currents of atrial and ventricular action potentials.[12] I_{Kr} and I_{Ks} reflect the expression of distinct molecular entities; the pore-forming α-subunit KvLQT1 (KCNQ1) coassembles with an accessory β subunit minK (KCNE1) to form the I_{Ks} channels, and the HERG (KCNH2) constitutes the pore-forming subunit of the channel that underlies the I_{Kr} 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.[13] I_{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_{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_{Ks} through a mechanism involving activation of the G protein–coupled 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.[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 NaH_2PO_4, 5.5 glucose, and 5.0 HEPES (pH adjusted to 7.4 with NaOH). The standard external solution for measuring I_{Ks} was normal Tyrode's solution supplemented with 0.4 μmol/L nisoldipine (a generous gift from Bayer AG, Wuppertal-Elberfeld, Germany) and 5 μmol/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), 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7, Seikagaku, Tokyo, Japan), bisindolylmaleimide I (Bis I, Sigma), phorbol 12-myristate 13-acetate (PMA; Sigma), 1-oleoyl-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_2PO_4, 1 $MgSO_4$, 3 Na_2-ATP, 0.1 Li_2-GTP, 5 EGTA, and 5 HEPES (pH adjusted to 7.2 with KOH). The concentration of free Ca^{2+} and Mg^{2+} in the pipette solution was calculated to be $\approx 1.5\times10^{-10}$ mol/L (pCa, 9.8) and 3.7×10^{-5} mol/L (pMg=4.4), respectively. In some experiments, 0.1 mmol/L GTP was replaced with 2 mmol/L GDPβS (trilithium salt, Roche), and 5 mmol/L EGTA was substituted with 20 mmol/L BAPTA (Sigma). To inhibit PLC, compound 48/80 (Sigma) was added to the pipette solution.

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 MΩ when filled with the pipette solution. I_{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 $I_{Ca,L}$ and I_{Kr} were blocked by nisoldipine (0.4 μmol/L) and E-4031 (5 μmol/L), respectively, added to the external solution for the measurement of I_{Ks}.[14] The effect of external application of Ang II or Sar^1–Ang II on I_{Ks} was tested after the initial rundown of I_{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 suprathreshold 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.

The concentration-response relationship for the potentiation of I_{Ks} by Ang II was drawn by least-squares fit of a Hill equation: $R=R_{max}/\{1+(EC_{50}/[agonist])^{nH}\}$, where R_{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_{Ks} activation was evaluated by fitting the normalized I-V relationship of tail currents to a Boltzmann equation: $I_{K,tail}=1/\{1+\exp[(V_{1/2}-V_m)/k]\}$, where $I_{K,tail}$ is the tail current amplitude normalized with reference to the maximum value measured at 50 mV, $V_{1/2}$ is the voltage at half-maximal activation, V_m is the test potential, and k is the slope factor. Time course for the decay of the I_{Ks} tail current was fitted with the sum of 2 exponential functions: $I_{K,tail}=A_f\exp(-t/\tau_f)+A_s\exp(-t/\tau_s)$, where A_f and A_s represent amplitudes of the fast and slow components, respectively, and τ_f and τ_s are time constants for the fast and slow components, respectively.

Time courses of changes in the amplitude of I_{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_{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_{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 μmol/L Ang II (Figure 1A) or 100 nmol/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_{Ks}, respectively. The potentiation of I_{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 μmol/L Ang II and 100 nmol/L Sar^1–Ang II increased the amplitude of I_{Ks} by 60.8±6.8% (n=8) and 100.7±16.4% (n=8), respectively. The percent increase in the amplitude of I_{Ks} tail current thus calculated was plotted against concentrations of Ang II (Figure 1D). The mean data could be well

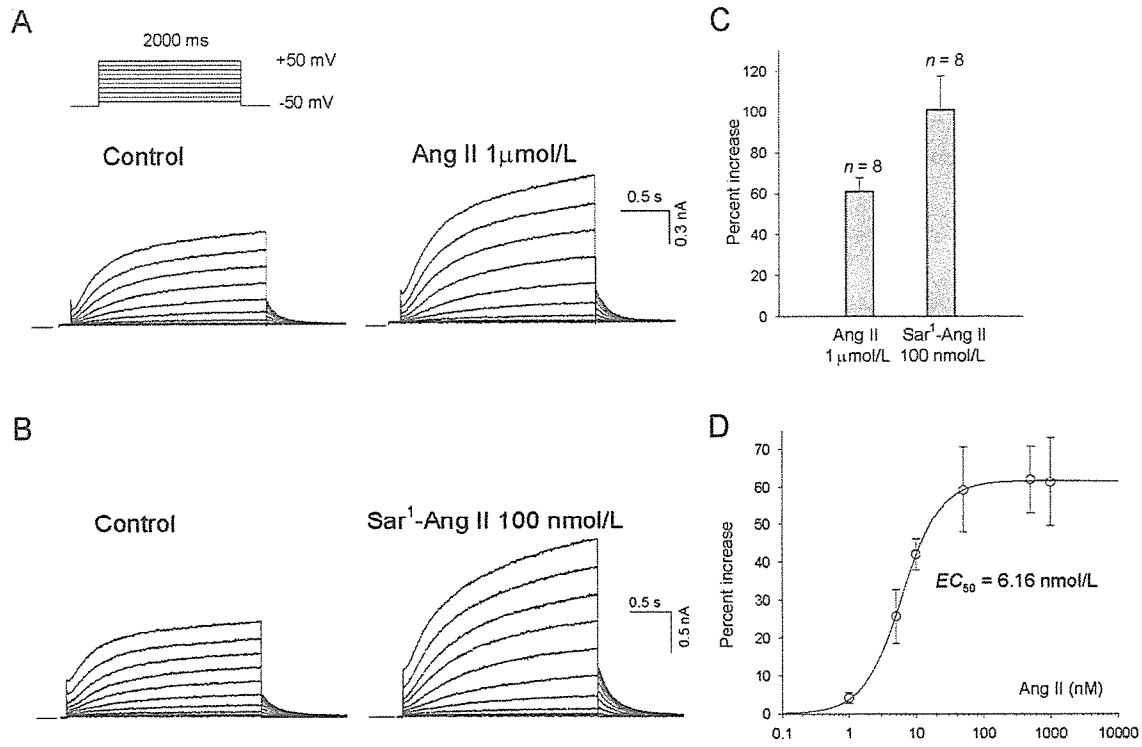

Figure 1. Enhancement of I_{Ks} by Ang II and Sar¹–Ang II. A, B, I_{Ks} was activated by 2000-ms depolarizing pulses to test potentials of −40 to 50 mV in 10-mV steps before (Control) and ≈4 minutes after exposure to 1 μmol/L Ang II (A) or 100 nmol/L Sar¹–Ang II (B). A and B were obtained from different myocytes. C, Percent increase in the amplitude of I_{Ks} tail current evoked by 1 μmol/L Ang II and 100 nmol/L Sar¹–Ang II (n=8 each), measured after 2000-ms depolarization to 30 mV. D, Concentration-response relationship for the increase of I_{Ks} tail current evoked by Ang II at concentrations between 1 and 1000 nmol/L. Each value is the mean, and error bars represent SEM of 4 to 6 measurements. Only 1 concentration of Ang II was examined in a given cell to exclude the influence of possible desensitization to the agonist.

described by a Hill equation with an EC_{50} of 6.16 nmol/L and n_H of 1.50.

We then examined whether Ang II and Sar¹–Ang II affected the voltage dependence of I_{Ks} 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 I_{Ks} 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 $V_{1/2}$ of 10.4±1.5 mV and k of 11.1±0.8 mV for control and $V_{1/2}$ of 12.2±2.5 mV and k of 10.3±1.1 mV for Ang II (n=6). Thus, the voltage dependence of I_{Ks} 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 I_{Ks} without appreciably affecting the voltage dependent activation of I_{Ks} ($V_{1/2}$ of 10.1±2.2 mV and k of 11.8±1.3 mV; n=6).

To examine whether the kinetics of I_{Ks} 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 (τ_f) and slow (τ_s) 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 τ_f and τ_s 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 AT_1 Receptor–Mediated Increase in I_{Ks}

We proceeded to explore the signal transduction pathways mediating the stimulatory action of Ang II and Sar¹–Ang II on I_{Ks}. To examine whether the I_{Ks} response to Ang II and Sar¹–Ang II was mediated through the AT_1 receptor, the effect of these agonists on I_{Ks} was examined in the presence of the selective AT_1 receptor antagonist valsartan.[15] 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 I_{Ks}. In a total of 8 myocytes, Sar¹–Ang II (100 nmol/L) potentiated I_{Ks} by 15.6±4.6% in the presence of valsartan (1 μmol/L), which is significantly smaller than the degree of the I_{Ks} potentiation in the absence of valsartan (100.7±16.4% increase; n=8, $P<0.05$; Figure 3C). Similarly, the potentiation of I_{Ks} 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;

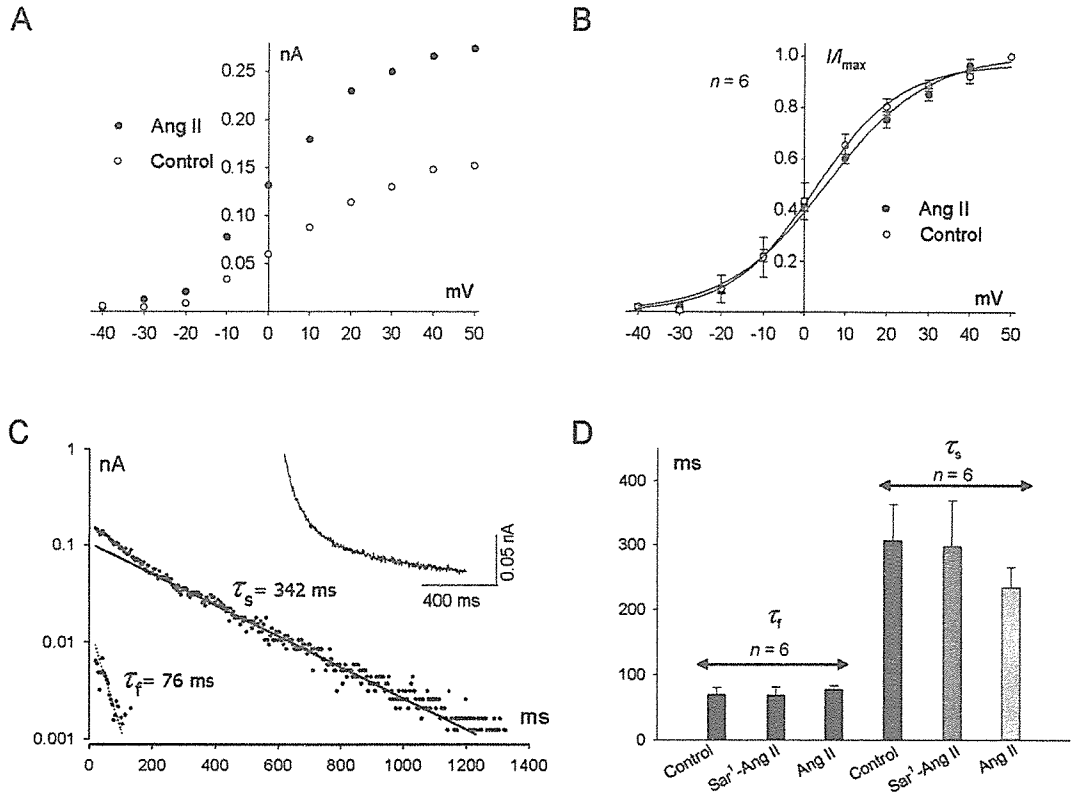

Figure 2. Effects of Ang II and Sar¹–Ang II on the voltage dependence of activation and the kinetics of deactivation. A, I-V relationships for I_{Ks} tail currents elicited on return to a holding potential of −50 mV after 2000-ms depolarization to various test potentials before and during exposure to 1 μmol/L Ang II. B, I-V relationships for normalized I_{Ks} tail currents. Smooth curves represent the least-squares fit of the data points (mean±SEM of 6 cells) to a Boltzmann equation. C, Semilogarithmic plot of I_{Ks} tail current elicited on repolarization to −50 mV after a 2000-ms voltage step to 30 mV under control conditions. Inset, Original current trace used for the analysis (τ_f=76 ms, τ_s=342 ms). D, Summarized data for τ_f and τ_s in control and during exposure to 1 μmol/L Ang II and 100 nmol/L Sar¹–Ang II.

