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CLINICAL PERSPECTIVE

The congenital long-QT syndrome (LQTS) diagnosed at perinatal life and through infancy is associated with high morbidity and mortality rates. However, data on the clinical presentation and genotype-phenotype correlation of this youngest age group of LQTS are limited. A nationwide survey was conducted in Japan, and 58 cases (18 fetuses, 31 neonates and 9 infants) were registered. Among them, the peak age at diagnosis was 0 to 2 days, and the 3 most frequent clinical presentations included sinus bradycardia, ventricular tachycardia/torsades de pointes, and atrioventricular block. The genotype was confirmed in 29 (71%) of 41 patients who underwent genotyping; the incidence resembled that of child LQTS. Patients who presented with early-onset ventricular tachycardia/torsades de pointes and atrioventricular block were almost exclusively those with LQT2 and LQT3 among the 3 major genes, but a considerable number of genetically unidentified ones were included. Sudden cardiac death/aborted cardiac arrest were prevalent in the latter. LQT1 patients tended to show only sinus bradycardia or positive family history of LQTS. These results mean that many life-threatening episodes observed in early-onset LQTS should be treated immediately and aggressively even without knowledge of the genotype. On the other hand, the present study was encouraging in that the outcome of patients was favorable with multiple pharmaceutical agents, typically with β-blockers, mexiletine, and magnesium and with pacemaker implantation/implantable cardioverter-defibrillator, independent of the genotype. Further application of gene testing is needed to establish the most appropriate genotype-specific strategy for these patients.

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Angiotensin II type 1 receptor mediates partially hyposmotic-induced increase of I_{Ks} current in guinea pig atrium

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Abstract A repolarizing conduction in the heart augmented by hyposmotic or mechanically induced membrane stretch is the slow component of delayed rectifier K^+ current (I_{Ks}). I_{Ks} upregulation is recognized as a factor promoting appearance of atrial fibrillation (AF) since gain-of-function mutations of the channel genes have been detected in congenital AF. Mechanical stretch activates angiotensin II type 1 (AT_1) receptor in the absence of its physiological ligand angiotensin II. We investigated the functional role of AT_1 receptor in I_{Ks} enhancement in hyposmotically challenged guinea pig atrial myocytes using the whole-cell patch-clamp method. In atrial myocytes exposed to hyposmotic solution with osmolality decreased to 70% of the physiological level, I_{Ks} was enhanced by 84.1%, the duration of action potential at 90% repolarization (APD_{90}) was decreased by 16.8%, and resting membrane potential was depolarized (+4.9 mV). The hyposmotic-induced effects on I_{Ks} and APD_{90} were significantly attenuated by specific AT_1 receptor antagonist candesartan (1 and 5 μM). Pretreatment of atrial myocytes with protein tyrosine kinase inhibitors tyrphostin A23 and A25 suppressed but the presence of tyrosine phosphatase inhibitor orthovanadate augmented hyposmotic stimulation of I_{Ks}. The above results implicate AT_1 receptor and tyrosine kinases in the hyposmotic modulation of atrial I_{Ks} and suggest acute antiarrhythmic properties of AT_1 antagonists in the settings of stretch-related atrial tachyarrhythmias.

Keywords Atrial myocytes · K^+ channel · I_{Ks} · Angiotensin II type 1 receptor · Membrane stretch

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Introduction

Cardiac electrical activity, a result of ionic fluxes through the sarcolemma, initiates contraction of cardiomyocytes through a process known as excitation–contraction coupling [2]. Conversely, mechanical forces acting on the heart chambers during every heart cycle continuously influence electrical processes: a phenomenon recognized as mechano-electric feedback, which in certain pathophysiological circumstances could generate arrhythmia [34].

It has been found that electrical disturbances in the atria could be either a result or a factor initiating atrial enlargement, particularly in the most frequently encountered sustained arrhythmia—atrial fibrillation (AF) [20, 30, 41]. Common cardiovascular diseases creating substrate for AF (systemic hypertension, heart failure, valvular disease) produce dilation or stretch of the atria—processes considered now an integral part of pathogenesis and atrial remodeling in the course of AF [1, 29].

In the settings of ischemic heart disease and spontaneous or therapeutic reperfusion, mechanical forces could be imposed on cardiomyocytes of the affected myocardial area due to an increased intracellular osmolality and subsequent cell volume changes. As significant variations of the volume of cardiomyocytes are deleterious, the cells activate sophisticated machinery to maintain their homeostasis: a process that includes ion movement through a number of channels and transporters thus affecting cardiac electrophysiology [9, 47].

Major repolarizing conductance in atrial and ventricular myocytes of human or other mammalian origin is slowly activating component of the delayed rectifier potassium current (I_{Ks}) [23, 48]. It has been demonstrated that I_{Ks} is upregulated by stretch or swelling of cardiomyocytes [42, 49]. Nevertheless, the underlying transduction mechanisms of this modulation are poorly understood. In canine and guinea pig ventricular myocytes, it is dependent on activity of protein tyrosine kinases (PTK) [26, 53], although it is not the case in COS7 mammalian cell line [22]. Current produced by reconstituted α-subunit of human I_{Ks} channel (KCNQ1) in *Xenopus* oocyte was reliant on intact actin cytoskeleton for swelling-triggered stimulation [16].

Furthermore, malfunction of I_{Ks} channel was implicated in pathogenesis of AF since gain-of-function mutation of KCNQ1 gene (S140G) was found in a family with autosomal-dominant hereditary AF [7]. Association between atrial stretch, I_{Ks}, and AF arrhythmogenesis was further highlighted by discovery of stretch-sensitive KCNQ1 mutation (R14C) in a different family with congenital AF. Only KCNQ1-R14C carriers with dilated atria developed the arrhythmia, and current recorded from KCNQ1-R14C channels expressed in mammalian cell line possessed enhanced sensitivity to hyposmotic-induced cell swelling in contrast to the wild-type KCNQ1 [31].

Renin–angiotensin system (RAS) maintains physiological integrity and also participates in pathogenesis of common disorders of cardiovascular system [43]. RAS is intimately incorporated into AF pathogenesis as its long-term stimulation provides substrate (fibrosis) for AF development, and activation of angiotensin II type 1 (AT_1) receptor-coupled pathways is essential for atrial remodeling after AF appearance [15]. In addition to angiotensin II, stretch also upregulates the upstream components of RAS in cardiomyocytes [24]. Moreover, AT_1 receptor, unlike other G protein-coupled receptors, appears to be one of the cellular stretch sensors. It has been found that mechanical stretch is sufficient stimulus for activation of AT_1 receptor and a number of coupled transduction pathways in the absence of its physiological ligand—angiotensin II [51, 54].

Based on the above facts, purpose of the present study was to investigate the potential role of AT_1 receptor in membrane stretch-induced I_{Ks} modulation in cardiac atrium and to make attempt to define underlying signaling mechanisms.

Materials and methods

Isolation of guinea pig atrial myocytes

The experimental procedures were conducted in accordance with the guidelines established by the Animal Care and Use Committee of Shiga University of Medical Science (Shiga, Japan). Single atrial myocytes were enzymatically dissociated from adult Hartley guinea pig hearts using a retrograde Langendorff perfusion method as has been described [11].

Solutions and chemicals

Normal Tyrode solution contained (in mM): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 5.5 glucose, and 5.0 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), pH was adjusted to 7.4 with NaOH. The isosmotic extracellular solution used for whole-cell I_{Ks} recording was normal Tyrode solution supplemented with 0.4 μM nisoldipine (a generous gift from Bayer AG, Wuppertal-Elberfeld, Germany) and 5 μM E-4031 (Wako, Osaka, Japan). The hyposmotic extracellular solution was prepared by simply reducing the NaCl concentration to 100 mM in the above solution. The osmolality of isosmotic and hyposmotic solution, measured using a freezing point depression osmometer (Fiske OS™, Friske Associates, Massachusetts, USA) averaged ~285 and ~210 mOsm/kg, respectively. Agents added to the external solutions included bisindolylmaleimide I (BIS I), PD98059 (Sigma, St Louis, MO, USA), candesartan (a generous gift from Takeda Pharmaceutical Chemical Industries, Osaka, Japan), U73122 (Wako, Japan), angiotensin II (human), AG 18 (tyrphostin A23), AG 82 (tyrphostin A25), AG 9 (tyrphostin A1), AG 490 (tyrphostin B42), PP2 (4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)-pyrazolo[3,4-*d*]pyrimidine), Y-27632 ((R)-(+)-*trans*-*N*-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide), all purchased from Calbiochem, San Diego, CA, USA. Prior to some experiments, atrial myocytes were incubated in normal Tyrode solution with rabbit polyclonal IgG antibody raised against amino acids 15–24 within the NH_2-terminal extracellular domain of human AT_1 receptor (N-10: sc-1173, Santa Cruz Biotechnology, CA, USA) or normal rabbit IgG (sc-2027, Santa Cruz Biotechnology). Both IgG were applied at concentration of 4 μg/ml for 30 min before electrophysiological recordings.

The control pipette solution contained (in mM): 70 potassium aspartate, 50 KCl, 10 KH_2PO_4, 1 $MgSO_4$, 3 Na_2-ATP, 0.1 Li_2-guanosine triphosphate (GTP), 5 ethyleneglycoltetraacetic acid, and 5 HEPES, pH adjusted to 7.2 with KOH. In a set of experiments, internal solution was supplemented with 500 μM sodium othovanadate (Sigma), or Li_2-GTP (0.1 mM) was replaced with 2 mM Li_3-GDPβS (Roche Diagnostics GmbH, Mannheim, Germany).

Electrophysiology

Isolated atrial myocytes were current- and voltage-clamped using the standard whole-cell patch-clamp technique [18] with an EPC-8 patch-clamp amplifier (HEKA, Germany).

