Effects on Atrial Fibrillation in Aged Hypertensive Rats by Ca²⁺-Activated K⁺ Channel Inhibition

Jonas G. Diness, Lasse Skibsbye, Thomas Jespersen, Emil D. Bartels, Ulrik S. Sørensen, Rie S. Hansen, Morten Grunnet

Abstract—We have shown previously that inhibition of small conductance Ca²⁺-activated K⁺ (SK) channels is antiarrhythmic in models of acutely induced atrial fibrillation (AF). These models, however, do not take into account that AF derives from a wide range of predisposing factors, the most prevalent being hypertension. In this study we assessed the effects of two different SK channel inhibitors, NS8593 and UCL1684, in aging, spontaneously hypertensive rats to examine their antiarrhythmic properties in a setting of hypertension-induced atrial remodeling. Male spontaneously hypertensive rats and the normotensive Wistar-Kyoto rat strain were divided in 2×3 groups of animals aged 3, 8, and 11 months, respectively. The animals were randomly assigned to treatment with NS8593, UCL1684, or vehicle, and open chest in vivo experiments including burst pacing–induced AF were performed. The aging spontaneously hypertensive rats were more vulnerable to AF induction both by S2 stimulation and burst pacing. Vehicle affected neither the atrial effective refractory period nor AF duration. SK channel inhibition with NS8593 and UCL1684 significantly increased the atrial effective refractory period and decreased AF duration in both the normotensive and hypertensive strains with no decline in efficacy as age increased. In conclusion, SK channel inhibition with NS8593 and UCL1684 possesses antiarrhythmic properties in a rat in vivo model of paroxysmal AF with hypertension-induced atrial remodeling. The present results support the notion that SK channels may offer a promising new therapeutic target in the treatment of AF. (Hypertension. 2011;57:1129-1135.) ● Online Data Supplement

Key Words: atrial fibrillation ■ small conductance Ca²⁺-activated K⁺ channel ■ spontaneously hypertensive rats ■ NS8593 ■ UCL1684 ■ ion channels ■ antiarrhythmia agents

Atrial fibrillation (AF) is the most common cardiac arrhythmia encountered in the clinic and contributes significantly to cardiac morbidity and mortality. There is a two-fold increase in the risk of death in patients with a history of AF, and the risk of embolic stroke is increased 4- to 5-fold.1,2

The use of conventional antiarrhythmic compounds has been limited by potentially fatal ventricular arrhythmias. Existing antiarrhythmic drugs approved for the treatment of AF exhibit moderate efficacy for AF termination and suppression and have significant associated adverse effects, resulting in poor patient tolerance. Ongoing drug development has focused on increasing safety by developing atrial-specific agents. Atrial selectivity can be achieved by targeting ion channels that are selectively expressed in the atria or by blocking Na⁺ channels in a state-dependent manner that favors blocking of atrial rather than ventricular action potentials. However, the number of such atrial-specific ion channels is limited. So far the ion channels responsible for the acetylcholine-activated current and the ultra rapid delayed rectifier potassium current have been the main targets in the search for an atrial-specific antiarrhythmic drug, but until now, no compound has been shown to exhibit exclusive selectivity for any of these currents.3

Within the last few years, the existence of cardiac small conductance Ca²⁺-activated K⁺ (SK) channels has been documented, and their functional role is gradually being elucidated.4-12 Three subtypes of SK channels (SK1–3) mediating a Ca²⁺-activated K⁺ current (I_KCa) exist, and biochemical evidence indicates that SK2 channels are predominantly expressed in the atria of human and mouse hearts as compared with the ventricles. Also, SK1 channels have been reported to be predominantly expressed in the atria of mouse hearts. SK3 channels, however, show similar levels of expression in atria and ventricles.4,5 Ellinor et al13 reported a multicenter trial, which established relationship between lone AF and common variants in the gene KCNN3, which codes for the SK3 channel.

