Analysis of the Vasorelaxant Action of Angiotensin II in the Isolated Rat Renal Artery

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Abstract. Taking into consideration that mechanisms involved in the vasodilator actions of angiotensin II have not yet been completely elucidated, the present study was undertaken in order to examine the mechanisms underlying the angiotensin II–induced relaxation of rat renal artery (RRA). Angiotensin II produced concentration-dependent and endothelium-independent relaxation of isolated RRA. Angiotensin II–induced relaxation was partially reduced by inhibitors of nitric oxide synthase and guanylyl cyclase. The remaining dilatation was inhibited by a potassium channel blocker, charybdotoxin. Precontraction of RRA with high concentration of K⁺ partially reduced angiotensin II-evoked relaxation, while indomethacin, glibenclamide, apamin and barium did not alter the angiotensin II concentration-response curve. Losartan had no effect on angiotensin II effect. Oppositely, HOE 140 and PD123319, separately or in combination, partially antagonized vasorelaxation induced by angiotensin II. Complete blockade of RRA response was obtained after simultaneous incubation of all three receptor antagonists HOE-140, PD123319, and losartan; L-NOARG plus HOE-140; or PD123319 plus charybdotoxin. These results indicate that angiotensin II produces endothelium-independent relaxation of RRA, which is most probably mediated by the interaction of the NO-cGMP pathway and K⁺ channels. Moreover, we can assume that AT₁, AT₂, and B₂ receptors are involved in the vasorelaxant effect of angiotensin II.

Keywords: angiotensin II, rat renal artery, vasorelaxation, K⁺ channel, AT receptor

Introduction

Angiotensin II is part of the complex renin-angiotensin system that has significant roles in regulation of blood pressure and vascular homeostasis. Two distinct subtypes of angiotensin II receptors, angiotensin AT₁ and AT₂, have been identified, based on their pharmacological and biochemical properties (1). Most of angiotensin II well-known actions, such as vasoconstriction, aldosterone release, stimulation of cell proliferation, are mediated through activation of AT₁-receptor subtype (2, 3). On the other hand, the physiological role of AT₂ receptors is still not completely understood. Previous studies have demonstrated that angiotensin II apart from its vasoconstrictor properties, may produce vasorelaxation through activation of AT₂ receptors (4–9). Angiotensin II–mediated vasodilatation has been observed in rabbit afferent arterioles (4), mesenteric artery of rats (5), rat aorta (6), rat carotid artery (7), canine renal and cerebral arteries (8), and human coronary microarteries (9). Experimental evidences suggest that angiotensin II can induce both endothelium-dependent (4, 9, 10) and endothelium-independent relaxation (6, 7, 11). However, the underlying mechanisms of angiotensin II vasodilator effect are still under investigation. Previous findings suggest involvement of the NO-cGMP pathway, prostaglandins, and kinin generation in angiotensin II–induced vasodilatation (12–14). Furthermore, studies performed on mesenteric arterial branches isolated from rats (11) provided evidence for the role of K⁺ channels, particularly large-conductance Ca²⁺-activated K⁺ channels (BKCa), in vasorelaxation.
mediated by angiotensin II.

More recently, it has been postulated that the role of angiotensin II–induced vasodilatation increases in different pathological conditions such as cardiovascular and renal diseases (15). Bautista et al. (16) have demonstrated that the number of the AT$_2$ receptors increased during development of kidney failure, which may account for enhanced angiotensin II–dependent vasodilatation. These authors suggested that increased vasodilator effects of angiotensin II may provide a protective mechanism that preserves renal perfusion in pathological conditions.

Taking into account the important role of renal artery in the maintenance of renal blood flow and renal function, as well as the possible protective role of the angiotensin II vasorelaxant effect, the present study was designed to investigate whether angiotensin II can induce relaxation of the rat renal artery (RRA). It was of particular interest to determine the role of intact endothelium and endothelial relaxing factors and to define the contribution of different K$^+$ channel subtypes in the angiotensin II–evoked vascular response. Furthermore, the aim of this study was also to examine the type of angiotensin II receptors involved in the relaxant actions of this peptide on isolated RRA.

