA. In matched experiments single CDRC to Ang II was constructed after a 60-minute rinsed period and then 1

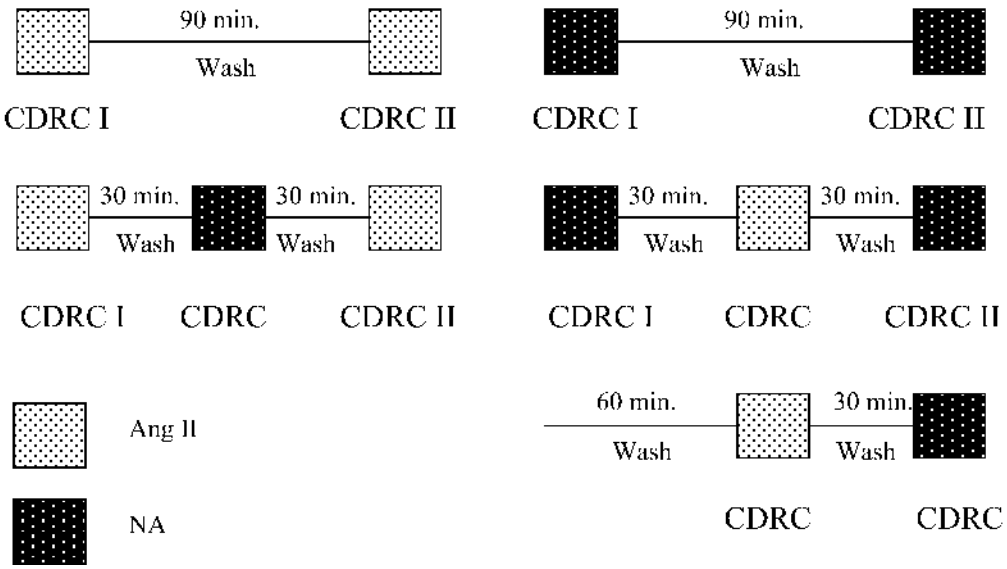

FIGURE 1. Graphic presentation of different protocols. The squares represent agonist stimulation. The protocols were performed both in rubbed and unrubbed arteries.

CDRC to NA was performed. To study the role of AT_1 receptors in the cross talk, losartan 10^{-7} M was added to the bath 30 minutes before the CDRC I to NA in arteries with endothelium. The AT_1 receptor antagonist was removed by washing before the CDRC to Ang II.

Results are expressed as mg of isometric contraction or a percentage of the maximal contractile force obtained during the CDRC I for Ang II or NA.

Statistical Analysis

Data are presented as mean values ± SEM and were analyzed by ANOVA with replications and Duncan test to evaluate CDRC. The pD_2 (negative log of molar concentration of Ang II inducing 50% of the maximal contraction) and the maximal contractile response were calculated using a curve-fitting analysis program. Student *t* test paired or unpaired were used to compare pD_2 values or maximal response. *P* less than 0.05 was considered statistically significant (two-tail test).

RESULTS

Effects of Ang II on Contractile Response of Arteries with and without Endothelium

The contractile response to Ang II (10^{-9} to $2.5.10^{-6}$ M) was dose dependent. The pD_2 values of CDRC I were similar in arteries with and without endothelium. However, a significant shift to the right of second exposure to Ang II was observed in unrubbed (Fig. 2A) and rubbed (Fig. 2B) arteries. The quantity of the rightward shift was similar (n.s., ANOVA and Duncan test). No significant differences were found in maximal response from arteries with and without endothelium both in CDRC II and I (Table 1).

NA treatment between the first and the second exposure to Ang II increased Ang II desensitization in unrubbed (Fig. 2C) but not in rubbed aortic rings (Fig. 2D). Nevertheless, the maximal contractile response to Ang II-CDRC II was not modified by NA-treatment in arteries with endothelium but was enhanced in endothelium-denuded preparations (Table 1).