The increasing evidence for atrial selectivity of SK1 and SK2 isoforms raises the possibility that drugs modulating
these channels may target cardiac disease in an atrial selective manner, thereby decreasing the risk of ventricular proarrhythmic effects. The precise role of SK channels in the heart still remains unanswered, but they have been suggested to play an important role in atrial repolarization and fibrillation, making them an interesting atrial-specific target in the treatment of AF.7,8

We have shown previously that inhibition of SK channels is antiarrhythmic in models of acutely induced AF.9 These models, however, do not take into account that AF derives from a wide range of predisposing factors, the most prevalent being hypertension.1,14,15 AF is secondary to underlying heart disease in 70% of patients, whereas lone AF patients with no detectable etiology constitute the remaining 30%.16

The spontaneously hypertensive rat (SHR) is a model of systemic hypertension that exhibits a progression of hypertension and left ventricular hypertrophy from a stable form with normal cardiac function. SHRs have been used extensively in combination with the normotensive Wistar-Kyoto rat (WKY), which were inbred as a normotensive control strain from the SHR, to study cardiac adaptation to increased afterload.17–23 The SHR shows a hypertension-induced remodeling of the left atrium involving atrial enlargement, interstitial fibrosis, and cellular electric remodeling, which, in the aging SHR, leads to increased vulnerability to burst pacing-induced atrial arrhythmias.18,24 Interstitial fibrosis can increase electrophysiological heterogeneity and slow conduction velocity by creating miniscule zigzagging circuits, thereby creating a substrate for multiple reentry.14,25

In this study we assessed the effects of two different SK channel inhibitors, NS8593 and UCL1684, in aging, hypertensive rats to examine their antiarrhythmic potential in a setting of hypertension-induced atrial remodeling.

Materials and Methods

All of the studies were performed under a license from the Danish Ministry of justice (license No. 2007/561-1299) and in accordance with the Danish guidelines for animal experiments according to the European Commission Directive 86/609/EEC.

Animals and Experimental Design

Male SHRs and WKYs (SHR/NHsd and WKY/NHs, respectively) aged 10 weeks were acquired from Harlan Laboratories in two batches with equal numbers of SHRs and WKYs, which were kept in in-house storage facilities. Each batch was divided into 2 groups of 10 rats with equal numbers of SHRs and WKYs. Before anesthetizing animals for the open chest in vivo experiments, noninvasive recordings of systolic blood pressure from all of the animals were obtained using the tail-cuff method without restraining the rats (ML125/R NIBP, ADInstruments). This also allowed for recordings of heart rate at sinus rhythm (SR), and blood pressure, weight, and heart rate, please see the online Data Supplement.

Blood Pressure, Weight, and Hypertrophy

For background data such as systolic blood pressure, weight, heart rate, and SR, please see the online Data Supplement.

AF Inducibility and aERP at Baseline

The absolute values for aERP separated for strain and age are shown in Figure 1A. SHR3 and SHR11 have significantly shorter aERPs than the age-matched WKY, and the WKY8 has significantly shorter aERP than WKY3. There is no statistically significant change in aERP for the SHR over time, but a slight tendency toward a decreased aERP as age increases can be observed.

Effects of $I_{\text{KCa}}$ Inhibition on aERP and AF Duration

For several years, multiple- and single-circuit re-entry have been widely accepted as major mechanisms in the perpetuation of AF. According to these hypotheses, drugs that increase aERP should be able to interfere with AF by preventing perpetuation of wavelets.26–28 Consistent with this, we observed a correlation between the aERP and AF in our experiments, which is best described by an exponential function as depicted in Figure 2.
We found that administration of 5 mg/kg of NS8593 increased the aERP from 34 ms [23–40 ms] to 46 ms [32–64 ms]. Similarly, 3 mg/kg of UCL1684 increased aERP from 30 ms [25–40 ms] to 46 ms [38–60 ms]. In comparison, vehicle did not change AERP significantly (from 30 ms [24–36 ms] to 35 ms [29–43 ms]; Figure 3A).

Regarding the AF duration, 5 mg/kg of NS8593 decreased it from 2.6 s [1.8–3.4 s] to 0.3 s [0.1–1.1 s], and a similar decrease in AF duration was caused by 3 mg/kg of UCL1684 from 2.6 s [2.2–3.5 s] to 0.6 s [0.2–1.1 s]. Vehicle did not change AF duration significantly (from 1.9 s [1.1–2.7 s] to 2.2 s [1.0–3.0 s]; Figure 3B). Thus, while vehicle affected neither aERP nor AF duration, the negative allosteric modulator of SK channels, NS8593, and the SK channel pore blocker, UCL1684, both significantly increased the aERP and decreased the AF duration in both the normotensive and hypertensive strains with no decline in efficacy as age increased (Figure 4).

