A Novel K<sub>ATP</sub> Current in Cultured Neonatal Rat Atrial Appendage Cardiomyocytes

Anne Baron, Laurianne van Bever, Dominique Monnier, Angela Roatti, Alex J. Baertschi

Abstract—The functional and pharmacological properties of ATP-sensitive K+ (K<sub>ATP</sub>) channels were studied in primary cultured neonatal rat atrial appendage cardiomyocytes. Activation of a whole-cell inward rectifying K+ current depended on the pipette ATP concentration and correlated with a membrane hyperpolarization close to the K+ equilibrium potential. The K<sub>ATP</sub> current could be activated either spontaneously or by a hypotonic stretch of the membrane induced by lowering the osmolality of the bathing solution from 290 to 260 mOsm/kg H<sub>2</sub>O or by the K+ channel openers diazoxide and cromakalim with EC<sub>50</sub> ≈ 1 and 10 nmol/L, respectively. The activated atrial K<sub>ATP</sub> current was highly sensitive to glyburide, with an IC<sub>50</sub> of 1.22±0.15 nmol/L. Recorded in inside-out patches, the neonatal atrial K<sub>ATP</sub> channel displayed a conductance of 58.0±2.2 pS and opened in bursts of 133.8±20.4 ms duration, with an open time duration of 1.40±0.10 ms and a close time duration of 0.66±0.04 ms for negative potentials. The channel had a half-maximal open probability at 0.1 mmol/L ATP, was activated by 100 μmol/L diazoxide, and was inhibited by glyburide, with an IC<sub>50</sub> in the nanomolar range. Thus, pending further tests at low concentrations of K<sub>ATP</sub> channel openers, the single-channel data confirm the results obtained with whole-cell recordings. The neonatal atrial appendage K<sub>ATP</sub> channel thus shows a unique functional and pharmacological profile resembling the pancreatic β-cell channel for its high affinity for glyburide and diazoxide and for its conductance, but also resembling the ventricular channel subtype for its high affinity for cromakalim, its burst duration, and its sensitivity to ATP. Reverse transcriptase–polymerase chain reaction experiments showed the expression of Kir6.1, Kir6.2, SUR1A, SUR1B, SUR2A, and SUR2B subunits, a finding supporting the hypothesis that the neonatal atrial K<sub>ATP</sub> channel corresponds to a novel heteromultimeric association of K<sub>ATP</sub> channel subunits. (Circ Res. 1999;85:707–715.)

Key Words: K<sub>ATP</sub> channel • sulfonylurea receptor • cardiac atrium • atrial natriuretic peptide • patch clamp

Atrial cardiomyocytes release atrial natriuretic peptide (ANP), a hormone that plays a major physiological role as an antihypertensive and cardioprotective agent.1,2 Both stretch and hypoxia are potent stimuli for ANP secretion.3 In isolated rat heart, the stretch- and hypoxia-induced ANP secretion are inhibited by K+ channel openers such as diazoxide or pinacidil.4 Regulatory effects of sulfonylureas have also been described that either potentiate or inhibit stretch-induced ANP secretion, depending on the experimental conditions and the concentrations used (References 4 and 5 and J.H. Jiao, P. Baumann, A. Baron, A. Roatti, R.A. Pence, A.J. Baertschi, unpublished data, 1995–1999). Taken together, these findings suggest that ATP-sensitive K+ (K<sub>ATP</sub>) channels might be important regulators of stimulated-ANP secretion.

