Angiotensin II Potentiates the Slow Component of Delayed Rectifier K⁺ Current via the AT₁ Receptor in Guinea Pig Atrial Myocytes

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**Background**—Angiotensin II (Ang II) has diverse actions on cardiac electrical activity. Little information is available, however, regarding immediate electrophysiological effects of Ang II on cardiac repolarization.

**Methods and Results**—The present study investigated the immediate effects of Ang II on the slow component of delayed rectifier K⁺ current (Iₖₛ) and action potentials in guinea pig atrial myocytes using the whole-cell patch-clamp technique. Bath application of Ang II increased the amplitude of Iₖₛ (EC₅₀, 6.16 nmol/L) concentration dependently. The stable analogue Sar¹–Ang II was also effective at increasing Iₖₛ. The voltage dependence of Iₖₛ activation and the kinetics of deactivation were not significantly affected by these agonists. The enhancement of Iₖₛ was blocked by the Ang II type 1 (AT₁) receptor antagonist valsartan (1 μmol/L) and was markedly attenuated by inclusion of GDPβS (2 mmol/L) in the pipette, indicating an involvement of G protein–coupled AT₁ receptor. The stimulatory effect was also significantly reduced by the phospholipase C inhibitor compound 48/80 (100 μmol/L) and the protein kinase C inhibitors bisindolylmaleimide I (200 mmol/L) and H-7 (10 μmol/L), suggesting that AT₁ receptor acts through phospholipase C–protein kinase C signaling cascade to potentiate Iₖₛ. As expected from its stimulatory action on Iₖₛ, Sar¹–Ang II markedly shortened the action potential duration, which could be reversed by valsartan.

**Conclusions**—The potentiation of Iₖₛ via AT₁ stimulation in atrial myocytes, accompanied by a shortening of the action potential duration, suggests a potential mechanism by which elevated levels of Ang II may promote atrial fibrillation in heart failure and warrants further investigation. (*Circulation*. 2006;113:1278-1286.)

**Key Words:** action potentials  ■ angiotensin  ■ atrium  ■ ion channels  ■ receptors

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**Clinical Perspective p 1286**

The renin-angiotensin system (RAS) plays a fundamental role in maintaining cardiovascular homeostasis, and disorders of the RAS are closely related to the development of hypertension, heart failure, atherosclerosis, cardiac hypertrophy, and myocardial and vascular remodeling.¹–⁵ An octapeptide angiotensin II (Ang II) is the principal effector of the RAS and produces its potent and diverse biological actions by interacting with specific membrane receptors, namely Ang II type 1 (AT₁) and type 2 (AT₂) receptors. The AT₁ receptors are widely distributed in a variety of cell and tissue types and mediate most of the known actions of Ang II, whereas the AT₂ receptor is expressed mainly in the embryonic and neonatal states but is upregulated in adult tissues under some pathological conditions.⁶ The various biological actions of Ang II via AT₁ receptor can be divided into the short-term effects that occur within minutes and the long-term effects that take place within hours or even later; vascular contraction constitutes the short-term events, whereas the long-term effects include a transcriptional response that leads to more physiological and functional alterations of cardiovascular systems such as cardiac hypertrophy, fibrosis, and remodeling.⁷

There is increasing evidence that the RAS is also associated with the occurrence of atrial and ventricular arrhythmias in experimental animals,⁸,⁹ and recent clinical studies haveproved that blockade of the RAS with ACE inhibitors or AT₁ antagonists is effective for the treatment of atrial fibrillation (AF).¹⁰,¹¹ The shortening of action potential duration (APD) and effective refractory period can be regarded as one of the main factors responsible for the occurrence of reentry-based tachyarrhythmias such as AF. Little information is available, however, regarding the effect of Ang II on repolarizing K⁺ currents and resultant changes in APD in cardiac myocytes.