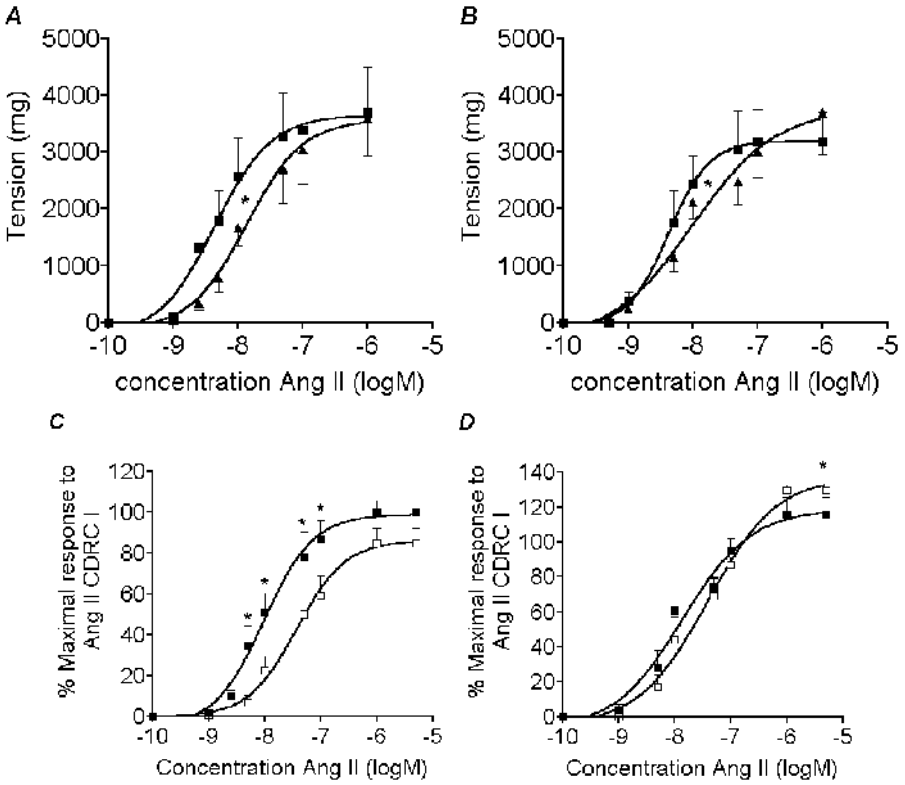

FIGURE 2. Cumulative doses-response curves (CDRC) to Ang II in rabbit aortic rings. A (with endothelium) and B (without endothelium): (■) first CDRC (▲) second CDRC performed after 90- minute washed period. Results are expressed as mg of isometric contraction. * $P < 0.05$ indicates a statistically significant difference in pD_2 between first and second CDRC (A: 8.27 ± 0.12 vs 7.94 ± 0.19; B: 8.17 ± 0.14 vs 7.89 ± 0.21). C (with endothelium) and D (without endothelium): (■) Control (□) NA-treated CDRC to Ang II. Results are expressed as a percentage of the maximal contractile response to Ang II of the first CDRC (CDRC I). * $P < 0.01$ indicates a statistically significant difference between control CDRC and NA-treated CDRC (ANOVA with replications and Duncan test). Values are means and vertical lines indicate SEM of 6 experiments.

TABLE 1. Maximal Response (R_{max}) to Ang II and NA in rabbit aortic rings

	R_{max} (mg)	
Ang II	**CDRC I**	**CDRC II**
E (+)	3703 ± 818	3696 ± 696
E (−)	3011 ± 645	3695 ± 822
E (+) + NA	3726 ± 1043	3235 ± 679
E (−) + NA	2770 ± 744	3717 ± 897*
NA		
E (+)	6434 ± 1276	7966 ± 1479*
E (−)	5097 ± 1140	6455 ± 1408*
E (+) + Ang II	7242 ± 1229	8223 ± 1491*
E (−) + Ang II	7611 ± 1479	8577 ± 1196*

E (+): arteries with endothelium; E (−): arteries without endothelium; + NA: arteries treated with noradrenaline between 2 CDRC to Ang II; + Ang II: arteries treated with angiotensin II between 2 CDRC to NA. *$P < 0.05$ indicates a statistically significant difference between CDRC I and CDRC II (paired t test). Results are expressed as mean ± SEM.

