Mechanism of agonistic angiotensin II type I receptor autoantibody-amplified contractile response to Ang II in the isolated rat thoracic aorta

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Abstract

Agonistic autoantibody to the angiotensin II type I receptor (AT1-AA) is highly associated with pre-eclampsia by increasing the sensitivity of Ang II during pregnancy in rats, thus leading to a pre-eclampsia-like syndrome. However, the mechanism underlying this phenomenon remains unclear. The purpose of this study was to observe AT1-AA amplification of Ang II-induced vasoconstriction in rat thoracic aortic rings. It was found that exposure to low concentrations of AT1-AA (0.4 nM) caused a contraction of <5% of the maximal response to 60 mM KCl. In addition, the Ang II-induced contractile response was amplified in the presence of a threshold contraction to AT1-AA, as manifested by a leftward shift of the midpoint of the concentration–response curve with no change in the maximal response. These results showed that preincubation with low AT1-AA could amplify the Ang II dose–response curve, and this amplification could be attenuated markedly by 0.1 µM heptapeptide AFHYESQ. In calcium-free Krebs solution, 10 µM of 2-aminoethoxydiphenyl borate (an IP3 receptor inhibitor) both blocked the AT1-AA base contraction and completely abolished the amplification. Both 5 µM of U-73122 (a phospholipase C inhibitor) and 10 µM of εV1–2 (an εPKC inhibitor) could partially inhibit the Ang II-induced contractile response. εV1–2, but not U-73122, could completely inhibit the amplification response of AT1-AA to Ang II. These results suggest that AT1-AA is able to cause amplification response to Ang II probably via the calcium-independent protein kinase C pathway, which may provide a new therapy strategy for preeclampsia.

Key words: agonistic angiotensin II type I receptor autoantibody, contractile response, isolated rat thoracic aorta, preeclampsia

Introduction

Several recent studies have suggested that the renin–angiotensin system (RAS) plays an important role in normal pregnancy and preeclampsia. During normal pregnancy, the activation of RAS components, such as angiotensinogen and renin, is increased, ultimately leading to an increase in angiotensin (Ang) II level, while angiotensin-converting enzyme is the only component that is decreased during normal pregnancy [1]. Although RAS components during normal pregnancy are up-regulated, their sensitivity to Ang II is decreased. Therefore,
pregnant women who are resistant to the pressor effect of this molecule require more Ang II to achieve a similar vasomotor response when compared with nonpregnant women [2]. Previous studies have demonstrated that in pregnant women who are resistant to the pressor effect of Ang II, blood pressure may be decreased [3]. In contrast, in patients with preeclampsia, the RAS is dysregulated when compared with healthy pregnant women. Many circulating RAS components, including plasma Ang I, Ang II, Ang-(1–7), and plasma renin activity, are down-regulated in preeclamptic women [2]. Despite the decrease in the expression of RAS components, preeclamptic patients have increased sensitivity to Ang II, showing an exaggerated pressor response to Ang II [4].

Over the last decade, many studies have also indicated that preeclamptic women produce a novel agonistic autoantibody to the Ang II type I receptor (AT1-AA) [5–7]. The exact binding site of AT1-AA to the seven amino acid stretches of the second extracellular loop of the AT1 receptor (the peptide AFHYEQ) exhibits an agonist-like activity similar to Ang II, which has a stimulatory positive chronotropic effect [5]. This antibody binds to the AT1 receptors in different tissues, triggering its pathological action, which can be attenuated with the administration of AT1-receptor antagonists [8]. It has been observed in animal models that the inoculation of AT1-AA from preeclamptic patients can reproduce the characteristics of the disease [9]. Although these novel findings imply that AT1-AA plays certain roles in the pathogenesis of hypertension during preeclampsia, the specific mechanism leading to excessive production and the mechanism whereby AT1-AA increases blood pressure during pregnancy remain unclear.