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 Sar¹–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 Sar¹–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

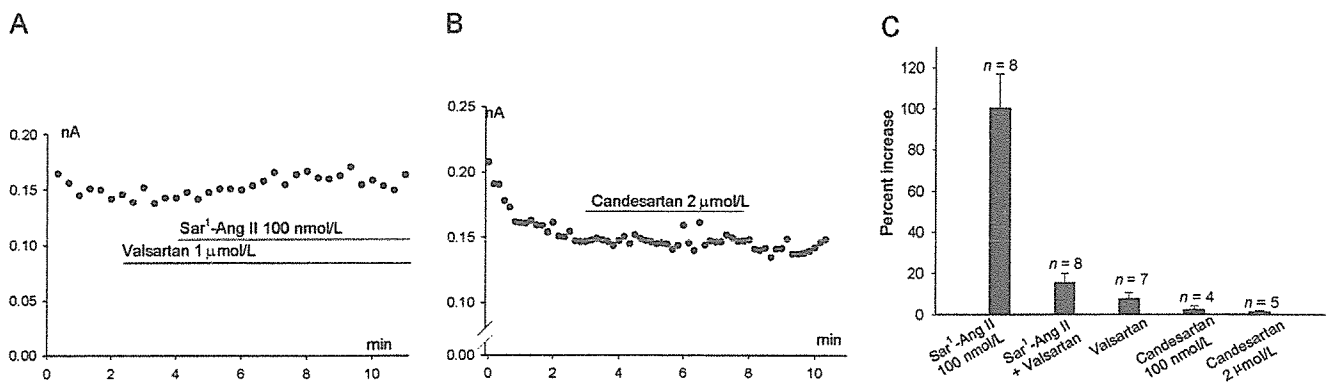

Figure 3. Potentiation of I_{Ks} by Sar¹–Ang II is mediated via AT_1 receptor. A, Effect of Sar¹–Ang II (100 nmol/L) on I_{Ks} in the presence of valsartan (1 μmol/L). B, Effect of candesartan (2 μmol/L) on I_{Ks} under baseline conditions. C, Summarized data for percent increase in the amplitude of I_{Ks} tail current evoked by Sar¹–Ang II (1 μmol/L), Sar¹–Ang II (1 μmol/L) plus valsartan (1 μmol/L), valsartan (1 μmol/L) alone, and candesartan alone at concentrations of 100 nmol/L and 2 μmol/L.

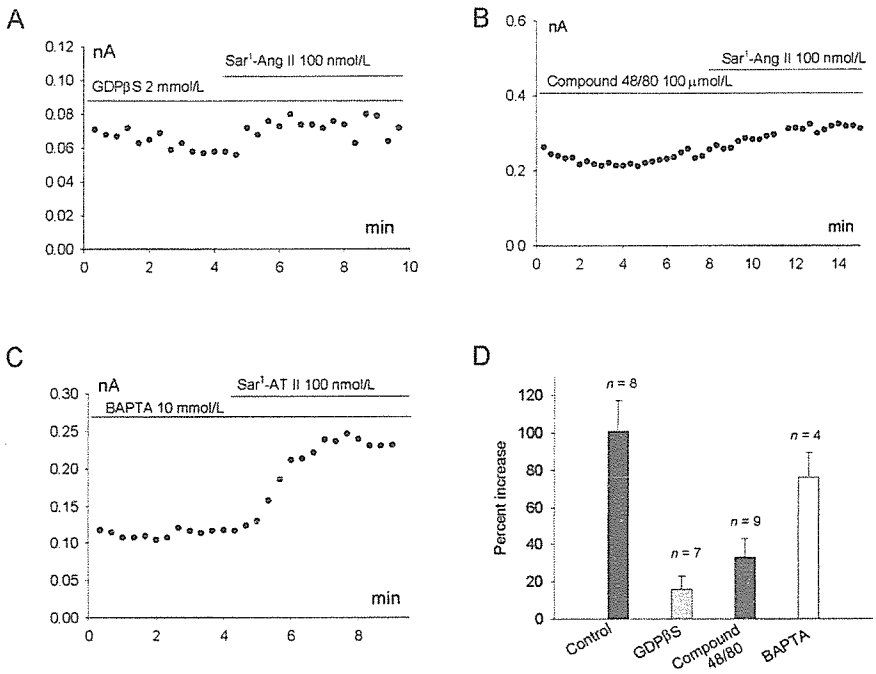

Figure 4. Evaluation of the involvement of G proteins, PLC, or intracellular free Ca^{2+} in the potentiation of I_{Ks} by AT_1 receptor stimulation. A–C, Effects of internal application of 2 mmol/L GDPβS (A), 100 μmol/L compound 48/80 (B), or 20 mmol/L BAPTA (C) on the response of I_{Ks} to 100 nmol/L Sar¹–Ang II. These reagents were allowed to dialyze into the cell for at least 4 minutes before bath application of Sar¹–Ang II. D, Summarized data for percent increase in I_{Ks} by 100 nmol/L Sar¹–Ang II in myocytes dialyzed with control pipette solution and pipette solutions containing GDPβS, compound 48/80, or BAPTA.

coupled to the activation of PLC via heterotrimeric G proteins, which results in production of inositol 1,4,5-trisphosphate ($InsP_3$), a Ca^{2+}-mobilizing second messenger, and diacylglycerol (DAG), an activator of PKC. Both an elevation in intracellular free Ca^{2+} and activation of PKC have been associated with an enhancement of I_{Ks} in guinea pig cardiac myocytes.[18] We therefore tested whether these signaling molecules are involved in an AT_1 receptor–mediated increase in I_{Ks}. Because the stable analogue Sar¹–Ang II evokes a larger increase in the amplitude of I_{Ks} than Ang II does (Figure 1C), we used Sar¹–Ang II as an agonist at the AT_1 receptor in subsequent experiments.

We examined whether G protein activation is involved in the signal transduction pathway by internally perfusing the nonhydrolysable GDP analogue GDPβS that irreversibly inhibits G protein activation. As illustrated in Figure 4A, the stimulatory effect of Sar¹–Ang II on I_{Ks} was greatly reduced in atrial myocytes dialyzed with 2 mmol/L GDPβS (control, 100.7±16.4% increase, n=8; GDPβS, 15.9±7.0% increase, n=7; $P<0.05$), indicating that G-protein activation mediates the potentiation of I_{Ks} via AT_1 receptor. As shown in Figure 4B, the potentiation of I_{Ks} by Sar¹–Ang II was also significantly attenuated by loading the myocytes with the PLC inhibitor compound 48/80 at 100 μmol/L (compound 48/80, 32.9±9.9% increase, n=9), supporting an involvement of PLC activation.

The finding that Ang II and Sar¹–Ang II potentiate I_{Ks} in myocytes dialyzed with a control pipette solution containing 5 mmol/L EGTA suggests that intracellular free Ca^{2+} does not play an essential role in mediating the potentiation of I_{Ks} via AT_1 receptor. This idea was further tested by dialyzing atrial myocytes with BAPTA in place of EGTA, which should provide more rapid and more efficient Ca^{2+}-buffering conditions inside the cells. As shown in Figure 4C, 100 nmol/L Sar¹–Ang II increased the amplitude of I_{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 Sar¹–Ang II was not significantly affected by an increased Ca^{2+}-buffering capacity achieved by BAPTA. This observation can be interpreted to indicate that intracellular free Ca^{2+} is not critically involved in the AT_1 receptor–mediated I_{Ks} increase under the present experimental conditions.

To test whether PKC mediates the I_{Ks} response to AT_1 receptor stimulation, we investigated the effect of PKC inhibitors and activators on the stimulatory action of Sar¹–Ang II. As illustrated in Figure 5A and 5B, the stimulatory action of Sar¹–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_{Ks} via the AT_1 receptor involves PKC activation. We also checked whether Sar¹–Ang II could further increase I_{Ks} after potentiation by maximal PKC activation. In guinea pig atrial myocytes, increasing the concentration of the nonspecific PKC activator PMA above 300 nmol/L produced no further increase in the amplitude of I_{Ks} (data not shown), indicating that a maximal potentiation of I_{Ks} was attained by 300 nmol/L PMA (44.5±5.6% increase, n=4). As illustrated in Figure 5C, Sar¹-Ang produced little further increase in I_{Ks} that was prestimulated maximally with 300 nmol/L PMA (7.7±2.3% increase, n=7; Figure 5E). When these reagents were applied in reverse order (first Sar¹–Ang II and then PMA), there was again only a little further increase in I_{Ks} during exposure to PMA (6.2±1.0% increase, n=5; data not shown). These observations suggest that Sar¹–Ang II and PMA activated the same signaling pathway to potentiate I_{Ks}. The involvement of PKC activation was supported further by the observation that Sar¹–Ang II caused only a small additional increase in I_{Ks} after a maximal potentiation by the selective PKC activator OAG at 20 μmol/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 AT_1-evoked potentiation of I_{Ks}, the effect of