**Effects of I_{KCa} Inhibition on Pacemaking Tissues**

In 2008, Zhang et al. examined the role of SK2 channels in pacemaking tissues in the heart. It was demonstrated that mice overexpressing the SK2 channel had shortened WCL and increased atrioventricular (AV)-node conduction when compared with wild-type mice. In the same study it was found that SK2 knockout mice had longer WCL and slower AV-node conduction than wild-type mice. Similar effects were seen in the sino-atrial node in which SK2 channel knockout led to sinus bradycardia, whereas overexpression led to shortening of the RR interval. We, therefore, examined the effects of I_{KCa} inhibition on SR and AV-node conduction by normalizing recordings of WCL and SR after drug administration to baseline values (Figure 5). The SR was decreased by I_{KCa} inhibition (Figure 5A). SR was decreased to 86.7 ± 1.7% of baseline value by 3 mg/kg of UCL1684, whereas 5 mg/kg of NS8593 did not reach statistical significance by reducing SR to 93.1 ± 1.4% when compared with control values of 96.5 ± 1.0%.
A 115.0 ± 5.1% of baseline value, whereas 5 mg/kg of NS8593 decreased AF duration from 2.6 s [1.8–3.4 s] to 0.3 s [0.1–1.1 s] (P < 0.001; n = 29). Wilcoxon matched-pairs signed-rank test with Bonferroni correction for multiple comparisons were used for the analysis of the effects on aERP and AF duration. Values of P < 0.05 are denoted by * vs age-matched WKY, and by † and ‡ vs corresponding 3-month-old and 8-month-old groups, respectively. In the same way, **, ††, and ‡‡ and ***, ††† and ‡‡‡ denote P < 0.01 and P < 0.001, respectively.

Figure 2. Plot of mean atrial fibrillation (AF) duration elicited by burst pacing as a function of atrial effective refractory period (aERP), including baseline values and values after Ca2+-activated K+ current (I_{KCa}) inhibition. The data have been fitted with a semilog line, showing a correlation between increasing aERP and decreasing AF durations.

The same pattern was observed with regard to AV-nodal effects; the WCL was increased by I_{KCa} inhibition (Figure 5B). Treatment with 3 mg/kg of UCL1684 increased WCL to 115.0 ± 5.1% of baseline value, whereas 5 mg/kg of NS8593 did not reach statistical significance (104.6 ± 2.1% of baseline) when compared with control (102.3 ± 2.2% of baseline).

Discussion

We here provide evidence that the two different SK channel inhibitors, NS8593 and UCL1684, increase aERP and impede AF in a setting of hypertension-induced atrial remodeling in aging rats.

Selectivity data obtained on NS8593 show no effects of the compound on other cardiac-relevant ion channels than SK channels in concentrations ≤10 μmol/L.9,29 Because the selectivity data include neuronal Na,1.2, but not cardiac Na,1.5, it cannot be ruled out that Na,1.5 inhibition interferes with the recordings of AF durations in our experiments. However, two potent SK channel pore blockers, structurally unrelated to NS8593 and with a different mode of action than NS8593, exert antiarrhythmic effects comparable to those of NS8593,9 and in combination with the selectivity data, this suggests that the antiarrhythmic effect of NS8593 is primarily a result of SK channel inhibition.

We have shown previously that these structurally and mechanistically different inhibitors of SK channels possess antiarrhythmic properties in models of acutely induced AF when no atrial remodeling has occurred.3 However, hypertension is the most prominent predisposing factor for the clinically encountered AF. Thus, the aging SHR constitutes a relevant preclinical model for addressing the potential antiarrhythmic properties of potential AF drugs, because it incorporates hypertension-induced atrial remodeling with atrial enlargement, interstitial fibrosis, and cellular electric remodeling.24

Theories of the mechanism of AF involve two main processes: one or more rapidly depolarizing foci that function as triggers of arrhythmia and a fibrillation-prone atrium allowing re-entry with one or more re-entrant circuits. Hypertension-induced atrial remodeling provides such a fibrillation-prone atrium. We demonstrate here the inhibition of I_{KCa} diminishes the substrate for arrhythmia by increasing aERP, thereby markedly reducing AF in this model. This further strengthens our previous conclusion, that SK channels represent a promising new therapeutic target in the treatment of AF.