Wenzel et al. [10] confirmed that an antibody obtained by immunization with a synthetic heptapeptide AFHYESQ may increase the sensitivity of angiotensin II during pregnancy in rats, leading to a preeclampsia-like syndrome, including hypertension, proteinuria, and intrauterine growth retardation. A recent study by Brewer et al. [7] reported that AT1-AA promoted an enhanced contractile response to Ang II in the afferent arteriole (AA) of pregnant rats. Despite the fact that AT1-AA is capable of enhancing the role of Ang II in the pathogenesis of preeclampsia, whether this effect is related to the potential amplifying effect of AT1-AA vascular response to Ang II remains unclear. In addition, if AT1-AA enhances the contractile response of Ang II, whether this synergism plays a role in the pathogenesis of preeclampsia remains unknown. The purpose of the present study was to determine whether AT1-AA amplifies the Ang II-induced contractile response in the normal rat thoracic aorta and explore its role and mechanism of action in the pathogenesis of preeclampsia.

Materials and Methods

Animal
All experimental procedures involving animals were approved by the Ethics Committee of Shanghai Jiao Tong University School of Medicine (Shanghai, China). Specific pathogen-free Wistar rats aged 12 weeks were raised in accordance with the institutional guidelines (Experimental Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine), and housed three per polypropylene plastic cage in a temperature-controlled room (23 ± 2°C) with a relative humidity of 60% ± 5% and a 12 h light–dark cycle, with free access to food and sterilized water throughout the experimental period. Animals without any abnormality after 1-week acclimatization were selected for the study.

Vascular ring preparation and protocol
Rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and killed by exsanguination. The thoracic aorta was rapidly dissected and placed in oxygenated Krebs bicarbonate solution, cleaned of extraneous fat and connective tissue, and cut into 3-mm rings. The composition of Krebs solution was (in mM): NaCl, 118.0; KCl, 4.6; CaCl2·2H2O, 2.5; MgSO4·7H2O, 5.7; NaHCO3, 25.0; KH2PO4·H2O, 1.1; and glucose, 11.0. In some experiments, calcium-free Krebs buffer was used. The composition was the same as described above except that CaCl2 was omitted and 1 mM EDTA was added. The endothelium was mechanically removed by gently rubbing the intimal surface of the rings with a metal rod. Two stainless-steel triangles were inserted through each vessel ring and suspended in 10-ml organ baths filled with Krebs solution at 37 ± 0.5°C, and bubbled with 95% O2 and 5% CO2. One triangle was attached to the bottom of the organ bath and the other connected to an isometric force transducer (Kent Scientific, Torrington, USA), which was connected to a computerized data acquisition system (PowerLab/SP, ADInstruments, Castle Hill, Australia) and recorded on a PC using Chart 5.0 software. The vascular rings were equilibrated for 1 h with the tension of 2.0 g and precontracted twice with KCl (60 mM) followed each time by a washout with Krebs solution. A 60 mM KCl Krebs’ solution was prepared by equimolar replacement of Na+ for K+.

The maximum contractile response elicited by the second KCl exposure was used as the standard response for all subsequent agonist-induced contractions. After washout and 30 min equilibration, the experimental protocol was initiated. In concentration response study, AT1-AA or Ang II (10 pM to 3 µM) was administered at 5 min intervals.

AT1-AA was extracted from the plasma of patients with PE according to our previously reported method [11]. AT1-AA was purified by MAb Trap Kit (Amersham, Piscataway, USA) according to the manufacturer’s instructions. Before use, the purified antibody was diluted with phosphate-buffered saline (3.2 mM Na2HPO4, 0.5 mM KH2PO4, 1.3 mM KCl, 135 mM NaCl, pH 7.4) to an antibody titer of greater than 1:640 detected by ELISA.

To block the vasoconstrictor effect of AT1-AA and Ang II, multiple inhibitors were administered 30 min in advance. They included 1 µM losartan (an AT1-receptor antagonist) [12], 1 µM heptapeptide (AFHYESQ, an AT1-AA specific inhibitor) [13], 10 µM U73122 (a phospholipase C inhibitor) [14], 10 µM 2-aminoethoxydiphenyl borate (2-APB, an IP3 receptor inhibitor) [15], 10 µM eV1–2 (protein kinase C (PKC)-ε inhibitor) [16], and 1 µM ML-7 (a myosin light chain kinase (MLCK) inhibitor) [17]. To investigate the mechanism underlying the magnifying effect of AT1-AA to Ang II, various inhibitors were administered 30 min before Ang II administration.