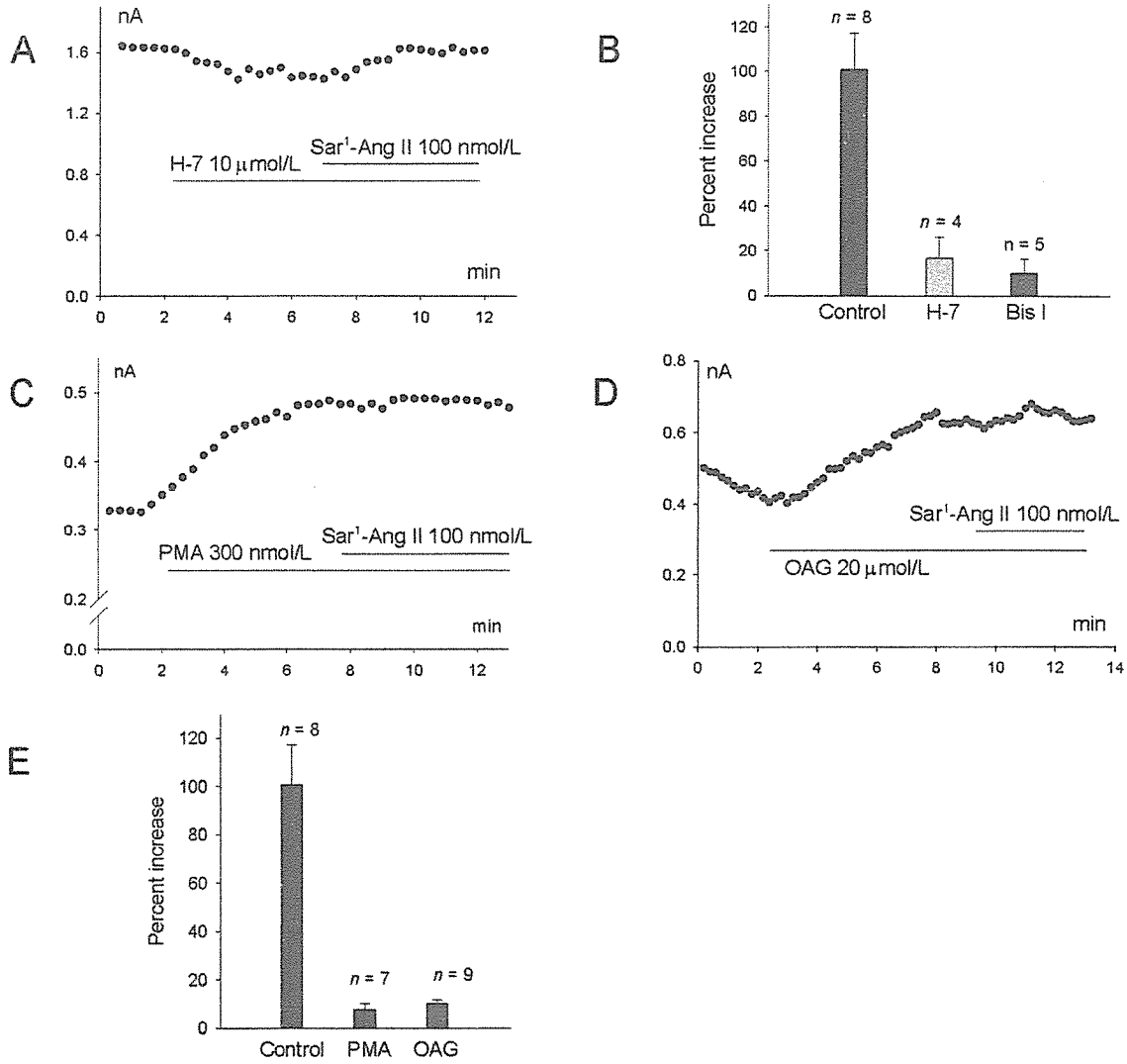

Figure 5. Enhancement of I_{Ks} via AT_1 receptor is mediated through PKC activation. A, C, D, Time course for changes in the amplitude of I_{Ks} tail current during exposure to Sar¹–Ang II (100 nmol/L) in the presence of 10 μmol/L H-7 (A), 300 nmol/L PMA (C), or 20 μmol/L OAG (D). B, E, Summarized data for percent increase in I_{Ks} evoked by Sar¹–Ang II in the presence of the PKC inhibitors H-7 and Bis I (B) and PKC activators PMA and OAG (E). $P<0.05$, H-7, Bis I, PMA, or OAG group vs control.

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 AT_1-mediated potentiation of I_{Ks}.

Taken together, our results indicate that I_{Ks} potentiation by AT_1 receptor is mediated primarily through the PKC activation.

Shortening of APD by AT_1 Receptor Stimulation

Because cardiac repolarization is shaped on a subtle balance of multiple ionic channel activities, an alteration in amplitude

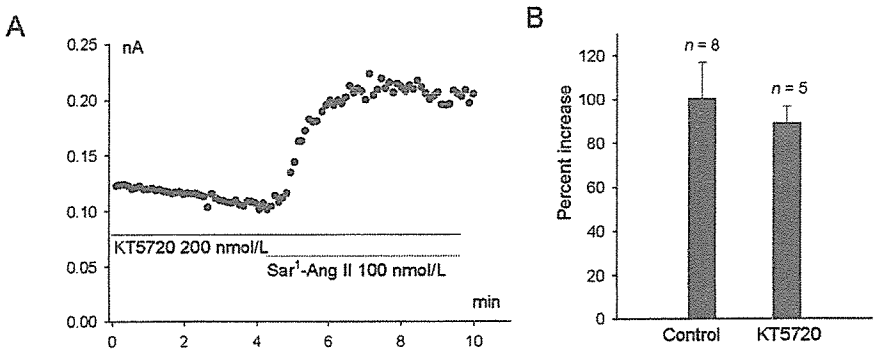

Figure 6. PKA activation is not involved in the enhancement of I_{Ks} via the AT_1 receptor. A, Time course of changes in the amplitude of I_{Ks} tail current during exposure to 100 nmol/L Sar¹–Ang II in the presence of 200 nmol/L KT5720. B, Percentage increase in I_{Ks} evoked by Sar¹–Ang II in the absence and presence of KT5720.

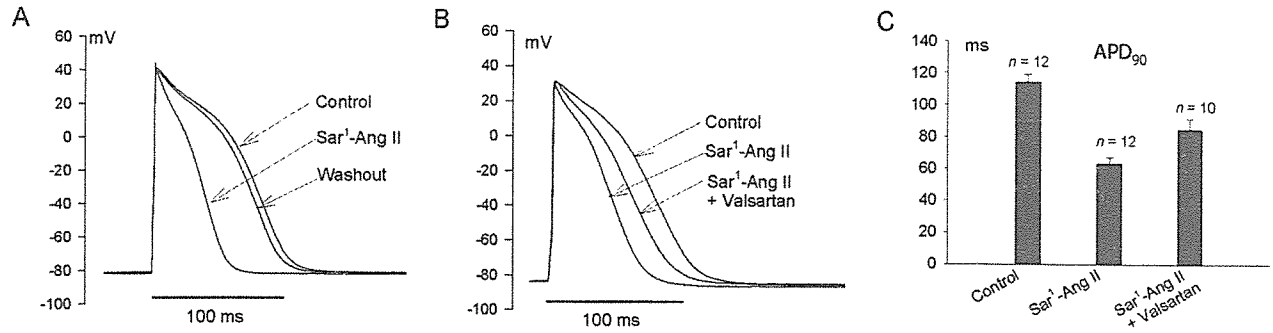

Figure 7. AT_1 receptor–mediated shortening of the APD in atrial myocytes. A, Superimposed action potentials recorded before and ≈2 minutes after exposure to 100 nmol/L Sar^1–Ang II and ≈5 minutes after the Sar^1–Ang II was washed out. B, Superimposed action potentials recorded before and during exposure to Sar^1–Ang II, initially without and then with 1 μmol/L valsartan. C, Summarized data for changes in APD_{90} by exposure to Sar^1–Ang II, without and then with valsartan.

of major repolarizing currents such as I_{Ks} would lead to substantial changes in the repolarization process. We therefore examined the net effect of Sar^1–Ang II on action potentials in guinea pig atrial myocytes during superfusion with normal Tyrode's solution with no added blockers for ionic channels. Figure 7A represents superimposed traces of action potentials recorded before and during exposure to Sar^1–Ang II (100 nmol/L) and after the agonist was washed out. Sar^1–Ang II markedly shortened APD, which was almost totally reversed on washing out of the agonist. In a separate set of experiments (Figure 7B), we confirmed that the Sar^1–Ang II (100 nmol/L)–induced shortening of APD was significantly reversed by the subsequently applied valsartan (1 μmol/L). In a total of 12 myocytes, APD_{90} was reduced from a control value of 113.1±8.8 to 63.1±5.8 ms during exposure to Sar^1–Ang II, which was partially recovered to 88.1±7.0 ms (n=10) by the subsequent application of valsartan (Figure 7C). The resting membrane potential (control, −84±3 mV; Sar^1–Ang II, −83±2 mV; n=8) and action potential amplitude (control, 120±9 mV; Sar^1–Ang II, 118±6 mV; n=8) remained unchanged during exposure to Sar^1–Ang II.

Discussion

The present experiments demonstrate that stimulation of the AT_1 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 ≥≈1 nmol/L (Figure 1D), which appears to be higher compared with the plasma level of Ang II in humans at baseline conditions (≈5 pmol/L).[19] However, Ang II is also stored in cardiomyocytes, is secreted by various stimuli such as mechanical stress, and acts as autocrine/paracrine factors.[20] A previous study found that the concentration of Ang II in the interstitial fluid space of dog heart is ≈6 nmol/L,[21] 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 AT_1 receptors are coupled predominantly to PLC via heterotrimeric G protein Gq, which leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce $InsP_3$ and DAG.[7] $InsP_3$ 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 AT_1 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,[22] the molecular constituents of human I_{Ks}.[13] 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 α, βII, γ, ε, and ζ isoforms of PKC.[23] The observation that I_{Ks} can be readily enhanced not only by PMA and OAG but also by AT_1 stimulation in a strong Ca^{2+} buffering of the cytoplasm (5 mmol/L EGTA) suggests the possibility that the Ca^{2+}-independent novel isoform PKCε, 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 PKCβII and PKCε.[24] It will be interesting to examine which isoform of PKC mediates the potentiation of I_{Ks} via AT_1 receptors in atrial myocytes.

It was previously demonstrated in guinea pig ventricular myocytes that Ang II decreases I_{Ks} but increases I_{Kr},[25] 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_K into its 2 components, I_{Kr} and I_{Ks}. Consistent with this, our preliminary results showed that Sar^1–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 AT_1 receptor might

be dissimilar between atrial and ventricular myocytes. It was also shown that in guinea pig hearts, AT_1 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 AT_1 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 AT_1 antagonist candesartan in dogs.[30] The AT_1 receptor–mediated shortening of APD via potentiation of I_{Ks} (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 AT_1 stimulation can be substantially reversed after addition of the AT_1 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_{Ks} 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 AT_1 antagonist irbesartan to amiodarone is more effective in maintaining sinus rhythm compared with treatment with amiodarone alone.[10] 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) recurrences of AF. The immediate reversal of APD shortening by AT_1 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.

Acknowledgments

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Disclosures

None.

