Remodeling of atrial ATP-sensitive K⁺ channels in a model of salt-induced elevated blood pressure

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tension is associated with the development of atrial fibrillation; however, the electrophysiological consequences of this condition remain poorly understood. ATP-sensitive K⁺ (KATP) channels, which contribute to ventricular arrhythmias, are also expressed in the atria. We hypothesized that salt-induced elevated blood pressure (BP) leads to atrial KATP channel activation and increased arrhythmia inducibility. Elevated BP was induced in mice with a high-salt diet (HS) for 4 wk. High-resolution optical mapping was used to measure atrial arrhythmia inducibility, effective refractory period (ERP), and action potential duration at 90% repolarization (APD90). Excised patch clamping was performed to quantify KATP channel properties and density. KATP channel protein expression was also evaluated. Atrial arrhythmia inducibility was 22% higher in HS hearts compared with control hearts. ERP and APD90 were significantly shorter in the right atrial appendage and left atrial appendage of HS hearts compared with control hearts. Perfusion with 1 μM glibenclamide or 300 μM tolbutamide significantly decreased arrhythmia inducibility and pro-
longed APD90 in HS hearts compared with untreated HS hearts. KATP channel density was 156% higher in myocytes isolated from HS animals compared with control animals. Sulfonylurea receptor 1 protein expression was increased in the left atrial appendage and right atrial appendage of HS animals (415% and 372% of NS animals, respectively). In conclusion, KATP channel activation provides a potential therapeutic approach for atrial arrhythmia inducibility (19, 24, 49, 53). However, the cellular mechanisms responsible for these effects remain poorly understood. Importantly, the contribution of ATP-sensitive K⁺ (KATP) channel currents (IK,ATP) to hypertension-induced electrophysiological remodeling has not been investigated.

KATP channels were first discovered in cardiac muscle (64) and couple the metabolic state of the cell with its electrical activity. These channels are expressed throughout the body in metabolically active cell types and are found in the sarcolemmal and mitochondrial membranes. Differences in the molecular composition of the channel determine its biophysical and pharmacological properties. The KATP channel complex is composed of inward rectifier K⁺ channel (Kir6.x) and sulfonylurea receptor (SURx) subunits (63). In ventricular myocytes, KATP channels are composed of Kir6.2 and SUR2A (3, 56, 79). Activation of ventricular KATP channels results in profound abbreviation of action potential (AP) duration (APD), which protects the heart during myocardial ischemia by limiting Ca²⁺ entry (9, 36, 51, 90). The reduction of Ca²⁺ entry is thought to reduce energy consumption and limit cellular damage. However, APD abbreviation shortens wavelength, which can facilitate the formation of ventricular arrhythmias (47, 73, 81, 82). Recent studies (29, 32) have demonstrated that atrial KATP channel complexes are predominantly composed of Kir6.2 and SUR1 subunits. Activation of these channels in isolated atrial preparations has been shown to be proarrhythmic (54). However, the physiological role and contribution to electrophysiological remodeling of atrial KATP channels in response to cardiac disease remain unknown.

Here, we investigated the electrophysiological consequences of salt-induced elevated blood pressure (BP) in adult mice. Our findings demonstrate that elevated BP is associated with a reduction in atrial effective refractory period (ERP), APD, and increased arrhythmia inducibility. These electrophysiological effects are reversible with KATP channel blockers. In addition, salt-induced elevated BP is associated with increased SUR1 levels in atrial myocytes and increased sarcosomal KATP channel density. The findings of this study highlight the KATP channel as a novel molecular link between salt-induced elevated BP and atrial arrhythmias.

MATERIALS AND METHODS

Mice. All studies were performed using 2- to 3-mo-old male CD-1 mice. Two groups of mice were fed ad libitum. The normal salt (NS) group received tap water, and the high-salt (HS) group received 1.0% NaCl and 0.3% KCl in the drinking water for a period of 4 wk (92). All procedures complied with the standards for the care and use of animal subjects as stated in the National Institutes of Health Guide for
the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996), and protocols were approved by the Institutional Animal Care and Use Committee of the New York University School of Medicine.