**Materials and Methods**

**Vascular preparations**

The animal procedures were in full agreement with guidelines from Good Laboratory Practice and European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. The renal artery was isolated from male Wistar rats weighing 220–280 g, carefully dissected from surrounding fat and connective tissue, and cut into 4-mm-long circular segments. All vessel segments were immediately placed in Krebs-Ringer bicarbonate solution. The endothelium was removed from some rings by gently rubbing the intimal surface with stainless-steel wire. Ring preparations were mounted between two stainless-steel triangles in an organ bath containing 15 ml Krebs-Ringer bicarbonate solution (37°C, pH 7.4), aerated with 95% O$_2$ and 5% CO$_2$. One of the triangles was attached to a displacement unit allowing a fine adjustment of tension and the other was connected to a force-displacement transducer (Hugo Sachs Elektronik F30 Type 372; Hugo Sachs Elektronik, Freiburg, Germany). Isometric tension was continuously recorded on a Rikadenki R-62 multi-pen electronic recorder (Rikadenki Kogyo Co., Ltd., Tokyo).

The preparations were allowed to equilibrate for 45 min in Krebs-Ringer bicarbonate solution. During this period the organ baths were washed with fresh (37°C) buffer solution every 15 min. After 45 min, each ring was gradually stretched to the previously established optimal point of the resting tension for RRA, 2 g (17). Once at their optimal length, the segments were allowed to equilibrate for 30 min before experimentation.

**Experimental procedure**

At the beginning of each experiment, endothelium functional integrity was examined by precontraction of isolated RRA with submaximal concentration (EC$_{70}$) of phenylephrine (1 µM), followed by addition of acetylcholine (1 µM) (17). This procedure was repeated three times in 20-min intervals. If the maximal relaxant effect was more than 80% of the initial contraction, it was considered that functional endothelium is present.

In order to study vasorelaxant properties of angiotensin II in the RRA with and without endothelium, the segments were initially contracted with phenylephrine (1 µM). When the contraction reached a steady state, cumulative concentration-response (CR) curves to angiotensin II were obtained by adding increasing concentrations of this peptide (0.0001 – 100 µM). A subsequent concentration was added to the organ bath after the previous concentration had produced its equilibrium response. Cumulative concentration-response curves were constructed to angiotensin II in the absence of any pharmacological blocker (control) or after the incubation of a specific antagonist with equilibration periods of 20 min for N$^\omega$-nitro-L-arginine (L-NOARG); 30 min for 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), apamin, glibenclamide, barium, losartan, D-Arg[Hyp$^\beta$,Thr$^\alpha$,D-Tic$^\gamma$,Oic$^\varepsilon$]bradykinin (HOE-140), S-[(+)-1-[(4-dimethylamino-3-methylphenyl)methyl]-5-[diphenylacetyl]-4,5,6,7-tetrahydro-1H imidazol-[4,5-C]pyridine-6-carboxylic acid (PD123319), and charybdoxin; or 40 min for indomethacin and tetraethylammonium (TEA). Only one CR curve was obtained per tissue, since preliminary studies had shown that marked tachyphylaxis developed if a second CR curve to angiotensin II was constructed (data not shown) (18, 19). Therefore, one vascular segment served as a time control, while with another vascular ring from the same rat, the incubation of specific pharmacological blocker was performed.

Relaxation produced by each concentration of angiotensin II was measured and expressed as a percentage of the maximum obtained relaxation after phenylephrine precontraction.

In some experiments, vascular rings were precontracted with K$^+$-rich Krebs-Ringer-bicarbonate solution prepared by equimolar replacement of 100 mM NaCl...
with 100 mM KCl.

Data analyses
The relaxation induced by each concentration of angiotensin II was expressed as a percentage of the maximum obtained relaxation (i.e., relaxation back to the baseline tension). The relaxation provoked by the highest angiotensin II concentration (100 μM) at the end of its cumulative addition was considered as a maximal response (\(E_{\text{max}}\)). The concentration of angiotensin II producing 50% of its own maximum response (EC\(_{50}\)) was determined for each curve by using a non-linear least square fitting procedure of the individual experimental data and presented as pD\(_2\) (pD\(_2\) = −log EC\(_{50}\)).

The results are expressed as means ± S.E.M., where \(n\) refers to the number of experiments. All calculations and graphics were done by using the computer program Graph Pad Prism (Graph Pad Software, Inc., San Diego, CA, USA). Statistical significance of differences between two mean values was determined with Student’s \(t\)-test for paired or unpaired observations. A value of \(P<0.05\) was considered to be statistically significant.