References

1. Griendling KK, Lassegue B, Alexander W. Angiotensin receptors and their therapeutic implications. *Annu Rev Pharmacol Toxicol.* 1996;36: 281–306.
2. Opie LH, Sack MN. Enhanced angiotensin II activity in heart failure: reevaluation of the counterregulatory hypothesis of receptor subtypes. *Circ Res.* 2001;88:654–658.
3. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med.* 1999; 340:115–126.
4. Higaki J, Aoki M, Morishita R, Kida I, Taniyama, Tomita N, Yamamoto K, Moriguchi A, Kaneda Y, Ogihara T. In vivo evidence of the importance of cardiac angiotensin-converting enzyme in the pathogenesis of cardiac hypertrophy. *Arterioscler Thromb Vasc Biol.* 2000;20: 428–434.
5. Paradis P, Dali-Youcef N, Paradis FW, Thibault G, Nemer M. Overexpression of angiotensin II type I receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. *Proc Natl Acad Sci U S A.* 2000; 97:931–936.
6. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger TH. International Union of Pharmacology, XXIII: the angiotensin II receptors. *Pharmacol Rev.* 2000;52:415–472.
7. Touyz RM, Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. *Pharmacol Rev.* 2000;52:639–672.
8. Kumagai K, Nakashima H, Urata H, Gondo N, Arakawa K, Saku K. Effects of angiotensin II type I receptor antagonist on electrical and structural remodeling in atrial fibrillation. *J Am Coll Cardiol.* 2003;41: 2197–2204.
9. Harada K, Komuro I, Hayashi D, Sugaya T, Murakami K, Yazaki Y. Angiotensin II type 1a receptor is involved in the occurrence of reperfusion arrhythmias. *Circulation.* 1998;97:315–317.
10. Madrid AH, Bueno MG, Rebollo JMG, Marín I, Pena G, Bernal E, Rodriguez A, Cano L, Cano JM, Cabeza P, Moro C. Use of irbesartan to maintain sinus rhythm in patients with long-lasting persistent atrial fibrillation: a prospective and randomized study. *Circulation.* 2002;106: 331–336.
11. Fletcher RD, Cintron GB, Johnson G, Orndorff J, Carson P, Cohn JN, for the V-HeFT II VA Cooperative Studies Group. Enalapril decreases prevalence of ventricular tachycardia in patients with chronic congestive heart failure. *Circulation.* 1993;87(suppl VI):VI-49–VI-55.
12. Wang Z, Fermini B, Nattel S. Rapid and slow components of delayed rectifier current in human atrial myocytes. *Cardiovasc Res.* 1994;28: 1540–1546.
13. Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell.* 2001;104:569–580.
14. Ding WG, Toyoda F, Matsuura H. Regulation of cardiac I_{Ks} potassium current by membrane phosphatidylinositol 4,5-bisphosphate. *J Biol Chem.* 2004;279:50726–50734.
15. Criscione L, de Gasparo M, Buhlmayer P, Whitebread S, Ramjoue HP, Wood J. Pharmacological profile of valsartan: a potent, orally active, nonpeptide antagonist of the angiotensin II AT1-receptor subtype. *Br J Pharmacol.* 1993;110:761–771.
16. Zou Y, Akazawa H, Qin Y, Sano M, Takano H, Minamino T, Makita N, Iwanaga K, Zhu W, Kudoh S, Toko H, Tamura K, Kihara M, Nagai T, Fukamizu A, Umemura S, Iiri T, Fujita T, Komuro I. Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol.* 2004;6:499–506.
17. Paul K, Ball NA, Dorn II GW, Walsh RA. Left ventricular stretch stimulates angiotensin II–mediated phosphatidylinositol hydrolysis and protein kinase Cε isoform translocation in adult guinea pig hearts. *Circ Res.* 1997;81:643–650.
18. Tohse N, Kameyama M, Irisawa H. Intracellular Ca^{2+} and protein kinase C modulate K^+ current in guinea pig heart cells. *Am J Physiol.* 1987; 253:H1321–H1324.
19. Bragat AC, Blumenfeld J, Sealey JE. Effect of high-performance liquid chromatography on plasma angiotensin II measurements in treated and untreated normotensive and hypertensive patients. *J Hypertens.* 1997;15: 459–465.
20. Sadoshima J, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell.* 1993;75:977–984.
21. Dell'Italia LJ, Meng QC, Balcells E, Wei CC, Palmer R, Hageman GR, Durand J, Hankes GH, Oparil S. Compartmentalization of angiotensin II generation in the dog heart: evidence for independent mechanisms in intravascular and interstitial spaces. *J Clin Invest.* 1997;100:253–258.
22. Kathöfer S, Röckl K, Zhang W, Thomas D, Katus H, Kiehn J, Kreye V, Schoels W, Karle C. Human β_3-adrenoreceptors couple to KvLQT1/MinK potassium channels in *Xenopus* oocytes via protein kinase C phosphorylation of the KvLQT1 protein. *Naunyn Schmiedebergs Arch Pharmacol.* 2003;368:119–126.
23. Takeishi Y, Jalili T, Ball NA, Walsh RA. Responses of cardiac protein kinase C isoforms to distinct pathological stimuli are differentially regulated. *Circ Res.* 1999;85:264–271.
24. Xiao GQ, Mochly-Rosen D, Boutjdir M. PKC isozyme selective regulation of cloned human cardiac delayed slow rectifier K current. *Biochem Biophys Res Commun.* 2003;306:1019–1025.
25. Daleau P, Turgeon J. Angiotensin II modulates the delayed rectifier potassium current of guinea pig ventricular myocytes. *Pflugers Arch.* 1994;427:553–555.

26. Baker KM, Singer HA. Identification and characterization of guinea pig angiotensin II ventricular and atrial receptors: coupling to inositol phosphate production. *Circ Res.* 1988;62:896–904.
27. Bosch RF, Zeng X, Grammer JB, Popovic K, Mewis C, Kühlkamp V. Ionic mechanisms of electrical remodeling in human atrial fibrillation. *Cardiovasc Res.* 1999;44:121–131.
28. Allessie M, Ausma J, Schotten U. Electrical, contractile and structural remodeling during atrial fibrillation. *Cardiovasc Res.* 2002;54:230–246.
29. Boldt A, Wetzel U, Weigl J, Garbade J, Lauschke J, Hindricks G, Kottkamp H, Gummert JF, Dhein S. Expression of angiotensin II receptors in human left and right atrial tissue in atrial fibrillation with and without underlying mitral valve disease. *J Am Coll Cardiol.* 2003;42:1785–1792.
30. Nakashima H, Kumagai K, Urata H, Gondo N, Ideishi M, Arakawa K. Angiotensin II antagonist prevents electrical remodeling in atrial fibrillation. *Circulation.* 2000;101:2612–2617.
31. Maggioni AP, Latini R, Carson PE, Singh SN, Barlera S, Glazer R, Masson S, Ceré E, Tognoni G, Cohn JN. Valsartan reduces the incidence of atrial fibrillation in patients with heart failure: results from the Valsartan Heart Failure Trial (Val-HeFT). *Am Heart J.* 2005;149:548–557.

Response to Letter Regarding Article, "Angiotensin II Potentiates the Slow Component of Delayed Rectifier K^+ Current via the AT_1 Receptor in Guinea Pig Atrial Myocytes"

We thank Dr Christ and his colleagues very much for their interest in our article.[1] We attempted to contend that the short-term enhancement of atrial I_{Ks} by angiotensin II (Ang II) shortens the action potential duration (APD) and may contribute to the very early phase of atrial electrical remodeling. We fully agree that the antifibrotic effects may represent the principal mechanism by which Ang II type 1 receptor blockers prevent atrial fibrillation (AF), but the difference between short-term and long-term phases of atrial remodeling should also be considered.

Dr Christ and colleagues cited a rapid pacing of the right atrium in a canine model used to induce long-term atrial structural remodeling and increased susceptibility to AF.[2] The animals were treated with candesartan from 1 week before the start of pacing, and the treatment was continued for 5 weeks. Although candesartan prevented structural remodeling and susceptibility to AF, it failed to affect the changes in atrial effective refractory period (AERP) produced by atrial tachycardia. On the other hand, the same authors[3] examined the effect of candesartan on short-term changes in AERP produced by rapid atrial pacing for just 180 minutes. The AERP shortening was completely prevented by candesartan, demonstrating a role for Ang II type 1 receptors in the early stage of electrical remodeling. In humans, irbesartan was found to significantly suppress the recurrence of AF.[4] The AF recurrence was efficiently inhibited during early days but not at more than 14 days. The most straightforward interpretation of the data would be that irbesartan did not prevent the development of atrial fibrosis but regressed the electrical remodeling. Our study, which examined the immediate action of Ang II and Ang II type 1 receptor blockers on a specific ion current regulating the APD, is consistent with a role for Ang II in early electrical remodeling.

In the studies of human atrial myocytes by Bertaso et al and Wang et al[5,6] cited by Dr Christ et al, the myocytes were isolated by using the "chunk" method, which has previously been demonstrated to be associated with damage to delayed rectifier currents.[7] Thus, we cannot take the absence of I_{Ks} from patch-clamp recordings of isolated human atrial myocytes as evidence of their lack of contribution to atrial repolarization in humans. Moreover, gain-of-function mutations of KCNQ1 are known to cause familial AF.[8] We therefore think that upregulation of human atrial I_{Ks} should be of substantial contribution in generating the AF, in which the shortening of APD is at least 1 of the major determinants of AF maintenance.

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Dimitar P. Zankov, MD
Mariko Omatsu-Kanbe, PhD
Futoshi Toyoda, PhD
Wei-Guang Ding, MD, PhD
Hiroshi Matsuura, MD, PhD
Department of Physiology
Shiga University of Medical Science
Shiga, Japan

Takahiro Isono, PhD
Central Research Laboratory
Shiga University of Medical Science
Shiga, Japan

Minoru Horie, MD, PhD
Department of Cardiovascular and Respiratory Medicine
Shiga University of Medical Science
Shiga, Japan

1. Zankov DP, Omatsu-Kanbe M, Isono T, Toyoda F, Ding WG, Matsuura H, Horie M. Angiotensin II potentiates the slow component of delayed rectifier K+ current via the AT1 receptor in guinea pig atrial myocytes. *Circulation.* 2006;113:1278–1286.
2. Kumagai K, Nakshima H, Urata H, Gondo N, Arakawa K, Saku K. Effects of angiotensin II type 1 receptor antagonist on electrical and structural remodeling in atrial fibrillation. *J Am Coll Cardiol.* 2003;41:2197–2204.
3. Nakashima H, Kumagai K, Urata H, Gondo N, Ideishi M, Arakawa K. Angiotensin II antagonist prevents electrical remodeling in atrial fibrillation. *Circulation.* 2000;101:2612–2617.
4. Madrid AH, Bueno MG, Rebollo JM, Marin I, Pena G, Bernal E, Rodriguez A, Cano L, Cano JM, Cabeza P, Moro C. Use of irbesartan to maintain sinus rhythm in patients with long-lasting persistent atrial fibrillation: a prospective and randomized study. *Circulation.* 2002;106:331–336.
5. Bertaso F, Sharpe CC, Hendry BM, James AF. Expression of voltage-gated K^+ channels in human atrium. *Basic Res Cardiol.* 2002;97:424–433.
6. Wang Z, Fermini B, Nattel S. Delayed rectifier outward current and repolarization in human atrial myocytes. *Circ Res.* 1993;73:276–285.
7. Yue L, Feng J, Li G-R, Nattel S. Transient outward and delayed rectifier currents in canine atrium: properties and role of isolation methods. *Am J Physiol.* 1996;270:H2157–H2168.
8. Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, Xu WY, Jin HW, Sun H, Su XY, Zhuang QN, Yang YQ, Li YB, Liu Y, Xu HJ, Li XF, Ma N, Mou CP, Chen Z, Barhanin J, Huang W. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science* 2003;299:251–254.