Results
Contracting action of AT1-AA and angiotensin II on the isolated aortic ring
As shown in Fig. 1A, either Ang II or AT1-AA administered alone resulted in a dose-dependent contraction in the rat endothelium-denuded thoracic aorta, with an EC50 value of 8.18 ± 0.83 or 4.75 ± 0.79 nM, respectively (n = 8 in each group). The Hill slope of the Ang II curve (1.2 ± 0.04) was steeper than that of the AT1-AA curve (0.6 ± 0.04) (P < 0.05). The Emax value of contractile response to AT1-AA was significantly smaller than that to Ang II (81.2% ± 5.3% vs. 114.7% ± 7.2%). As shown in Fig. 1B, AT1-AA caused a slow-developing and long-lasting increase in vascular tension, while Ang II induced a fast-developing and transient vasoconstriction. Addition of low concentrations of Ang II to the Krebs’ solution elicited vasoconstriction consistently. The vasoconstriction tended to be suppressed
when Ang II concentration was raised to 1 µM. The EC10 value of contractile response induced by Ang II and AT1-AA was 0.87 ± 0.12 and 0.46 ± 0.10 nM, respectively.

Comparison of vasoconstrictor actions of Ang II and AT1-AA

To analyze the different contractile response induced by Ang II and AT1-AA, the differences in intracellular signaling pathways between Ang II and AT1-AA were compared by administrating different antagonists before 1 µM Ang II or 1 µM AT1-AA administration. In the endothelium-denuded aorta, the contractile effect of Ang II was completely inhibited by 1 µM of losartan (an AT1-receptor antagonist), but not by 0.1 µM of seven peptide (an AT1-AA specific inhibitor, AFHYESQ). Meanwhile, 10 µM U-73122 (a phospholipase C inhibitor) and 10 µM 2-APB (an IP3 receptor antagonist) significantly reduced the contractile effect of Ang II by more than 60%, and this effect was partially attenuated by 10 µM εV1-2 (a PKC-ε inhibitor) and 1 µM ML-7 (an MLCK inhibitor), suggesting that the phospholipase C-dependent intracellular Ca2+ release pathway may play a crucial role in the Ang II-induced contractile response. AT1-AA also caused vasoconstriction of the isolated rat thoracic aorta, and this effect was completely blocked by losartan and the heptapeptide. Unlike Ang II, U-73122 partially attenuated the contractile effect of AT1-AA, while εV1-2 and ML-7 significantly attenuated the contractile effect of AT1-AA, suggesting that the noncalcium-dependent PKC-ε pathway may play a key role in the AT1-AA-induced contractile response of isolated rat aorta (Fig. 2).

Amplification response of AT1-AA to Ang II

To observe the amplification response of AT1-AA to Ang II, a concentration of less than EC10 AT1-AA was obtained before the Ang II concentration–response curve. This dose induced base contraction of the isolated rat thoracic aorta which was not more than 8% of the maximum KCl response. Ang II concentration–response curve was obtained by cumulative additions of Ang II (1 pM to 1 µM) to the bath solution, when the tissue reached one sustained response. Our results showed that addition of AT1-AA (0.4 nM) to the rat thoracic aorta caused a minimal base contraction (5.2% ± 0.43% of maximum KCl response), which shifted the Ang II dose–response curve to the left, and EC50 was reduced significantly when compared with the control group, though no increase in the maximal response was observed (Fig. 3A). This amplification response was still observed after reduction of the AT1-AA induced base contraction from the total contractile response at each concentration of Ang II (Fig. 3B).

To determine whether the amplification response was due to a precontraction induced by low-concentration AT1-AA, a contraction of less than EC10 Ang II (0.7 nM, base contracted value is 4.9% ± 0.35%) was tested to enhance the Ang II concentration–response curve. As shown in Fig. 4, when the Ang II precontraction was subtracted from each point on the Ang II-pretreated curve, the concentration–response curve in the control and Ang II-pretreated tissues was superimposed at all concentrations, showing no significant differences in the mean EC50 and Emax values.

