Original Article

Effects of Angiotensin II on the Action Potential Durations of Atrial Myocytes in Hypertensive Rats

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Angiotensin II (Ang II) has been reported to indirectly influence atrial electrical activity and to play a critical role in atrial arrhythmias in hypertensive patients. However, it is unclear whether Ang II has direct effects on the electrophysiological activity of the atrium affected by hypertension. We examined the effects of Ang II on the action potentials of atrial myocytes enzymatically isolated from spontaneous hypertensive rats (SHRs). The action potentials were recorded by the perforated patch-clamp technique and the atrial expression of the receptors AT_{1a} and AT_2 was measured by radioimmunoassay. Ang II significantly shortened the action potential durations (APDs) of SHRs without changes in the resting membrane potentials (RMPs). Pretreatment with selective AT_{1a} blockers abolished the Ang II-induced reduction of atrial APDs of SHRs; however, a selective AT_2 blocker did not, which was consistent with the results of the receptor assay. Pretreatment with phosphatidylinositol 3 (PI3)-kinase inhibitor, phospholipase C inhibitor, or protein kinase C (PKC) inhibitor abolished the Ang II-induced shortening of atrial APDs, but pertussis toxin and protein kinase A (PKA) inhibitor did not. To study the effects of chronic AT_{1a} inhibition on Ang II-induced shortening of atrial APD, SHRs were treated with AT_{1a} blocker for 4 weeks. AT_{1a} blocker abolished the Ang II-induced reduction of atrial APDs of SHRs and also significantly lowered their blood pressure. In conclusion, Ang II shortened atrial APDs of SHRs *via* AT_{1a} coupled with the Gq-mediated inositol triphosphate (IP3)-PKC pathway. Our findings indicated that Ang II caused atrial arrhythmias in hypertensive patients by shortening the effective refractory period of the atrium. (*Hypertens Res* 2005; 28: 173–179)

***Key Words*: angiotensin II type 1a receptor, atrial myocytes, action potential, CS-866, spontaneous hypertensive rats**

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Introduction

Angiotensin II (Ang II) plays a central role in blood pressure as well as in water and electrolyte homeostasis (*1*). Ang II also has a direct effect on cardiac function and structure *via* its actions on the angiotensin type 1 receptor (AT_1), and the activation of a local intracardiac angiotensin system may participate in certain aspects of cardiac pathophysiology, such as myocardial hypertrophy and dilatation (*2–4*). Previous experimental and clinical studies (*5–13*) have reported that the activation of the atrial renin-angiotensin system may contribute to the occurrence of atrial arrhythmias. Goette *et al*. (*5*) reported that activation of AT_1 in the atrium promoted Ang II-dependent progressive atrial fibrosis. Nakashima *et al*. (*8*) demonstrated in the canine atrium that AT_1 blockers prevent shortening of the effective refractory period during high rate pacing, suggesting that activation of the AT_1 receptor shortens the atrial action potential duration (APD), which in turn causes atrial arrhythmias. Hypertension is the most common cardiovascular cause of atrial fibrillation (*11*). The activation of AT_1 in the atrium is likely to be proarrhythmic in hypertensive patients, since several recent reports (*12*, *13*) have indicated that AT_1 blockers significantly reduce the occurrence of atrial fibrillation in hypertensive patients. However, whether stimulation of AT_{1a} has a direct influence on the electrophysiological properties of the atrium affected by hypertension remains unclear. Ang II binds to both AT_1 and AT_2, each of which is coupled with a subset of G proteins and activates multiple signaling pathways, including the conventional Ca^{2+}/phospholipase C (PLC)/protein kinase C (PKC) (*14*) and cAMP/protein kinase A (PKA) (*15*) signaling pathways. In the present study, we examined the effects of Ang II on the action potentials of atrial myocytes from spontaneous hypertensive rats (SHRs), and found that Ang II shortened the atrial APD by activating the Gq-inositol triphosphate (IP3)-PKC pathway *via* AT_{1a}.