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トピックス

I. 病態と診断の進歩
2. 不整脈と原因遺伝子

伊藤　英樹　　堀江　　稔

要　旨

ここ10年の間で不整脈に関係する原因遺伝子が数多く報告されてきた．その多くは心筋細胞膜に発現するイオンチャネルか，これらのチャネルの膜発現に必要な構成蛋白である．発現実験による機能解析から，遺伝子異常が引き起こす機能変化と臨床像との関連が次第に明らかとなってきた．今後，さらに新たな不整脈の原因遺伝子が発見されることが期待される．　〔日内会誌　95：203～208，2006〕

Key words：不整脈，遺伝子，QT延長，突然死

はじめに

近年の分子遺伝学あるいは電気生理学的アプローチによって，遺伝性不整脈疾患の遺伝子異常とその異常がもたらす臨床病態に関する情報は爆発的に増えている．また近年，ポストゲノム時代を先取りした形でこれらの遺伝性不整脈疾患の病態を明らかとするために，発現実験を駆使した異常チャネルの機能解析が精力的に行われている．

本稿では，現在までに見出されている遺伝性不整脈疾患の原因遺伝子を疾患別に概説するとともに，臨床病態を裏付けるこれらの機能解析の成果に関しても簡単に述べたい．なお，詳細な記述はイタリアのPrioriらのグループによりインターネット上に公開されており，参照されたい（http://pc4.fsm.it:81/cardmoc/main.htm）．

いとう　ひでき，ほりえ　みのる：滋賀医科大学呼吸循環器内科

1. 先天性QT延長症候群（LQTS）

LQTSは，心電図QT間隔の延長を特徴とする疾患で多形性心室頻拍（torsades de pointes）などの重篤な心室性不整脈により失神発作や突然死を引き起こす症候群である．先天性聾を伴い常染色体劣性遺伝を示すJervell and Lange-Nielsen症候群と先天性聾を伴わず常染色体優性遺伝を示すRomano-Ward症候群があり，病像は一般に前者のほうが重篤である．QT延長症候群の原因遺伝子は現在までに8つが見出されており，LQT1からLQT8に分類分けされている（表）．但し，LQT7とLQT8は他の身体所見も多彩であり，LQTSとして分離するには問題があるとする立場もある．サブタイプによって心電図QT波形の特徴が異なったり，不整脈発生時の特徴が違うことが特徴として挙げられる．例えば，心イベントはLQT1では運動時，LQT2では電話のベルなどの聴覚刺激や驚き，LQT3では安静時や睡眠時に多い．このようにサブタイプによって臨床像が違うことから予想されるように，治療方法も各サブタイプで異なる．LQT1はβブロッカー

表．遺伝性不整脈疾患の責任遺伝子

疾患名	染色体	分類	責任遺伝子	障害部位と電流
QT 延長症候群	11p15.5	LQT1	KCNQ1	遅延整流性外向きＫチャネル（遅い成分）I_{Ks}
	7q35-q36	LQT2	KCNH2	遅延整流性外向きＫチャネル（速い成分）I_{Kr}
	3p21-p23	LQT3	SCN5A	電位依存性 Na チャネル I_{Na}
	4q25-q27	LQT4	ANK-B	Ankyrin-B, $I_{Na\ Ca}$
	21q22.1-p22	LQT5	KCNE1	遅延整流性外向きＫチャネル（遅い成分）I_{Ks}
	21q22.1-p22	LQT6	KCNE2	遅延整流性外向きＫチャネル（速い成分）I_{Kr}
	17q23	LQT7	KCNJ2	内向き整流性Ｋチャネル $I_{Kir2.1}$
	12p13.3	LQT8	CACNAIC	Ｌ型 Ca チャネル I_{CaL}
Brugada 症候群	3p21-p23	BRU1	SCN5A	I_{Na}
Lenegre-Lev 病	3p21-p23	CCD1	SCN5A	I_{Na}
先天性洞不全症候群	3p21-p23	SSS1	SCN5A	I_{Na}
	15q24-q25	SSS2	HCN4	過分極誘発陽イオンチャネル
不整脈源性右室異形性症	1q42-42	ARVD2	hRyR2	リアノジン受容体
	6p24	ARVD8		Desmoplakin
	12p13	ARVD9		Plakophillin
Naxos 病	17q21	NAXOS		Plakoblobin
家族性心房細動	11p15.5	PAF1	KCNQ1	I_{Ks}
	21q22.1-p22	PAF2	KCNE2	I_{Kr}
	3p21-p23	PAF3	SCN5A	I_{Na}
カテコラミン感受性心室頻拍	1q42-42	CPVT1	hRyR2	リアノジン受容体
	1p13.3-p11	CPVT2	CASQ2	Calsequestrin
QT 短縮症候群	7q35-q36	SQTS1	KCNH2	I_{Kr}
	11p15.5	SQTS2	KCNQ1	I_{Ka}
	17q23	SQTS3	KCNJ2	$I_{Kir2.1}$

の効果が期待できるが，一方LQT2，LQT3 はβブロッカーの効果が弱く，逆にLQT3 はI群抗不整脈薬の効果がある．我々は周期性四肢麻痺，形態異常，心室性不整脈を３主徴とするAndersen 症候群にKCNJ2 の遺伝子変異を報告した．前胸部誘導でのU波を特徴とし，LQT7 に分類されている[1]（図 1）．またLQT8 として，Timothy症候群に初めて心筋Caチャネルの遺伝子異常が報告された[2]．

QT延長症候群の特徴として遺伝子異常のキャリアーであっても，その多く（約 70%）が無症状であることであり，家族内の浸透率は低い．これは不整脈の発生には遺伝的要因だけでなく，修飾因子の関与が大きく，QT延長を来す薬剤や低カリウム血症，徐脈，脱水などで容易にQT延長が顕在化することがあり注意が必要である[3]．

2. Brugada症候群

Brugada症候群は器質的な心疾患がないにもかかわらず，致死性不整脈を認める８症例に共通する特徴的な心電図波形（V1 からV2 もしくはV3 誘導のST上昇，図 2）を，スペインのBrugada兄弟らが発見したことからその名がついている．臨床的には東南アジアや日本に比較的多いこと，男性症例が多いこと，不整脈発作は夜間に多いことなどが挙げられる．家族内突然死の多いこれらの症例に，Chenらは心筋Naチャネルのαサブユニットをコードする SCN5Aに遺伝子異常を見出した．これらの遺伝子変異によりNa電流が減少することが本症候群の病態である

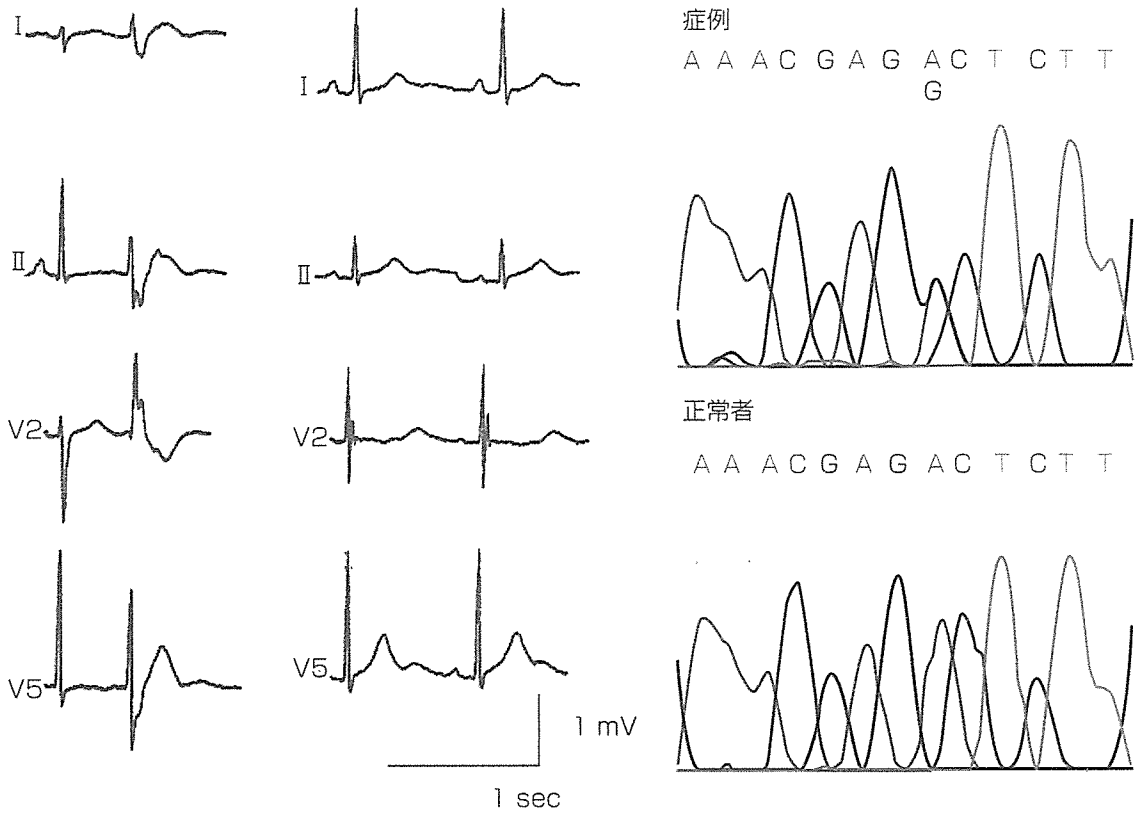

図 1．Andersen 症候群にみられた心電図異常と KCNJ2 の遺伝子異常（文献 1 を改変引用）．**姉弟に多発する心室性期外収縮と著明な QT 延長を認め，両症例は KCNJ2 の 192 番目のスレオニン（ACT）がアラニン（GCT）へ変化するヘテロ接合と判明した．**

と理解されている．ただSCN5Aに遺伝子異常が見出される症例は本症候群の 20% 前後と低く，他の原因遺伝子が存在することが予想されている．現在のところ有効な治療方法は植え込み型徐細動器しかないが，キニジンなど一過性カリウム電流（I_{to}）を抑制する薬剤が有用であるとする報告もある．

3．進行性心臓伝導障害，先天性洞不全症候群

本症候群はLenègre病とも呼ばれ，刺激伝導系の進行障害により脚ブロック，完全房室ブロックをきたす疾患で，SCN5Aの遺伝子異常が報告されている．先天性洞不全症候群はSCN5AとHCN4 の遺伝子変異が報告されている．ただ注意が必要なのは完全に独立した疾患ではなく，特にSCN5Aの遺伝子変異の場合，Brugada症候群やQT延長症候群との合併を認めることがあり，互いに臨床像がオーバーラップすることがある．

4．不整脈源性右室異形成（ARVD）

ARVDは右室心筋の脂肪変性により，心室性不整脈を認める疾患で，現在までに 10 個の原因遺伝子が報告されている．特にGerullらは 120 家系のARVD症例中 32 例もの症例に膜構成蛋白の 1 つであるplakophilin-2 に遺伝子異常を見出した[1]．この蛋白がARVDの発症の中心的役割を担うことに疑う余地はないが，その他の蛋白との関連に関してさらに検討が必要である．

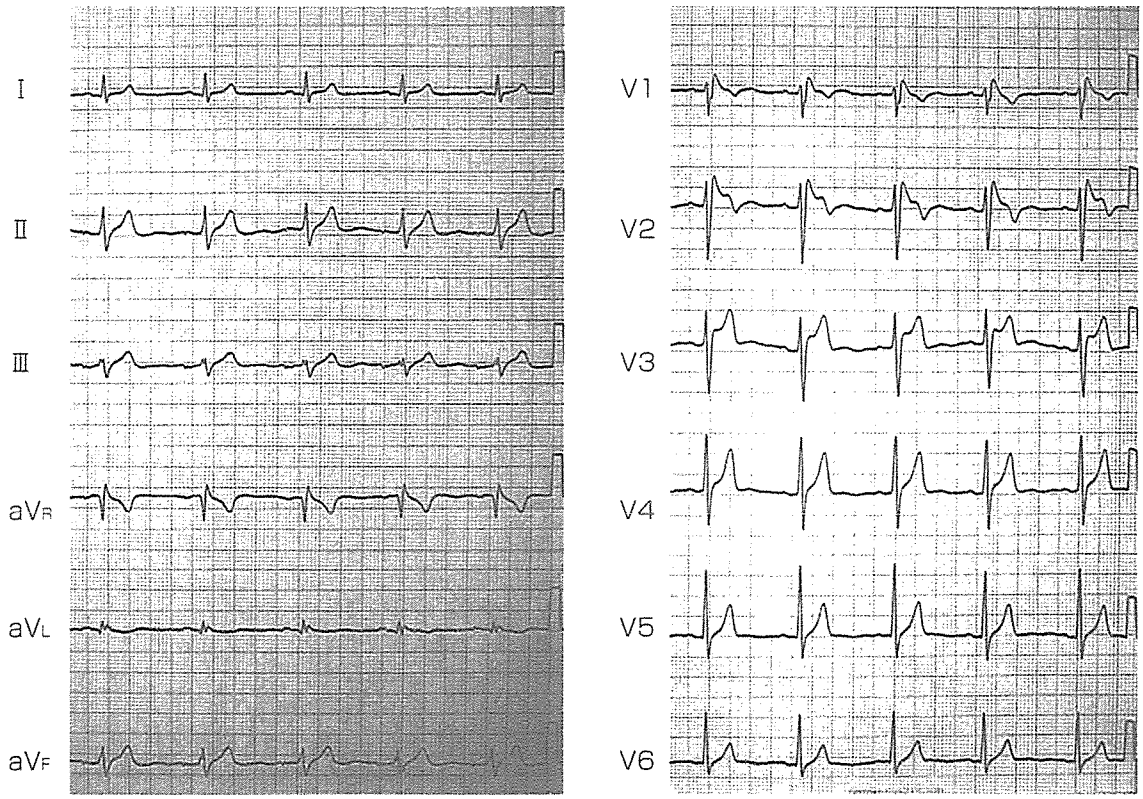

図2. 24歳で失神発作を認めたBrugada症候群の心電図. 胸部誘導V1からV3誘導でcovedタイプと呼ばれる典型的なST上昇を認めた. 本症例は翌年突然死した.