Because K<sub>ATP</sub> channels are inhibited by physiological concentrations of cytosolic ATP, they are thought to couple membrane excitability to the metabolic state of the cell. These widely distributed channels are involved in various physiological functions, including secretory processes such as the glucose-stimulated insulin secretion by pancreatic β-cells,5,6 and the release of neurotransmitters, growth hormone, and renin.6,8–10

The K<sub>ATP</sub> channel is formed by the association of the following 2 types of protein subunits: (1) inward rectifying K+ channel subunits (Kir), constituting the pore of the channel and containing the major ATP binding site, and (2) sulfonylurea receptor (SUR) regulatory subunits.11–14 Until now and regardless of animal species, 2 Kir subunits, Kir6.1 and Kir6.2,12,15 and 5 SUR subunits, SUR1A, SUR1B (GenBank accession No. AF039595), SUR2A, SUR2B, and SUR2C,14,16–19 have been cloned. The association SUR1A/Kir6.2 has been shown to form K<sub>ATP</sub> channels with a 1-for-1 stoichiometry, the functional channel being an octamer.20,21 The association Kir6.2/SUR1A is found in the pancreatic β-cell K<sub>ATP</sub> channel involved in insulin secretion, whereas SUR2A and SUR2B subunits have been proposed as components of the ventricular and the vascular smooth muscle K<sub>ATP</sub> channels, respectively.

Concerning the heart, discrepancies persist regarding the pharmacological properties of ventricular K<sub>ATP</sub> channels (see Discussion), and very few data have been published on atrial...
K<sub>ATP</sub> channels. Zünkl et al<sup>23</sup> described an opening effect of cromakalim and a low affinity for tolbutamide on human atrial myocytes, whereas Hamada et al<sup>23</sup> and Song et al<sup>24</sup> reported a high-affinity inhibitory effect of glyburide on guinea pig atrial myocytes. Interestingly, van Wagoner<sup>25</sup> showed that rat atrial K<sub>ATP</sub> channels were stretch-activated, a phenomenon that could play a major physiological role in these stretch-sensitive secretory cells.

We report here novel functional and pharmacological properties of the atrial appendage K<sub>ATP</sub> channel. These were investigated in primary cultured neonatal rat atrial appendage myocytes by means of patch-clamp recordings of cellular and unitary K<sub>ATP</sub> currents. The expression of Kir and SUR isoforms was determined by performing reverse transcriptionase–polymerase chain reaction (RT-PCR) on atrial appendage cardiomyocyte mRNA. A preliminary account of the work has been published in abstract form.<sup>26</sup>

Materials and Methods

Atrial Myocyte Cultures

Atrial appendage myocytes from 2- to 3-day-old rats were cultured as described previously<sup>27</sup> for 2 to 4 days in the presence of 1 μmol/L dexamethasone and 1 μmol/L triiodothyronine in a 5% CO<sub>2</sub> incubator. Cultured atrial myocytes were shown to synthesize ANP by immunostaining (not shown).

Patch-Clamp Recording of K<sub>ATP</sub> Current

K<sub>ATP</sub> currents were recorded on culture days 2 to 4 at room temperature from initially beating atrial myocytes using the whole-cell and the inside-out configurations of the patch-clamp technique<sup>28</sup> with hardware and software from Axon Instruments. Borosilicate glass pipettes had a resistance of 2 to 4 MΩ for whole-cell recordings and 5 to 10 MΩ for inside-out recordings. For whole-cell recordings, the standard pipette solution contained (in mmol/L) KCl 121, CaCl<sub>2</sub> 1.5, MgCl<sub>2</sub> 1.3, ATP 0 to 5, glucose 10, KOH 34, and HEPES 10 (pH 7.45 with KOH), and the bathing solution contained (in mmol/L) KCl 5, MgCl<sub>2</sub> 3, MgCl<sub>2</sub> 1, NaCl 118, glucose 10, and HEPES 10 (pH 7.5 with NaOH). The osmolality of the hypotonic solution (stimulus) was 260 mOsm/kg H<sub>2</sub>O, and sucrose was added to yield a solution of 290 mOsm/kg H<sub>2</sub>O. Currents were filtered at 2 kHz and sampled at a frequency of 0.8 kHz. For inside-out recordings, the pipette solution contained (in mmol/L) KCl 140, CaCl<sub>2</sub> 1, MgCl<sub>2</sub> 1, NaCl 118, MgCl<sub>2</sub> 1, EGTA 5 (pH 7.3 with KOH), whereas the bathing solution contained (in mmol/L) KCl 5, KOH 5, NaCl 115, MgCl<sub>2</sub> 1, EGTA 5, glucose 10, and HEPES 5 (pH 7.3 with KOH). Currents were filtered at 1 kHz and sampled at 6 kHz. Burst kinetics analysis and open and close time distribution histograms were only performed where a single channel was present on the membrane patch.