Amplification response of AT1-AA to Ang II was reduced by heptapeptide AFHYESQ

Next, we performed experiments with tissues preexposed to 0.5 nM heptapeptide AFHYESQ, an AT1-AA-specific inhibitor, to determine AT1-AA-induced amplification response. After a 30-min preincubation
with heptapeptide, the AT1-AA-induced amplification response of Ang II was completely inhibited. As a result, the curve was no longer different from that in the absence of AT1-AA, and the two concentration–response curves overlapped completely. When AT1-AA concentration was increased to 1.2 nM in the presence of the heptapeptide, the AT1-AA-induced amplification response of Ang II was rescued. These results suggest that AT1-AA was able to cause amplification response to Ang II (Fig. 5).

**Mechanism analysis of amplification response of AT1-AA to Ang II**

To determine the role of intracellular Ca\(^{2+}\) in the amplification response of AT1-AA to Ang II, the Krebs buffer was replaced by calcium-free buffer with 1 mM EDTA, and buffer containing 2-APB (10 μM) to deplete the intracellular calcium stores. The results showed that pretreatment with 2-APB decreased the vasoconstrictor effect of Ang II significantly, and the amplification response of AT1-AA to Ang II still existed (Fig. 6). There was significant difference in the concentration–response curve of Ang II between the group pretreated with AT1-AA and 2-APB and the group retreated with AT1-AA. The mean EC\(_{50}\) value of the concentration–response curve of Ang II was significantly larger than other two groups, but the \(E_{\text{max}}\) value was significantly smaller than the other two groups. These results suggested that the amplification response of AT1-AA to Ang II was calcium-independent.

To ascertain the role of noncalcium-dependent PKC-ε pathway in the AT1-AA-amplified Ang II response, we evaluated the effect of 10 μM εV1–2 and found that it had no significant effect on 0.4 nM AT1-AA-induced base contraction. It was 5.1% ± 0.42% of the maximum KCl response in the presence of εV1–2 vs. 4.9 ± 0.39% in the absence of εV1–2 (n = 8). εV1–2 caused a decrease in the maximal contractile response induced by Ang II, from 94.7% ± 8.7% to 63.6% ± 6.3%, but EC\(_{50}\) values did not differ significantly. Simultaneously, εV1–2 also significant weakened the AT1-AA-induced amplification response of the Ang II response, and the concentration–response curve was very close to that in the absence of AT1-AA, so that the

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**Figure 3.** The concentration–response curves for Ang II in the presence and absence of a base contraction to AT1-AA in the rat thoracic aorta rings Responses are presented as a percentage of the maximum potassium contraction. (A) The presence and absence of a base contraction to AT1-AA. (B) The value of the AT1-AA + Ang II (5.2%) minus from each point on the AT1-AA + Ang II curve. n = 10, \(*P<0.05.\)

**Figure 4.** The concentration–response curves for Ang II in the presence or absence of a base contraction to Ang II Responses are presented as a percentage of the maximum potassium contraction. The value of the Ang II base contraction (4.9%) was subtracted from each point on the Ang II + Ang II curve, n = 8.

**Figure 5.** The effect of heptapeptide AFHYESQ on Ang II concentration–response curves in the presence or absence of a base contraction to AT1-AA in the rat thoracic aorta rings The concentration–response curves to Ang II were obtained in the presence and absence of AT1-AA, after incubation with 0.5 nM AFHYESQ. Responses are presented as a percentage of the maximum potassium contraction. The value of the AT1-AA precontraction (4.7% ± 0.38%) was subtracted from every point on the AT1-AA + Ang II curve. n = 6.
on the AT1-AA + Ang II curve. The concentration–response curves to Ang II were obtained in the presence and absence of AT1-AA, after incubation with 1 μM 2-APB. Responses are presented as a percentage of the maximum potassium contraction. The value of the AT1-AA precontraction (4.7% ± 0.38%) was subtracted from every point on the AT1-AA + Ang II curve. n = 6.