Methods

Cell Isolation

Single atrial myocytes were prepared as described previously (*16*). SHRs weighing 120 to 180 g (8 weeks) were anesthetized with pentobarbital sodium and the heart was quickly removed. Using a Langendorff apparatus, the excised heart was perfused with modified Tyrode's solution to wash out the residual blood at a temperature of 37°C. The perfusate was then switched to Ca^{2+}-free Tyrode's solution to stop the heartbeat. The quiescent heart was then perfused with Ca^{2+}-free Tyrode's solution containing 0.4 mg/ml collagenase (Type I, PKF1629; Wako Pure Chemicals, Tokyo, Japan) for 20 min and rinsed and stored with Kraftbruhe (KB) medium (*17*) of the following composition (in mmol/l): 25 KCl, 10 KH_2PO_4, 116 KOH, 0.5 EDTA, 80 glutamic acid, 10 taurine, 14 oxalic acid, 11 glucose, and 10 HEPES. The pH was adjusted to 7.4 with KOH. The composition of Tyrode's solution (in mmol/l) was 140 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 5 HEPES, and 5.5 glucose, and the pH was adjusted to 7.4 with NaOH. The atrium was minced with scissors in KB medium and the cells were collected by filtering through a stainless steel mesh and stored in KB medium. All procedures were performed in accordance with the Tottori University Animal Care Guidelines, which conform to the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985).

Measuring the Action Potential

Isolated single atrial myocytes were dispersed in the recording bath and superfused with normal Tyrode's solution. The action potential was recorded by the current clamp techniques under the perforated patch clamp mode (*18*). For the perforated patch-clamp, the pipette solution contained (in mmol/l) 140 K aspartate, 5 $MgCl_2$, 5 K_2ATP, 5 EDTA, 5 HEPES, and 250 μg/ml amphotericin B, and the pH was adjusted to 7.2 with KOH. The capacitance was corrected, and perforation was monitored by changes in membrane potential observed in current (I)=0 mode on the patch-clamp amplifier and changes in series resistance. Recordings were not made until the series resistance was <15 MΩ to minimize voltage errors. The series resistance was further minimized by electronic compensation. The data were acquired and analyzed using a computer (PC98XL, NEC, Tokyo, Japan) *via* an analog/digital converter (PCM-DP16; SHOSHIN EM Corp., Okazaki, Japan).

Membrane Preparation and Receptor Assay

The rats were killed by decapitation, and the atria were dissected out and minced with scissors. Membrane fractions were prepared from pooled samples (n=10) as previously described (*19*). Membrane fractions were incubated with different concentrations (0.05–1 nmol/l) of ^{125}I Sar1, Ile8-Ang II or ^{125}I CGP42112A for 120 min at 20°C for the saturation experiment. Specific binding of ^{125}I Sar1, Ile8-Ang II and ^{125}I CGP42112A was determined from the difference between counts in the absence and presence of 10 μmol/l AT_1 blocker (losartan) and 1 μmol/l AT_2 blocker (CGP42112A), respectively. The K_d and B_{max} values were estimated by Rosenthal analysis of the saturation data, and AT_1 and AT_2 densities were calculated from B_{max} values.

Chronic Treatment with CS-866

Eight-week-old male SHRs were randomly divided into 2 groups. The control group received no treatment (n=8). The olmesartan group (n=8) received CS-866 (10 mg/kg/day) orally. CS-866 was orally administered by gastric gavage once a day for 4 weeks. During the test period, systolic and