Signals were low-pass filtered at 5 kHz, acquired at 2 kHz through a LIH-1600 analog-to-digital converter (HEKA) and stored on hard disc drive, using PATCHMASTER software (HEKA). Borosilicate glass electrodes had a resistance of 2.5–3.5 MΩ when filled with the pipette solution. I_{Ks} was elicited by various depolarizing test potentials given from a holding potential of −50 mV, a voltage that inactivated Na^+ current (I_{Na}). L-type Ca^{2+} current ($I_{Ca,L}$) and rapid component of I_K (I_{Kr}) were blocked by externally added 0.4 μM nisoldipine and 5 μM E-4031, respectively. Action potentials were evoked in current-clamp mode at a rate of 0.2 Hz by suprathreshold current pulses of 2–5 ms duration applied via the patch electrode. The action potential duration was measured at 90% repolarization (APD_{90}). All experiments were performed at 36±1°C. Voltage dependence of I_{Ks} activation was evaluated by fitting the current–voltage (I–V) relationship of tail currents to the Boltzmann equation: $I_{K,tail} = I_{max}/\left(1+\exp\left(\left(V_{1/2}-V_m\right)/k\right)\right)$, where $I_{K,tail}$ is the tail current amplitude, I_{max} is fitted maximal tail current amplitude, $V_{1/2}$ is the voltage at half-maximal activation, V_m is the test potential, and k is the slope factor. Deactivation kinetics of I_{Ks} was examined by fitting the time course of the decaying tail current to the sum of two 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, and τ_f and τ_s time constants for the fast and slow components, respectively. Cell membrane capacitance (C_m) was calculated on the basis of the capacitive transients during 20 ms voltage-clamp steps (±5 mV), using the equation $C_m = \tau_C I_0/\Delta V_m(1 - I_{ss}/I_0)$, where τ_C is the time constant of the capacitive transient, I_0 is the initial peak current amplitude, I_{ss} is the steady-state current value, and ΔV_m is the amplitude of the voltage step (5 mV). Variations of I_{Ks} amplitude in the presence of different reagents were determined by measuring the amplitude of tail currents elicited upon repolarization to a holding potential of −50 mV following 2 s depolarization to +30 mV every 10 s.

Light microscopy images of atrial myocytes were taken by a charge-coupled device (CCD) camera (Ds-Fi1, Nikon, Tokyo, Japan) mounted on an inverted microscope (Eclipse TE2000-U Nikon), and length and width of myocytes were measured using a Nikon DS-L2 camera control unit. In ~10% of cases, atrial myocytes swelled spontaneously in isotonic conditions, or hyposmotic challenge did not produce any effect on myocyte volume. The data obtained from these myocytes were not included in the analysis.

The period of exposure to various reagents is denoted by a horizontal bar in the figures, and the original current traces recorded at a time pointed by arrows are illustrated in the inset. The zero-current level is indicated on the left of current traces by a horizontal line.

Immunostaining of AT_1 receptor

Isolated atrial myocytes were immobilized on glass coverslips with biological glue Cell-Tak™ (BD Biosciences, NJ, USA), fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS), washed with 1% glycine/PBS and PBS, then blocked/permeabilized with PBS containing 10% BSA and 0.2% Triton X-100. The cells were subsequently incubated in the blocking/permeabilizing solution with rabbit polyclonal anti-AT_1 IgG antibody (Santa Cruz Biotechnology, 1:1,000) at room temperature for 30 min and at 5°C overnight. The cells were then washed with PBS and incubated with Alexa Fluor 546-conjugated goat anti-rabbit IgG (1:1,000) at room temperature for 3 h. After washing with PBS, each cover slip was mounted on a glass slip in a 1:1 solution of PBS/glycerol. Fluorescent signals were detected with a confocal laser-scanning microscope (LSM META 510, Carl Zeiss, Germany) with a ×60 oil immersion objective (Carl Zeiss, Inc.), 543 nm excitation filter, and 560 nm long-path emission filter. Exactly the same procedure was repeated with normal rabbit IgG (Santa Cruz Biotechnology, 1:1,000) for negative control experiment.

Statistical analysis

All presented values are mean±SEM. A statistical difference between two groups of numerical data was determined by paired or unpaired Student's t test and one-way analysis of variance followed by Dunnett or Tukey post hoc analysis in the case of multiple comparisons. A value of $P<0.05$ was considered statistically significant.

Results

Hyposmotic challenge upregulates I_{Ks} in guinea pig atrial myocytes

Figure 1 summarizes hyposmotic cell swelling-induced regulation of guinea pig atrial I_{Ks}. Exposure of atrial myocytes to hyposmotic solution (Hypo-S) markedly increased the amplitude of I_{Ks} at all test potentials (Fig. 1a, b), and magnification of tail currents after +30 mV depolarization (the test potential used consistently in this study for evaluation of the effects of various drugs and solutions on I_{Ks}) was measured to be 84.1±8.2% (n=12). For assessment of I_{Ks} amplitude, we quantified tail currents because they reflect the exact degree of I_{Ks} activation by preceding voltage-clamp command.

To elucidate whether hyposmotic stimulation shifted voltage dependence of I_{Ks} activation, the average amplitudes of tail currents elicited after depolarizations between −40 and +50 mV (10 mV increment) were fitted with the

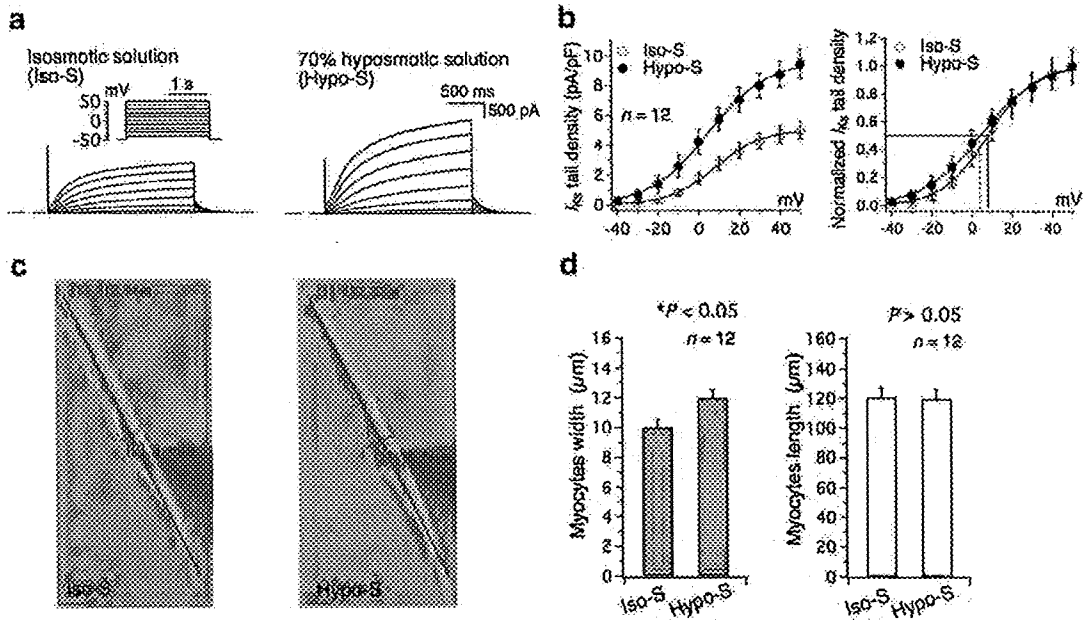

Fig. 1 Hyposmotic swelling-induced enhancement of I_{Ks} in guinea pig atrial myocytes. **a** Family of I_{Ks} traces, evoked by 2-s depolarizing steps given from a holding potential of −50 mV to potentials ranging from −40 to +50 mV (voltage-clamp protocol is drawn in the *inset*) in isosmotic solution (Iso-S) and after 3 min exposure to hyposmotic solution (Hypo-S). **b** *I–V* relationships for mean amplitude of I_{Ks} tail current (expressed as current density) recorded under isosmotic conditions (*open circles*) and during exposure to Hypo-S (*filled circles*). Smooth curves through the data points denote the least-squares fit with the Boltzmann equation, yielding $V_{1/2}$ and k (see text). No significant shift in voltage dependence of I_{Ks} activation was measured. *Right panel* presents the same *I–V* relation when the values were normalized to the maximal current. *Solid and dashed lines* connect points of half-activation voltages on the fitting curves and their respective values on *both axes*. **c** CCD camera images of whole-cell patch-clamped guinea pig atrial myocytes, taken before and after 5 min exposure to Hypo-S. **d** Mean effect of Hypo-S on myocyte width (*left*) and length (*right*)

Boltzmann equation (Fig. 1b). Mean half-activation voltage ($V_{1/2}$) and slope factor (k) of the fitting curves for I_{Ks} in control isosmotic solution (Iso-S) were 8.2±0.8 and 11.6±0.6 mV, respectively, vs. 4.7±0.9 and 14.5±0.7 mV in Hypo-S exposed cells (n=12). There was no significant difference in the values of $V_{1/2}$ (P=0.89) and k (P=0.93) between isosmotic and hyposmotic conditions suggesting that Hypo-S had little effect on the voltage dependence of I_{Ks} activation. Correspondingly, the kinetics of I_{Ks} deactivation in the cells exposed to Hypo-S was similar to that in the cells superfused with Iso-S: fast and slow time constants for decaying tail currents, respectively, were 94.9±9.1 and 299.5±28.3 ms (Iso-S) vs. 101.7±6.5 and 347.8±38.7 ms (Hypo-S, n=14, P>0.05; not shown).

The atrial myocytes responded to hyposmotic challenge with a significant increase in the cell width (10.0±0.4 μm in Iso-S and 12.0±0.5 μm after 5 min in Hypo-S, n=14, P< 0.05) but not with substantial change in the cell length (120.4±6.6 and 119.8±6.5 μm in the same order, n=14, Fig. 1c, d). This observation was in agreement with the reported descriptions of hyposmotic cell swelling in atrial myocytes of different mammalian species [38, 40]. On the other hand, average cell membrane capacitance, measured in 34 atrial myocytes, was identical in Iso-S and Hypo-S: 45.3±3.4 and 44.6±3.7 pF, respectively (P>0.05).