A single dose of NA 5.10^{-7} M was not able to modify either the contractile response or the pD_2 in Ang II-CDRC both in arteries with and without endothelium (Table 2).

Effect of Prazosin Treatment on Contractile Response to Ang II in Arteries with and without Endothelium

In the presence of prazosin, a significant shift to the right of Ang II-CDRC I was observed in rubbed ($pD_2 = 8.02 \pm 0.16$, control; $pD_2 = 7.58 \pm 0.09$, prazosin, $P < 0.01$, $n = 6$) and unrubbed arteries ($pD_2 = 8.28 \pm 0.08$, control; $pD_2 = 7.35 \pm 0.17$ prazosin, $P < 0.01$, $n = 6$). Maximal contractile response was not modified. However, prazosin treatment was able to avoid the rightward shift of the second CDRC that has been observed in aortic rings with intact endothelium without any effect on the maximal response (Fig. 3). Furthermore, prazosin inhibited the maximal contractile response increase in endothelium- denuded arteries (Table 3).

TABLE 2. Effect of Pre-Treatment With NA 5.10^{-7} M on pD_2 and maximal response (R_{max}) to Ang II in Rabbit Aortic Rings

	pD_2	R_{max} (mg)
E (+)	8.24 ± 0.12	3562 ± 421
E (−)	8.15 ± 0.15	4242 ± 562
E (+) + NA	8.37 ± 0.06	3967 ± 424
E (−) + NA	8.28 ± 0.08	4186 ± 413

E (+): arteries with endothelium; E (−): arteries without endothelium; + NA: arteries treated with a single dose of NA 5.10^{-7} M prior to one CDRC to Ang II. Statistical analyses were performed with ANOVA and Duncan test. Values are expressed as mean ± SEM of 8 experiments.

Effects of NA on Contractile Response of Arteries with and without Endothelium

The contractile response to NA (10^{-8} to $2.5\ 10^{-5}$ M) was dose dependent. The pD_2 values of CDRC I were similar in arteries with (6.37 ± 0.10) and without endothelium (5.93 ± 0.10). In addition, no significant differences in pD_2 were observed in the second exposure to Ang II both in unrubbed (6.37 ± 0.08, n.s, $n = 8$; Fig. 4A) and rubbed arteries (5.97 ± 0.12, n.s, $n = 8$). However, the maximal contractile response was increased both in aortic rings with and without endothelium (Table 1).

Ang II treatment between the first and the second exposure to NA induced a significant rightward shift of the second CDRC to NA in unrubbed arteries (pD_2= 1st: 6.37 ± 0.08; 2nd: 6.15 ± 0.06, $P < 0.001$, $n = 14$; Fig. 4B). However, in rubbed aortic rings no differences were found in pD_2 values (1st: 6.21 ± 0.14; 2nd: 6.22 ± 0.06, n.s., $n = 8$). Furthermore, a maximal contractile response increase was also observed in rubbed and unrubbed aortic rings (Table 1).

Increase in contractile response to NA 5.10^{-7} M during the second stimulation was observed in aortic rings with endothelium (1st: 2226 ± 572 mg; 2nd: 3520 ± 715 mg, $P < 0.001$, $n = 8$) and without endothelium (1st: 2204 ± 640 mg; 2nd: 3287 ± 666 mg, $P < 0.001$, $n = 8$). This effect was not modified by treatment with Ang II between the 2 stimulations in arteries with endothelium (1st: 1780 ± 432 mg; 2nd: 2965 ± 540 mg, $P < 0.001$, $n = 8$) and denuded-endothelium (1st: 1962 ± 690 mg; 2nd: 3219 ± 864 mg, $P < 0.001$, $n = 8$).