Chemicals and Drugs

EGTA, ATP, glyburide, diazoxide, and cromakalim were all purchased from Sigma.

RT-PCR Analysis for K<sub>ATP</sub> Channel Subunits

Total RNA from 4-day-old cultured neonatal atrial appendage myocytes was extracted by Trizol reagent (GIBCO-BRL) and subjected to RQI DNase (Promega) digestion. cDNAs were synthesized with oligo(dT) primers and Superscript II RT (200 units, GIBCO-BRL) at 42°C. PCR was carried out in a Biometra personal cycler in a final volume of 50 μL containing 1 μL of the RT reaction, 1.5 units of Taq polymerase (GIBCO-BRL), 1.5 μmol/L MgCl<sub>2</sub>, 250 μmol/L of each dNTP, and 20 pmol of each primer. The PCR conditions were the following: initial denaturation at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension step at 72°C for 10 minutes. The nature of the PCR products (see also legend to Figure 7) was confirmed by double-strand sequencing on both strands.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results

K<sub>ATP</sub> Whole-Cell Current on Neonatal Atrial Appendage Myocytes

Whole-cell K<sub>ATP</sub> currents were recorded on initially beating atrial appendage cardiomyocytes during a 10-second voltage ramp (from –70 or –80 mV to +90 mV), with a holding potential of –40 mV, to prevent the activation of voltage-dependent channels. The EGTA-buffered low Ca<sup>2+</sup> concentration in the patch pipette (10 mmol/L estimated free Ca<sup>2+</sup>) prevented the activation of Ca<sup>2+</sup>-dependent currents. The membrane potential at 0 pA current was measured after each current recording.

Whole-cell capacitance was measured to estimate the changes in atrial myocyte membrane surface in culture. Assuming a specific capacitance of 1 μF/cm<sup>2</sup>, the membrane surface increased from a mean value of 1180 μm<sup>2</sup> on culture day 2 to 1600 μm<sup>2</sup> on day 3 and 2000 μm<sup>2</sup> on day 4. In the presence of 2 mmol/L internal ATP, K<sub>ATP</sub> current densities were 105±12 pA/pF (n=21), 77±8 pA/pF (n=16), and 78±9 pA/pF (n=19) on days 2, 3, and 4, respectively.

In the absence of ATP in the pipette, a weakly inward rectifying outward current developed 1 to 2 minutes after breaking the patch membrane (Figure 1A and 1B, ●), correlating with a cell hyperpolarization (Figure 1B, ○) reaching a mean maximal value of –64.6±3.2 mV (n=18; for resting potentials, see online Materials and Methods, http://www.circresaha.org). The slow activation is probably due to the long time constant for the diffusion of cellular ATP into the pipette. This activation was usually transient and the current amplitude progressively decayed with time. Figure 1C shows the saturable relation between the current amplitude and the subsequent membrane hyperpolarization, with ~20% of the maximal current inducing a maximal hyperpolarization.