Candesartan and anti-AT_1 receptor antibody attenuated hyposmotic I_{Ks} activation

It is generally accepted that hyposmotic swelling imposes mechanical forces on the cell membranes in many different types of cells including cardiac myocytes [47]. Membrane stretch activates AT_1 receptor without the presence of angiotensin II [51, 54]. Therefore, we examined whether I_{Ks} increase observed during hyposmotic cell swelling would be affected by pharmacological blockade of AT_1 receptor. In the experiment shown in Fig. 2a, atrial myocyte was initially superfused with Iso-S containing AT_1 antagonist candesartan (1 μM) for 10 min. Subsequent exposure of the cell to Hypo-S in the continuous presence of candesartan resulted in a much smaller enhancement of I_{Ks}. Figure 2b illustrates *I–V* relationships for I_{Ks} tail currents recorded in candesartan-supplemented Iso-S and Hypo-S. The degree of swelling-induced I_{Ks} increase at test potential of +30 mV in the presence of 1 and 5 μM candesartan averaged 48.0±4.1% (n=10) and 47.2±2.7% (n=9), respectively: the values were significantly smaller than measurements in the absence of candesartan (84.1±8.2%, P<0.01; Fig. 2c). These results provide an evidence that restraining the transition of AT_1 receptor to the membrane stretch-produced active conformation could

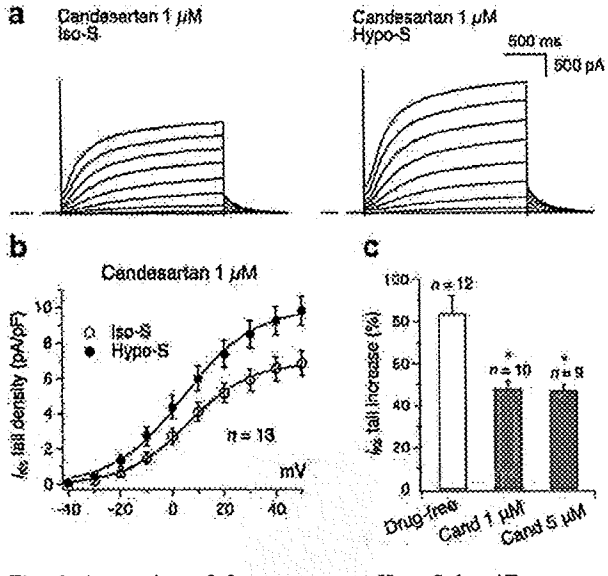

Fig. 2 Attenuation of I_{Ks} response to Hypo-S by AT_1 receptor antagonist candesartan. **a** The atrial myocyte was initially superfused with control Iso-S containing 1 μM candesartan for 10 min and then with Hypo-S in the continuous presence of candesartan. I_{Ks} was activated by depolarizing voltage-clamp steps to test potentials between −40 and +50 mV. **b** *I–V* relationships for mean I_{Ks} tail currents recorded during superfusion with isosmotic and hyposmotic external solutions in the continuous presence of 1 μM candesartan (n= 13). Data points were fitted with the Boltzmann equation, yielding $V_{1/2}$ of 6.0±0.8 mV and k of 13.6±0.8 mV for Iso-S and $V_{1/2}$ of 4.6± 1.1 mV and k of 12.1±0.6 mV for Hypo-S. There were no significant differences in $V_{1/2}$ and k values between Iso-S and Hypo-S. **c** Graph summarizing the percentage of I_{Ks} tail current increase induced by Hypo-S without and with AT_1 receptor antagonist candesartan (1 and 5 μM, abbreviated "*Cand*"); P<0.05 vs. drug-free (control) Hypo-S

suppress considerably I_{Ks} modulation during hyposmotic cell swelling.

Richards et al. [36] have shown that anti-AT_1 receptor antiserum was able to prevent effectively the physiological action of angiotensin II mediated through AT_1 receptor. We examined whether the interaction between AT_1 receptor and anti-AT_1 receptor IgG antibody (AT_1-IgG, see "Materials and methods") could also antagonize I_{Ks} modulation by hyposmotic challenge. To demonstrate clearly the specific effect of AT_1-IgG, we at the same time conducted experiments with Iso-S and Hypo-S containing unspecific IgG (negative control experiment). Average swelling-induced I_{Ks} upregulation was not affected significantly by unspecific IgG in the test solutions (73.2±5.8% vs. 84.1± 8.2% in IgG-free solutions, P>0.05; Fig. 3a, c). However, in atrial myocytes preincubated with AT_1-IgG (Fig. 3b, c), Hypo-S produced only 38.9±5.7% increase of I_{Ks} tail amplitude: a significantly smaller enhancement than that in IgG-free or unspecific IgG-containing solutions. Interaction between AT_1 receptor and AT_1-IgG suppressed hyposmotic response of I_{Ks} in a way comparable to the pharmacological block of the receptor with candesartan (Figs. 2c and 3c).

Single atrial myocytes loaded with unspecific IgG or AT_1-IgG were immunostained with secondary anti-IgG antibody labeled with Alexa Fluor 546 (see "Materials and methods") and then analyzed with fluorescence and differential interference contrast (DIC) microscopy. There was no detectable fluorescence in the images recorded with nonspecific IgG (Fig. 3d), but fluorescent signals were observed in the periphery of the cells treated with AT_1-IgG (Fig. 3e), indicating the presence of AT_1 receptors in the plasma membranes of guinea pig atrial myocytes.

Repeated hyposmotic challenge of the same atrial myocyte

According to the data reported by Sasaki et al. [42], I_{Ks} response to Hypo-S reaches a maximum within 1 min and reverses completely with similar to the onset time course after switching back to Iso-S. In addition, we found that second exposure to Hypo-S in an interval of 4–6 min augmented I_{Ks} equally to the initial application (Fig. 4a). The last observation allowed us to compare the degree of I_{Ks} enhancement by Hypo-S before and during superfusion with AT_1 receptor antagonist candesartan in the same atrial myocyte. Figure 4b shows a typical experiment: the atrial myocyte was exposed twice to Hypo-S, initially without and then with 1 μM candesartan. The drug significantly reduced the degree of I_{Ks} increase during the second exposure to Hypo-S by 31.7±4.1% (n=16, P<0.05 vs. the first I_{Ks} enhancement; summarized in Fig. 4b, right panel). These results confirmed again that AT_1 receptor activation participated in I_{Ks} regulation after hyposmotic swelling of guinea pig atrial myocytes.

I_{Ks} modulation in the presence of angiotensin II

The experimental results shown so far propose that AT_1 receptor is essential part of transduction mechanisms that enhance I_{Ks} after hyposmotic cell swelling in guinea pig atrial myocytes (Figs. 2, 3, and 4). We have found that in the same cardiac cells, stimulation of AT_1 receptor with its physiological ligand angiotensin II potentiated I_{Ks} through the G protein–phospholipase C (PLC)–protein kinase C (PKC) pathway [52]. To examine the possible involvement of common receptor and/or postreceptor signals in the angiotensin II- and Hypo-S-induced regulation of I_{Ks}, we measured the extent of I_{Ks} response to Hypo-S in the presence of angiotensin II. In the experiments like the one shown in Fig. 4c, initial administration of Iso-S containing 300 nM angiotensin II (the maximally effective concentration) enhanced I_{Ks} by 57.9±10.3% (n=9), approximating the results already reported [52]. In the continuous presence of angiotensin II, bath solution was then switched to Hypo-S,

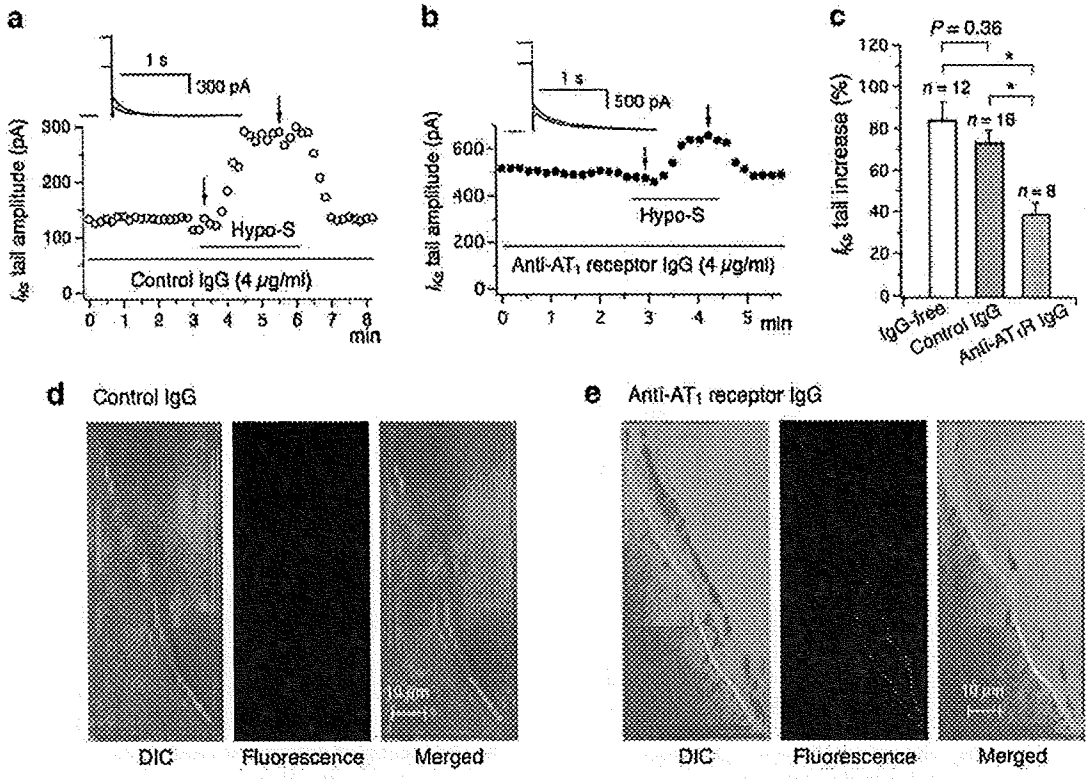

Fig. 3 Suppression of I_{Ks} modulation by IgG targeting AT_1 receptor. **a** Representative time course of changes in the amplitude of I_{Ks} tail current during exposure to Hypo-S in atrial myocyte pretreated for 30 min with unspecific (control) IgG at concentration 4 μg/ml. Traces indicated with *arrows* are shown superimposed in the *inset*. **b** Similar to **a** experiment, but guinea pig atrial myocytes were preincubated with anti-AT_1 receptor IgG antibody (4 μg/ml). **c** Summary bar graph of average I_{Ks} tail increase in IgG-free Hypo-S, in Hypo-S supplemented with control IgG, and in Hypo-S containing anti-AT_1 receptor antibody. Only specific AT_1-receptor antibody prevented significantly I_{Ks} modulation. **d**, **e** Fluorescence, DIC, and merged images from guinea-pig atrial myocytes treated with control and anti-AT_1 receptor IgG, respectively, and immunostained with Alexa Fluor 546-labeled secondary antibody. Fluorescent signal could be detected from cells treated with anti-AT_1 receptor antibody (**e**) but not with control IgG (**d**) illustrating specific binding of the anti-AT_1 receptor IgG and the presence of AT_1 receptor in the membrane of atrial myocytes

which further augmented I_{Ks} by 45.0±5.3% (n=9). However, the magnitude of this hyposmotic I_{Ks} regulation was significantly smaller than that induced by Hypo-S in the absence of angiotensin II (84.1±8.2%, P<0.05; Fig. 4c). Besides, the sum of I_{Ks} enhancement evoked by initial application of angiotensin II and subsequent exposure to Hypo-S (99.5±17.2%, n=9) was not statistically larger than the degree produced by Hypo-S alone (P=0.42; Fig. 4c). Taken together, these results imply that some signaling molecule(s) mediating hyposmotic I_{Ks} increase was activated during initial application of angiotensin II and are consistent with the results supporting the involvement of AT_1 receptor in Hypo-S-induced I_{Ks} modulation.