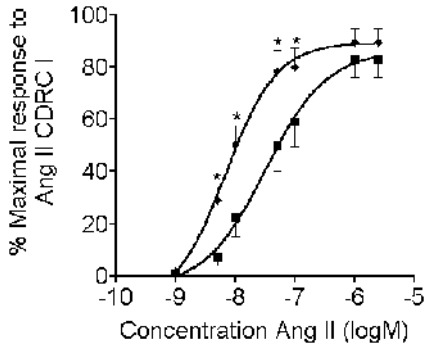

FIGURE 3. Cumulative doses-response curves (CDRC) to Ang II in rabbit aortic rings with endothelium treated with one CDRC to NA (■) Control: NA-treated CDRC to Ang II. (♦) Control plus prazosin 10^{-6} M treatment during the first CDRC to Ang II. Results are expressed as a percentage of the maximal contractile response to Ang II of the CDRC I. * $P < 0.01$ indicates a statistically significant difference between control CDRC and prazosin-treated CDRC (ANOVA with replications and Duncan test). Values are means and vertical lines indicate SEM of 6 experiments.

TABLE 3. Effect of NA and Prazosin Treatment on the Contractile Response to Ang II in Arteries Without Endothelium

Ang II Dose	Control (%)	+ NA (%)	+ Prazosin (%)
10^{-9} M	4 ± 3	0	2 ± 1
5×10^{-9} M	28 ± 10	17 ± 7	29 ± 7
10^{-8} M	61 ± 8	44 ± 13	49 ± 11
5×10^{-8} M	74 ± 6	69 ± 15	69 ± 9
10^{-7} M	95 ± 7	86 ± 9	75 ± 8
10^{-6} M	115 ± 12	129 ± 3	92 ± 8*

+ NA: arteries treated with 1 CDRC to NA between 2 CDRC to Ang II. + prazosin: arteries treated with prazosin 10^{-6} M on the first CDRC to Ang II. Results are expressed as a percentage of the maximal contractile response obtained during the first CDRC to Ang II. *$P < 0.05$ indicates statistically significant difference at Ang II 10^{-6} M between aortic rings treated with NA and arteries treated with prazosin. Statistical analyses were performed with ANOVA and Duncan test. Values are mean ± SEM of 6–8 experiments.

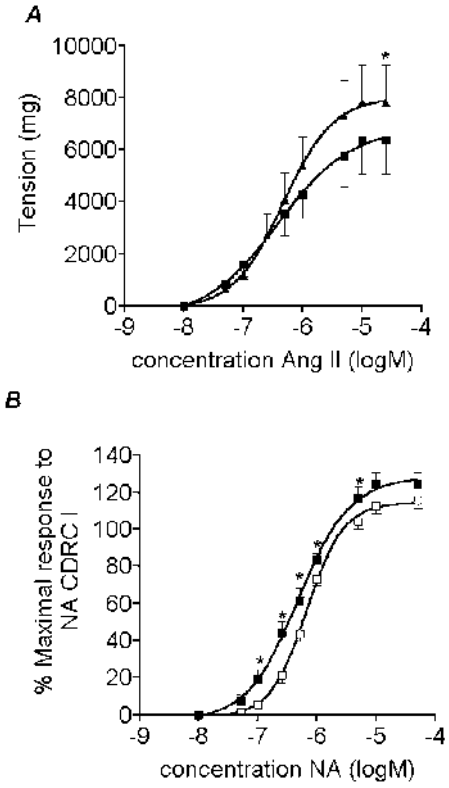

FIGURE 4. Cumulative doses-response curves (CDRC) to NA in rabbit aortic rings with endothelium. A, (■) first CDRC (▲) second CDRC performed after 90-minute washed period. Results are expressed as mg of isometric contraction. * $P < 0.01$ indicates a statistically significant difference in maximal response between first and second CDRC. B, (■) control (□) Ang II-treated CDRC to NA. Results are expressed as a percentage of the maximal contractile response to NA of the CDRC I. * $P < 0.01$ indicates a statistically significant difference between control CDRC and Ang II-treated CDRC (ANOVA with replications and Duncan's test). Values are means and vertical lines indicate SEM of 6-8 experiments.