Drugs and solutions
The Krebs-Ringer bicarbonate solution had the following composition: 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), 1.2 mM KH\(_2\)PO\(_4\), 25.0 mM NaHCO\(_3\), 0.026 mM Ca-EDTA, and 11.1 mM glucose. The solution was continuously bubbled with 95% O\(_2\) and 5% CO\(_2\), resulting in a pH of 7.4, and the temperature was kept at 37°C. The following drugs were used: angiotensin II, L-phenylephrine, indomethacin, glibenclamide, ODQ, HOE-140, PD123319, losartan, acetylcholine iodide, apamine, charybdotoxin, methacin, glibenclamide, ODQ, HOE-140, PD123319, 2.5 mM CaCl\(_2\), a nonselective potassium-channel blocker (500 μM), losartan (1 μM), an ATP-sensitive K\(^+\) channel (K\(_{\text{ATP}}\)) blocker had no effect (Table 3, \(n = 9\), \(P>0.05\)).

Results

Effect of angiotensin II on RRA precontracted with phenylephrine
Angiotensin II (0.1 nM – 100 μM) produced concentration-dependent relaxation of intact RRA rings precontracted with phenylephrine (pD\(_2\) = 7.33 ± 0.28, \(E_{\text{max}} = 97.0 ± 3.5\%\), \(n = 15\)) (Fig. 1A). After removal of endothelium, the vascular response to angiotensin II was not affected (pD\(_2\) = 7.08 ± 0.27, \(E_{\text{max}} = 95.5 ± 7.3\%\), \(n = 15\); \(P>0.05\)). Since no significant difference was found between endothelium-intact and endothelium-denuded rings, the rest of the experiments were conducted in denuded vascular segments.

Mechanisms underlying angiotensin II–induced relaxation
Angiotensin II–induced relaxation was unaffected (\(P>0.05\)) by indomethacin (10 μM, \(n = 7\)) (pD\(_2\) = 7.15 ± 0.27, \(E_{\text{max}} = 90.0 ± 3.5\%\) vs pD\(_2\) = 7.33 ± 0.19, \(E_{\text{max}} = 96.3 ± 7.1\%\) in the control). Pretreatment of RRA rings with l-NOARG (10 μM, \(n = 7\)), a non-selective nitric oxide (NO)–synthase inhibitor, or ODQ (1 μM, \(n = 6\)), a guanylyl cyclase inhibitor, produced significant reduction in the RRA response to angiotensin II (\(P<0.01\)) (Table 1 and Fig. 1: B and C).

Precontraction of RRA with K\(^+\)-rich Krebs-Ringer bicarbonate solution (KCl = 100 mM, \(n = 5\)) partially inhibited the RRA response to angiotensin II (\(P<0.05\)) (Table 2 and Fig. 2A). Similarly, in the presence of TEA, a nonselective potassium-channel blocker (500 μM, \(n = 6\)), or charybdotoxin (100 nM, \(n = 8\)), a selective blocker of large-conductance Ca\(^2+\)-activated K\(^+\) channels, angiotensin II–mediated dilatation was partially reduced (Table 2 and Fig. 2: B and C). After concomitant application of l-NOARG and charybdotoxin, complete block of the vasorelaxant effect of angiotensin II has been observed (\(E_{\text{max}} = 7.0 ± 0.3\%\) vs \(E_{\text{max}} = 95.7 ± 3.5\%\) in control, \(n = 6\)) (Fig. 2D). Incubation with apamin (1 μM), a selective blocker of low-conductance Ca\(^2+\)-activated K\(^+\) channels, barium (1 μM), a selective blocker of inward rectifying K\(^+\) channels, or glibenclamide (1 μM), an ATP-sensitive K\(^+\) channel (K\(_{\text{ATP}}\)) blocker had no effect (\(P>0.05\)) on RRA relaxation. (Table 3, \(n = 5 – 8\)).