It has been demonstrated in various mammalian species, including humans, that the delayed rectifier K⁺ current (Iₖ) consists of rapidly and slowly activating components (Iₖᵣ and Iₖₛ, respectively), which are the major repolarizing outward...
currents of atrial and ventricular action potentials.\textsuperscript{12} \(I_{\text{Ks}}\) and \(I_{\text{Ca}}\) reflect the expression of distinct molecular entities; the pore-forming \(\alpha\)-subunit KvLQT1 (KCNQ1) coassembles with an accessory \(\beta\) subunit minK ( KCNE1) to form the \(I_{\text{Ks}}\) channels, and the HERG ( KCNHH2) constitutes the pore-forming subunit of the channel that underlies the \(I_{\text{Ks}}\) channels. Mutations in genes encoding these channel proteins are responsible for the long-QT syndrome in humans, an inherited cardiac arrhythmia characterized by abnormal cardiac repolarization and a high risk for sudden death.\textsuperscript{13} \(I_{\text{Ks}}\) also represents a relevant target for modulation by autonomic neurotransmitters and hormones and thereby mediates the regulation of cardiac electrical activity and contraction by these extracellular signaling molecules.

The present study was designed to examine the possible regulation of \(I_{\text{Ks}}\) by Ang II and its associated signaling pathways in isolated guinea pig atrial myocytes using the whole-cell patch-clamp technique. Our results show for the first time that Ang II in nanomolar concentrations markedly potentiates \(I_{\text{Ks}}\) through a mechanism involving activation of the G protein–coupled \(\text{AT}_1\) receptor linked to the phospholipase C (PLC)–protein kinase C (PKC) pathway.

**Methods**

**Preparation of Atrial Myocytes**

The experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH publication 85–23, revised 1996). Single atrial myocytes were enzymatically dissociated from the heart of adult Hartley guinea pigs as described previously.\textsuperscript{14}

**Solutions and Chemicals**

Normal Tyrode’s solution contained (in mmol/L) 140 NaCl, 5.4 KCl, 1.8 CaCl\(_2\), 0.5 MgCl\(_2\), 0.33 Na\(_2\)HPO\(_4\), 5.5 glucose, and 5.0 HEPES (pH adjusted to 7.4 with NaOH). The standard external solution for measuring \(I_{\text{Ks}}\) was normal Tyrode’s solution supplemented with 0.4 mmol/L nisoldipine (a generous gift from Bayer AG, Wuppertal-Elberfeld, Germany) and 5 mmol/L E-4031 (Wako, Osaka, Japan). Agents added to the external solution included Ang II (human; Calbiochem, San Diego, Calif, and Sigma, St Louis, Mo), Sar\(^1\)-Ang II (Sigma), I-(5-isouquinolinesulfonfyl)-2-methylpirerazin (H-7, Seikagaku, Tokyo, Japan), bisindolylmaleimide I (Bis I, Sigma), phorbol 12-myristate 13-acetate (PMA; Sigma), 1-octyl-2-acetyl-sn-glycerol (OAG; Sigma), KT5720 (Alomone Labs, Jerusalem, Israel), valsartan (a generous gift from Novartis, Basel, Switzerland), and candesartan (a generous gift from Takeda Pharmaceutical Chemical Industries, Osaka, Japan). The control pipette solution contained (in mmol/L) 70 potassium aspartate, 50 KCl, 10 KH\(_2\)PO\(_4\), 1 MgSO\(_4\), 3 Na\(_2\)ATP, 0.1 Li\(_2\)-GTP, 5 EGTA, and 50 mmol/L angiotensin II (E-4031; Sigma). The concentration-response relationship for the potentiation of \(I_{\text{Ks}}\) by Ang II was drawn by least-squares fit of a Hill equation: 

\[
R = \frac{R_{\text{max}}}{1 + (EC_{50}/[\text{agonist}])^n_h},
\]

where \(R_{\text{max}}\) represents the maximal degree of potentiation expressed as a percentage, \(EC_{50}\) is the concentration giving half-maximal potentiation, and \(n_h\) is the Hill coefficient. Voltage dependence of \(I_{\text{Ks}}\) activation was evaluated by fitting the normalized I-V relationship of tail currents to a Boltzmann equation: 

\[
I_{\text{Ks}} = I_{\text{Ks,0}} - I_{\text{Ks,tail}} = I_{\text{Ks,0}} - A_t \exp(-\tau_r/\tau),
\]

where \(A_t\) and \(\tau_r\) represent amplitudes of the fast and slow components, respectively, and \(\tau\) is time constant for the fast and slow components.