5. カテコラミン感受性心室頻拍(CPVT)

運動により, 失神発作, 突然死を引き起こす疾患で, 運動誘発性心室頻拍とも呼ばれる. これらの症状の原因は運動時に多源性心室期外収縮, 2方向性心室頻拍, 多形成心室頻拍から心室細動にいたることが原因である. 特に心室頻拍は2方向性のものが特徴である[5] (図3). 運動時に不整脈が出現する臨床病型はQT延長症候群のLQT1に類似しているがCPVTはQT延長を伴わない. Prioriらは筋小胞体に存在する膜蛋白であるリアノジン受容体の遺伝子変異を2003年に報告した. リアノジン受容体は筋小胞体からのカルシウムの放出を制御しており, 遺伝子変異によりこの制御が効かなくなり, 細胞内のカルシウム濃度が上昇することが不整脈の原因とされる. また, 心筋リアノジン受容体遺伝子(RyR2)とその関連蛋白でcalsequestrin (CSQ2) 遺伝子に変異が見出されている. 治療薬としてはβブロッカーが第一選択であるが, 無効例も多い.

6. 家族性心房細動

1997年にBrugadaらが初めて家族性心房細動の遺伝解析を報告したが, 現在までに明らかになっている原因遺伝子は2つで, 共にLQTSの原因遺伝子でもあるKCNQ1とKCNJ2の遺伝子異常である. LQTSとは逆にこれらの変異は機能亢進(gain of function)をきたすことが知られている. 心房細動は我々臨床医が日常診療でよく遭遇する不整脈のひとつであり, 今後さらに遺伝子解析が精力的に進められることが期待される. 一部の心房細動の原因遺伝子が発見されたことは今後心房細動の発症メカニズムあるいは治療を考える上で福音となるであろう.

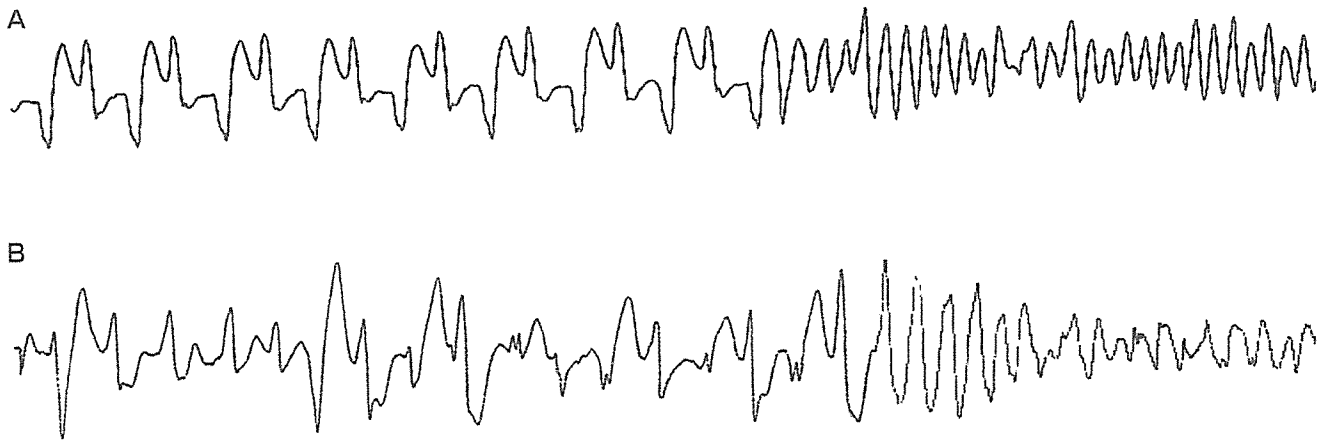

図3. カテコラミン感受性心室頻拍に特徴的な2方向性の心室頻拍（bidirectional VT）から心室細動への移行.

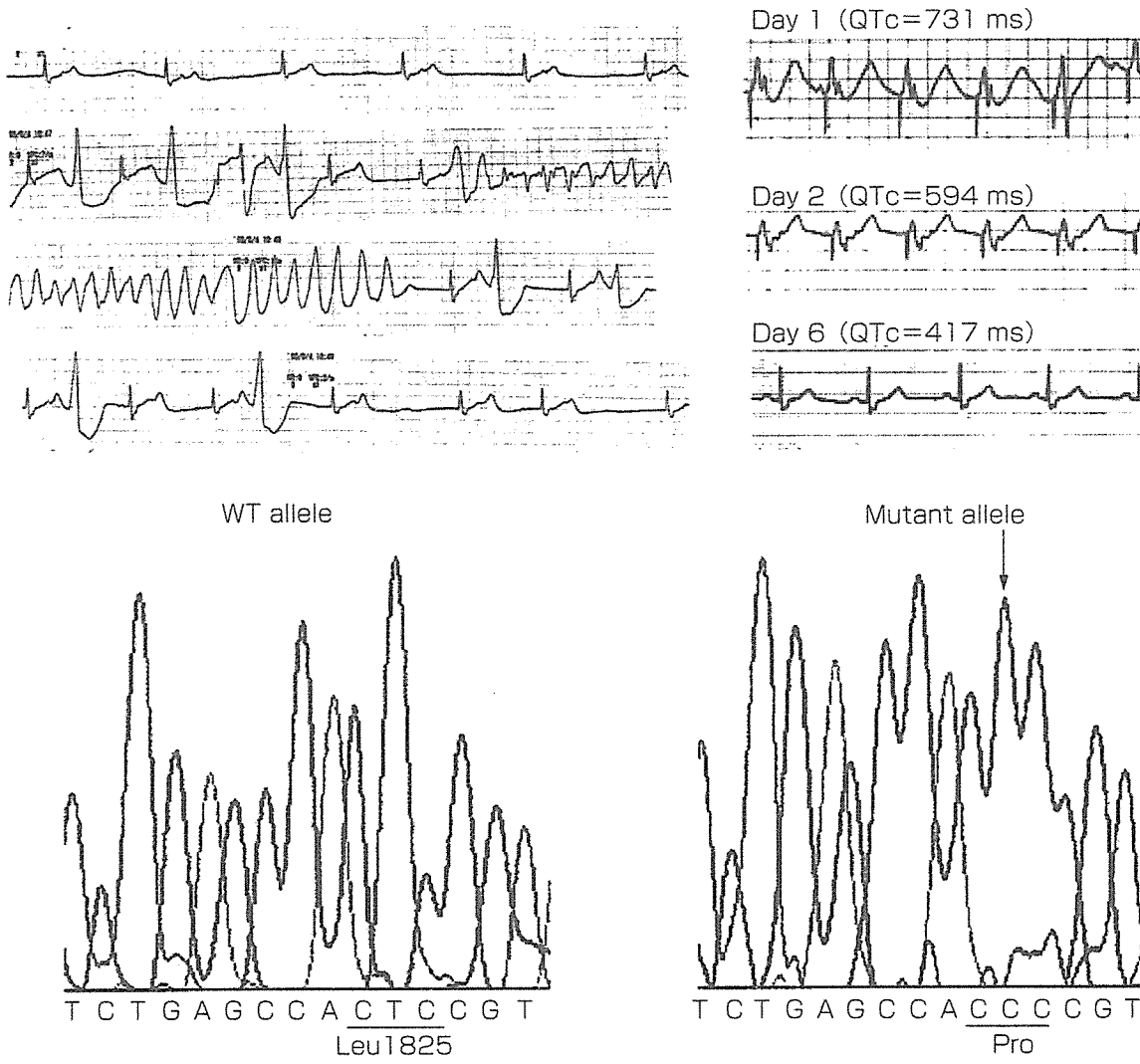

図4. 薬剤誘発性QT延長症候群の心電図変化（上段）と遺伝子異常（下段）. 高齢女性で胃腸薬として知られるシサプリドを内服後，QT延長と多形性心室頻拍による失神発作を認めたが，薬剤内服を中止後QT間隔は正常化した（上段）. 本症例の遺伝子検索の結果，心筋Naチャネルをコードする SCN5A の1,825番目のロイシンがプロリンへ変化する遺伝子変異が検出された.

7. QT短縮症候群

QT短縮症候群は2000年にGussakらが初めて症例報告したもので，比較的発見されてから歴史の浅い疾患である．器質的心疾患がないにもかかわらず恒常的なQT短縮（QTc 300ms以下）を認める症候群で心臓突然死の家族歴や不整脈症状と関連する．PrioriらはQT延長症候群の原因遺伝子でもあるKCNH2がQT延長症候群の原因遺伝子でもあることを2002年に初めて報告した．以後，KCNQ1，KCNJ2遺伝子にも変異を認めた家系が報告されてきている．QT短縮はこれらのチャネルのgain of functionが原因で再分極過程が速まり活動電位が短くなることが原因と考えられている．心房細動を合併することが多いのも特徴で，先に示したように家族性心房細動とQT短縮症候群の原因遺伝子とその機能障害は類似する点が多く，互いにオーバーラップする症候群といえる．現在のところ硫酸キニジンや植え込み型徐細動器が有用とされるが，報告例が十分でなく今後の検討が必要である．

まとめ

遺伝性不整脈疾患の原因遺伝子の解析は精力的に行われ，数多くの遺伝子異常が発見されてきた．その遺伝解析は1990年代にQT延長症候群にはじまり，10年余りで“common disease”である心房細動にまで研究の手は及んでいる．一方，おびただしい程の遺伝子異常が報告されてきたにもかかわらず，Brugada症候群の10～20%にしか遺伝子異常が見出せないように，その多くの原因遺伝子は未だ不明であるのも事実である．

遺伝子異常の機能解析が進むにつれて，同じ遺伝子座の異常であってもその機能変化によってはその表現型が異なる場合があることが明らかとなってきた．また臨床的側面からみるとQT延長症候群がKチャネルやNaチャネル，Caチャネルの遺伝子異常が原因で起こるように，同一疾患でも原因遺伝子はさまざまである．これらの事実は，遺伝性不整脈疾患は予想以上に複雑な疾患群をなしており，各疾患毎に境界線を引くことは難しいことを示唆する．

従来“後天性”と呼ばれてきた不整脈疾患の原因遺伝子も次第に明らかにされてきている[6]（図4）．一方，イオンチャネル研究の過程で，アミノ酸変化を引き起こすSNPは，機能的な変化も伴う場合があることが，近年，続々と報告され，遺伝子多型と疾患との関連が注目されている．今後これらの不整脈疾患の新たな原因遺伝子が益々発見されるとともに，未知の遺伝子と不整脈発症の関連が，従来の方法に加えて，model animalやコンピュータモデルを通して明らかにされると思われる．

文　献

1) Ai T, et al : KCNJ2 mutation in familial periodic paralysis with ventricular dysrhythmia. Circulation 105 : 2592–2595, 2002.
2) Splawski I, et al : Ca (V) 1.2 caldium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell 119 : 19–31, 2004.
3) 堀江　稔：遺伝性不整脈と心臓突然死．循環器科　55 : 305–307, 2004.
4) Gerull B, et al : Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. Nat Genet 36 : 1162–1164, 2005.
5) Priori SG, et al : Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation 106 : 69–74, 2002.
6) Makita N, et al : Drug-induced long-QT syndrome associated with a subclinical SCN5A mutation. Circulation 106 : 1269–1274, 2002.