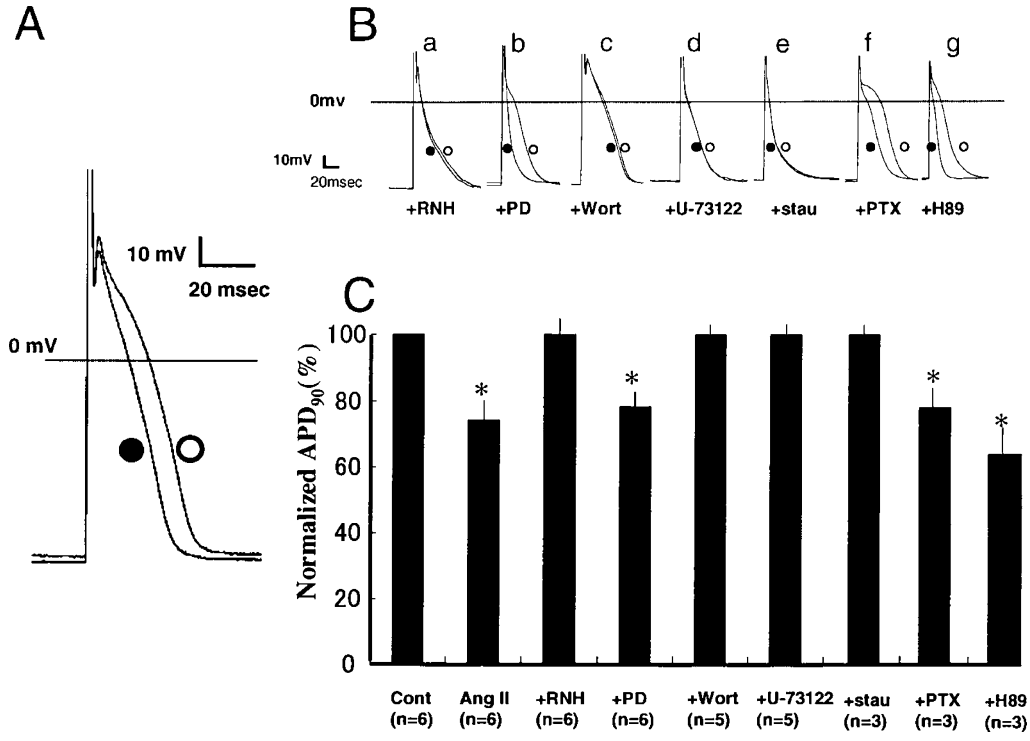

Fig. 1. *Effects of Ang II on the atrial action potentials of SHRs in the presence of various agents. A: Representative traces of action potentials of atrial myocytes before (open circle) and after (closed circle) perfusion with Ang II at 100 nmol/l. B: Effects of various agents on the Ang II-induced shortening of APD. The following agents were administered prior to the application of 100 nmol/l Ang II. a: +RNH, RNH6270 (10 nmol/l); b: +PD, PD123319 (1 μmol/l); c: +Wort, wortmannin (20 μmol/l); d: +U-73122 , U-73122 (1 μmol/l); e: +stau, staurosporin (100 nmol/l); f: +PTX, pertussis toxin (5 g/l), applied to the KB solution in which the cells were preincubated for more than 2 h at 37°C; or g: +H89, H89 (1 μmol/l), administered for 30 min prior to the application of 100 nmol/l Ang II. Open circles indicate before and closed circles indicate after the administration of Ang II (100 nmol/l). C: Summary of the effects of various agents on the Ang II-induced shortening of APD_{90}. The ordinate indicates the % of APD_{90} normalized to the control (Cont). Each bar represents the mean±SEM and the number of experiments performed is given in parenthesis.*

diastolic blood pressure (SBP/DBP) were also monitored by the tail-cuff method.

Drugs

RNH6270 and CS-866 (*20*) were supplied by Sankyo Co. (Tokyo, Japan). PD123319 was from Warner-Lambert Co. (Morris Plains, USA) and pertussis toxin from Seikagaku Co. (Tokyo, Japan). All other reagents were purchased from Sigma Chemical Co. (St. Louis, USA) and Wako Industries, Ltd. (Osaka, Japan).

Statistical Analysis

One-way ANOVA test and Fisher's exact test for post hoc analysis were carried out for multiple comparisons among groups. All data are expressed as the mean±SEM. Values of $p<0.05$ were regarded as statistically significant.

Results

Effects of Ang II on Atrial Action Potentials in SHRs

Figure 1A shows a representative action potential tracing from atrial myocytes of SHRs before and 5 min after the administration of Ang II (100 nmol/l). Administration of Ang II (100 nmol/l) shortened action potential duration at the 50% repolarization (APD_{50}) by 30% and that of 90% repolarization (APD_{90}) by 26%, although Ang II did not affect either the action potential amplitude or the resting membrane potential (RMP). Table 1 summarizes the observed effects of Ang II on action potential in the 6 experiments. Ang II significantly shortened both APD_{50} and APD_{90} without changes in RMPs.

Table 1. Effects of Angiotensin II (Ang II) on the Parameters of Atrial Action Potentials of SHRs

	RMP(mV)	APD_{50} (ms)	APD_{90} (ms)
Control	−64.8±7.1	28.6±3.3	38.4±3.9
Ang II	−69.8±3.3	17.1±1.3*	25.8±5.5*

Control: before perfusion with Ang II; Ang II: after perfusion with Ang II (100 nmol/l). SHR, spontaneous hypertensive rats; RMP, resting membrane potential; APD_{50}, action potential duration at the 50% repolarization; APD_{90}, action potential duration at 90% repolarization. *$p<0.05$.