**BP and systolic function.** BP and fractional shortening (FS) measurements were obtained from a subset of animals before euthanization. BP measurements were obtained from conscious, restrained mice using tail-cuff plethysmography after a period of training on a warmed (37°C) platform (Kent Scientific) as previously described (95). At least five measurements were obtained from each animal and averaged. FS measurements were obtained from anesthetized animals (1.5% inhaled isoflurane, VetEquip) using a Philips HDI 5000 ultrasound sound system and a 15-MHz probe. The heart was visualized at the short axis at the level of the papillary muscle in the two-dimensional mode, and FS measurements were calculated from M-mode recordings as the percent decrease from diastolic to systolic diameter at the level of the papillary muscle (20). Heart and body weight measurements were performed immediately after euthanization.

**In vivo electrophysiological experiments.** Animals were anesthetized with inhaled isoflurane. The jugular vein was dissected and cannulated with a 1.1-Fr octopolar electrophysiological catheter (Millar Instruments), which was advanced and positioned in the right heart chambers. The esophagus was cannulated with a 4-Fr octopolar electrophysiological catheter (St. Jude Medical) and positioned adjacent to the left atrium (LA). Electrode locations were determined from the surface ECG while the heart was paced with different pairs of electrodes. Atrial pacing was performed using 1-2 ms stimuli at twice diastolic threshold. ERP measurements were obtained using an S1–S2 protocol consisting of a 10-second S1 drive cycle at a basic cycle length (BCL) of 100 ms followed by the S2 stimulus. The S1–S2 coupling interval was reduced by 2 ms until the S2 stimulus failed to elicit a conducted response. ERP was defined as the shortest S1–S2 interval that resulted in successful capture. This process was performed twice for each atrium, and an average ERP for each atrium was obtained. Animals were considered inducible if an arrhythmic episode lasting longer than 1 s was observed during the ERP measurement protocol.

**Isolated heart preparation.** Hearts were Langendorff perfused as previously described (60, 86). Briefly, mice were administered heparin (1.0 U/g body weight) and killed by exposure to 2% CO2 (37°C) Tyrode solution at a constant pressure of 68–74 mmHg. Hearts and hearts were excised, cannulated, and Langendorff perfused with a modified Tyrode solution containing (in mM) 1.8 CaCl2, 1.0 MgCl2, and 10.0 HEPES (pH 7.4). The bath solution contained (in mM) 113.0 NaCl, 1.2 KH2PO4, 130.0 NaCl, 4.7 KCl, 0.6 KHPO4, 0.6 NaHPO4, 1.2 MgSO4, 12.0 NaHCO3, 10.0 KHCO3, 10.0 HEPES, 10.0 tauroctone, 10.0 β-2,3-butanedione monoxime, and 10.0 glucose for 2 min. Next, hearts were perfused with Tyrode solution containing 1.8 MgCl2 and 5% bovine calf serum at 4°C, and used for patch clamp experiments within 90 min of isolation.

**Patch-clamp experiments.** Myocytes isolated from the LAs of NS and HS animals were plated on laminin-coated coverslips, mounted in a recording chamber, and placed on the stage of an inverted microscope (Nikon TE2000-V). Single channel recordings were performed in the inside-out configuration at room temperature using standard patch-clamp techniques (38). Patch pipettes were pulled using borosilicate glass and had resistances between 3 and 4 MΩ when filled with pipette solution, which contained (in mM) 140.0 KCl, 2.0 CaCl2, 1.0 MgCl2, and 10.0 HEPES (pH 7.4). The bath solution contained (in mM) 140.0 KCl, 1.0 EGTA, 10.0 HEPES, and 1.2 MgCl2 (pH 7.2). Current was filtered (low-pass Bessel response with a cutoff frequency of ~3 dB at 1 kHz), digitized at 5 kHz, and stored for offline analysis with pCLAMP software (Clampex 9.0, Axon Instruments). Unless otherwise stated, the pipette potential was +80 mV (membrane potential of ~80 mV). At this potential, current passing through the KATP channel out of the pipette (inward membrane currents) is represented as downward deflections in all figures. Unitary conduc-
tance measurements were made in 100 or 300 μM ATP in the bath solution from −80 to −20 mV. K_{ATP} channel unitary conductance has been shown to be unaffected by ATP concentration (85). Recordings were made while slowly changing the patch potential from −100 to 100 mV. ATP sensitivity was determined by applying ATP from 1,000 to 1 μM using a rapid solution changer. Exposure to ATP ramps was bracketed by recordings in zero ATP to ensure the absence of significant rundown, which was corrected if necessary. Current was normalized to the maximal channel current amplitude in zero ATP and the minimum current amplitude in 1,000 μM ATP. The IC_{50} was defined as the ATP concentration that resulted in the half-maximal inhibition of excised patch current. Patches typically contained several channels, and I_{K,ATP} was calculated as the mean patch current relative to zero current in the presence of 1,000 μM ATP when all K_{ATP} channels were blocked. I_{K,ATP} was plotted as a function of ATP concentration, and ATP-inhibitory curves were obtained by fitting a pseudo-Hill function to the experimental data using a nonlinear least-squares method. The apparent number of channels per patch was estimated as the maximal number of coincident channel openings observed in the absence of bath ATP, as determined from a multiple Gaussian fit to all-points current amplitude histograms. In patches that contained too many channels to perform this procedure, the number of channels per patch was estimated immediately after patch excision as the mean patch current divided by the average measured unitary conductance.