Discussion

Recent animal experiment and clinical studies have demonstrated that AT1-AA is closely associated with the incidence of preeclampsia, not only in the presence of high titers of plasma AT1-AA in preeclamptic women but also in the animal model of preeclampsia established by intravenous injection AT1-AA, suggesting that AT1-AA plays a potential role in the pathogenesis of preeclampsia. The study of Zhou et al. [9] suggested that AT1-AA is a circulating component stimulated in response to placental ischemia. Wenzel et al. [10] generated and purified activating antibodies against the AT1 receptor (AT1-AB) by immunizing rabbits against the AHYYESQ epitope of the second extracellular loop, and found that it increased Ang II sensitivity to induce the preeclampsia-like syndrome, including hypertension, proteinuria, and intrauterine growth retardation in pregnant rats. Previous studies [4] reported that Ang II level did not undergo significant change in the serum of patients with preeclampsia; rather, the vascular sensitivity to Ang II was increased. Whether such a change is associated with AT1-AA needs further investigation.

A recent study by Yang et al. [12] demonstrated that human AT1-AA caused vascular constriction in the isolated rat thoracic aorta rings, middle cerebral artery, and coronary artery segments in a concentration-dependent manner. In the present study, we also observed that AT1-AA caused constriction of the rat thoracic aortic rings. However, we found that while AT1-AA caused a slow-developing and long-lasting increase in vascular tension, and with the effect sustaining for 30 min, Ang II induced a fast-developing and transient vasoconstriction that lasted only for 5 min. The vasoconstrictive effect induced by AT1-AA and Ang II in the rat isolated thoracic aorta was completely blocked by losartan, an AT1-receptor antagonist. The heptapeptide AHYYESQ suppressed the AT1-AA-induced contractile responses but did not suppress the contractile response to Ang II, suggesting that the contractile mechanism of AT1-AA and Ang II may be associated with receptor activation, though the characteristics of the contractile response are substantially different. The Ang II binding site of the AT1 receptor was Lys199 and His256 but AT1-AA binding site is the second extracellular loop of AT1 receptor. We believe that it is because of the different binding sites in the AT1-receptor sites, Ang II and AT1-AA could induce vascular smooth muscle cell contraction through different intracellular signaling pathways, eventually leading to different vasoconstrictive properties. The role of AT1-AA in the process of the pathogenesis of preeclampsia has attracted much attention and needs to be clarified by further studies.

Our experiments showed that both Ang II and AT1-AA could cause vasoconstriction, but their characteristics of vasoconstriction are completely different. Unlike Ang II, AT1-AA causes a slow-developing and long-lasting increase in vascular tension, and is not easily removed by washing with Krebs solution. According to the characteristics of vasoconstriction induced by AT1-AA, AT1-AB is believed to be an important factor contributing to enhanced contractile response to Ang II. First, we observed that the effect of low concentration of AT1-AA on the vascular contractile response was induced by Ang II, and that pretreatment with low dose of AT1-AA significantly increased the vascular contractile response induced by low concentration of Ang II. But with the increasing dose of Ang II, the amplification effect was weakened. The concentration–response curve shifted to the left, and the EC50 value was significantly decreased. However, the Emax remained unchanged. The amplification response was still observed even when the AT1-AA-induced base contraction was subtracted from the total contractile response at each concentration of Ang II. Our results suggested that pretreatment with AT1-AA not only induced precontraction but also produced an amplification effect on Ang II.

Smooth muscle contraction and relaxation are tightly coupled with the phosphorylation and dephosphorylation of the regulatory myosin...
light chain. The state of myosin light chain phosphorylation is deter-
mined by the relative activities of MLCK and myosin light chain
phosphatase (MLCP). MLCK phosphorylates MLC20 leading to con-
traction, and MLCP dephosphorylates MLC20 leading to relax-
atation. Both MLCK and MLCP activities are highly regulated. Phos-
phorylation of MLC20 by MLCK is generally considered as the pri-
mary mechanism for regulating the contraction of the smooth mus-
cle. Phosphorylation can be simply described as an interaction of Ca2+
with CaM that induces a conformational change of MLCK and acti-
vates MLCK. MLCP activity is regulated by multiple signaling path-
ways, leading to MLC20 dephosphorylation that inhibits muscle con-
traction. When MLCP activity is inhibited, the phosphorylated form of MLC20 is maintained and smooth muscle remains contracted.