Effect of Single CDRC to Ang II or NA on the Affinity and Maximal Response of Subsequent CDRC to NA or Ang II, Respectively

One CDRC to NA before single CDRC to Ang II caused a shift to the right in aortic rings with endothelium. pD_2 values either in aortic rings without (control) and with previous exposure to NA were 8.48 ± 0.07 and 8.22 ± 0.08, respectively ($P < 0.05$, n = 14). In rubbed aortic rings no differences were found in pD_2 (8.20 ± 0.16 vs 8.03 ± 0.08, n.s, n = 14). There were no significant differences in the maximal contractile response.

Nevertheless, 1 CDRC to Ang II before single CDRC to NA caused no differences in either affinity or the maximal contractile response in arteries with endothelium (pD_2 = 6.26 ± 0.07 vs 6.37 ± 0.08, n.s., n = 8) and without endothelium (pD_2 = 6.35 ± 0.12 vs 6.21 ± 0.06, n.s., n = 8).

Effect of Losartan Treatment on Contractile Response to NA in Arteries with and without Endothelium

Losartan did not modify either the pD_2 (6.00 ± 0.27, control; 6.11 ± 0.25; losartan, n.s, $n = 8$) or the maximal contractile response (6034 ± 978 mg, control; 6228 ± 839, losartan, n.s, $n = 8$) of the first CDRC to NA. However, losartan treatment was able to avoid the rightward shift that has been observed in the second exposure to NA after Ang II treatment (Fig. 5). Increase in the maximal contractile response was not modified (1^{st}: 6228 ± 839; 2^{nd}: 7633 ± 998, $P < 0.01$, $n = 8$).

DISCUSSION

Results obtained in the present study showed that desensitization to Ang II contractile response in rabbit aortic rings (rubbed and unrubbed) is not reversible after a 90-minute recovery period after the first exposition. As early as 1980, Gunther et al[26] reported the first direct evidence, by radioligand binding assay, that Ang II regulates the number of its own receptors in resistance vasculature. Further investigations revealed that AT_1 receptor activation is subject to a negative feedback, in that increased levels of Ang II diminish and decreased Ang II concentrations enhance AT_1 receptor activation.[27,28]

In a previous work we demonstrated in rabbit aorta that the presence of an intact endothelium increased Ang II–desensitization.[25] Furthermore, there are 2 mechanisms involved in the development of Ang II-tachyphylaxis; one involves endothelium influence and one occurs at the level of the smooth muscle and is endothelium-independent. The endothelium-dependent tachyphylaxis is related with the intrinsic contractile property and the endothelium-independent tachyphy-

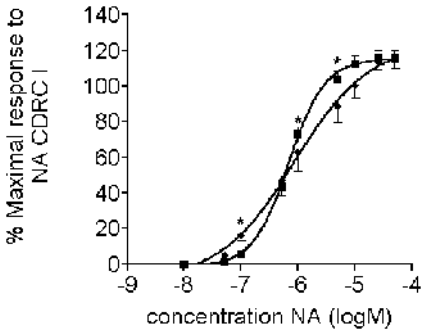

FIGURE 5. Cumulative doses-response curves (CDRC) to NA in rabbit aortic rings with endothelium treated with one CDRC to Ang II. (■) Control: Ang II-treated CDRC to NA. (♦) Control plus losartan 10^{-6} M treatment during the first CDRC to NA. Results are expressed as a percentage of the maximal contractile response to NA of the CDRC I. * $P < 0.01$ indicates a statistically significant difference between control CDRC and losartan treated CDRC (ANOVA with replications and Duncan test). Values are means and vertical lines indicate SEM of 8 experiments.

laxis is related with the loss of affinity. In the present work the recovery time after the first CDRC to Ang II was larger and no differences were found in the maximal contractile response between the first and the second CDRC to Ang II. This result suggests the endothelium-dependent desensitization disappearance. However, a shift to the right of the CDRC II to Ang II was observed in rubbed and unrubbed arteries. This would mean that the endothelium-independent tachyphylaxis can not be reversed by increasing the recovery time. This result is in agreement with the previous finding of Kuttan and Sim[12] and Gruetter et al[29] in rat aorta. Tachyphylaxis is associated with changes at the receptor level (ie, change in affinity and coupling efficiency). The factors involved can be derived from the endothelium or the smooth muscle cell. Kai et al[15] found in intact vascular smooth muscle cells homologous desensitization of the IP_3 response to the subsequent stimulation, but not downregulation of $AT_{1A}R$ or protein $G_{\alpha q}/G_{\alpha 11}$ after short-term exposure (stimulation with single dose during 10 minutes) to Ang II.