**Histology.** Animals were euthanized, and hearts were immediately excised via a midline sternotony. Aortas were cannulated, and hearts were perfused with Tyrode solution to clear the blood. Spontaneously contracting tissue was arrested in diastole by perfusing 50 mM KCl at 20°C. The solution was then switched to 4% paraformaldehyde and kept at 4°C for 8 h. Hearts were stored in PBS for 1 h (4°C), dehydrated in ethanol (20°C), fixed in xylene (20°C), and embedded in paraffin. Tissue was sectioned (5 μm) using a microtome. Sections were stained with Gomori’s trichrome, following the manufacturer’s instructions. Fibrosis content was determined from four-chamber views in a blinded fashion using Image-Pro Plus 5.0 software (Media Cybernetics). Data were acquired from at least 5 sections/heart, which were analyzed by quantifying blue pixel content as a percentage of total tissue area.

**Western blot analysis.** Purification and enrichment of membrane proteins were performed as previously described (78). Briefly, atria were minced, snap frozen in liquid nitrogen, and stored at −80°C. A total of 15 animals/group were analyzed in 3 independent preparations. For each preparation, five atria were pooled and homogenized in 2 ml of cold lysis buffer containing (in mM) 250.0 sucrose, 2.0 EGTA, 20.0 HEPES, 1.0 NaVO₄, 50.0 NaF, and 1.0 PMSF (pH 7.4) with 2× Complete protease inhibitor (Roche) using a mechanical tissue homogenizer (Ultra-Turrax T8, IKA Labortechnik). The homogenate was dounced on ice and centrifuged at 4,000 g for 5 min at 4°C. Supernatants were centrifuged at 190,000 g and 4°C for 1 h. Membrane pellets were dissolved in 25 μl of SDS buffer containing 62.5 mM Tris HCl, 3% SDS, and 10% glycerol (pH 6.8) and sonicated for 10 s on ice. Protein concentrations were determined using the Lowry method. β-Mercaptoethanol (5%) and bromphenol blue (0.01%) were added, and equal amounts of protein (100 μg) were subjected to SDS-PAGE on 10% acrylamid gels and transferred to polyvinylidine difluoride membranes. Blots were blocked for 1.5 h in blocking solution, which contained 0.085 mM Tris HCl, 0.17 mM Tris base, 0.5 mM NaCl, 0.1% Tween 20, 5% and low-fat milk, and incubated with primary antibodies diluted in blocking buffer overnight at 4°C. Immunoreactive proteins were visualized by species-specific secondary horseradish peroxidase-conjugated antibodies and subsequent enhanced chemiluminescence (Supersignal West Pico or Femto Chemiluminescent Substrate, Pierce Chemical) as recommended by the manufacturer. To confirm equal loading, signals were normalized to N-cadherin levels using computer-assisted densitometry (Scion Image Software, Scion, NIH). Values for HS LAs and RAs were normalized to the same chambers in NS animals. Additionally, equal loading was confirmed with Ponceau staining. Briefly, data were collected for three Ponceau-stained signals of different molecular weights and averaged. Densitometric values for respective K_{ATP} channel subunits were normalized to these signals.

**Antibodies.** Primary antibodies used for immunoblot analyses included sera raised in the rabbit (1:250 SURI, custom made by Dr. William Coetsee) and goat (1:200 gKIR6.2, G-16, Santa Cruz Biotechnology). Secondary antibodies included horseradish peroxidase-conjugated donkey anti-rabbit (1:5,000, Santa Cruz Biotechnology) and donkey anti-goat (1:5,000, Santa Cruz Biotechnology).