**Whole-Cell Patch-Clamp Techniques and Data Analysis**

Isolated atrial myocytes were current and voltage clamped using the standard whole-cell patch-clamp technique with an EPC-8 patch-clamp amplifier (HEKA Electronics, Lambrecht, Germany). Borosilicate glass electrodes had tip resistances of 2.5 to 4.0 M\(\Omega\) when filled with the pipette solution. \(I_{\text{Ks}}\) was elicited by depolarizing voltage-clamp steps given from a holding potential of −50 mV to various test potentials under conditions in which the Na\(^+\) current was inactivated by setting the holding potential to −50 mV, and \(I_{\text{Ca}}\) and \(I_{\text{Ks}}\) were blocked by nisoldipine (0.4 \(\mu\)mol/L) and E-4031 (5 mmol/L), respectively, added to the external solution for the measurement of \(I_{\text{Ks}}\).\textsuperscript{14} The effect of external application of Ang II or Sar\(^1\)-Ang II on \(I_{\text{Ks}}\) was tested after the initial rundown of \(I_{\text{Ks}}\) within 3 to 5 minutes of patch rupture was allowed to reach a steady-state level, and control records were obtained immediately before drug exposure in each experiment. Action potentials were evoked at a rate of 0.2 Hz with suprathermal current pulses of 2- to 3-ms duration applied via patch electrode in the current-clamp mode. The APD was measured at 90% repolarization (APD\(_{90}\)). All experiments were performed at 36±1°C.

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\[
I_{\text{Ks}} = I_{\text{Ks,0}} - I_{\text{Ks,tail}},
\]

where \(I_{\text{Ks,0}}\) and \(I_{\text{Ks,tail}}\) represent amplitudes of the fast and slow components, respectively.

Time courses of changes in the amplitude of \(I_{\text{Ks}}\) in the presence of various reagents were determined by measuring the amplitude of tail currents elicited on repolarization to a holding potential of −50 mV after 2000-ms depolarization to 30 mV every 10 or 20 seconds.

**Statistical Analysis**

All averaged values presented are mean ±SEM. Statistical comparisons were made by Wilcoxon signed-rank test for paired data. Wilcoxon rank-sum test was used to compare unpaired data between 2 groups; the Kruskal-Wallis test was applied to compare data among ≥3 groups. A value of \(P<0.05\) was considered statistically significant.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

**Stimulatory Action of Ang II and Sar\(^1\)-Ang II on \(I_{\text{Ks}}\) in Guinea Pig Atrial Myocytes**

Figure 1A and 1B demonstrates the representative examples for the stimulatory effect of Ang II and its stable analogue Sar\(^1\)-Ang II, respectively, on \(I_{\text{Ks}}\) in guinea pig atrial myocytes. Atrial myocytes were depolarized from a holding potential of −50 mV to test potentials of −40 to 50 mV for 2000 ms, before and during exposure to 1 \(\mu\)mol/L Ang II (Figure 1A) or 100 mmol/L Sar\(^1\)-Ang II (Figure 1B). Both Ang II and Sar\(^1\)-Ang II markedly increased the slowly activating outward currents during depolarizations and the decaying tail currents on return to the holding potential, which represented the activation and deactivation of \(I_{\text{Ks}}\), respectively. The potentiation of \(I_{\text{Ks}}\) by Ang II and Sar\(^1\)-Ang II was quantitatively evaluated by measuring the amplitude of tail currents elicited on return to the holding potential after a 2000-ms test pulse to 30 mV. As demonstrated in Figure 1C, 1 \(\mu\)mol/L Ang II and 100 mmol/L Sar\(^1\)-Ang II increased the amplitude of \(I_{\text{Ks}}\) by 60.8±6.8% (n=8) and 100.7±16.4% (n=8), respectively. The percent increase in the amplitude of \(I_{\text{Ks}}\) tail current thus calculated was plotted against concentrations of Ang II (Figure 1D). The mean data could be well
described by a Hill equation with an EC₅₀ of 6.16 nmol/L and nₕ of 1.50.