Activation of AT_1 receptor–G protein–PLC–PKC pathway was not essential for hyposmotic I_{Ks} increase

To examine whether the cell swelling- and angiotensin II-induced activation of AT_1 receptor share the same intracellular pathway(s) to enhance I_{Ks}, we tested the effect of pharmacological blockade of G proteins, PLC and PKC. Figure 5 demonstrates typical examples of I_{Ks} response to Hypo-S in atrial myocytes dialyzed internally with G protein inhibitor GDPβS, 2 mM (Fig. 5a) or PLC blocker U73122, 3 μM (Fig. 5b) and in the cells superfused with PKC inhibitor BIS I, 200 nM (Fig. 5c). Summarized data in Fig. 5d illustrate that none of these interventions significantly affected the extent of I_{Ks} regulation by Hypo-S. Average increase of I_{Ks} tails in the presence of GDPβS, U73122, and BIS I was 67.4±4.3% (n=21), 90.2±7.1% (n=10), and 77.0±5.1% (n=10), respectively. G proteins–PLC–PKC cascade did not appear to be critical signaling pathway linking AT_1 receptor activation and I_{Ks} increase during hyposmotic cell swelling.

PTK activity is necessary for hyposmotic-induced I_{Ks} regulation

It has been found that activation of PTK is among the earliest responses to hyposmotic volume increase in various

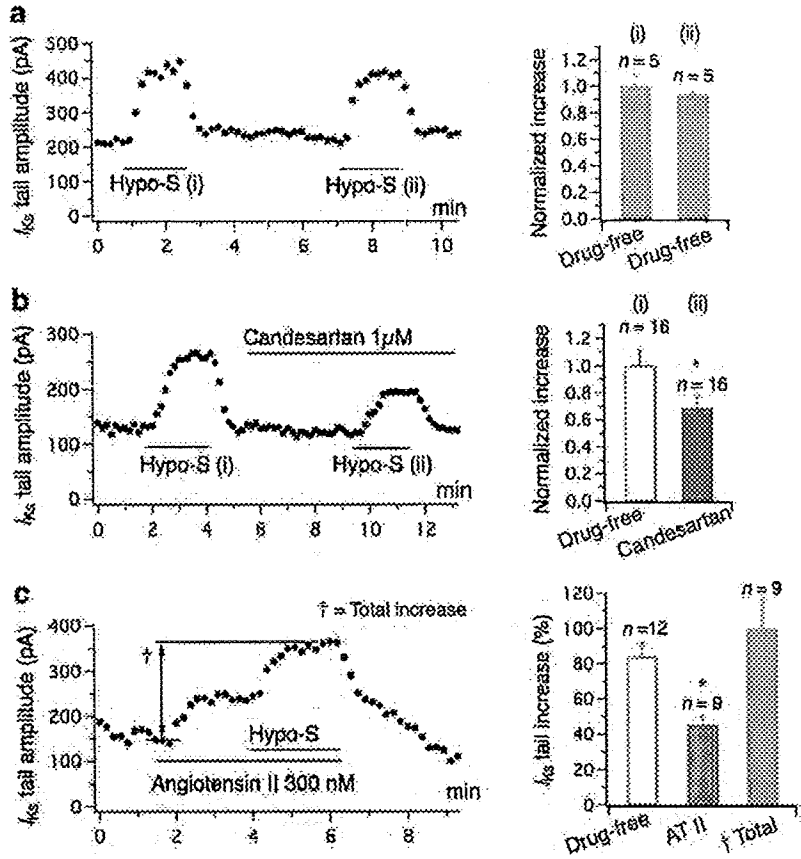

Fig. 4 I_{Ks} regulation *(i)* during repetitive application of Hypo-S and *(ii)* after pretreatment with angiotensin II. **a** Typical time course (*left*) and bar graph outlining the mean values (*right*) of the changes in the amplitude of I_{Ks} tail current during the first and second exposures to Hypo-S in the same atrial myocyte. **b** Pretreatment with AT_1 receptor blocker candesartan (1 μM) following initial exposure and withdrawal of Hypo-S produced smaller I_{Ks} current response in the course of second hyposmotic challenge. Representative time course of I_{Ks} tail current amplitude is shown on the *left*. Candesartan inhibited I_{Ks} enhancement with 31.7±4.1% (n=16, P<0.05, *right*). The values in the bar graphs (**a** and **b**) are normalized to the mean I_{Ks} increase during first Hypo-S administration. **c** Time course of I_{Ks} tail amplitude during exposure to Hypo-S in the continuous presence of 300 nM angiotensin II (*left*). Pretreatment with angiotensin II significantly reduced hyposmotic modulation of I_{Ks} from 84.1±8.2% to 45.0± 5.3%, P<0.05, although the combined increase (bar named "*Total*" in the *right panel* of **c**) was comparable to that in drug-free solution, 99.5±17.2% (P>0.05 vs. control enhancement). These results suggest that common transduction mechanism was partly shared between the two I_{Ks} regulatory signals—angiotensin II and hyposmotic stress

types of cells as well as in cardiomyocytes [8, 39]. PTK are large family of kinases with broad functions including regulation of ion channels [10]. PTK are related to I_{Ks} current modulation during hyposmotic-induced swelling in canine and guinea pig ventricular myocytes [26, 53] and sophisticated network of signaling coupled to AT_1 receptor includes various PTK (such as c-Src, focal adhesion kinase, Janus kinases) [45]. Therefore, we investigated the effect of PTK inhibitors tyrphostin A23 and A25 on the hyposmotic I_{Ks} increase in guinea pig atrial myocytes. Tyrphostins A23 and A25 had dual effect on I_{Ks} tail current amplitude (Fig. 6a, b). Following initial application, I_{Ks} was down-regulated by 58.7±4.1 % (n=13) and 55.1±3.5% (n=12), respectively, P<0.05 (similar to the observations of Missan et al. [26]). In the presence of tyrphostin A23 and A25, swelling of the myocytes elicited smaller increase of I_{Ks} current: 42.0±9.5% (n=13) and 52.7±4.7% (n=12), respectively (P<0.05 vs. 84.1±8.2% in Iso-S). None of these actions were found when inactive analog tyrphostin A1 was used (I_{Ks} was enhanced by 72.3±7.7%, n=8, P>0.05 vs. increase in Iso-S; Fig. 6c).

Described reduction of I_{Ks} enhancement by PTK block implies that inhibition of protein tyrosine phosphatases (PTP) is expected to have opposite effect or at least to delay the recovery of current amplitude after readministration of Iso-S. PTP inhibition by orthovanadate (500 μM) loaded into atrial myocytes through the pipette solution altered hyposmotic I_{Ks} modulation in several ways (Fig. 6d): orthovanadate (1) elevated basic I_{Ks} current amplitude with 53.0± 17.9% (n=4, P<0.05), (2) augmented Hypo-S-induced

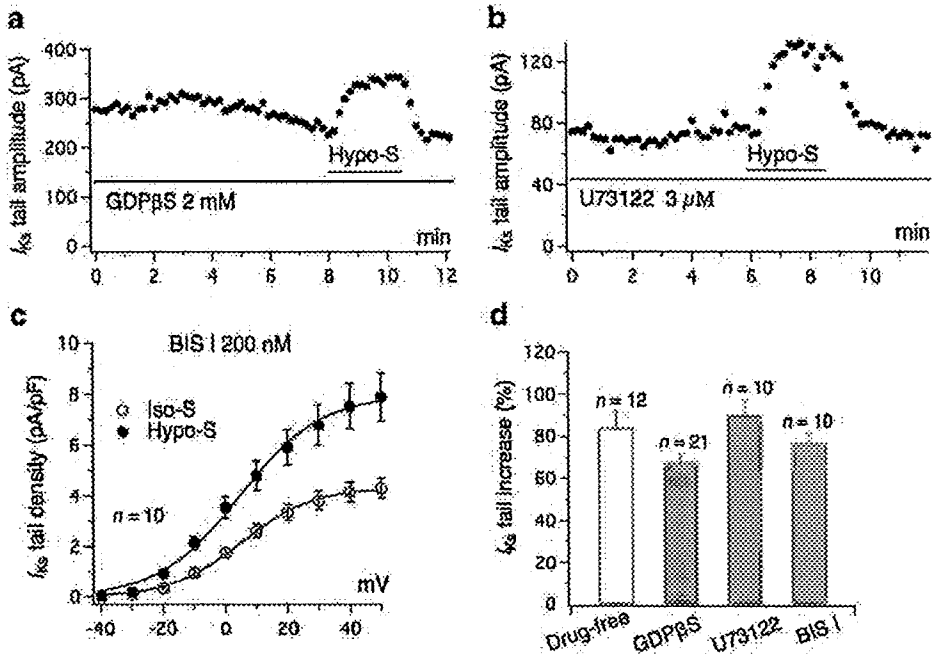

Fig. 5 Investigation of G protein–phospholipase C–protein kinase C pathway. **a**, **b** Typical time courses of I_{Ks} tail current increase by Hypo-S when the atrial myocytes were perfused with intracellular solution supplemented with G protein blocker GDPβS (2 mM) or PLC inhibitor U73122 (3 μM), respectively. **c** Mean current–voltage relationship of I_{Ks} tail currents before and during hyposmotic challenge in the presence of PKC inhibitor BIS I (200 nM) in the bath solution. Smooth curves are fit to the Boltzmann equation. Average $V_{1/2}$ and k in Iso-S were 5.2±0.7 and 11.4±0.5 mV, respectively, vs. 4.5±1.3 and 13.0±1.0 mV in Hypo-S. There was no significant difference between corresponding values in Iso- and Hypo-S. **d** Summary of experiments shown in **a**–**c**. Modulation of I_{Ks} tail amplitudes in the presence of GDPβS, U73122, and BIS I was similar to that in control conditions (see text)

stimulation of I_{Ks} to 124.6±10.6% (n=8, P<0.05), and (3) significantly decreased the recovery rate of tail current amplitude after Hypo-S withdrawal. Kinetics of recovery was evaluated by fitting the time course of tail amplitudes to a single exponential function starting from the point of readministration of Iso-S (Fig. 6d, inset). Time constants were 1.8±0.2 s (n=8) and 7.1±0.3 s (n=7) in control state and after PTP inhibition, respectively, P<0.05. Figure 6e outlines the mean values of I_{Ks} magnification in the course of inhibition of PTK and PTP.