**Statistical analysis.** Ex vivo arrhythmia inducibility data were compared using the Freeman-Halton extension of the Fisher’s exact test. In vivo arrhythmia inducibility data were compared without the Freeman-Halton extension. The Mann-Whitney rank-sum test was used for the comparison of the number of channels per patch. Two-way ANOVA with replication was used for the comparison of atrial fibrosis followed by two-tailed Student’s t-tests. Two-tailed Student’s t-tests were used for all other comparisons. Values are reported as means ± SE. Differences were considered significant if P < 0.05.

**RESULTS**

**BP and systolic function.** Systolic BP was quantified using tail-cuff plethysmography, and cardiac function was assessed using echocardiography. BP was significantly elevated (P = 0.003) in HS animals (121.2 ± 4.3 mmHg, n = 11) compared with NS animals (103.4 ± 3.5 mmHg, n = 11). Average FS was not significantly different between groups. The ratio of heart weight to body weight was significantly increased (P = 0.013) by 22.6% in HS animals compared with NS animals (n = 10).

**Atrial interstitial fibrosis.** The development of atrial interstitial fibrosis has been demonstrated in several animal models of hypertension (19, 49, 53). Figure 1 shows representative NS and HS sections stained with Gomori’s trichrome. In HS hearts, the percentage of interstitial fibrosis was significantly higher (P = 0.006) in the LA (9.97 ± 0.70%, n = 5) compared with NS hearts (6.75 ± 0.51%, n = 5). The percentage of interstitial fibrosis was not significantly changed in the RA.

**Atrial arrhythmia inducibility.** To determine whether salt-induced elevated BP is associated with atrial electrophysiological changes and increased arrhythmia inducibility in vivo, electrophysiological experiments were conducted on intact anaesthetized animals. Figure 2 shows representative surface and intracardiac electrograms and average LA and RA ERP measurements. Using transesophageal and transvenous pacing techniques for stimulating the LA and RA, significantly more atrial arrhythmias were observed in HS animals (6 of 11) compared with NS animals (1 of 10, P < 0.05). Average ERP values in HS animals were significantly shorter in the LA (46.1 ± 10.1% of NS animals, P = 0.009) and RA (67.9 ± 4.3% of NS animals, P = 0.001). These data demonstrate that salt-induced elevated BP is associated with a significant reduction in the duration of refractoriness and increased atrial arrhythmia inducibility in vivo.

**Atrial arrhythmia dynamics.** Atrial arrhythmia dynamics were evaluated in isolated Langendorff-perfused hearts. Atrial arrhythmias were induced in a small percentage of NS hearts (5 of 36). In contrast, programmed electrical stimulation induced atrial arrhythmias in a significantly higher (P = 0.04) percentage of HS hearts (16 of 45). Figure 3, A and B, shows
representative voltage maps showing the progression of polymorphic reentrant activity in the RAA of NS and HS hearts, respectively. Reentrant activity cycle length in the NS and HS hearts were 32 and 20 ms, respectively. The power spectra for these atrial arrhythmias are shown in Fig. 3, and Fig. 3, D and E, shows the respective DF maps. Arrhythmias in NS and HS atria had average DFs of 28 and 41 Hz, respectively. Figure 3F shows the average DFs for atrial arrhythmias recorded from NS and HS atria. These data indicate that arrhythmias in HS hearts are characterized by significantly higher (P < 0.008) DFs (45.1 ± 2.7 Hz, n = 12) compared with NS hearts (31.2 ± 3.5 Hz, n = 5).

Atrial CV and repolarization. CV and ERP represent important parameters for arrhythmia inducibility and complexity (14, 17, 18, 50). Figure 4 shows representative activation maps and average CV and ERP measurements obtained from the LAA and RAA of isolated NS and HS hearts. Atrial CVs were not found to be significantly altered in HS hearts compared with NS hearts in either the LAA or RAA. Average ERP values were significantly shorter in HS hearts for both the LAA (53.6 ± 5.0% of NS animals, P = 2.68 × 10⁻⁷) and RAA (59.7 ± 5.3% of NS animals, P = 1.67 × 10⁻⁵). These data are consistent with the in vivo electrophysiological changes and demonstrate that salt-induced elevated BP is associated with minimal changes in conduction parameters and a significant reduction in the duration of refractoriness.