We then examined whether Ang II and Sar¹–Ang II affected the voltage dependence of IKₛ activation by measuring the amplitude of tail currents elicited on return to a holding potential of −50 mV after 2000-ms depolarizing pulses to test potentials of −40 to 50 mV. Figure 2A illustrates a representative example of I-V relationships for IKₛ tail currents recorded before and during exposure to 1 μmol/L Ang II obtained from the experiment of Figure 1A. The tail current amplitude at each test potential was then normalized with reference to the maximum value at 50 mV, and mean values for the normalized tail currents, obtained from 6 experiments, were plotted against test potentials (Figure 2B). The data points were reasonably well fitted by a Boltzmann equation, with V₁/₂ of 10.4 ± 1.5 mV and k of 11.1 ± 0.8 mV for control and V₁/₂ of 12.2 ± 2.5 mV and k of 10.3 ± 1.1 mV for Ang II (n=6). Thus, the voltage dependence of IKₛ activation was found to be affected little, if at all, by Ang II.

In a separate set of experiments, it was also confirmed that Sar¹–Ang II increased the amplitude of IKₛ without appreciably affecting the voltage dependent activation of IKₛ (V₁/₂ of 10.1 ± 2.2 mV and k of 11.8 ± 1.3 mV; n=6).

To examine whether the kinetics of IKₛ deactivation was affected by Ang II and Sar¹–Ang II, the tail currents elicited on return to −50 mV after depolarizing pulses were evaluated by fitting to the sum of 2 exponential functions (Figure 2C). The time constants for the fast (τᵢ) and slow (τₛ) components averaged 69.6 ± 10.8 and 305.6 ± 57.6 ms for control, 77.7 ± 6.2 and 233.0 ± 31.9 ms for Ang II, and 69.4 ± 12.0 and 297.2 ± 71.7 ms (n=6) for Sar¹–Ang II, respectively (Figure 2D). There are no significant differences in the values of τᵢ and τₛ among control, Ang II, and Sar¹–Ang II groups, suggesting that the kinetics of current deactivation at −50 mV was not significantly affected by Ang II and Sar¹–Ang II.

**Signal Transduction Pathways Involved in AT₁ Receptor–Mediated Increase in IKₛ**

We proceeded to explore the signal transduction pathways mediating the stimulatory action of Ang II and Sar¹–Ang II on IKₛ. To examine whether the IKₛ response to Ang II and Sar¹–Ang II was mediated through the AT₁ receptor, the effect of these agonists on IKₛ was examined in the presence of the selective AT₁ receptor antagonist valsartan. As illustrated in Figure 3A, pretreatment of atrial myocytes with 1 μmol/L valsartan almost totally prevented the stimulatory action of 100 nmol/L Sar¹–Ang II on IKₛ. In a total of 8 myocytes, Sar¹–Ang II (100 nmol/L) potentiated IKₛ by 15.6 ± 4.6% in the presence of valsartan (1 μmol/L), which is significantly smaller than the degree of the IKₛ potentiation in the absence of valsartan (100.7 ± 16.4% increase; n=8, P<0.05; Figure 3C). Similarly, the potentiation of IKₛ by 1 μmol/L Ang II was almost totally abolished by pre-exposure to 1 μmol/L valsartan (control, 60.8 ± 6.8% increase, n=8;
valsartan, 8.5±3.4% increase, n=8; P<0.05). These observations support the view that the potentiation of $I_{Ks}$ by Ang II and Sar$^1$–Ang II is mediated through the AT$_1$ receptor. Moreover, valsartan alone had minimal effect on baseline $I_{Ks}$ (7.8±2.9% increase, n=7; Figure 3C), suggesting that valsartan prevents the stimulatory action of Ang II and Sar$^1$–Ang II by blocking the binding of these agonists to the AT$_1$ receptor.

To explore whether the AT$_1$ receptor is tonically activated to potentiate $I_{Ks}$ in guinea pig atrial myocytes, we examined the effect of candesartan, the inverse agonist of the AT$_1$ receptor,$^{16}$ on $I_{Ks}$ in basal conditions. As demonstrated in Figure 3B and 3C, the baseline $I_{Ks}$ was not appreciably affected by exposure to candesartan at concentrations of 100 nmol/L and 2 μmol/L, which suggests that there is little, if any, tonic activation of AT$_1$ receptor leading to the enhancement of $I_{Ks}$ in baseline conditions of guinea pig atrial myocytes.