Increasing the external K<sup>+</sup> concentration from 5 to 30 mmol/L (substitution of Na<sup>+</sup> by K<sup>+</sup>) shifted the current reversal potential from –80 to –40 mV, thus following the predicted Nernst equilibrium potential for K<sup>+</sup> (Figure 1D). The probability of K<sup>+</sup> current activation depended on the pipette ATP concentration: 65.4% (18/28), 57% (69/121), 40% (26/65), and 13.1% (8/61) of the recorded cells in the presence of 0, 1, 2, and 5 mmol/L internal ATP, respectively. According to the χ<sup>2</sup> test, the probability of K<sub>ATP</sub> current activation is significantly (P<0.001) tilted toward low percentages at high internal ATP. The mean maximal subtracted current amplitude, measured at +50 mV, and the mean maximal potential, measured at 0 pA, were not significantly different for ATP concentrations ranging from 0 to 2 mmol/L, 1065±107 pA and –64.6±3.2 mV (n=18) in the absence of ATP, but significantly decreased in the presence of 5 mmol/L internal ATP to reach 32.8±1.1% (current) and 52.8±2.5% (potential), respectively, of the control values measured in the absence of ATP (P=0.001, n=8). These results show that the atrial appendage K<sub>ATP</sub> channel is influenced by ATP. How-
ever, the $K_{ATP}$ current activation in the presence of 5 mmol/L ATP may reflect some cellular heterogeneity of $K_{ATP}$ channel properties, a minority of myocytes being less sensitive to ATP. Alternatively, it may reflect nonspecific effects of the whole-cell configuration, such as dilution of cytosolic regulators or membrane stretch by the pipette. The accurate ATP sensitivity of the atrial $K_{ATP}$ channel, determined on unitary currents, is shown in Figure 6D.

**Activation of Neonatal Atrial Appendage $K_{ATP}$ Current by a Hypotonic Stretch**

When no current activation occurred after 5 to 6 minutes of whole-cell recording with ATP in the pipette, $K_{ATP}$ currents could be activated by a hypotonic stretch of the membrane, with the osmolality of the bathing solution being reduced from 290 to 260 mOsm/kg H$_2$O (Figure 2A and 2B). Figure 2C shows the saturable relation between the current amplitude and the subsequent membrane hyperpolarization. The mean maximal subtracted current, measured at $+50$ mV, and the mean maximal membrane potential, measured at 0 pA, were $1159 \pm 155$ pA and $-64.3 \pm 2.5$ mV ($n=17$) with 1 mmol/L ATP. In 5 of the 17 cells, glyburide (10 $\mu$mol/L) was applied and fully inhibited the stretch-activated current (example in Figure 2B). Figure 2D shows the mean control current amplitude, in the presence of 1 mmol/L internal ATP, and its inhibition after application of glyburide (0.1 to 1 $\mu$mol/L).

**Inhibition of Neonatal Atrial Appendage $K_{ATP}$ Current by Glyburide**

Figure 3A and 3B shows the effect of 1 and 10 nmol/L glyburide on the atrial $K_{ATP}$ current spontaneously activated in the presence of 1 mmol/L internal ATP. This glyburide-sensitive current showed a saturable relation with membrane potential measured at 0 pA (Figure 3C), similar to that seen for spontaneously activated or hypotonic stretch-activated
K<sub>ATP</sub> currents (Figures 1C and 2C). Figure 3D illustrates the percentage of remaining current as a function of glyburide concentration and shows a high sensitivity of the atrial appendage K<sub>ATP</sub> current to glyburide, with IC<sub>50</sub> = 1.22 ± 0.15 nmol/L. The affinity for glyburide was similar whether the K<sub>ATP</sub> current was activated spontaneously ( ), or by a hypotonic stretch ( ). In the presence of 1 nmol/L glyburide, the K<sub>ATP</sub> current was 49.9 ± 10.2% (n = 8) and 47.9 ± 3.8% (n = 5) of the control current, when activated spontaneously or by a hypotonic stretch, respectively, in the presence of 1 mmol/L internal ATP.

### Activation of Neonatal Atrial Appendage K<sub>ATP</sub> Current by K<sup>+</sup> Channel Openers

The K<sub>ATP</sub> current could be activated in the presence of 1 to 5 mmol/L internal ATP by diazoxide (Figure 4A and 4B, b current) and cromakalim (not shown). 2 K<sup>+</sup> channel openers.