ERP abbreviation suggests that APD may be reduced in HS atria. Figure 5 shows representative optical AP traces from NS and HS atria and average APD₉₀ values. The optical traces (Fig. 5, A and B) and average APD₉₀ values (Fig. 5, E and F) recorded at a 100-ms S1–S2 coupling interval showed significantly shorter (P < 0.003) APDs in the LAA of HS hearts (15.63 ± 0.73, n = 26) compared with NS hearts (19.30 ± 1.02 ms, n = 20). The reduction in APD was not observed in the RAA. APD₉₀ values measured at an S1-S2 coupling interval of 25 ms (Fig. 5, C, D, G, and H) were significantly shorter in both the LAA (13.04 ± 0.67, n = 16) and RAA (12.63 ± 0.55, n = 17) in HS hearts compared with the LAA (17.29 ± 1.56, n = 8, P = 0.009) and RAA (15.14 ± 0.53, n = 8, P =
amide significantly prolonged LAA (the 25-ms S1-S2 coupling interval, glibenclamide and tolbutamide-treated HS atria. This effect was not observed in the RAA. At 0.02) significantly prolonged LAA APDs compared with untreated HS hearts. Similar differences were obtained at APD_{50} and APD_{70} (data not shown).

Effects of K_{ATP} channel blockers on arrhythmia inducibility and APD. The contribution of I_{K,ATP} to atrial arrhythmia inducibility was determined in NS and HS hearts after perfusion with the K_{ATP} channel blocker glibenclamide. Atrial arrhythmia inducibility was significantly reduced (P = 0.04) in HS hearts during glibenclamide perfusion (2 of 21) compared with untreated hearts (16 of 45). A similar decrease in arrhythmia inducibility was also observed for HS hearts treated with tolbutamide (P = 0.006). Arrhythmia inducibility was unaffected by glibenclamide or tolbutamide in the NS group.

The contribution of I_{K,ATP} to atrial APD was evaluated during perfusion with glibenclamide and tolbutamide. Consistent with a previous study (32), atrial APD values in NS hearts were unaffected by perfusion of either drug (data not shown), suggesting that atrial K_{ATP} channels play a limited role in membrane excitability under physiological conditions. Figure 6 shows average APD values in HS mice during perfusion of glibenclamide and tolbutamide. At an S1-S2 coupling interval of 100 ms, glibenclamide (P = 0.004) and tolbutamide (P = 0.02) significantly prolonged LAA APDs compared with untreated HS atria. This effect was not observed in the RAA. At the 25-ms S1-S2 coupling interval, glibenclamide and tolbutamide significantly prolonged LAA (P = 0.007 and 0.001, respectively) and RAA APD values (P = 3.59 \times 10^{-5} and 0.008, respectively) compared with untreated HS hearts. These data demonstrate that activation of atrial I_{K,ATP} contributes to elevated BP-induced APD shortening.

Sarcolemmal K_{ATP} channel properties and density. Figure 7 shows K_{ATP} channel biophysical properties and channel densities evaluated in isolated LA myocytes from NS and HS animals measured using patch-clamp techniques. Inside-out single channel recordings showed unitary deflections with inward rectification properties (Fig. 7A). The slope conductance of the unitary current (Fig. 7B) was consistent with a previous study (71) of K_{ATP} channels and was not significantly different in HS myocytes (77.2 ± 1.37 pS, n = 6) compared with NS myocytes (75.8 ± 0.71 pS, n = 7). Channel activity in excised patches from both NS and HS myocytes decreased with increasing concentrations of ATP (Fig. 7C). The IC_{50} for ATP (Fig. 7D) was not significantly different in patches from HS myocytes (14.6 ± 2.13 μM, n = 6) compared with NS myocytes (17.7 ± 3.31 μM, n = 12). The average number of functional K_{ATP} channels per patch (Fig. 7E) was significantly greater (P = 0.046) in HS myocytes (41 ± 17.2, n = 7) compared with NS myocytes (16 ± 5.7, n = 18). Excised patch membrane areas, as estimated from pipette resistances, were unchanged between the groups (3.2 ± 0.13 and 3.3 ± 0.22 MΩ for the NS and HS group, respectively).