Recently, it has been shown that multiple agonists other than Ang II modulate AT_1 receptor function.[30] This phenomenon, referred to as heterologous AT_1 receptor regulation, is induced by various factors (eg, glucocorticoids, aldosterone, forskolin, nitric oxide, etc.), including NA, all of which downregulate AT_1 receptor expression. Yang et al[8] demonstrated that negative feedback regulation of Ang II receptors by NA is a result of a decrease in AT_1 receptor gene expression in WKY brain neurons. In addition, neurons of SHR brain lack this downregulatory mechanism. The action of NA on AT_1 receptors involves the α_{1A}-adrenergic receptor and thus provides an example of the cross talk between these 2 receptors in the neurons.

Taking account of these data about the heterologous desensitization of the response to Ang II produced by NA prolonged infusion, we investigated the effects of interpolating one CDRC to NA after the first CDRC to Ang II. The result was an increase of the Ang II-desensitization in unrubbed arteries. This is in agreement with Seasholtz et al.[16] However, in aortic rings without endothelium the maximal contractile response was enhanced. A single dose of NA 5.10^{-7} M did not modify either the affinity or the maximal contractile response to Ang II both in rubbed and unrubbed arteries. That would mean this is a time- dependent phenomenon. Therefore, these results demonstrate endothelium-dependent enhancement of Ang II-contractile response desensitization induced by NA. The role of endothelium in this phenomenon will be discussed later. On the other hand, the increased intrinsic activity may be due to the fact that no counteracting action of endothelium-relaxing factors was observed and the equilibrium was displaced by release of vasoconstrictors; for that reason, the maximal response observed was greater.

A significant shift to the right in CDRC I to Ang II was observed in rubbed and unrubbed aortic rings treated with prazosin. This result is in agreement with Zimmerman et al[31] and Zimmerman,[2] who demonstrated that Ang II can facilitate peripheral sympathetic function through multiple mechanisms both at level of the central nervous system and peripherally at the level of the sympathetic nerve ending. In the latter case, it also may be the result either of an increased release of NA from sympathetic nerves, or the inhibition of NA uptake by sympathetic nerves,[32] or a direct effect on the excitation-coupling vascular smooth muscle mechanism.[33] In the present study, when the α_1-AR was blocked with prazosin during the first stimulation with Ang II, NA released from nerve ending by Ang II could not interact with its receptor. Therefore, the results obtained mean that α_1-AR stimulation would enhance Ang II, not only in contractile response but affinity. These data are in agreement with Majewsky et al.[4] However, prazosin was able to avoid the shift to the right of Ang II-CDRC II in arteries with endothelium. Furthermore, the increase in the maximal contractile response observed in rubbed aortic rings was blocked. These data prove a role of α_1-AR in the desensitization to Ang II because α_1-AR blocking during the first stimulation with Ang II eliminated completely the loss of affinity observed in the second stimulation. In addition, the inhibitory effect of prazosin in the improvement of the maximal response observed in rubbed arteries suggests the sensitization of the contractile mechanism caused by α_1-AR stimulation during the first CDRC to Ang II and the subsequent CDRC to NA. This effect was not observed in unrubbed arteries on account of the endothelium-counteracting action. To check the heterologous desensitization induced by NA without previous stimulation of AT_1 receptor, a single CDRC to NA was performed before 1 CDRC to Ang II. Results showed that NA stimulation shifted to the right the Ang II CDRC in unrubbed arteries. That

means no previous AT_1 receptor stimulation was necessary to the heterologous desensitization. Taken together, these findings would suggest on the one hand the presence of cross talk between α_1-AR and Ang II receptors and on the other hand an endothelium influence in such phenomenon.