薬剤性QT延長症候群における遺伝子背景

堀江　稔(滋賀医科大学呼吸循環器内科)

はじめに

最近10年あまりの間に，家族性QT延長症候群の病態生理が解明され，心室筋の再分極を担うイオン・チャネルやその調節蛋白をコードする遺伝子の異常により発症することが示された．現時点で6つの異なる遺伝子が同定されている．AndersenおよびTimothy症候群は少し概念が異なるためここでは入れない(表1)．一方，臨床的には，薬剤性を含む二次性のQT延長症候群が圧倒的に多い．このような明らかなトリガーにより発症するQT延長症候群症例の中にも，軽微な遺伝的異常が存在し，そのために，なんらかのトリガーが存在すると，一気に致命的な心室性不整脈まで惹起される可能性が当初から指摘されていた．われわれを含めて世界中のいくつかの研究室で，このような仮説のもとに，薬剤性などsporadic caseのQT延長症候群症例を対象にして遺伝子検索が始められ，現在までに，単一遺伝子多型(single nucleotide polymorphism)を含めて多くの知見が集積されている．本稿では，とくに薬剤性QT延長症候群でわかってきた遺伝子異常について解説する．

家族性と薬剤性QT延長症候群

QT延長症候群は，心電図上，著しいQT時間の延長と特異な多形性心室頻拍(torsade de pointes：TdP)(図1)により失神や心臓突然死を起こす恐ろしい病態である[1]．心電図が臨床に普及するようになって，それまで，家族性に失神や突然死が集積する家系においてQT時間が延長している症例があることがわかってきた．1990年代になって，分子遺伝学あるいは電気生理学的なアプローチが駆使されて，本症候群の原因遺伝子が同定された．表1にこれらの遺伝子とその障害されるチャネル機能についてまとめるが，Romano-Ward症候群はこれらの遺伝子のヘテロ接合の異常で，Jervell Lange-Nielsen(JLN)症候群の多くの場合は，*KCNQ1*あるいは*KCNE1*のホモ接合で発症することがわかってきた．

表1　遺伝性不整脈症候群の原因遺伝子とイオン・チャネル機能

タイプ	遺伝子座	原因遺伝子	イオン電流など
QT延長症候群			
先天性(Romano-Ward症候群)常染色体優性			
LQT1	11p 15.5	*KCNQ1*	I_{Ks}
LQT2	7q 35-36	*KCNH2*	I_{Kr}
LQT3	3p 21-24	*SCN5A*	I_{Na}
LQT4	4q 25-27	*Ankyrin-B*	Na/Ca exchanger, I_{Na}, $[Ca^{2+}]i$
LQT5	21q22.1-q22.2	*KCNE1*	I_{Ks}
LQT6	21q22.1-q22.2	*KCNE2*	I_{Kr}
先天性(Jervell & Lange-Nielsen syndrome)常染色体劣性			
JNL1	11p 15.5	*KCNQ1*	I_{Ks}
JNL2	21q22.1-q22.2	*KCNE1*	I_{Ks}

一方，二次性特に薬剤性QT延長症候群は，日常診療では家族性よりも圧倒的に高頻度で経験する．表2にQT延長を来す可能性のある薬剤リストを挙げる．Mitcheson，Sanguinettiらの実験[2]で，表2の薬剤の多くが心室筋の再分極を担う遅延整流Kチャネルのうち，早いコンポーネントのI_{Kr}電流を選択的に阻害する結果，QT延長を起こすことがわかってきた．したがって，薬剤性QT延長症候群の病像は，家族性のうち，I_{Kr}チャネルをコードしている*KCNH2*の変異で招来されるLQT2(表1)に類似している．

なお，薬剤のQT延長作用についての詳細な情報は，インターネット上で知ることができる(http://

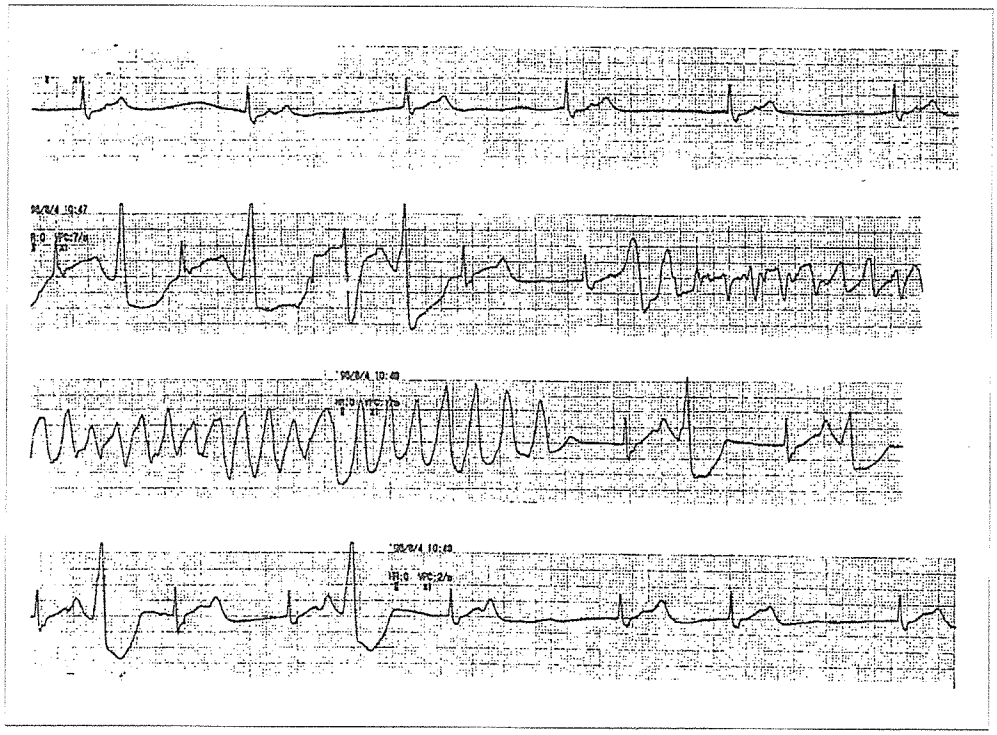

図1
シサプリド(現在発売中止)内服中に，高度の徐脈とTdPを来した70歳女性のモニター心電図(文献[7]より改変)
その後の遺伝子検索で*SCN5A*遺伝子の変異L1825Pが発見された．

www/qtdrugs.org)．また，アリゾナ大学のホームページにアクセスすると，QT延長の可能性のある薬剤について検索することができる(http://www.torsades.org)．ともに日常診療で利用頻度の高い情報源である．

薬剤性QT延長症候群と遺伝子異常

さて，**表2**に一部を掲げた薬剤は，非常に多くの患者に処方される一方，実際に，(病的な)QT延長あるいはTdPまで発症するのは，ほんの一部である．すなわち，薬剤だけでなく，投与されるホスト側の体質あるいは素質といった問題が想定される．家族性QT延長症候群では，**表1**のように原因遺伝子が発見され，たとえばSchwartzスコア[1]が4点以上の確診症例では，その50～60%の症例で原因遺伝子が同定されていること[3][4]から，われわれは，これらの遺伝子の変異あるいはSNPがあって，ごく軽度にチャネル機能が障害され，その結果，いわゆる「潜在性」QT延長症候群といった病態があるのではない

表2　QT延長を来す可能性のある薬剤

抗不整脈薬	Ia群，Ic群，Ⅲ群薬，ベプリジル
抗うつ薬・向精神薬	アミトリプチリン，イミプラミン，クロールプロマジン，フェノチアジン，ドロペリドール，ハロペリドール，リスペリドン，チオリダジン，フロキセチン，フルボキサミン，セルトラリン
抗生物質・抗真菌薬	マクロライド系，ニューキノロン系，ケトコナゾール，フルコナゾール，イトラコナゾール，メトロニダゾール，ST合剤
抗ウイルス薬・抗がん剤	リトナビル，インジナビル，サキナビル，アマンタジン，フォスカルネット，タモキシフェン
免疫抑制剤	タクロリムス
高脂血症薬	プロブコール
抗アレルギー薬	テルフェナジン，アステミゾール
消化管運動改善薬	シサプリド
H_2遮断薬	シメチジン，ラニチジン，ファモチジン

表3　二次性(薬剤性を含む)QT延長症例で発見された遺伝子異常

Gene	Base pair change	Amino acid change	Drugs	Age (years)	Sex	Additional risk factors	Symptoms
KCNE1	253G→A	D85N	Sotalol	80	Female	−	TdP
	253G→A	D85N	Quinidine	71	Male	Hypokalaemia	TdP
KCNE2	22A→G	T8A	Amiodarone	12	Male	−	TdP
	22A→G	T8A	Quinidine	n.s.	n.s.	−	TdP
	22A→G	T8A	Sulfametoxazole	45	Male	−	QTc＞600ms
	25C→G	Q9E	Clarithromycin	76	Female	Hypokalaemia, diabetic, history of stroke	TdP, VF
	161T→C	M54T	Procainamide	n.s.	n.s.	−	TdP
	170T→C	I57T	Oxatomide	n.s.	n.s.	−	TdP
	347C→T	A116V	Quinidine, Mexiletine	55	Female	History of cardiac arrest	Syncope with TdP
KCNH2	1039C→T	P347S	Cisapride, Clarithromycin	77	Female	−	TdP
	1048C→T	R328C	n.s.	45	Male	−	TdP
	2350C→T	R784W	Amiodarone	n.s.	n.s.	−	TdP
KCNQ1	944A→G	Y315C	Cisapride	77	Female	Hypokalaemia	Cardiac arrest
	1663C→T	R555C	Terfenadine	38	Female	cLQTS family	Sudden death
	1747C→T	R583C	Dofetilide	n.s.	n.s.	−	TdP
SCN5A	1844G→A	G615E	Quinidine	n.s.	n.s.	−	TdP
	1852C→T	L618F	Quinidine	n.s.	n.s.	−	TdP
	3748T→C	F1250L	Sotalol	n.s.	n.s.	−	TdP
	5474T→C	L1825P	Cisapride	70	Female	−	TdP

かとの仮説を立てて，1996年当時から，家族性のみならず，薬剤性QT延長症候群の症例に対しても遺伝子検索を行っている[5)~7)]．このような可能性は，実は20年以上も前にMossらによって提唱されていた[8)]．