It has been demonstrated in various cell types, including guinea pig cardiac myocytes,$^{7,17}$ that the AT$_1$ receptor is
coupled to the activation of PLC via heterotrimeric G proteins, which results in production of inositol 1,4,5-trisphosphate (InsP3), a Ca2+-mobilizing second messenger, and diacylglycerol (DAG), an activator of PKC. Both an elevation in intracellular free Ca2+ and activation of PKC have been associated with an enhancement of \(I_{\text{Ks}}\) in guinea pig cardiac myocytes. We therefore tested whether these signaling molecules are involved in an AT1 receptor–mediated increase in \(I_{\text{Ks}}\). Because the stable analogue Sar\(^{1-}\)Ang II evokes a larger increase in the amplitude of \(I_{\text{Ks}}\) than Ang II does (Figure 1C), we used Sar\(^{1-}\)Ang II as an agonist at the AT1 receptor in subsequent experiments.

We examined whether G protein activation is involved in the signal transduction pathway by internally perfusing the nonhydrolysable GDP analogue GDP\(\beta\)S that irreversibly inhibits G protein activation. As illustrated in Figure 4A, the stimulatory effect of Sar\(^{1-}\)Ang II on \(I_{\text{Ks}}\) was greatly reduced in atrial myocytes dialyzed with 2 mmol/L GDP\(\beta\)S (control, 100.7±16.4% increase, \(n=8\); GDP\(\beta\)S, 15.9±7.0% increase, \(n=7\); \(P<0.05\)), indicating that G-protein activation mediates the potentiation of \(I_{\text{Ks}}\) via AT1 receptor. As shown in Figure 4B, the potentiation of \(I_{\text{Ks}}\) by Sar\(^{1-}\)Ang II was also significantly attenuated by loading the myocytes with the PLC inhibitor compound 48/80 at 100 \(\mu\)mol/L (compound 48/80, 32.9±9.9% increase, \(n=9\)), supporting an involvement of PLC activation.

The finding that Ang II and Sar\(^{1-}\)Ang II potentiate \(I_{\text{Ks}}\) in myocytes dialyzed with a control pipette solution containing 5 mmol/L EGTA suggests that intracellular free Ca2+ does not play an essential role in mediating the potentiation of \(I_{\text{Ks}}\) via AT1 receptor. This idea was further tested by dialyzing atrial myocytes with BAPTA in place of EGTA, which should provide more rapid and more efficient Ca2+-buffering conditions inside the cells. As shown in Figure 4C, 100 mmol/L Sar\(^{1-}\)Ang II increased the amplitude of \(I_{\text{Ks}}\) even in the myocyte loaded with 20 mmol/L BAPTA to an extent similar to that in the controls (BAPTA, 76.0±11.5% increase, \(n=4\)), indicating that the stimulatory effect of Sar\(^{1-}\)Ang II was not significantly affected by an increased Ca2+-buffering capacity achieved by BAPTA. This observation can be interpreted to indicate that intracellular free Ca2+ is not critically involved in the AT1 receptor–mediated \(I_{\text{Ks}}\) increase under the present experimental conditions.

To test whether PKC mediates the \(I_{\text{Ks}}\) response to AT1 receptor stimulation, we investigated the effect of PKC inhibitors and activators on the stimulatory action of Sar\(^{1-}\)Ang II. As illustrated in Figure 5A and 5B, the stimulatory action of Sar\(^{1-}\)Ang II was largely abolished by pretreatment of atrial myocytes either with the nonspecific PKC inhibitor H-7 (16.0±9.2% increase, \(n=4\)) or with the specific PKC inhibitor Bis I (9.8±6.4% increase, \(n=5\)). These results strongly suggest that the potentiation of \(I_{\text{Ks}}\) via the AT1 receptor involves PKC activation. We also checked whether Sar\(^{1-}\)Ang II could further increase \(I_{\text{Ks}}\) after potentiation by maximal PKC activation. In guinea pig atrial myocytes, increasing the concentration of the nonspecific PKC activator PMA above 300 mmol/L produced no further increase in \(I_{\text{Ks}}\) during maximal PKC activation. As illustrated in Figure 5C, Sar\(^{1-}\)Ang produced little further increase in \(I_{\text{Ks}}\), that was prestimulated maximally with 300 mmol/L PMA (7.7±2.3% increase, \(n=7\); Figure 5E). When these reagents were applied in reverse order (first Sar\(^{1-}\)Ang II and then PMA), there was again only a little further increase in \(I_{\text{Ks}}\) during exposure to PMA (6.2±1.0% increase, \(n=5\); data not shown). These observations suggest that Sar\(^{1-}\)Ang II and PMA activated the same signaling pathway to potentiate \(I_{\text{Ks}}\). The involvement of PKC activation was supported further by the observation that Sar\(^{1-}\)Ang II caused only a small additional increase in \(I_{\text{Ks}}\) after a maximal potentiation by the selective PKC activator OAG at 20 mmol/L (Figure 5D; 10.1±1.5% increase, \(n=9\); Figure 5E).