K_{ATP} subunit protein levels. Atrial tissue from NS and HS animals was analyzed to determine K_{ATP} channel subunit levels using immunoblot analysis (Fig. 8). SUR1 protein expression was significantly increased in HS LAs (414.6 ± 87.6% of NS LAs, n = 3, P = 0.02) and RAs (371.7 ± 10.5% of NS RAs, n = 3, P = 0.001; Fig. 8, A and B). Kir6.2 protein expression was unchanged (Fig. 8, C and D). N-cadherin expression was not significantly altered in HS atria compared with untreated hearts (16 of 45). A similar decrease in arrhythmia inducibility was also observed for HS hearts treated with tolbutamide (P = 0.006). Arrhythmia inducibility was unaffected by glibenclamide or tolbutamide in the NS group.

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with NS atria and was used as a loading control. Similar results were obtained using three Ponceau-stained signals of different molecular weights as a loading control (data not shown).

**DISCUSSION**

Salt-induced hypertension is highly relevant given the dramatic rise of salt consumption in Western countries over the last three decades (12). Increased risk of cardiovascular morbidity and mortality due to chronic high-salt intake has been attributed to both BP-dependent and -independent effects on the heart and vasculature (59). Moreover, hypertension is widely recognized as an independent risk factor for AF (7, 46); however, the electrophysiological consequences of this condition are poorly understood. This is the first study to demonstrate elevated BP with a HS diet leads to significant atrial electrophysiological remodeling and an increase in atrial arrhythmia inducibility.

After the induction of elevated BP with a HS diet, we observed a modest increase in LAA interstitial fibrosis, consistent with previous observations using other models of hypertension (19, 49, 53). On average, HS animals and isolated hearts were more susceptible to atrial arrhythmias induced by programmed stimulation compared with NS animals. Atrial arrhythmias were characterized by higher DFs in HS hearts compared with NS hearts. CV was unaffected by salt-induced

**Fig. 4. Decreased atrial ERP.** A and B: representative activation maps from the LAA (A) and RAA (B) of a NS heart. C and D: representative activation maps from the LAA (C) and RAA (D) of a HS heart. E and F: average conduction velocity (CV; E) and ERP (F) values. Numbers in bars indicate numbers of animals in each group. Bar = 1 mm.*P < 0.05.

**Fig. 5. Decreased atrial action potential (AP) duration (APD).** A and B: representative LAA (A) and RAA (B) single-pixel optical AP traces obtained at an S2 coupling interval of 100 ms. C and D: representative LAA (C) and RAA (D) single-pixel optical AP traces obtained at an S2 coupling interval of 25 ms. E and F: average LAA (E) and RAA (F) APD at 90% repolarization (APD90) values obtained at an S2 coupling interval of 100 ms. G and H: average LAA (G) and RAA (H) APD90 values obtained at an S2 coupling interval of 25 ms. Numbers in bars indicate numbers of animals in each group. *P < 0.05.
elevated BP. LAA and RAA ERPs were significantly shorter in the HS group. In HS animals, APD was decreased in the LAA when measured at a 100-ms cycle length and decreased in both the LAA and RAA when measured at a 25-ms cycle length. Differences in RAA APDs measured at the two coupling intervals suggest that salt-induced elevated BP may lead to electrical remodeling, which includes altered ion channel gating kinetics. Importantly, perfusion with glibenclamide and tolnbutamide resulted in the prolongation of APD and a reduction in atrial arrhythmia inducibility in HS animals, suggesting the basal activity of K\textsubscript{ATP} channels. Analysis of the biophysical characteristics of atrial sarcolemmal K\textsubscript{ATP} channels demonstrated that sur1 levels were significantly increased in HS atria, suggesting that sur1 is responsible for the increase in functional K\textsubscript{ATP} channels.

The atrial myocardium has been shown to remodel in response to AF (1, 62). Structural remodeling has been extensively documented and is characterized by atrial dilation and an increase in interstitial fibrosis (66). Additionally, patients with AF show electrophysiological changes, including shorter atrial ERP, increased heterogeneity of atrial CV, and decreased local electrogram amplitude (77). Similar changes in electrophysiological parameters have been described in tachypaced animal models (1, 61, 74, 76). The principal cellular mechanisms that have been associated with AF-induced electrical remodeling include reduced L-type Ca\textsuperscript{2+} current (I\textsubscript{CaL}), transient outward K\textsuperscript{+} current (I\textsubscript{to}), and ACh-activated K\textsuperscript{+} current (I\textsubscript{K,ACH}) and increased levels of inward rectifier K\textsuperscript{+} current (I\textsubscript{K1}) and the constitutively active component of I\textsubscript{K,ACH} (16, 25, 26, 93). Several studies have examined the role of the K\textsubscript{ATP} channel in humans and animal models of AF. Although one study (91) found increased I\textsubscript{K,ATP} density associated with chronic AF, others have demonstrated that K\textsubscript{ATP} subunit transcripts (13) and current density (4) are downregulated in AF patients. Studies (34, 43, 89) using animal models of rapid atrial pacing have suggested the electrophysiological changes are unaffected by K\textsubscript{ATP} channel blockade.