We made an attempt to identify specific PTK or PTK-associated transduction that might mediate I_{Ks} stimulation and is downstream of AT_1 receptor. Src-PTK regulates volume-sensitive chloride current in human atrial myocytes and in rabbit ventricular myocytes [4, 13]. One of the mitogen-activated protein kinases (MAPK) named extracellular signal-regulated kinase (ERK) is stimulated during stretch [37, 54]. ERK is known to be specifically activated by MAPK/ERK kinase (MEK), a tyrosine and threonine protein kinase [37]. Inhibition of Src and MEK in guinea pig atrial myocytes by 10 μM PP2 [4] and 20 μM PD 98059 [3], respectively, did not affect swelling-induced I_{Ks} increase (Fig. 6f).

Tyrosine kinase JAK-2 is coupled to AT_1 receptor stimulation [25] and is also activated by stretch [54]. JAK-2 inactivation by tyrphostin B42 [50] in our experiments did not have significant effect on I_{Ks} modulation (Fig. 6f).

Activation of RhoA small GTP binding proteins and RhoA-associated coiled coil-containing kinase (ROCK) signaling could be stretch-induced phenomenon [44]. In addition, RhoA/ROCK pathway is involved in regulatory volume decrease responses, [32] and RAS can activate RhoA/ROCK [12]. Nevertheless, in guinea pig atrium, selective ROCK antagonist Y-27632 at concentration 10 μM had no influence on Hypo-S-related I_{Ks} modulation. The mean values of swelling-induced I_{Ks} tail current increase during exposure of myocytes to PP2, PD 98059, tyrphostin B42, and Y-27632 are presented in Fig. 6f.

Candesartan attenuated swelling-induced action potential shortening

All transsarcolemmal ionic conductances are integrated into the action potential, the trace presenting voltage change during depolarization and repolarization of cardiomyocyte membrane. Mechano-electric feedback process during stretch and swelling affects key ion channels and transporters [47], and therefore, alterations in atrial and ventricular action potential shape have been reported [6, 21, 33]. Figure 7a shows typical results in our experimental settings.

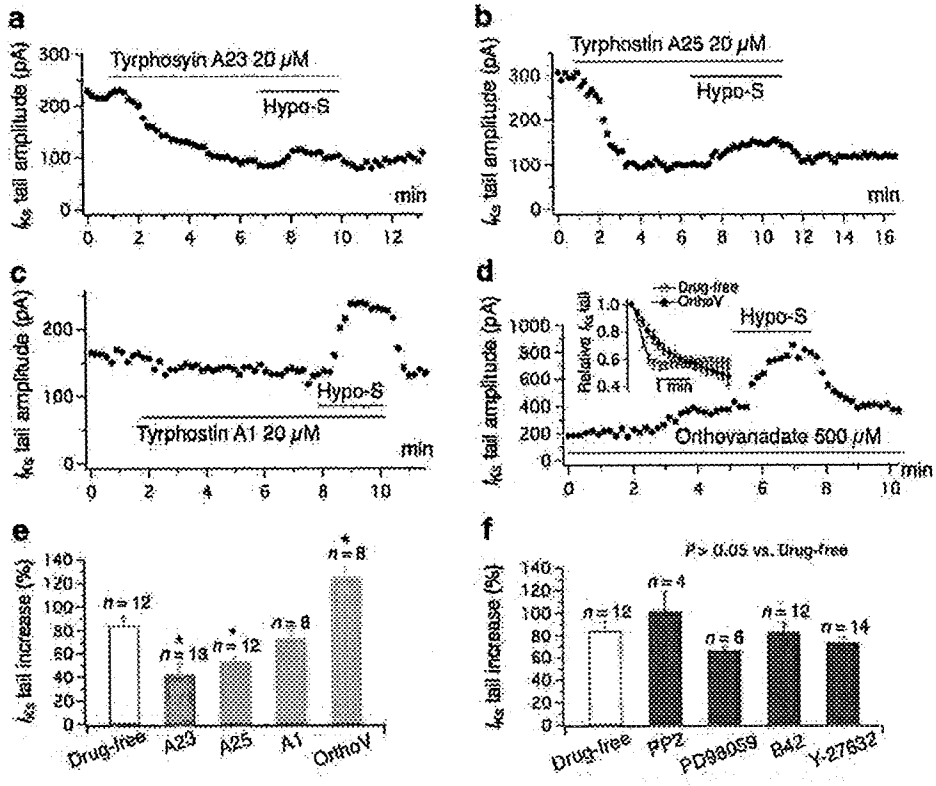

Fig. 6 Protein tyrosine kinase activity and I_{Ks} modulation. **a**, **b** Effects of PTK inhibitors tyrphostin A23 and A25 (each at 20 μM) on I_{Ks} tail amplitude and hyposmotic modulation. Tyrphostin A23 and A25 initially diminished I_{Ks} amplitude, and following hyposmotic challenge evoked weakened I_{Ks} response. **c** Inactive analog tyrphostin A1 (20 μM), on the other hand, did not produce significant changes in I_{Ks} amplitude or hyposmotic stimulation. **d** Representative experiment with intracellular orthovanadate (500 μM) demostrating (1) the increase of basic level of I_{Ks} tail current, (2) enhancement of swelling-induced response, and (3) slowing down the rate of recovery of I_{Ks} tail amplitude upon return to Iso-S. *Inset* shows initial 3 min fraction of average time courses of I_{Ks} tail amplitudes from the point of discontinuation of Hypo-S. Values are normalized to the maximal I_{Ks} amplitude and fitted to the single exponential function. Kinetics was significantly slower when orthovanadate was used (see text). **e** Summary of mean I_{Ks} current activation by Hypo-S in the course of action of PTK inhibitors, inactive analog tyrphostin A1, and PTP inhibitor. Mean amplitudes are compared to control I_{Ks} increase in drug-free Hypo-S. $P<0.05$ for tyrphostin A23, A25 and orthovanadate (abbreviated "*OrthoV*") vs. drug-free Hypo-S. **f** Bar graph of average I_{Ks} tail increase in the presence of pharmacological inhibitors of Src-PTK (PP2, 10 μM), MEK (PD98059, 20 μM), JAK-2 (tyrphostin B42, 20 μM), and RhoA kinase (Y-27632, 10 μM). I_{Ks} tail current was augmented by 102.1±17.1% (n=4), 66.1±3.4% (n=6), 82.9±8.7% (n=12), and 73.3±4.9% (n=14), respectively. These values were not statistically different from the control increase of 84.1±8.2% in drug-free Hypo-S

Hypo-S-induced swelling of guinea pig atrial myocytes abbreviated APD_{90} from 114.1±8.3 ms to 95.0±6.4 ms (n=9, $P<0.05$) and depolarized resting membrane potential from −79.4±0.2 to −74.5±0.6 mV (n=9, $P<0.05$).

We tested the effect of AT_1 receptor blocker candesartan during hyposmotic challenge of atrial myocytes (Fig. 7b). When myocytes were pretreated with 1 μM candesartan for 4–6 min, reduction of APD_{90} was decreased from 16.8± 1.3% to 9.08±1.5% ($P<0.0001$). In the presence of candesartan, APD_{90} before and after application of Hypo-S was 115.1±7.3 and 103.7±5.9 ms, respectively (n=18, $P<0.05$). Resting membrane potential, however, was depolarized to a similar degree as control: from −79.4±0.1 to −76.0±0.3 mV (n=18, $P<0.05$). Figure 7a, b shows typical action potential traces in control state and in the course of candesartan action. Figure 7c outlines changes of APD_{90} and resting membrane potential.

Caballero et al. [5] have shown that candesartan could prolong ventricular APD in guinea pig (although the difference was not significant statistically) and directly interacts with several human cardiac ion channels reconstituted in mammalian cell line. This raises the question about the mechanisms of action of candesartan on atrial APD_{90} (Fig. 7b) during Hypo-S exposure: direct modulation of ion channels or AT_1 receptor inhibition. In our experimental conditions, we did not observe any measurable effect on APD_{90} in guinea pig atrial myocytes after superfusion with Iso-S containing 5 μM candesartan—Fig. 7d, e. In addition, the average values of APD_{90} in Iso-S from Fig. 7a (drug-free) and b (candesartan 1 μM)

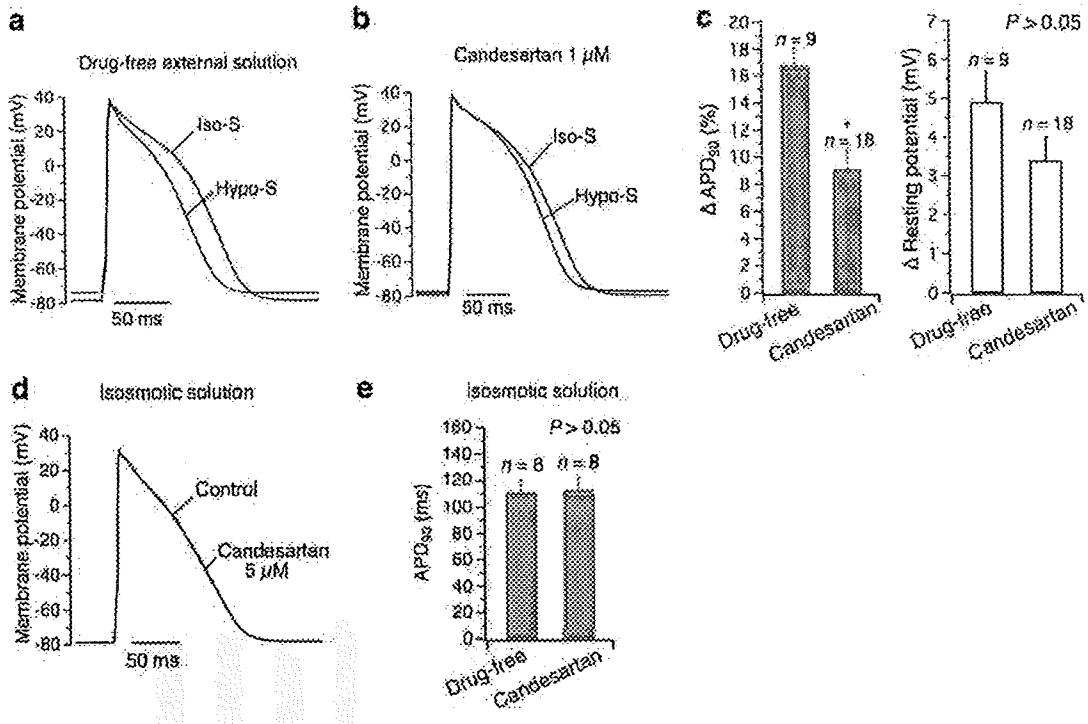

Fig. 7 Candesartan attenuated swelling-induced contraction of atrial action potential. **a** Superimposed traces of guinea pig atrial action potential demonstrates Hypo-S-induced effects: shortening of action potential duration at 90% repolarization and reduction of resting membrane potential. **b** Typical traces of atrial action potential in Iso-S and Hypo-S, recorded from myocytes pretreated with AT_1 receptor blocker candesartan (1 μM). **c** Mean reduction of APD_{90} (*left*) and changes of resting membrane potential (*right*) in control, drug-free state and during AT_1 receptor blockade. While effect on APD was significant ($P<0.0001$), no statistical difference was measured between the values of resting membrane potential, although they also decreased. **d**, **e** Representative traces of guinea pig atrial action potential and mean values of APD_{90} in control Iso-S before and after superfusion with 5 μM candesartan. There was no significant difference in the average value of APD_{90} in the presence of the drug

were similar. Thus, it seems that candesartan acted through inhibition of AT_1 receptor-coupled signaling mechanisms to reduce the shortening of APD_{90} by Hypo-S in guinea pig atrial myocytes.