Effects of I_{KCa} Inhibition on Pacemaking Tissues

In the present study, we also examined the effects of I_{KCa} inhibition on SR and WCL and observed small but statistically significant effects on both parameters. The magnitude of the effects on pacemaking tissues was not equal for the two different SK channel inhibitors; the effects of UCL1684 were greater than the effects of NS8593, although the effects on
aERP prolongation and AF reduction were comparable. The spontaneous depolarization of nodal tissue is controlled by influx of Ca\(^{2+}\) via t-type Ca\(^{2+}\) channels. A conjectural explanation for the difference in magnitude of effect could, therefore, be that an SK channel pore blocker such as UCL1684 would have more pronounced effects than a negative modulator such as NS8593 if the intracellular-free Ca\(^{2+}\) concentration in pacemaking tissue is sufficiently great to overcome the negative modulation. However, because data on the free plasma concentrations of the compounds and data on intracellular Ca\(^{2+}\) concentrations are not available, a direct comparison of the efficacy of these two compounds cannot be meaningfully made.

The SK channels in the AV-nodal tissue may play a role under certain pathological conditions; it may be speculated that, during AF, as in the rest of the atrial tissue, the rapid depolarization increases intracellular Ca\(^{2+}\) and potentiates the \(I_{\text{KCa}}\) and thereby the AV-nodal conduction. Inhibition of SK channels may, therefore, represent an attractive method of modulating ventricular rate during atrial tachyarrhythmias. The same could be argued for the sinus-nodal effects under a condition of sinus-nodal tachycardia. However, this will need to be addressed in future studies.

**Conclusions**

In this study it has been confirmed that two chemically and mechanistically different SK channel inhibitors possess antiarrhythmic properties in a rat in vivo model of paroxysmal AF with hypertension-induced atrial remodeling in aging rats. Hypertension-induced atrial remodeling in aging rats did not reduce the efficacy of \(I_{\text{KCa}}\) inhibition, which prolonged the
aERP and thereby impeded AF, with equal efficacy in normotensive and hypertensive strains at all ages.

**Perspectives**

AF is the most common form of arrhythmia in humans, and current options for antiarrhythmic therapy of AF are limited by marginal efficacy and toxicity, including proarrhythmia, making new drug development crucial. We have demonstrated previously that inhibition of SK channels is effective in preventing and terminating AF in several animal species, providing the first experimental evidence that pharmacological targeting of SK channels may be a promising novel therapeutic approach for treatment of AF in humans. The present study demonstrates for the first time that SK channel inhibitors show efficacy in a hypertension-remodeled animal heart, which is characterized by a higher susceptibility to AF. Although further studies are warranted to address the clinical relevance of these findings, the present results support the notion that SK channels may offer a promising new therapeutic target in the treatment of AF.

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**Disclosures**

None.

**References**


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Effects on Atrial Fibrillation in Aged Hypertensive Rats by Ca2+-activated K+ channel inhibition

Running title: Effects on AF in Aged SHR by $I_{KCa}$ inhibition

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Expanded Materials and Methods

In vivo experiments were conducted as previously described with a few modifications (1). The rats were mebumal-anaesthetized (50mg/kg, i.p.), artificially respirated and kept at 37°C. Tail and pod reflexes were tested frequently to assure effective anaesthesia. At the end of each experiment the hearts were excised and weighed. Following thoracotomy, a pacing electrode was placed on the right atrium (RA), monophasic action potential (MAP) recordings were obtained with a MAP electrode placed on the RA and an electrocardiogram (ECG) was obtained with subcutaneous needle electrodes, two near the forelimbs and one near the left hind limb. After a period of stabilization without pacing, the aERP was measured by continuously pacing the RA at a rate of 500 beats per minute (BPM) and applying 10 basic stimuli (S1) followed by premature S2 stimuli applied with 1-10 ms decrements. The aERP was defined as the longest S1-S2 coupling interval failing to elicit an extra action potential. The Wenckebach cycle length (WCL) was measured by pacing the RA with increasing frequencies, and the WCL was defined as the slowest pacing rate failing to conduct 1:1 to the ventricles.