PKC is a phospholipid-dependent protein kinase. Ca2+ is not re-
quired in the activation of PKC-ε [17]. In this study, we observed
that the vasoconstriction effect of AT1-AA was weakened after either
PKC-inhibitor or MLCK-inhibitor treatment, suggesting that a noncal-
cium-dependent pathway may be involved in the vasoconstriction
induced by AT1-AA, and that 2-APB at a concentration of 10 μM
might limit the intracellular calcium level below the threshold, which
could cause cell contraction so that the intracellular calcium level of vas-
cular smooth muscle cells would not be elevated by activators. 2-APB is
not only an inhibitor of IP3 receptor to prevent calcium ions release
from the sarcoplasmic reticulum but also an intracellular calcium deple-
tion agent [17]. We observed that the vasoconstriction effect of Ang II
was still amplified by AT1-AA treatment in the calcium-free Krebs solu-
tion with 2-APB, but the amplification effect was reduced by 67% when
compared with that in the solution containing calcium ions, suggesting
that the vasoconstriction effect of Ang II amplified by AT1-AA is partly
modulated by the noncalcium pathway.

In conclusion, our experimental results showed that low dose of
AT1-AA did not cause significant vasoconstriction; but amplified
the vascular contractile response induced by Ang II. The possible
mechanism may lie in the fact that low dose of AT1-AA induces an in-
crease in intracellular free calcium level, which activates PKC or PLC
in smooth muscle cells. This finding may provide a useful reference for
the treatment of cardiovascular diseases.

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References
1. Anton L, Brosnihan KB. Systemic and uteroplacental renin-angiotensin sys-
tem in normal and preeclamptic pregnancies. Ther Adv Cardiovasc Dis
2. Pipkin FB, Baker PN. Angiotensin II has depressor effects in pregnant and
16: 213–220.
et al. The uterine placental bed renin-angiotensin system in normal and pre-
Baur E, et al. Patients with preeclampsia develop agonistic autoantibodies
against the angiotensin AT1 receptor. J Clin Invest 1999, 103: 945–952.
6. LaMarca B, Wallace K, Granger J. Role of angiotensin II type 1 receptor ag-
gonistic autoantibodies (AT1-AA) in preeclampsia. Curr Opin Pharmacol
Endothelin-1, oxidative stress, and endogenous angiotensin II: mechanisms
of angiotensin II type 1 receptor autoantibody-enhanced renal and
blood pressure response during pregnancy. Hypertension 2013, 62:
886–892.
8. Dechend R, Müller DN, Wallukat G, Homuth V, Krause M,
Dudenhausen J, Luft FC. AT1 receptor agonistic antibodies, hypertension,
Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in
10. Wenzel K, Rajakumar A, Haase H, Geusens N, Hubner N, Schulz H,
Brewer J, et al. Angiotensin II type 1 receptor antibodies and increased
angiotensin II sensitivity in pregnant rats. Hypertension 2011, 58:
77–84.
ure to AT1 receptor autoantibodies during pregnancy increases suscepti-
bility of the maternal heart to postpartum ischemia-reperfusion injury in rats.
Autoantibody against AT1 receptor from preeclamptic patients induces
vasoconstriction through angiotensin receptor activation. J Hypertens
13. Irani RA, Zhang Y, Zhou CC, Blackwell SC, Hicks MJ, Ramin SM,
Kellems RE, et al. Autoantibody-mediated angiotensin receptor activation
contributes to preeclampsia through tumor necrosis factor-α signaling.
cological studies of tentacle extract from the jellyfish Cyanea capillata in iso-
15. Egan CG, Wanswinkel CL, Wadsworth RM, Nixon G. FPDGF-induced sig-
aling in proliferating and differentiated vascular smooth muscle: effects of
16. Wang XY, Ding YJ, Zhu YZ, Shi Y, Yao T, Zhu YC. Role of PKC in the
novel synergistic action of urotensin II and angiotensin II in urotensin
H348–H359.
17. Goyal R, Galffy A, Field SA, Gheorghe CP, Mittal A, Longo LD. Matu-
ration and the role of PKC-mediated contractility in ovine cerebral arteries.