Discussion

Major findings in the present work are: (1) AT_1 receptor activation participates in hyposmotic-provoked upregulation of I_{Ks} in guinea pig atrial myocytes; (2) biochemical pathways stimulating the function of I_{Ks} channel involve PTK; (3) AT_1 receptor antagonist candesartan and anti-AT_1 receptor antibody attenuate enhancement of I_{Ks}; candesartan, in addition, reduced the shortening of atrial action potential during myocyte swelling.

Our data provide for the first time an evidence of involvement of AT_1 receptor in the pathways mediating hyposmotic I_{Ks} regulation in atrial myocytes (Figs. 2, 3, and 4). Since the discovery of I_{Ks} modulation by mechanical stretch or hyposmotic swelling of cardiomyocytes, very little of underlying mechanisms are clarified. Consistent are negative findings for the independence of I_{Ks} modulation in native cardiomyocytes from activity of protein kinases A, C, or G, concentration of intracellular free Ca^{++}, or integrity of cytoskeleton [26, 42, 49, 53]. However, it was found that PTK blockers diminished considerably hyposmotic I_{Ks} increase in both canine and guinea pig ventricular myocytes [26, 53]. In the case of reconstituted KCNQ1/KCNE1 channels, regulatory mechanisms for stretch-induced activation are most likely dependent on the cell type and expression system. In *Xenopus* oocyte [16], KCNQ1 channel modulation was completely prevented by cytochalasin D, an inhibitor of actin filament polymerization, and in COS7 cell line, hyposmotic enhancement of KCNQ1/KCNE1 current was not affected by the PTK blocker genistein [22]. Our results in guinea pig atrial myocytes, like other reports in native cardiomyocytes, confirmed the importance of PTK for hyposmotic I_{Ks} regulation. PTK inhibitors, candesartan, and anti-AT_1 receptor IgG considerably reduced response of I_{Ks} to swelling of the myocytes (Figs. 2, 3, 4, and 6). As AT_1 receptor is coupled to a dense network of intracellular signaling pathways [45], the PTK involved in hyposmotic I_{Ks} upregulation could be downstream to activated AT_1 receptor, although this sequence of events has to be demonstrated.

In addition to its high-affinity interaction with AT_1 receptor, candesartan possesses properties of ion channel blocker. The drug was shown to affect reconstituted hKv1.5, HERG, KvLQT1/minK, Kv4.3 channels, and prolong guinea pig ventricular action potential (although the change in APD was not statistically significant) [5]. This makes probable the hypothesis that hyposmotic-induced stimulation of I_{Ks} in our experiments might have been prevented by the direct inhibition of the I_{Ks} channel by candesartan and not by the blockade of AT_1 receptor. However, as we had observed before [52], candesartan at concentration 0.1–5 μM had no measurable effect on guinea pig atrial I_{Ks} (visible also in Fig. 4b) or atrial action potential (Fig. 7a, b, d), suggesting that attenuation of hyposmotic I_{Ks} modulation was mediated by AT_1 receptor antagonism and not by direct drug–channel interaction in guinea pig atrial myocytes.

In our previous report [52], we demonstrated that I_{Ks} enhancement by angiotensin II in guinea pig atrial myocytes was dependent on the activation of PKC. During hyposmotic increase of atrial I_{Ks} in the same animal, PKC seems to have little, if any, role (as found in earlier studies for ventricular myocytes) [27, 49]. One explanation for this discrepancy could be that the "mode" of AT_1 receptor activation is dependent on the nature of the stimulus: angiotensin II-evoked conformational change activates G protein–PLC–PKC (and maybe simultaneously many other) pathways, but mechanically activated AT_1 receptor predominantly relays the signal through different biochemical cascades. The existence of angiotensin II- and stretch-induced active conformations of AT_1 receptor was proposed recently [51].

Angiotensin II- and stretch-elicited modulation of I_{Ks} appeared to be not entirely independent events because pretreatment of atrial myocytes with 300 nM angiotensin II suppressed hyposmotic I_{Ks} increase to a significant extent (Fig. 4c). This observation suggests that some signaling molecule(s) essential for I_{Ks} stimulation, but not PKC, is at least partly activated by angiotensin II or the transition to stretch-related active conformation of AT_1 receptor might be affected by the presence of angiotensin II. These hypotheses, however, require experimental confirmation in a further investigation. Nevertheless, the above observation does not exclude the possibility that angiotensin II and cell swelling are independent activators of I_{Ks} (Fig. 5). In addition, hyposmotic stimulation of I_{Ks} was not totally abolished by candesartan, anti-AT_1 receptor IgG, and PTK inhibitors (Figs. 2, 3, 4, and 6), a fact suggesting that other cellular components involved in mechanical stretch sensing and transduction in cooperation with AT_1 receptor and PTK upregulate atrial I_{Ks} in guinea pig atrium.

Intricate system of PTK signaling is among the earliest responses in the process of regulatory volume decrease (RVD) after hyposmotic cell swelling [8, 39]. Src-PTK has been shown to control the augmented expulsion of chloride ions during RVD through volume-sensitive chloride channel in human atrial myocytes [13]. Stretch-stimulated AT_1 receptor translates the signal (at least) to G proteins, JAK-2, and ERK [54]. According to our experimental data, activation of these regulatory proteins seems to be not obligatory for I_{Ks} modulation in guinea pig atrial myocytes during hyposmotic challenge. Furthermore, activity of RhoA/ROCK pathway was known to be dependent on stretch or swelling in mouse fibroblasts and neonatal rat ventricular myocytes [32, 44], although in our investigation we could not detect any relation of this pathway to hyposmotic I_{Ks} regulation. Thus, specific PTK together with additional intracellular regulatory components explaining I_{Ks} modulation in atrial myocytes remain to be defined.

The Hypo-S-induced shortening of APD and depolarization of resting membrane potential observed in guinea pig atrial myocytes are findings consistent with earlier reports [14, 21, 46]. The basis of these changes is modulation of activities of many ion channels and transporters (the detailed description of the swelling-induced alterations of these channels are not the focus of this study, see e.g. [21]). Among them, there are two channels which are both regulated by hyposmotic swelling or stretch, and the mechanism of their activation include RAS components: swelling-activated Cl^- current ($I_{Cl,swell}$) [17, 35, 40] and, according to our data, atrial I_{Ks} (already discussed above). Contribution of $I_{Cl,swell}$ to APD shortening and membrane depolarization in stretched or swelled cardiomyocytes has been shown to be considerable [14, 46]. Therefore, partial recovery of APD_{90} and resting potential (although the latter was not statistically significant) in the presence of candesartan (Fig. 7b) can be explained at least to some extent by interfering of the drug with Hypo-S-induced mechanisms of I_{Ks} and $I_{Cl,swell}$ stimulation.

In summary, hyposmotic modulation of I_{Ks} in guinea pig atrium requires membrane stretch-induced activation of AT_1 receptor and stimulation of PTK. AT_1 receptor antagonists among other protective effects in cardiovascular system might also possess acute antiarrhythmic activity (particularly candesartan [19, 28]) in the cases of stretch-related atrial arrhythmias.

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Disclosures The authors have no potential conflict of interests.

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Optimal Treatment Strategy for Patients With Paroxysmal Atrial Fibrillation

— J-RHYTHM Study —

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Background Although previous clinical trials demonstrated the non-inferiority of a rate control to rhythm control strategy for management of atrial fibrillation (AF), the optimal treatment strategy for paroxysmal AF (PAF) remains unclear.

Methods and Results A randomized, multicenter comparison of rate control vs rhythm control in Japanese patients with PAF (the Japanese Rhythm Management Trial for Atrial Fibrillation (J-RHYTHM) study) was conducted. The primary endpoint was a composite of total mortality, symptomatic cerebral infarction, systemic embolism, major bleeding, hospitalization for heart failure, or physical/psychological disability requiring alteration of treatment strategy. In the study, 823 patients with PAF were followed for a mean period of 578 days. The primary endpoint occurred in 64 patients (15.3%) assigned to rhythm control and in 89 patients (22.0%) to rate control (P=0.0128). No significant differences between the treatment strategies were observed in the incidences of death, stroke, bleeding and heart failure. Meanwhile, significantly fewer patients requested changes of assigned treatment strategy in the rhythm control vs the rate control group, which was accompanied by improvement in AF-specific quality of life scores.

Conclusion The J-RHYTHM study showed that rhythm control was associated with fewer primary endpoints than rate control. However, mortality and cardiovascular morbidity were not affected by the treatment strategy (umin-CTR No. C000000106). (*Circ J* 2009; 73: 242–248)

Key Words: Antiarrhythmic agents; Atrial fibrillation (AF); Mortality; Morbidity

Atrial fibrillation (AF), which is associated with increased mortality and morbidity[1–3] is a growing public health problem that has reached epidemic proportions[4,5] There has long been controversy concerning the basic question of whether reestablishment and maintenance of sinus rhythm or control of heart rate alone is better in the management of AF[6–9] Many randomized clinical trials have been conducted to answer this question, including the Pharmacological Intervention in Atrial Fibrillation (PIAF), Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM), Rate Control vs Electrical Cardioversion (RACE) and Strategies of Treatment of Atrial Fibrillation (STAF) trials[10–13] but none has shown superiority of rhythm control to rate control with respect to the mortality and morbidity of AF patients[10–14]

Editorial p 229

Nevertheless, in certain patients, there is an actual need to maintain sinus rhythm, not to reduce mortality and morbidity but to improve quality of life (QOL), which is quite important from the AF patient's viewpoint[15] Moreover, previous clinical trials, focusing on AF patients with expected high mortality, under-represented certain patient groups, younger patients without risk factors for stroke, patients with severe symptoms, and, particularly, those with paroxysmal AF (PAF)[9–13] Therefore, efforts should be continued to determine the optimal management of AF for various endpoints[16] and in a variety of patients.