Effect of receptor antagonists on the angiotensin II–induced relaxation
Angiotensin II–induced relaxation was unaffected by losartan (1 μM), a selective AT\(_1\)-receptor antagonist, (pD\(_2\) = 7.55 ± 0.10, \(E_{\text{max}} = 98.4 ± 4.5\%\) in the absence and pD\(_2\) = 7.25 ± 0.23, \(E_{\text{max}} = 95.2 ± 6.3\%\) in the presence of losartan, \(n = 9\), \(P>0.05\)). PD123319 (0.03 –
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1 µM, a selective AT2-receptor antagonist, produced significant (P<0.01) rightward shift of the concentration-response curves to angiotensin II with suppression of the maximal response. Concurrent incubation of vascular rings with PD123319 and losartan did not further inhibit vasorelaxation induced by angiotensin II (pD2 = 6.08 ± 0.35, Emax = 40.23 ± 4.58% in the presence of 1 µM PD123319 and pD2 = 6.19 ± 0.18, Emax = 41.2 ± 3.2% in the presence of PD123319 + losartan, n = 6, P<0.01).

HOE-140 (1 µM), a selective antagonist of bradykinin B2 receptor, reduced significantly, but not completely, the RRA response to angiotensin II (Table 1 and Fig. 3B). The administration of exogenous bradykinin (0.1 mM – 10 µM) on phenylephrine-precontracted endothelium-denuded RRA rings failed to produce relaxation (Emax = 7.6 ± 4.0%, n = 10).

In the presence of HOE-140, losartan, or PD123319 elicited further reduction of the angiotensin II–induced vasorelaxation (pD2 = 6.11 ± 0.11, Emax = 55.4 ± 6.3%...
and pD2 = 6.05 ± 0.12, Emax = 40.5 ± 3.9%, respectively, vs pD2 = 6.10 ± 0.36, Emax = 69.4 ± 3.0% in the presence of HOE-140, n = 6 for each, P<0.01) (Fig. 3B). In the presence of all three receptor antagonists (HOE-140 + losartan + PD123319), even high concentrations of angiotensin II failed to produce significant RRA relaxation (Emax = 4.6 ± 0.2% vs Emax = 93.5 ± 6.6% in the control, n = 5; Fig. 3D). On the other hand, neither concurrent incubation with HOE-140 and charybdotoxin nor with PD123319 and L-NOARG elicited any further reduction compared to application of these antagonists alone (pD2 = 6.35 ± 0.13, Emax = 60.4 ± 5.3% and pD2 = 6.09 ± 0.10, Emax = 53.4 ± 5.9%, respectively, n = 6 for each).

Discussion

The results of our experiments have shown that angiotensin II produced concentration-dependent and endothelium-independent relaxation of isolated RRA. Removal of the vascular endothelium did not produce any significant change in angiotensin II–induced relaxation, indicating that its vasodilator effect does not depend on endothelial functional integrity. Previous studies concerning the role of endothelium in angiotensin II–induced relaxation are not consistent. It has been shown that angiotensin II leads to an endothelium-independent release of NO.
The relaxation of fowl aorta (20), rat uterine artery (10), rat mesenteric artery (21), and human coronary microarteries (9). On the other hand, it has been demonstrated that angiotensin II relaxes rat aorta (7) and rat carotid artery (6) in an endothelium-independent manner, which is consistent with our results.

Although it has been proposed that prostacyclin (PGI2) may be involved in vasodilatation mediated by angiotensin II (5, 14), the present results have shown that indomethacin, a cyclooxygenase inhibitor, even at high concentration of 10 µM, failed to produce any significant change in the RRA response to angiotensin II. The experimental data from this part of our study are in agreement with the results of Kimura et al. (22) and Batenburg et al. (9).

Earlier reports suggested that angiotensin II–evoked vasorelaxation is mediated by the release of nitric oxide (6, 7, 23). It is well known that NO causes relaxation by stimulation of soluble guanylate cyclase with a consequent rise in the intracellular levels of cyclic 3,5-guanosino monophosphate (cGMP) (24, 25). Synthesis of NO can be inhibited by an analogue of L-arginine, L-NOARG (26), while synthesis of cGMP can be blocked with ODQ, a selective inhibitor of guanylyl cyclase enzyme (27). In our experiments, both L-NOARG and ODQ significantly reduced the concentration-response

Table 4. Effect of PD123319 (0.03 – 1 µM) on the angiotensin-induced relaxation of rat renal artery rings without endothelium