No homologous desensitization of the contractile response to NA was observed with the present protocol. However, an increase endothelium-independent of the maximal contractile response was observed. This phenomenon was not time-dependent because it was also observed with single doses to NA. Previous reports have shown that exposure to elevated catecholamines or other receptor agonists in vivo or in vitro result in desensitization of α_1-AR–mediated vascular smooth muscle contraction in rat or rabbit aorta.[10, 34] The disagreement with the present results may be due to the different NA incubation and recovery periods used in the experiments. Treatment with 1 CDRC to Ang II after the first CDRC to NA caused a rightward shift of the second CDRC in unrubbed arteries. Losartan added during the first stimulation with NA blocked this effect. However, 1 CDRC to Ang II performed before 1 CDRC to NA was not able to induce NA desensitization both in rubbed and unrubbed arteries. These findings may suggest that cross talk between α_1-AR and AT_1-receptor during the first CDRC to NA is necessary for inducing heterologous desensitization. These data, with the data mentioned previously, support the view that endothelium plays an important role in the cross talk between Ang II and adrenergic receptors. According to the literature, cardiovascular signaling cross talk mediates both short- and long-term events, and coordination of the individual contributory pathways is regulated at various signaling junctions, particularly the G protein, AC, PK, and MAPK levels.[35] It has been previously reported that endothelium influence vascular smooth muscle intrinsic activity. However, endothelium influence on the hormone-receptor affinity has not yet been established. Oriowo et al[36] proposed that factors in the microenvironment of the receptor could alter the affinity of the receptor. The possible involvement of such factors is also suggested from the observation that the endothelium may be a source of endothelium-derived modulating factors, which influence the affinity of the angiotensin receptor.[12]

Griendling et al[37] reported that when Ang II binds to its AT_1 receptor in vascular smooth muscle, it initiates a biphasic response activating phospholipase C (PLC) and later on phospholipase D (PLD). In contrast to the PLC response, PLD activation does not appear to desensitize significantly for as long as 1 hour. This pathway is the most important source of phosphatidic acid and diacylglycerol and probably represents the major pathway by which protein kinase C (PKC) remains activated. On the other hand, potassium channels that are inhibited by internal ATP (K_{ATP} channels) provide a critical link between metabolism and cellular excitability. Light et al[38] demonstrated that PKC acts on K_{ATP} channels to regulate diverse cellular processes, including cardioprotection by ischemic preconditioning and pancreatic insulin secretion. PKC action decreases the Hill coefficient of ATP binding to cardiac K_{ATP} channels, thereby increasing their open probability at physiological ATP concentrations. In a previous paper[25] we found that Glibenclamide (K_{ATP}. blocker) was the only one potassium channels blocker able to avoid the loss of affinity on the second CDRC to Ang II. Furthermore, the K_{ATP} channels opener cromakalin increased the desensitization to Ang II in aortas without endothelium. Taking into account these data from the literature, we hypothesize that endothelium-dependent hyperpolarization induced by K_{ATP} channels-activation might modify Ang II affinity. That means a role of K_{ATP} channels in the endothelium-dependent cross talk between α_1-AR and Ang II receptor is possible. Nevertheless, further studies are necessary to establish the mechanisms involved in the endothelium-dependent cross talk.

In summary, we have provided the first direct evidence that there is an endothelium-dependent cross talk between α_1-AR and Ang II receptors in smooth muscle of rabbit aortic rings. Although is well established that the renin-angiotensin system is implicated in the development and maintenance of blood pressure elevation, numerous aspects about the role of Ang II in pathogenesis of essential hypertension are unclear. Cross talk between the renin-angiotensin system and sympathetic nervous system has received some attention in the literature and a more thorough appraisal of recent articles in this area is required. An altered regulation of Ang II at the cellular and molecular level could be fundamental in the pathology of essential hypertension. Both in vitro studies in cultured cells and data from whole animal experiments indicate that Ang II signaling in hypertension is upregulated. Lack of cross talk between the renin-angiotensin system and sympathetic nervous system might account for this phenomenon. By extension, it seems reasonable to assume that endothelial dysfunction associated with hypertension could play an important role in this interaction.

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