The Japanese Rhythm Management Trial for Atrial Fibrillation (J-RHYTHM study) was designed to determine the optimal strategic approach for AF patients, including those usually under-represented in previous trials[17] The study emphasized patient-reported experience and perception of AF-specific disability, in addition to mortality and

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Table 1 Baseline Patient Characteristics

	Overall (n=823)	Rate control group (n=404)	Rhythm control group (n=419)	P value
Age (years)	64.7±11.3	64.5±12.3	64.9±10.3	0.6525
Male (%)	570 (69.3)	281 (69.6)	289 (69.0)	0.88
Coronary artery disease (%)	61 (7.4)	31 (7.7)	30 (7.2)	0.7913
Valvular disease (%)	46 (5.6)	26 (6.4)	20 (4.8)	0.3623
Cardiomyopathy (%)	13 (1.6)	5 (1.2)	8 (1.9)	0.5788
History of CHF (%)	30 (3.6)	16 (4.0)	14 (3.3)	0.7174
History of TIA/thromboembolism (%)	52 (6.3)	23 (5.7)	29 (6.9)	0.4782
Hypertension (%)	352 (42.8)	165 (40.8)	187 (44.6)	0.2907
Diabetes mellitus (%)	96 (11.7)	48 (11.9)	48 (11.5)	0.9136
EF (%)	68.3±8.9	68.0±10.3	68.7±7.4	0.3798
LAd (mm)	38.4±7.0	38.4±7.1	38.4±6.9	0.9797
CHADS2 score[12] (%)				
0	356 (43.3)	184 (45.5)	172 (41.1)	0.659
1	286 (34.8)	134 (33.2)	152 (36.3)	
2	121 (14.7)	55 (13.6)	66 (15.8)	
3	36 (4.4)	17 (4.2)	19 (4.5)	
4	18 (2.2)	11 (2.7)	7 (1.2)	
5	6 (0.7)	3 (0.7)	3 (0.7)	

Data are mean±SD or n (%).
CHF, congestive heart failure; TIA, transient ischemic attack; EF, ejection fraction; LAd, left atrial diameter

morbidity[18,19] We herein present new data concerning rate and rhythm control strategies in patients with PAF.

Methods

Study Design

The J-RHYTHM study was a randomized multicenter comparative study of patients with PAF treated by either rate or rhythm control. Study design details are published elsewhere[17] PAF was defined as AF expected to convert spontaneously to sinus rhythm within 48 h of onset[20] Exclusion criteria included initial AF episodes, contraindication for anticoagulation, and AF occurring during the acute phase of myocardial infarction or cardiac surgery.

Patients were randomly assigned to either the rate control or rhythm control treatment group. In the rate control group, control of heart rate itself was by β-blockers, calcium-channel blockers, and digitalis. In the rhythm control group, antiarrhythmic drugs were selected according to "The Japanese Guideline for Atrial Fibrillation Management"[21]

Oral antithrombotic therapy was used in both rate and rhythm control arms according to a protocol modified from that used in the AFFIRM study[9,17] Factors for assessment of stroke risk included age >65 years, hypertension, diabetes mellitus, congestive heart failure, history of stroke/transient ischemic attack/systemic embolism, left atrial diameter >50 mm, fractional shortening <25%, and ejection fraction <40%. In patients with 1 or more factors, warfarin was prescribed to maintain the prothrombin time–international normalized ratio between 1.6 and 3.0. Anticoagulant therapy was continued throughout the study, even if sinus rhythm appeared to be maintained by rhythm control therapy. Institutional review boards in each participating clinical site approved the study protocol, and all patients gave written informed consent for the study.

Endpoints

The primary endpoint was a composite of total mortality, symptomatic cerebral infarction, systemic embolism, major bleeding, hospitalization for heart failure requiring intravenous administration of diuretics, and physical/psychological disability requiring alteration of the assigned treatment strategy. This is the first study in which patient reluctance to continue the assigned strategy accompanied by their spontaneous desire to move to the other strategy was also included as an endpoint to represent patient disability under an assigned strategy The cross-over would likely result from uncontrollable symptoms, hesitation to repeat cardioversion, or anxiety about the adverse effects of drugs without any life-threatening consequences, and could not be avoided without movement from 1 assigned strategy to the other. The reasons were reported by patients themselves.

Secondary endpoints were patient QOL scores, and the efficacy and safety of drugs required in AF treatment. Patient QOL was evaluated using the Japanese Society of Electrocardiology's Atrial Fibrillation Quality of Life Questionnaire (AFQLQ), which comprises 3 subsets that include 26 questions concerning frequency of occurrence of 6 symptoms (palpitations, dizziness, shortness of breath, chest discomfort, irregular pulse, and pulse deficit) (AFQLQ1), the severity of these symptoms (AFQLQ2), and anxiety and limitation of daily activities related to AF and AF treatment (AFQLQ3)[22]

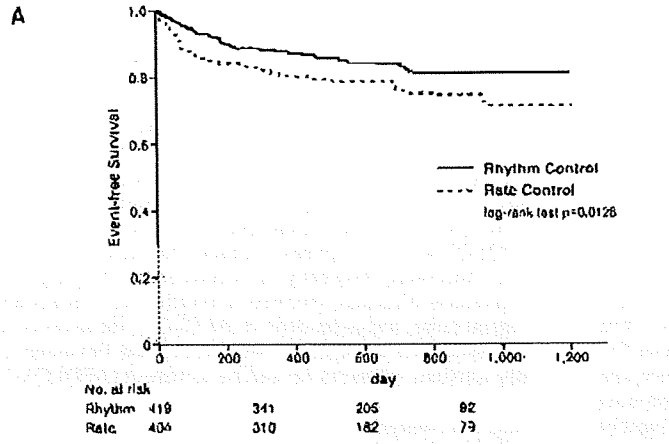
Statistical Analysis

The primary analysis was an intention-to-treat comparison between groups of time to first event of any of those forming the composite primary endpoint. Baseline patient characteristics were compared using chi-square tests and unpaired Student's t-test. Rates for all time-to-event analyses were estimated by the Kaplan-Meier method[23] and were compared by the log-rank test. Secondary analyses were conducted to evaluate results within subgroups. Unadjusted hazard ratios for primary endpoint with rhythm control vs rate control were estimated in each subgroup. Covariates of age, sex, presence or absence of congestive heart failure, and presence or absence of hypertension were used to construct a multivariate Cox proportional hazards survival model by a stepwise procedure. Covariates significantly associated with primary endpoint were then used to adjust the primary treatment comparison. A 2-tailed P-value of <0.05 was considered statistically significant.

Table 2 Drugs for Initial Therapy in the Rate Control and the Rhythm Control Groups

	No. of patients (%)		
	Rate control group (n=404)	Rhythm control group (n=419)	P value
Drug			
β-blocker	208 (51.5)		
*CCB**	107 (26.5)		
Digoxin	77 (19.1)		
Pilsicainide		136 (32.5)	
Cibenzoline		87 (20.8)	
Propafenone		49 (11.7)	
Disopyramide		37 (8.8)	
Flecainide		34 (8.1)	
Aprindine		30 (7.2)	
Pirmenol		4 (1.0)	
Bepridil		28 (6.7)	
Amiodarone		2 (0.5)	
Other drugs			
ACEI	17 (4.2)	30 (7.3)	0.07
ARB	70 (17.3)	89 (21.2)	0.16
Other CCB	73 (18.1)	80 (19.1)	0.79
Sicily	52 (12.9)	49 (11.7)	0.67
Nitrate	14 (3.5)	14 (3.3)	1.00
Drugs for DM	33 (8.2)	31 (7.4)	0.37
Diuretics	35 (8.7)	30 (7.2)	0.77
Warfarin	240 (59.4)	252 (60.1)	0.83

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockers; CCB, calcium-channel blockers; DM, diabetes mellitus.
*Verapamil, diltiazem.



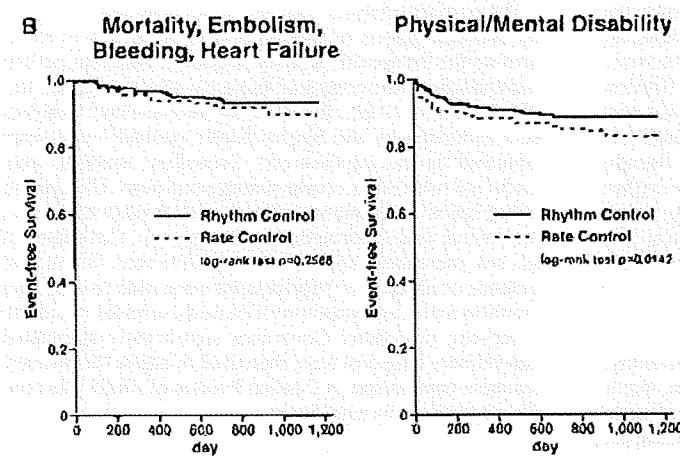


Fig 1. (A) Kaplan-Meier estimate of event-free survival (composite endpoints). (B) Event-free survival curves from mortality, embolism, major bleeding, and heart failure (Left) and from cross-over for physical/mental disability (Right)

Table 3 Components of the Primary Endpoint

Event	Overall (n=823)	Rate control group (n=404)	Rhythm control group (n=419)
All (%)	153 (18.6)	89 (22.8)	64 (15.3)
Total mortality (%)	7 (0.9)	3 (0.7)	4 (1.0)
Symptomatic stroke (%)	20 (2.4)	11 (2.7)	9 (2.1)
Systemic embolism (%)	2 (0.2)	1 (0.2)	1 (0.2)
Major bleeding (%)	3 (0.4)	1 (0.2)	2 (0.5)
Heart failure (%)	8 (1.0)	6 (1.5)	2 (0.5)
Physical/psychological disability (%)	113 (13.7)	67 (16.6)	46 (11.0)

Data are n (%)