<table>
<thead>
<tr>
<th>Groups</th>
<th>pD2 ± S.E.M.</th>
<th>max. % relaxation ± S.E.M.</th>
<th>Significant versus control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.46 ± 0.29</td>
<td>95.40 ± 5.10</td>
<td></td>
</tr>
<tr>
<td>PD123319 (0.03 µM)</td>
<td>6.97 ± 0.24</td>
<td>70.56 ± 6.34</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>PD123319 (0.1 µM)</td>
<td>6.89 ± 0.40</td>
<td>70.03 ± 3.72</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>PD123319 (0.3 µM)</td>
<td>6.83 ± 0.41</td>
<td>63.10 ± 5.83</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>PD123319 (1 µM)</td>
<td>6.08 ± 0.35</td>
<td>40.23 ± 4.58</td>
<td></td>
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</tbody>
</table>
curve for angiotensin II, suggesting that NO and cGMP are involved in its vasodilator effect. Comparable results were obtained in investigations of angiotensin II–induced relaxation of rat aorta, rat carotid artery, and rat uterine artery (5 – 7, 9). Since we have demonstrated that angiotensin II induces endothelium-independent relaxation of isolated RRA, it may be assumed that NO and cGMP derived from smooth muscle cells contribute to the relaxant effects of angiotensin II. This presumption is consistent with previous findings, that indicate the ability of vascular smooth muscle cells to produce NO (28, 29) followed by the increase in cGMP content (30). In addition, it has been shown that nNOS may play important role in the control of renal vascular resistance (31, 32). It has been also demonstrated by using a selective inhibitor of the neural isoform of NO synthase, 7-nitroindazole, that activation of AT2 receptors stimulates renal production of nitric oxide in conscious rats by activating neuronal NOS (33).

It is established that the role of potassium flow across the cellular membrane related to the smooth muscle relaxation can be investigated by precontraction of isolated vessels with a buffer containing high concentration of K+ (34). In the present study, we have shown that in the presence of elevated extracellular K+ concentration, angiotensin II–mediated relaxation was significantly reduced. On the basis of these findings, it could be assumed that RRA relaxation induced by angiotensin II partially depends upon increased smooth muscle potassium conductance.

The role of K+ channels in angiotensin II–mediated vasorelaxation has been already described (10, 21). Our results have shown that apamin, a selective blocker of the low-conductance Ca2+-activated K+ channels (SKCa), glibenclamide, a selective blocker of ATP-sensitive K+ (KATP) channels, or barium, a selective blocker of inward rectifying K+ channels, consistently failed to change control angiotensin II relaxation. Thus, we may suggest that SKCa, Kir, and KATP channels most probably do not participate in RRA response to angiotensin II. In accordance to this finding, Dimitroupoulou et al. (11) and De Moura et al. (21) found that activation of SKCa, Kir, and KATP are not involved in the angiotensin II-induced rat mesenteric artery relaxation. On the other hand, we have shown that administration of TEA or charybdotoxin (a blocker of large-conductance Ca2+-activated K+ channels) produced significant but not complete inhibition of angiotensin II–induced relaxation. Although, TEA and charybdotoxin are not specific blockers of the BKCa channels, in our experiments they were used in the concentrations selective to block BKCa channels (35 – 37). Thus, our results favor the conclusion that BKCa channels participate in vasorelaxant effect of angiotensin II. The role of BKCa channels in angiotensin II–induced relaxation has been previously demonstrated in studies on rat mesenteric artery (11) and rat aorta (7). Since complete reduction of the concentration-response curve was obtained only after simultaneous incubation of L-NOARG and charybdotoxin, we could assume that the NO-cGMP pathway and BKCa-channel activation are independent and both participate to a similar extent in angiotensin II–induced RRA relaxation. Comparable observations were obtained in rat cerebral arteries, where only the presence of both L-NOARG and K+ channel blockers fully antagonized the angiotensin II relaxant effect, suggesting that these pathways are independent (14).