Having determined the aERP and WCL, continuous pacing was stopped and short episodes of AF were induced every 2 minutes by open-chest burst pacing (83 Hz) of the RA for 10 s followed by 110 s of intrinsic heart rhythm. Animals were divided into three groups receiving i.v. injections of either NS8593 (5 mg/kg), UCL1684 (3 mg/kg), or vehicle subsequent to 30 minutes of baseline recordings. The total duration of AF at all 110 s interburst intervals was measured over a period 30 minutes before injection in order to establish the baseline AF duration. After injection, aERP and WCL were measured as well as the total duration of AF over another period of 30 minutes.
Supporting information

Blood pressure, weight, and hypertrophy

Background data such as sBP, weight, heart weight, and SR can be seen in Table S1. As expected, the recordings of sBP revealed consistently higher values in the SHRs compared to the WKYs at all ages (p<0.001 for all ages of SHR compared with all ages of WKY). In the SHR the sBP increased between ages 3 and 8 months (192±9mmHg and 212±12mmHg, respectively), whereas the sBP decreased in the WKY between ages 3 and 8 months (134.9±2.6mmHg and 120.7±3.0mmHg). No age-related changes in sBP were observed between 8 and 11 months of age within the two strains.

The WKYs generally weighed more than the age-matched SHRs and they gained weight at a more rapid rate than the SHRs. Two-way ANOVA reveals that strain, age, and interaction accounts for 21.1%, 62.0%, and 3.7%, respectively, of the total variance seen in the weight measurements (p<0.0001 in all cases). The Bonferroni post hoc test showed statistically significant differences between the strains at the age of 8 and 11 months with p-values below 0.001, whereas the strain-related weight difference at 3 months was not statistically significant.

The heart weight:body weight ratios demonstrated that hearts from SHRs were hypertrophied in comparison with WKY rats which is consistent with previous reports (2-4). Recordings of SR from conscious and anaesthetized animals differed somewhat; in the conscious animals the recordings showed slightly faster heart rates in the SHR as compared to age-matched WKY. Only one statistically significant difference in SR was observed in the awake animals, namely a significantly faster heart rate in the SHR8 than in the WKY8.

The baseline recordings of SR from anaesthetized animals revealed faster heart rates in the SHR3 as compared to WKY3, no difference in SR between SHR8 and WKY8, and slower hearts rates in SHR11 than WKY11. The SHR11 had significantly slower heart rates at the baseline recordings than both the WKY11 and the younger groups of SHR. Previous recordings from isolated atrial preparations from WKY and SHR aged 5-6 months show no differences in basal heart rates. However, a hyperresponsiveness to norepinephrine-induced increase in SR was observed in SHR as compared to WKY, which might explain these differences in heart rate between anaesthetized and awake animals (5).
## Table S1
Background data for the different strain and age groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3 months</th>
<th>8 months</th>
<th>11 months</th>
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<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
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<tr>
<td>WKY</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sBP (mmHg)</td>
<td>134.9 2.6</td>
<td>192.9 5.6***</td>
<td>120.7 3.0†</td>
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<td>Weight (g)</td>
<td>291.4 3.9</td>
<td>248.0 3.4</td>
<td>479.4 13.7</td>
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<td>Heart weight (g)</td>
<td>1.49 0.06</td>
<td>1.41 0.04</td>
<td>1.51 0.06</td>
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<td>Heart weight/body weight (g/kg)</td>
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<td>5.63 0.16†</td>
<td>3.16 0.10†</td>
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<td>SR awake (BPM)</td>
<td>379.7 8.12</td>
<td>391.4 11.05</td>
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<td>SR baseline (BPM)</td>
<td>326.1 11.4</td>
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<td>364.1 8.9</td>
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<td>Strain, age, and treatment</td>
<td>Baseline aERP (ms)</td>
<td>Treatment aERP (ms)</td>
<td>Baseline AF (s)</td>
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<td>---------------------</td>
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<tr>
<td>SHR3 NS8593</td>
<td>30.2 ± 9.8</td>
<td>60.3 ± 9.6</td>
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<td>57.0 ± 28.7</td>
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<td>57.0 ± 23.6</td>
<td>3.11 ± 0.67</td>
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<td>47.0 ± 18.4</td>
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<td>54.5 ± 15.4</td>
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<td>55.8 ± 3.0</td>
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<td>2.63 ± 0.48</td>
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<td>36.8 ± 12.6</td>
<td>1.65 ± 0.63</td>
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</tbody>
</table>

**Table S2**
AF durations and aERP values presented individually for each animal strain, age group and treatment group.
Reference List