Table 2. Effects of RNH6270 on the Parameters of Atrial Action Potentials of SHRs before and during Perfusion with Angiotensin II (Ang II)

	RMP(mV)	APD_{50} (ms)	APD_{90} (ms)
Control	−62.2±3.8	30.9±3.1	37.5±6.6
RNH6270	−59.0±2.0	28.4±7.2	36.4±6.4
Ang II+RNH6270	−61.1±1.1	31.7±5.2	35.1±6.0

Control: before treatment; RNH6270: during perfusion with RNH6270 (10 nmol/l); Ang II+RNH6270, during perfusion with Ang II (100 nmol/l) in the presence of RNH6270. SHR, spontaneous hypertensive rats; RMP, resting membrane potential; APD_{50}, action potential duration at the 50% repolarization; APD_{90}, action potential duration at 90% repolarization.

As shown in a in Fig. 1B, Ang II did not affect the APDs of atrial myocytes in the presence of the AT_{1a} blocker RNH6270 (10 nmol/l). Table 2 provides a summary of the observed effects of RNH6270 on the action potential and the subsequent effects of Ang II in the presence of RNH6270 in the 6 experiments. Ang II did not shorten either the APD_{50} or APD_{90} of atrial myocytes in the presence of RNH6270 (10 nmol/l), while RNH6270 alone did not affect the parameters of action potential. Ang II shortened both the APD_{50} and APD_{90} of atrial myocytes from SHRs in the presence of PD123319 at 1 μmol/l (b in Fig. 1B), indicating that the significant Ang II-induced shortening of atrial APDs of SHRs was mediated by activation of AT_{1a}. The radioimmunoassay showed that atrial myocytes of SHRs exhibited a significantly higher maximum binding (21±1.3 fmol/mg protein) for Ang II receptors with an affinity (K_d of 0.9±0.05 nmol/l; $n=10$) higher than that of atrial myocytes from normotensive rats (maximum binding of 11±0.6 fmol/mg protein and K_d of 1.5±0.07 nmol/l, respectively; $n=10$). The percentages of AT_{1a} and AT_2 in the atrium of SHRs were 82±5% and 18±7%, respectively, indicating that AT_{1a} was predominant AT isoform expressed in the atrium of SHRs. Taken togerther, these results indicate that Ang II shortened the APDs *via* activation of the AT_{1a} predominantly expressed in the atrium of SHRs.

To further elucidate the signaling pathway of this Ang II-induced shortening of APDs, we examined the effects of various blockers on the reduction of APDs of the atrial myocytes obtained from SHRs by Ang II at 100 nmol/l (Fig. 1B and C). c–g in Fig. 1B show representative action potential tracings from atrial myocytes of SHRs before and after administration of Ang II in the presence of blockers, and Fig. 1C summarizes the effects of the indicated agents on the reduction of APD_{90} of the atrial myocytes obtained from SHRs by Ang II. Wortmannin (20 μmol/l: c in Fig. 1B), an inhibitor of PI3-kinase, as well as U-73122 (1 μmol/l: d in Fig. 1B), an inhibitor of the PLC-dependent process, staurosporin (100 nmol/l: e in Fig. 1B), and calphostin C (100 nmol/l: data not shown), an inhibitor of the PKC-dependent process, abolished the Ang II-induced shortening of APD_{90} in SHRs, while neither 30-min preincubation with pertussis toxin (5 g/l; f in Fig. 1B) nor 30-min preincubation with H89 (1 μmol/l: g in Fig. 1B), an inhibitor of Gi-mediated PKA-dependent process, prevented the Ang II effect. These results indicated that the Gq stimulation that generated IP3 and PKC was responsible for the Ang II-induced reduction of atrial APDs in SHRs.

Chronic Treatment with CS-866 Abolished the Ang II-Induced Shortening of Atrial APDs in SHRs

The effect of chronic treatment with CS-866 (10 mg/kg) on the Ang II-induced shortening of APDs of atrial myocytes in SHRs was examined. Treatment with CS-866 for 4 weeks significantly decreased SBP and DBP from 175±7 and 100±6 mmHg to 124±13 and 90±12 mmHg, respectively, without changes in heart rate (from 361±18 to 369±24 beat/min). The action potentials were recorded 1 h after cell isolation. Table 3 summarizes the protective effects of 4-week pretreatment with CS-866 on the parameters of the atrial action potentials of SHRs. Pretreatment with CS-866 completely abolished the effect of Ang II on the action potentials, indicating that pretreatment with CS-866 for 4 weeks abolished the Ang II-induced shortening of the atrial APDs in SHRs.