この「潜在性」QT延長症候群の関連で，Prioriらは，家族内で変異の詳細なスクリーニングを行い，その浸透率が非常に低いこと，また，キャリアであっても，無症状やQT時間が正常範囲内である症例が多いことを報告した[9)]．しかし，このような症例は，薬剤を含め電解質(低カリウム血症[10)]など)の異常や徐脈[5)]などのトリガーによって，TdPが誘発される危険性の高いことは容易に理解できる．当初の薬剤性QT延長症候群と遺伝子変異に関する記述は症例報告が多かった[7)11)~15)]が，最近では，薬剤性のTdPを起こした患者をコホートとして検索した論文も散見されるようになってきた[16)~20)]．**表3**に，薬剤性QT延長症例で発見された遺伝子異常の主な報告をリストとして掲示する．

薬剤性QT延長症候群：特にSNPについて

SNP(single nucleotide polymorphism)は，数ある遺伝子多型の中でも単一遺伝子多型と呼ばれるもので，エクソン・イントロンに関係なく発見され，通常，ある母集団に1％以上の高い確率でみつかる．従来，疾患との関連は少ないとされたが，多くの多因子疾患，たとえば糖尿病や高血圧などでは病態との関連が示唆されるものもある．さらにSNPはゲノム上での数がほかの遺伝子多型に比べて圧倒的に多いので，全体として疾患感受性に対する寄与が大きい．先に，Iwasaら[21)]は，家族性QT延長症候群の関連遺伝子上のSNPを日本人コホートで調べて報告している．薬剤性QT延長との関連で，この中の，*KCNE1*/D85NというSNPが最近注目されている．

*KCNE1*は，アミノ酸120個あまりの短い蛋白をコー

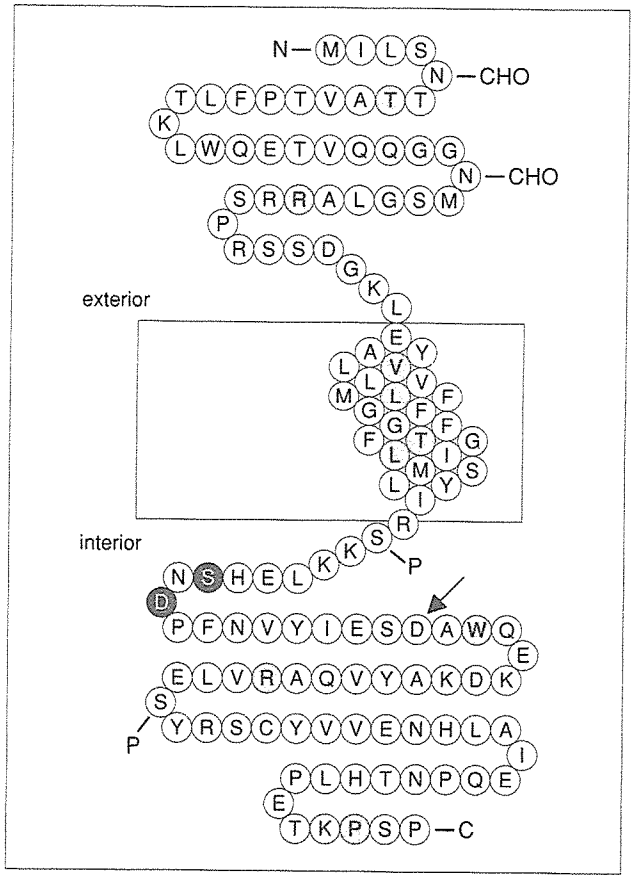

図2　*KCNE1*がコードするMinK蛋白のトポロジーを示す
形質膜を一回貫通する短い蛋白で，網がけしてあるアミノ酸の変異が報告されており，さらに濃い網がけのS74L，D76Nについては機能解析もされている．
矢印がD85である．

ドする遺伝子[22]（**図2**）であり，遅延整流Kチャネルのうち遅いコンポーネントであるI_{Ks}チャネルをコードする*KCNQ1*と一緒になって機能的なチャネルを形成する[23][24]．このチャネルのいくつかの変異はQT延長症候群を惹起することが報告されている[25]～[27]（**図2**で網がけにしてあるアミノ酸に報告あり）．

さて，この*KCNE1*/D85Nは，85番目のアスパラギン酸がアスパラギンに置換するミスセンスSNPであり，本邦では2％にこのSNPが認められたとのこと[21]であるが，Sanguinettiらのカエル卵母細胞を用いた機能解析[28]により，*KCNE1*/D85Nは，I_{Ks}チャネルの機能低下（loss-of-function）を起こすことが示されたため，いわゆる，functional SNPであることが判明した．われわれも，姻戚関係にない151名のQT延長症候群の発端者で，この*KCNE1*/D85N SNPの検索を行ったところ，8名（5.3％）に発見した（未発表）．この陽性率は，Iwasaら[21]の健常人での頻度（2％）より高く，したがって，このSNPは，いわゆる「潜在性」を含め軽症のQT延長症候群と，関連する可能性が高いようである．

この関連で，Paulussenら[29]はヨーロッパで薬剤性QT延長症例32名を調査しているが，2名（6.3％）に同じ*KCNE1*/D85Nが発見されたとのことであり，本SNPは病態と関連すると思われる．しかしながら，いまだ検索数が少ないため，より大きなコホートでの検討が必要である．

おわりに

薬剤性QT延長において，どの程度遺伝的な背景が，TdP発症に寄与しているのかは，今後，明らかにしなければいけない課題の一つである．しかしながら，本症ではその発症に電解質の異常やほかの心疾患，脳血管障害，徐脈，加齢など複数のトリガーが重複することにより発症することがほとんどである．予期せぬ心臓性突然死を避けるためにも，薬剤性QT延長症候群の患者やその家族での遺伝子検索が勧められる．

文　献

1) Schwartz PJ, Moss AJ, Vincent GM, Crampton RS : Diagnostic criteria for the long QT syndrome. An update. *Circulation* 1993 ; 88 : 782-784
2) Mitcheson JS, Chen J, Lin M, et al : A structural basis for drug-induced long QT syndrome. *Proc Natl Acad Sci USA* 2000 ; **97** : 12329-12333
3) Splawski I, Shen J, Timothy KW, et al : Spectrum of mutations in long-QT syndrome genes. KvLQT1, *HERG*, *SCN5A*, *KCNE1*, and *KCNE2*. *Circulation* 2000 ; **102** : 1178-1185
4) Mohler PJ, Schott JJ, Gramolini AO, et al : Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 2003 ; **421** : 634-639
5) Yoshida H, Horie M, Otani H, et al : Bradycardia-induced

long QT syndrome caused by a *de novo* missense mutation in the S2-S3 inner loop of *HERG*. *Am J Med Genet* 2001 ; **98** : 348-352
6) Kubota T, Horie M, Takano M, et al : Evidence for a single nucleotide polymorphism in the *KCNQ1* potassium channel that underlies susceptibility to life-threatening arrhythmias. *J Cardiovasc Electrophysiol* 2001 ; **12** : 1223-1229
7) Makita N, Horie M, Nakamura T, et al : Drug-induced long-QT syndrome associated with a subclinical *SCN5A* mutation. *Circulation* 2002 ; **106** : 1269-1274
8) Moss AJ, Schwartz PJ : Delayed repolarization (QT or QTU prolongation) and malignant ventricular arrhythmias. *Mod Concepts Cardiovasc Dis* 1982 ; **51** : 85-90
9) Priori SG, Napolitano C, Schwartz PJ : Low penetrance in the long-QT syndrome : clinical impact. *Circulation* 1999 ; **99** : 529-533
10) Kusano KF, Hata Y, Yumoto A, et al : Torsade de pointes with a normal QT interval associated with hypokalemia : A case report. *Jpn Circ J* 2001 ; **65** : 757-760
11) Donger C, Denjoy I, Berthet M, et al : KvLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. *Circulation* 1997 ; **96** : 2778-2781
12) Piquette RK : Torsade de pointes induced by cisapride/clarithromycin interaction. *Ann Pharmacother* 1999 ; **33** : 22-26
13) Napolitano C, Schwartz PJ, Brown AM, et al : Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. *J Cardiovasc Electrophysiol* 2000 ; **11** : 691-696
14) Piippo K, Holmstrom S, Swan H, et al : Effect of the antimalarial drug halofantrine in the long QT syndrome due to a mutation of the cardiac sodium channel gene *SCN5A*. *Am J Cardiol* 2001 ; **87** : 909-911
15) Mohammad S, Zhou Z, Gong Q, January CT : Blockage of the *HERG* human cardiac K^+ channel by the gastrointestinal prokinetic agent cisapride. *Am J Physiol* 1997 ; **273** : H2534-2538
16) Abbott GW, Sesti F, Splawski I, et al : *MiRP1* forms I_{Kr} potassium channels with *HERG* and is associated with cardiac arrhythmia. *Cell* 1999 ; **97** : 175-187
17) Sesti F, Abbott GW, Wei J, et al : A common polymorphism associated with antibiotic-induced cardiac arrhythmia. *Proc Natl Acad Sci USA* 2000 ; **97** : 10613-10618
18) Chevalier P, Rodriguez C, Bontemps L, et al : Non-invasive testing of acquired long QT syndrome : evidence for multiple arrhythmogenic substrates. *Cardiovasc Res* 2001 ; **50** : 386-398
19) Yang P, Kanki H, Drolet B, et al : Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. *Circulation* 2002 ; **105** : 1943-1948
20) Wei J, Wang DW, Alings M, et al : Congenital long-QT syndrome caused by a novel mutation in a conserved acidic domain of the cardiac Na^+ channel. *Circulation* 1999 ; **99** : 3165-3171
21) Iwasa H, Kurabayashi M, Nagai R, et al : Twenty single-nucleotide polymorphisms in four genes encoding cardiac ion channels. *J Hum Genet* 2002 ; **47** : 208-212
22) Murai T, Kakizuka A, Takumi T, et al : Molecular cloning and sequence analysis of human genomic DNA encoding a novel membrane protein which exhibits a slowly activating potassium channel activity. *Biochem Biophys Res Commun* 1989 ; **161** : 176-181
23) Barhanin J, Lesage F, Guillemare E, et al : KvLQT1 and lsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature* 1996 ; **384** : 78-80
24) Sanguinetti MC, Curran ME, Zou A, et al : Coassembly of KvLQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature* 1996 ; **384** : 80-83
25) Splawski I, Tristani-Firouzi M, Lehmann MH, et al : Mutations in the hminK gene cause long QT syndrome and suppress I_{Ks} function. *Nat Genet* 1997 ; **17** : 338-340
26) Tesson F, Donger C, Denjoy I, et al : Exclusion of KCNE1 (IsK) as a candidate gene for Jervell and Lange-Nielsen syndrome. *J Mol Cell Cardiol* 1996 ; **28** : 2051-2055
27) Tyson J, Tranebjaerg L, Bellman S, et al : IsK and KvLQT1 : mutation in either of the two subunits of the slow component of the delayed rectifier potassium channel can cause Jervell and Lange-Nielsen syndrome. *Hum Mol Genet* 1997 ; **6** : 2179-2185
28) Westenskow P, Splawski I, Timothy KW, et al : Compound mutations : A common cause of severe long-QT syndrome. *Circulation* 2004 ; **109** : 1834-1841
29) Paulussen AD, Gilissen RA, Armstrong M, et al : Genetic variations of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* in drug-induced long QT syndrome patients. *J Mol Med* 2004 ; **82** : 182-188