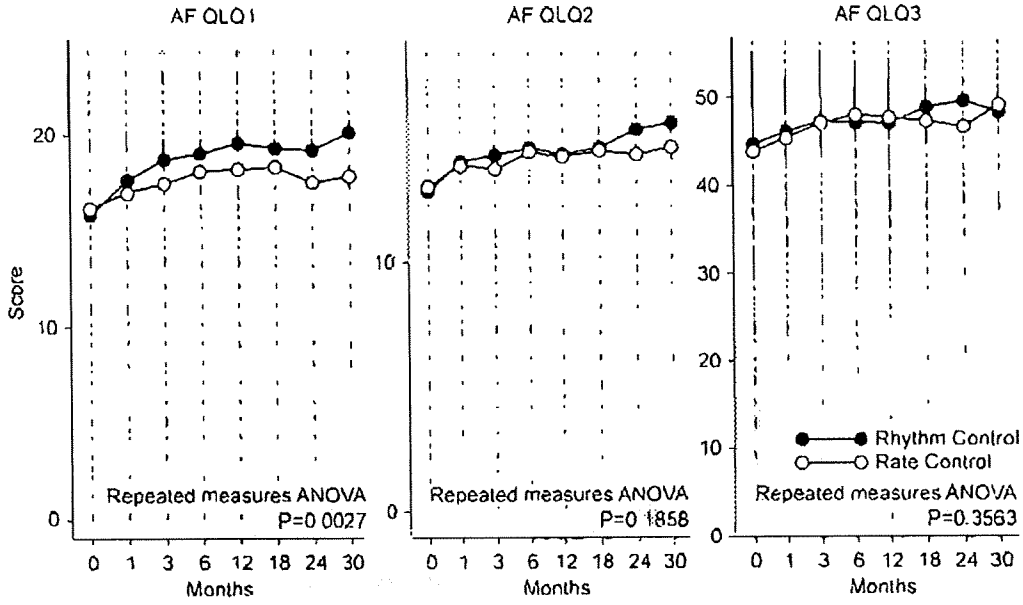


Fig 2. Atrial fibrillation (AF) quality of life questionnaire (QLQ) subset scores for AF-specific quality of life. Scale=0–24 (AF QLQ1), 0–8 (AF QLQ2), 0–56 (AF QLQ3), higher is better.

Results

Baseline Patient Characteristics

A total of 885 patients with PAF were enrolled in the study; rate control group, n=442; rhythm control group, n=443. The average follow-up period was 578 days, and 62 patients dropped out (7.0%) during the study; rate control group, n=38; rhythm control group, n=24. Mean age was 64.7±11.3 years. Baseline clinical data are summarized in Table 1. The proportion of patients with structural heart diseases was low compared with that in trials in Western countries.[10–13] Only approximately 20% of the patients were considered at high risk for stroke. Low CHADS2 scores[14] of 0 and 1 were observed in 43.3% and 34.8% of the patients, respectively. Patient clinical characteristics were not significantly different between the rate and rhythm control groups.

Treatment

Drugs for initial therapy in the 2 groups are outlined in Table 2. Beta-blockers were used in over 50% of patients in the rate control group. Antiarrhythmic drugs used in the rhythm control group were different from those used in previous clinical trials.[10–13] More than 85% of patients were started on class I drugs in accordance with the "The Japanese Guideline for Atrial Fibrillation Management".[2] Amiodarone was prescribed in only 0.5% of the patients as initial therapy. The prescription rate of warfarin was high and not significantly different between groups.

Sinus Rhythm Maintenance

In the rhythm control group, sinus rhythm was observed in 87.2% of the patients at 6 months, 88.9% at 1 year, 84.3% at 2 years, and 72.7% at 3 years on periodic ECGs. In contrast, the rate control group showed significantly lower proportions of patients with sinus rhythm: 74.0% at 6 months, 69.2% at 1 year, 65.6% at 2 years, and 43.9% at 3 years.

Primary Endpoint

Event-free survival was significantly higher in the rhythm than in the rate control group (hazard ratio 0.664, 95% confidence interval 0.481–0.917; P=0.0128, Fig 1A). Components of the primary endpoint are shown in Table 3. The primary endpoint occurred in 64 (15.3%) of the 419 rhythm control patients and in 89 (22.0%) of the 404 rate control patients. Total mortality, however, was low and was not significantly different between groups (1.0% in the rhythm and 0.7% in

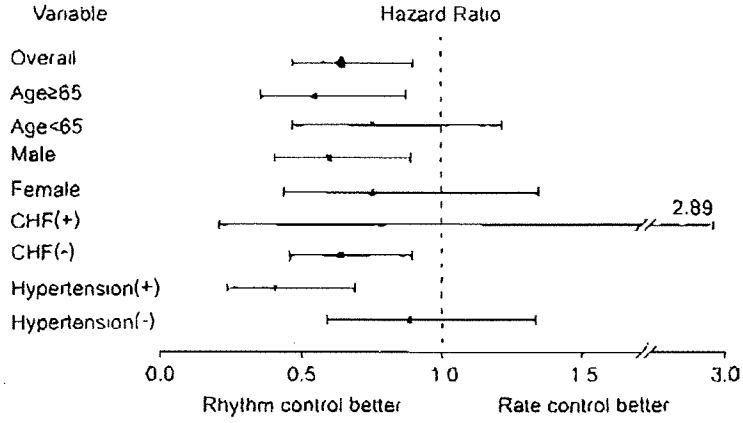


Fig 3 Subgroup hazard ratio analyses for the primary endpoint. CHF, congestive heart failure

the rate control group). The incidence of symptomatic stroke, systemic embolisms, major bleeding or heart failure was not significantly different between groups. Consequently, there were no significant differences between the groups in the total occurrences of mortality, embolism, major bleeding and heart failure (Fig 1B). Most of the primary endpoints resulted from the patients' desire to move to the alternate treatment strategy because of physical/psychological disability caused by their current treatment (Fig 1B). In the rhythm control group, 46 patients (11.0%) requested a change to rate control, whereas 67 rate control patients (16.6%) requested a change to rhythm control. Reasons for this endpoint differed between the groups: in the rhythm control group, it was uncontrollable symptoms in 19, anxiety over drug adverse events in 14, hesitation concerning electrical cardioversion in 6 and others in 7 patients; in the rate control group, it was uncontrollable symptoms in 56, anxiety over drug adverse events in 8 and others in 3.

Secondary Endpoints

The AFQLQ1 (frequency of symptoms) subset scores were higher (higher=better) in the rhythm control than in the rate control group, whereas the AFQLQ2 (severity of symptoms) and AFQLQ3 (AF-related anxiety and limitation of daily activities) subset scores improved with both treatment strategies and were not significantly different between groups (Fig 2).

Drug-related adverse events occurred very rarely: syncope in 2, ventricular tachycardia in 1, atrial flutter in 9, and symptomatic bradycardia in 4 patients. There were no significant differences in incidences between groups.

Primary Endpoint Hazard Ratios in Subgroups

Hazard ratios for primary endpoints in subgroups are shown in Fig 3. Rhythm control was associated with a better rate of event-free survival than was rate control among patients aged ≥65 years, male patients, hypertensive patients, and those without previous history of congestive heart failure.

Discussion

The present study shows that in PAF patients the mortality and cardiovascular morbidity are not affected by treatment strategy, and also that rhythm control is associated with fewer occurrences of the patient's desire of cross-over than rate control strategy. The study population was quite different from that in past clinical trials completed in the US and Europe,[10–13] comprising relatively young and symptomatic patients with PAF. The present findings substantiate the importance of individualizing PAF therapy.

Cardiovascular Mortality and Morbidity in PAF

For clinicians, AF type (paroxysmal, persistent, or permanent) may be a simple surrogate marker for designing a therapeutic strategy, as reflected in many guidelines published previously.[19,25,26] The present study focused on PAF, which is different from previous clinical trials. In the present study, total mortality and cardiovascular morbidity rates were remarkably low compared with those in the previous studies, in which most or all patients had persistent AF.[10–13] However, low mortality and morbidity rates should be attributed to patient comorbidities rather than AF type. The J-RHYTHM study patients were characterized by PAF, young age, no structural heart diseases, and no previous history of congestive heart failure, all of which may predict good prognosis,[27–30] and only approximately 20% of patients were at high risk for stroke. These low comobidities would lessen the role of the treatment strategy in the mortality and cardiovascular morbidity of these PAF patients.

Disability Requiring Alternation of Assigned Strategy

In these lower risk patients, safe and effective control of symptoms should be the clinician's primary goal, and thus an important guide when selecting a therapeutic strategy. Although disability requiring cross-over was more frequently observed in the rate control group, more important was that the reasons for the movement between the strategies were different. In rate control patients, the reason was mainly uncontrollable symptoms. In contrast, in rhythm control patients, the reasons were mostly hesitation over drug-related adverse events or electrical cardioversion, indicating the presence of some conservative patients.[31] These diversities in this study population emphasize the importance of individualizing PAF therapy.

We should realize that the endpoint representing patient acceptance in our study may be biased by the impressions of both patients and physicians who were unblinded to treatment strategy. To substantiate this subjective assessment, we used the AF-specific questionnaire, the AFQLQ.[32] Both treatment strategies significantly improved QOL scores in the 3 AFQLQ subsets, with a significant difference only in

the AFQLQ1 scores between groups. These facts would support the validity for the occurrence of the cross-over in view of AFQLQ1, and at the same time, could imply possible information bias by the physicians in view of AFQLQ2 and AFQLQ3. Therefore, we should not overestimate the beneficial effects of rhythm control strategy on the QOL of the patients, but should focus upon individualizing therapy. Conversely, it is noteworthy that approximately 80% of the PAF patients undergoing rate control were satisfied with the assigned strategy.

Subgroup Analysis

Subgroup analysis, showing a relationship between the occurrence of the primary endpoints and clinical background, indicated that the lower incidence of cross-over in the rhythm control group was not uniformly observed in the present PAF patients. The rhythm control strategy resulted in better composite primary endpoints in elderly and male patients, whereas the 2 strategies were not significantly different in young and female patients. These results suggested that age and gender might affect the psychological perception of AF therapy.

Study Limitations

First, in the present study, therapeutic strategies were not blinded to physicians and patients, which could lead to biases in the occurrence of endpoints. Second, in the assessment of control of PAF, transtelephonic monitoring could not be used to evaluate drug effects on PAF, such as prevention of episodes or heart rate control during episodes. Selection of drugs and dosage was dependent primarily on the evaluation of patient symptom reports by attending physicians. Third, most patients in the present study had no structural heart disease. Although limited, the present study, by clarifying the role of the rhythm or rate control strategy in the mortality, morbidity and QOL of PAF patients, supports the importance of individualizing PAF therapy.

Conclusion

The J-RHYTHM study showed that a rate control strategy of PAF is equivalent to a rhythm control strategy in terms of mortality and morbidity. However, event-free survival of the primary endpoint in this study was significantly higher with the rhythm control than with the rate control strategy, implying the importance of individualizing PAF therapy according to the patient's background and QOL.

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