Discussion

The present study revealed several features of the effects of Ang II on the atrial action potentials of SHRs. 1) Ang II significantly reduced the atrial APDs without changes in the RMPs. 2) Ang II reduction of atrial APDs was abolished by the antagonist of AT_{1a} but not by the antagonist of AT_2 ; AT_{1a} receptors were expressed more significantly than AT_2 in the atrial myocytes, and the expression of Ang II receptors in SHRs was significantly greater and the receptors showed significantly higher affinity to Ang II than in wild rats. 3) Pretreatment with either wortmannin, U-73122, calphostin C or staurosporin abolished the effect of Ang II on atrial APDs, while pretreatment with either pertussis toxin or H89 did not affect atrial APDs. 4) Treatment with CS-866 for 4 weeks to lower their blood pressures significantly abolished the effect of Ang II on atrial APDs. These findings may support the notion that activation of the atrial renin-angiotensin system

Table 3. Effects of Angiotensin II (Ang II) on the Atrial Action Potentials of SHRs Chronically Treated with CS-866 for 4 Weeks

	RMP(mV)	APD_{50} (ms)	APD_{90} (ms)
Control	−56.2±4.8	27.1±1.0	41.3±4.9
Ang II	−60.0±5.6	26.3±2.1	39.5±4.1

Control: before perfusion with Ang II; Ang II: during perfusion with Ang II (100 nmol/l). SHR, spontaneous hypertensive rats; RMP, resting membrane potential; APD_{50}, action potential duration at the 50% repolarization; APD_{90}, action potential duration at 90% repolarization.

can induce proarrhythmic activity, and that administration of AT_{1a} blockers can confer protection against atrial arrhythmias in hypertensive patients, as reported elsewhere (*13*, *14*).

Mechanism of Ang II-Induced Shortening of the Atrial Action Potentials of SHRs

There have been several, somewhat conflicting reports about the effects of Ang II on cardiac action potentials. Bonnardeaux *et al*. (*21*) showed that Ang II lengthened the plateau phase of the action potential and prolonged the APD in isolated rabbit atria. In another study, Ang II induced spontaneous action potentials in partially depolarized preparations of rabbit atria and increased the rate of diastolic depolarization and spontaneous discharge in human atrial tissues (*22*). In rabbit sinoatrial node cells, Ang II depolarized the RMPs accompanied by a reduction of the action potential amplitude and a negative chronotropic effect (*23*). In isolated rabbit ventricular myocytes, Morita *et al*. (*24*) reported that Ang II decreased the APD after 3 min of application, but prolonged the APD after application for 7 min. These reports indicated that Ang II prolongation of the atrial and ventricular APDs was accompanied by membrane depolarization. On the other hand, other investigators reported that Ang II did not affect any parameters of the action potentials in rat (*25*) and guinea pig (*26*) atria and ventricles. The present study is the first to demonstrate that Ang II significantly shortened the atrial APDs in SHRs without changes in the RMPs (Table 1). This result, taken together with the observed negative effects of AT_2 blockers, and the finding that AT_{1a} blocker abolished the effects of Ang II on atrial APDs, indicated that Ang II-induced shortening of atrial APDs was mediated through the activation of AT_{1a} of SHRs (Table 2). Both the predominant expression of AT_{1a} and their higher affinity for Ang II in atrium of SHRs may facilitate the actions of Ang II on the APDs of SHRs. It is well known that Ang II receptors couple to the major phosphorylation systems; namely, that they couple negatively to PKA and positively to PKC (*1*, *14*, *27*). In the present study, inhibition of the Gq-IP3-PKC pathway abolished the Ang II effects on atrial APDs of SHRs, but inhibition of the Gi-adenylate cyclase-PKA-dependent pathway had no influence on the effects of Ang II, indicating that Ang II-induced shortening of atrial APDs was involved in the Gq-IP3-PKC-dependent pathway in SHRs (Fig. 1B). Interestingly, chronic treatment with CS-866 for 4 weeks significantly attenuated the Ang II-induced shortening of atrial APDs of SHRs (Table 3), and this attenuation was associated with a significant lowering of the SBP and DBP. These results suggested that chronic treatment with CS-866 continuously disrupted the Ang II signaling to stimulate the AT_{1a}-Gq-IP3-PKC pathway, and thereby prevented Ang II-induced shortening of the APDs of SHRs. There are two possible mechanisms (*28*) for the prolonged preventive effects of RNH-6270 on Ang II-induced shortening of the APDs of atrial myocytes after isolation: 1) binding of RNH-6270 covalently to AT_{1a} followed by a slow dissociating from receptors; and 2) a continuous block of RNH-6270 that affects the ability of Ang II to transduce subsequent signaling. The inhibitory effect of RNH-6270 on AT_{1a} has been reported to persist for 90 min after washout, which can at least partly explain the continuous blockade of AT_{1a} by RNH-6270. Further investigations are needed to clarify this mechanism.