It has been previously shown that mechanisms other than the NO-cGMP pathway and K+ channels, such as a kinin generation, participate in the angiotensin II vasorelaxant effect (33, 38). In the present study, HOE-140, a selective antagonist of B2 kinin receptors, significantly reduced angiotensin II–induced relaxation, indicating that activation of B2 receptors contribute to the relaxation of isolated RRA. The precise mechanism underlying interaction between angiotensin II and B2 receptors is still not clear. Previous results of Tsutsumi et al. (39) have shown that angiotensin II causes intracellular acidosis through inhibition of amiloride-sensitive Na+/H+ exchanger activity, resulting in enhancement of kininogenase activity in vascular smooth muscle cells and bradykinin generation. According to these authors, released bradykinin exerts vasodilatation through paracrine activation of B2 receptors. In order to verify this hypothesis we applied bradykinin exogenously on phenylephrine-precontracted denuded RRA rings. Since our experiments have shown that bradykinin can not relax RRA in an endothelium-independent manner, it may be proposed that bradykinin generation after angiotensin administration is not responsible for B2-receptor activation. Therefore, we can assume that some other mechanisms rather than bradykinin formation underlie B2-receptor activation. According to the findings of AbdAlla et al. (40), a heterodimerization between AT1 and B2 receptors on vascular smooth muscle cells may be involved.

Angiotensin II controls vascular tone by activating angiotensin type 1 (AT1) and type 2 (AT2) receptors (1). In the present study, we have shown that PD123319, a selective antagonist of the AT2 receptors, but not losartan, a selective antagonist of the AT1 receptors, significantly reduced angiotensin II–mediated relaxation. Taking into account that even the highest concentrations of PD123319 did not fully antagonize relaxation, it may be suggested that AT2 receptors are not the only receptor type involved in RRA relaxation. Incubation of both
PD123319 and HOE-140 or losartan and PD123319 led to further inhibition of the angiotensin II response. However, the angiotensin II–induced vasodilatation was fully antagonized only after administration of all three receptor antagonists at the same time. On the basis of this finding, we may propose that interaction between AT₁, AT₂, and B₂ receptors on the vascular smooth muscle cell membrane underlies vasodilatation mediated by angiotensin II. The role of both AT₁- and AT₂-receptor subtypes in angiotensin II–induced relaxation has been already shown in studies on rat cerebral artery (14). Moreover, since we have demonstrated that RRA response does not depend on the functional integrity of the endothelium, it could be assumed that AT₂ receptors are present on the vascular smooth muscle cells of rat renal blood vessels.

The obtained concentration-response curves for angiotensin II in the presence of increasing concentrations of PD123319 clearly indicate the presence of irreversible competitive antagonism, which further suggests possible involvement of receptors other than AT₂. This is consistent with our results that AT₁ and B₂ receptors may be partly responsible for the angiotensin II–induced effect on RRA. In order to further analyze mechanisms involved in angiotensin II–mediated relaxation, a combination of antagonists and inhibitors was used. In the presence of both HOE-140 and L-NOARG or both PD123319 and charybdotoxin, the RRA response was completely antagonized. In contrast, incubation with HOE-140 and charybdotoxin or with PD123319 and L-NOARG did not cause any further reduction compared to incubation with any of these antagonists alone. Thus, it seems that B₂-receptor stimulation is probably followed by BK⁺Ca²⁺-channel activation. Additionally, it may be also proposed that NO contributes to RRA dilatation in response to AT₂-receptor activation. Similar results were obtained in the investigation of Baranov et al. (41). Furthermore, it was also reported that angiotensin II–induced relaxation of rat anococcygeus smooth muscle seems to be mediated by stimulation of AT₂ receptors, which triggers activation of the nNOS-soluble guanylyl cyclase pathway (42).

In conclusion, the results of the present study indicate that angiotensin II induces endothelium-independent relaxation of isolated RRA. These findings could be of clinical relevance since endothelium-independent angiotensin II–induced relaxation could constitute a protective mechanism to preserve renal tissue perfusion when endothelium is damaged. The vasorelaxant effect of angiotensin II is dependent on activation of AT₁, AT₂, and B₂ receptors on vascular smooth muscle cells. The mechanism of angiotensin II–induced RRA relaxation probably involves the NO-cGMP pathway and activation of BKCa channels. Its ability to relax RRA even in the presence of L-NOARG, ODQ, and K⁺-channel blockers suggest that these mechanisms are independent and probably separately contribute to the angiotensin II vasorelaxant effect. It may be proposed that stimulation of AT₂ receptors is the trigger for the NO-cGMP pathway activation, while B₂-receptor activation is followed by BKCa activation.

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