To rule out the possible involvement of protein kinase A (PKA) in the AT1-evoked potentiation of \(I_{\text{Ks}}\), the effect of
Sar₁–Ang II (100 nmol/L) was examined in the presence of the selective PKA inhibitor KT5720. As demonstrated in Figure 6A and 6B, there were no significant differences in the degree of $I_{Ks}$ potentiation by Sar₁–Ang II in the absence and presence of KT5720 (200 nmol/L), thus supporting the view that PKA activation is not involved in the AT₁-mediated potentiation of $I_{Ks}$.

Taken together, our results indicate that $I_{Ks}$ potentiation by AT₁ receptor is mediated primarily through the PKC activation.

**Shortening of APD by AT₁ Receptor Stimulation**

Because cardiac repolarization is shaped on a subtle balance of multiple ionic channel activities, an alteration in amplitude...
The present experiments demonstrate that stimulation of the AT1 receptor evokes a marked increase in the amplitude of $I_{Ks}$ in guinea pig atrial myocytes. Ang II is effective at potentiating $I_{Ks}$ at concentrations of $\cong 1$ nmol/L (Figure 1D), which appears to be higher compared with the plasma level of Ang II in humans at baseline conditions ( $\cong 5$ pmol/L). However, Ang II is also stored in cardiomyocytes, is secreted by various stimuli such as mechanical stress, and acts as autocrine/paracrine factors. A previous study found that the concentration of Ang II in the interstitial fluid space of dog heart is $\cong 6$ nmol/L, which seems to be comparable to the concentration needed to affect $I_{Ks}$ in cardiac myocytes.

It has been shown in various tissue and cell types that AT1 receptors are coupled predominantly to PLC via heterotrimeric G protein Gq, which leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce InsP3 and DAG. InsP3 stimulates its receptors on the sarcoplasmic reticulum to mobilize intracellular Ca$^{2+}$ stores; DAG activates Ca$^{2+}$-dependent (conventional) and Ca$^{2+}$-independent (novel) isoforms of PKC. The present results are consistent with activation of the AT1 receptor linked to a G protein (probably Gq)–PLC signaling pathway to mediate the stimulatory action of Ang II on $I_{Ks}$ (Figures 3 and 4). The involvement of resultant activation of PKC in the action of Ang II is supported by the experiments using the inhibitors and activators of PKC; the stimulatory action of Ang II was greatly reduced by the presence of Bis I and H-7 and was masked by previous application of PMA and OAG (Figure 5).

At present, the precise mechanism by which PKC regulates $I_{Ks}$ remains to be fully elucidated. The recent mutagenesis study has detected PKC phosphorylation sites (S409, S464, T513, and S577) in the C-terminus of KCNQ1 protein, responsible for potentiating the KCNQ1/KCNE1 channel, the molecular constituents of human $I_{Ks}$. However, it is also possible that PKC acts on nonchannel substrate(s) to enhance $I_{Ks}$. Further studies are thus required to clarify the molecular basis for PKC-mediated regulation of $I_{Ks}$.