Ang II has been reported to exert effects on various cardiac ion currents (*29*). Ang II increases the T-type Ca^{2+} current in neonatal rat cardiac cells, the L-type Ca^{2+} current in neonatal rat hearts, the Na^+ current in neonatal rat cardiac ventricular cells, the Cl^- current in rat myocytes, the Cl^- current in rabbit sino-atrial cells, and the delayed rectifier K^+ current in guinea pig ventricular myocytes (*29*). On the other hand, Ang II has been shown to decrease the L-type Ca^{2+} current in rabbit sinus nodal cells, the isoproterenol-induced Cl^- current in guinea pig ventricular myocytes, the I_{to} in canine ventricular myocytes (*30*), and the ATP-sensitive K^+ current in guinea pig ventricular myocytes (*31*). Since the cardiac APDs can be determined by the balances between net outward and inward currents, either a block of the Ca^{2+} current, a block of the steady state Na^+ current, or activation of the K^+ current can explain Ang II-induced shortening of the atrial APDs. Therefore, the ion currents responsible for Ang II-induced shortening of the APDs in SHRs warrant further investigation.

Clinical Implications of AT_{1a}-Mediated Shortening of Atrial APDs in SHRs

It has been reported that activation of AT_1 in the atrium promotes Ang II-dependent progressive shortening of the effective refractory period (*8*) and Ang II-dependent progressive atrial fibrosis (*5*). These results, together with the recent findings that AT_{1a} antagonists significantly suppressed the occurrence of atrial fibrillation in hypertensive patients (*12*, *13*), indicate that activation of AT_{1a} in the atrium can cause arrhythmias in hypertensive patients. The present radioimmunoassay data indicated that the expression of Ang II receptors in the atrium was significantly larger in SHRs, which were found to have a smaller mean K_d value, than in normotensive rats. Moreover, AT_{1a} was the predominant isoform expressed

in the atrium of SHRs, suggesting that the effects of Ang II on APDs may be more likely to occur in the atrium affected by hypertension. Ang II-induced shortening of atrial APDs in SHRs *via* AT_{1a} might be relevant to the increased incidence of atrial fibrillation in hypertension, perhaps by shortening the wavelength of excitation and encouraging re-entrant arrhythmias or by increasing the heterogeneity of repolarization within the atrium. The fact that chronic treatment with CS-866 as well as acute treatment with RNH-6270 abolished the Ang II-induced shortening of atrial APDs in SHRs can account for the effectiveness of selective AT_{1a} blockers in halting the trigger of atrial arrhythmia in hypertensive patients. In addition to Ang II-induced shortening of the atrial APDs in SHRs, Ang II may enhance augment the progression of atrial fibrosis and thereby reduce the conduction velocity of the atrium. This Ang II-induced fibrosis could reduce the atrial conduction velocity and cause re-entrant atrial arrhythmias. The mechanical stretch in hypertension induces the stored Ang II in cardiomyocytes to activate many protein kinases, including extracellular-signal-regulated kinase (ERKs), reprogramming of gene expression, cardiomyocyte hypertrophy, and cardiac fibrosis (*32*, *33*). It has most recently been reported that without involvement of Ang II, mechanical stretch alone induces the activation of ERKs and increased phosphoinositide production with Janus kinase 2 and translocation of $G\alpha q_{11}$ proteins into cytosol to promote cardiomyocytes hypertrophy and cardiac fibrosis (*34*). Therefore, stimulation with either Ang II or mechanical stretch to activate the Gq-signal may play a pivotal role in causing the re-entrant circuit in the atrium by cardiac fibrosis.

In conclusion, Ang II shortened the atrial APDs of SHRs *via* AT_{1a} coupled with the Gq-mediated IP3-PKC pathway. Our findings indicated that Ang II can cause atrial arrhythmias in hypertensive patients at least in part by shortening the effective refractory period of the atrium.

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