A comparatively small number of studies have examined the structural and electrophysiological consequences of hypertension (19, 49, 53). In the ventricles, structural remodeling associated with hypertension is characterized by myocyte hypertrophy, increased myofibrillar disarray, and interstitial fibrosis (40). Macroscopic electrophysiological changes associated with hypertension include prolongation and increased dispersion of ventricular repolarization (40, 52, 57), which are thought to be mediated by reductions in I\textsubscript{K1} and I\textsubscript{to} (58).

Choisy et al. (19) investigated electrophysiological remodeling and susceptibility to atrial arrhythmias in spontaneously hypertensive rats at 3 and 11 mo. Comparisons of hypertensive and control animals indicated that APD and atrial ERP values were not significantly different. Arrhythmia inducibility was unchanged in 3-mo-old hypertensive animals and significantly increased in 11-mo-old animals compared with control animals. Patch-clamp recordings of LA myocytes demonstrated that I\textsubscript{CaL} densities were decreased in 3-mo-old hypertensive hearts compared with age-matched control hearts. These data indicate that acute hypertension induces electrophysiological remodeling in the LA myocardium that is consistent with APD shortening. A more recent study (24) demonstrated that atrial ERP is significantly decreased in spontaneously hypertensive rats compared with control animals, and this was associated with an increase in AF duration. Pharmacological studies have suggested that these changes may be associated with an increase in the Ca\textsuperscript{2+}-activated K\textsuperscript{+} current. Another study by Lau et al. (53) characterized the atrial electrophysiological and structural changes using the one-kidney, one-clip ovine hypertensive model. After 7 wk of hypertension, significant hypertrophy and interstitial fibrosis were evident in the atria. Electrophysiological alterations were observed in the LA and RA and included increased atrial ERP, reduced and more heterogeneous CVs, and greater AF inducibility. None of the above studies investigated the contribution of I\textsubscript{K,ATP} to electrophysiological remodeling. There are several factors that could account for the model-specific differences in electrophysiological remodeling. The interventions used to increase BP may have different effects on the renin-angiotensin-aldosterone system. Circulating levels of angiotensin II can affect electrophysiological characteristics through direct interactions with ion channels (84) and transcriptional mechanisms (41), possibly resulting in the differences observed. Recent evidence has also suggested that differences in sympathetic tone can alter atrial repolarization kinetics through K\textsubscript{ATP} channel-dependent mechanisms (42). Together, these studies suggest that the mode of hypertension induction may determine whether atrial repolarizing currents are remodeled.

Mechanoelectric feedback has long been suspected to modulate the hypertensive AF substrate (10, 69, 94). Acute atrial stretch has been demonstrated to affect ERP (10, 11, 48). Such changes can be spatially heterogeneous, contributing to ar-

Fig. 6. Glibenclamide and tolbutamide increase APD. A and B: average LAA (A) and RAA (B) APD\textsubscript{90} values obtained at an S2 coupling interval of 100 ms. C and D: average LAA (C) and RAA (D) APD\textsubscript{90} values obtained at an S2 coupling interval of 25 ms, respectively. HS-GLY, HS group perfused with 1 μM glibenclamide; HS-TOLB, HS group perfused with 300 μM tolbutamide. The dashed lines indicate untreated NS average APD\textsubscript{90} values. Numbers in bars indicate numbers of animals in each group. *P < 0.05.
rhythmogenesis by shortening the reentrant wave length and
favoring functional block (89). Stretch-activated channels are
the primary ionic mediators of mechanoelectric feedback and
have been found in the cardiac tissue of various species,
including humans (37, 72, 83). It has been shown that activa-
tion of stretch-activated channel current accelerates repolariza-
tion during the AP plateau, resulting in APD shortening and
afterdepolarizations (30, 39, 69). Despite the fact that irrevers-
ible atrial structural remodeling has been well documented in
hypertension, the electrophysiological alterations caused by
atrial stretch (decreased APD and atrial ERP) have been shown
to be quickly reversible with the release of stretch (10). The
present study demonstrated that altered electrophysiological
parameters persist in isolated nonworking Langendorff-per-
fused hearts, where afterloads in the atria are negligible and
would have been similar in all groups studied. Another possi-
bile effect of mechanical stretch would be through the modu-
lation of SUR1 expression. The FOXO subfamily of forkhead
transcription factors has been shown to regulate KATP channel
gene transcription (68). As a downstream target of Akt (2),
FOXOs are subject to regulation by the complex interplay of
angiotensin II expression (70) and mechanical stress (67).