Adult guinea pig myocardium has been demonstrated to express the $\alpha$, $\beta$II, $\gamma$, $\epsilon$, and $\zeta$ isoforms of PKC. The observation that $I_{Ks}$ can be readily enhanced not only by PMA and OAG but also by AT1 stimulation in a strong Ca$^{2+}$-buffering of the cytoplasm (5 mmol/L EGTA) suggests the possibility that the Ca$^{2+}$-independent novel isoform PKCe, rather than Ca$^{2+}$-dependent conventional PKC isoforms, is preferentially involved in the $I_{Ks}$ response under the present experimental conditions. It has recently been reported that the KCNQ1/KCNE1 channel heterologously expressed in Xenopus oocytes is potentiated by both PKC$\beta$II and PKCe. It will be interesting to examine which isoform of PKC mediates the potentiation of $I_{Ks}$ via AT1 receptors in atrial myocytes.

It was previously demonstrated in guinea pig ventricular myocytes that Ang II decreases $I_{Ks}$, but increases $I_{K}$, which is apparently in contrast to the present results concerning the effect of Ang II on $I_{Ks}$. One possible explanation could be the different method of dissecting $I_{Ks}$ into its 2 components, $I_{Kr}$ and $I_{Ko}$. Consistent with this, our preliminary results showed that Sar1–Ang II (100 nmol/L) did not evoke any appreciable inhibitory effect on $I_{Ks}$ in guinea pig ventricular myocytes when evaluated with the present experimental protocol shown in Figure 1 (unpublished observation). Alternatively, intracellular signaling pathways coupled to the AT1 receptor might...
be dissimilar between atrial and ventricular myocytes. It was also shown that in guinea pig hearts, AT1 receptor in atria has a higher affinity for Ang II than that in ventricles.26

Previous studies have shown that AF itself causes progressive electrophysiological remodeling (shortening of effective refractory period) in the atria by affecting the expression and function of several ion channels.27,28 It has recently been demonstrated that an upregulation of AT1 receptors, which occurs in the left atrium of patients with lone AF and AF with mitral valve disease, is closely related to the remodeling process and stabilization of AF.29 Consistent with this notion, it was also reported that electrical remodeling during experimental AF is prevented by the AT1 antagonist candesartan in dogs.30 The AT1 receptor–mediated shortening of APD via potentiation of \(I_K\) (Figure 7) might be the another way through which Ang II participates in electrophysiological perturbation in the atria during AF. On the other hand, the present observation that a drastic shortening of atrial APD by AT1 stimulation can be substantially reversed after addition of the AT1 antagonist valsartan could explain why the incidence of newly developed AF is decreased in patients (with heart failure) who receive the drug (Val-HeFT trial).31 It should be noted, however, that a possible direct blockade of repolarizing currents other than \(I_K\), by valsartan could also contribute to the reversal of APD shortening observed in this study (Figure 7B and 7C).

In recent years, a prospective, randomized trial has demonstrated that in patients with persistent AF cardioverted to sinus rhythm, adding the AT1 antagonist irbesartan to amiodarone significantly improves Sinus rhythm maintenance compared with treatment with amiodarone alone.19 In this trial, the benefit of irbesartan is largely ascribed to the reduction of the immediate and so-called subacute (during 1 hour and the first weeks after cardioversion, respectively) recoveries of AF. The immediate reversal of APD shortening by AT1 blockade (Figure 7) may again contribute at least partly to this advantage of irbesartan in preventing relapses of AF in the initial short-term phase.

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Disclosures
None.

References
Heart failure is associated with activation of the renin-angiotensin system and with atrial fibrillation (AF). We investigated the effects of angiotensin (Ang) II and its stable analogue Sar₁–Ang II on I_Ks that contributes to repolarization in the atrium and ventricle. In guinea pig atrial myocytes, Ang II and Sar₁–Ang II increased the amplitude of I_Ks by activating the Ang II type 1 receptor coupled to a G protein–phospholipase C–protein kinase C signaling pathway. As expected from this stimulatory action on I_Ks, Sar₁–Ang II markedly shortens the action potential duration, and this electrophysiological effect is reversed by the Ang II type 1 receptor antagonist valsartan. Because shortening of refractoriness promotes reentry, these results suggest a potential mechanism by which elevated levels of Ang II may promote AF in heart failure that warrants further investigation. Reversal of these electrophysiological effects might be a mechanism through which Ang II antagonists can reduce AF.
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