Our Western blot analysis data suggest that increased SUR1
expression may be sufficient to increase the density of func-
tional K_ATP channels, since Kir6.2 expression was unchanged.
The notion that SUR subunits are stoichiometrically limiting
for the formation of cardiac K_ATP channels was first introduced
by the work of Du et al. (27). Using a transgenic mouse where
SUR2A expression was under the control of a cytomegalovirus
promoter, they demonstrated that overexpression of SUR2A
(in the absence of altered mRNA expression of other KATP
channel proteins) resulted in increased sarcolemmal expression
of functional K_ATP channels in ventricular myocytes (27).
Moreover, available mRNA expression data suggest that SUR1
may be stoichiometrically limiting in the atria (31, 68), imply-
ning that overexpression of this subunit alone may be sufficient
to increase the density of functional K_ATP channels. Other
recent evidence has suggested that SUR1-containing K_ATP chan-

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**Fig. 7. Increased atrial ATP-sensitive K⁺ (K_ATP) channel density.**

A: representative single channel currents from NS and HS atrial myocytes recorded during a slow ramp (−100 to 100 mV). Inset: representative single channel recording and activity histogram. B: average current (I)-voltage (V) curve. C: representative responses of excised patches to decreasing concentrations of ATP. D: average responses to varying concentrations of ATP. E: average numbers of K_ATP channels per patch. Numbers in bars indicate numbers of animals in each group. *P < 0.05.

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**Fig. 8. Elevated sulfonylurea receptor 1 (SUR1) protein levels.**

A and B: representative immunoblot (A) and average SUR1 expression levels (B). N-cadherin (N-Cad) was used as a loading control. Protein expression levels were normalized to the NS group for each chamber. C and D: representative immunoblot (C) and average Kir6.2 expression levels (D). Numbers in bars indicate numbers of animals in each group. *P < 0.05.
nels are preferentially localized to the plasmalemma, whereas SUR2 causes localization to intracellular vesicles (5). It is possible that the increased expression of SUR1 that we observed resulted in enhanced trafficking to the plasma membrane from an endosomal reservoir.

Despite the expression of functional K$_{ATP}$ channels, excised hearts from NS animals did not demonstrate ERP or APD prolongation with K$_{ATP}$ channel blockade. This suggests that, as in the ventricles, atrial K$_{ATP}$ channels contribute little to repolarizing current under basal conditions, which is consistent with a previous study (32). However, APD in hypertensive animals was prolonged with K$_{ATP}$ channel blockade, implying that, in addition to overexpression of sarcolemmal K$_{ATP}$ channels, a HS diet also leads to increased channel function. There are a number of potential explanations for this observed K$_{ATP}$ channel activation. It is possible that salt-induced hypertension results in a sufficient metabolic challenge, resulting in K$_{ATP}$ channel activation. In addition, changes in the levels of phospholipid (phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate) (6), G protein (80), adenylyl kinase (15), creatine kinase (22), and M-LDH (21) as well as GAPDH activity (44, 45) may play a role in K$_{ATP}$ channel activation in HS animals. Although the patch-clamp data provide direct evidence supporting that sarcolemmal K$_{ATP}$ channels contribute to the electrophysiological remodeling in hypertensive animals, a possible role of mitochondrial K$_{ATP}$ channels was not excluded. Several studies (65, 87, 88) of ischemic preconditioning have suggested that mitochondrial K$_{ATP}$ channels are involved in myocardial protection.

We have provided evidence supporting K$_{ATP}$ channel activation as a mechanistic link between salt-induced elevated BP and atrial arrhythmia inducibility. These data also suggest that elevated systolic BP may be an important early risk factor for electrical remodeling leading to AF and emphasize the need for aggressive treatment at early stages. The findings of this study have important implications for the treatment and prevention of atrial arrhythmias in the setting of elevated BP and may lead to new therapeutic approaches.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


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