Maximal cromakalim and diazoxide-activated currents were of similar amplitude, and both were fully inhibited by glyburide (Figure 4A and 4B, c current), with an affinity in the nanomolar range (Figure 3D, ). Figure 4C and 4D shows the mean current amplitude activated by various concentrations of cromakalim (Figure 4C) and diazoxide (Figure 4D) in the presence of 2 mmol/L internal ATP. In the presence of either cromakalim or diazoxide, the mean maximal activated current, measured at +50 mV, was 1010 ± 101 pA and the mean maximal membrane potential, measured at 0 pA, was −64.6 ± 1.6 mV (n = 39). Although the exact EC<sub>50</sub> was not determined, both cromakalim and diazoxide activated the K<sub>ATP</sub> current with approximate EC<sub>50</sub> values of 10 and 1 nmol/L, respectively, corresponding to a mean current amplitude of 444 ± 149 pA (n = 6) and 401 ± 84 pA (n = 7), with
P<0.05 compared with the mean maximal current amplitude. DMSO was required to dissolve these drugs, but its final concentration in the perfusion medium was never >1 mmol/L. At 1 μmol/L or 1 mmol/L, DMSO by itself had no effect on KATP current activation. In fact, 100 μmol/L diazoxide activated a mean maximal KATP current of 875 ± 166 pA (n = 9) after 2 to 3 minutes of bath application, whereas the mean subtracted current amplitude was 4 ± 14 pA (n = 4) after 2 to 4 minutes of application of the corresponding DMSO concentration (1 mmol/L).

Unitary Neonatal Atrial Appendage KATP Current

Although usually closed in the cell-attached configuration, KATP channels were activated on excision of the membrane patch (inside-out configuration) in the absence of ATP in the bath solution. The channel activity ran down in 2 to 3 minutes and could be refreshed on washout of an ATP-containing solution. Figure 5A shows the I-V curve and the original current recordings in the absence of ATP, with 140 mmol/L K+ in the pipette solution and a Nernst K+ equilibrium of +48 mV. The KATP channel opened in long-lasting bursts of 133.8 ± 20.4 ms (n = 15 patches), with a unitary conductance of 58 ± 2 pS (n = 11) for negative potentials. A similar conductance was measured on the rare openings recorded on cell-attached patches, 58 ± 4 pS (n = 6). Within bursts, the channel showed a flickering activity, with rapid openings and closings (Figure 5B). The mean opening duration and the mean closing duration within bursts were estimated to be 1.40 ± 0.10 ms and 0.66 ± 0.04 ms (n = 15) respectively, between −120 and −70 mV and in the absence of ATP.

The sensitivity to ATP of the channel has been examined after the channel activity was refreshed by an ATP-containing medium. After refreshment, the channel showed a high open probability (Po), ≈0.7, which could be abolished by 1 mmol/L ATP, whereas 0.1 mmol/L only exerted a partial inhibition (Figure 6A). The effects of ATP concentration ranging from 0 to 5 mmol/L on the atrial KATP channel Po are represented on Figure 6B. The half-maximal inhibition is
obtained in the presence of 0.1 mmol/L cytosolic ATP, the K\textsubscript{ATP} channel Po being reduced from 0.49±0.11 (n=6) to 0.23±0.09 (n=6).

The atrial appendage K\textsubscript{ATP} channel could be activated first by diazoxide, reaching a mean maximal Po of 0.31±0.1 (n=8), and then inhibited by nanomolar concentrations of glyburide (Figure 6C and 6D). The channel Po is reduced by diazoxide, reaching a mean maximal Po of 0.31±0.05% (n=8), and then inhibited by nanomolar concentrations of glyburide (Figure 6C and 6D). The channel Po is reduced to 0.11±0.05% (n=3) and 16.17±5.58% (n=4) of the control value, in the presence of 1 and 10 nmol/L glyburide, respectively, thus confirming the affinity for glyburide measured on whole-cell current. The inhibition by glyburide could be reversed (not shown), but usually required a 4- to 10-minute washout, especially for high concentrations.

### K\textsubscript{ATP} Channel Subunits Expressed by Neonatal Atrial Appendage Cardiomyocytes

RT-PCR was performed on RNA extracts from primary cultured atrial cardiomyocytes (Figure 7). To reveal the expression of the splice variants SUR1A and SUR1B, SUR2A and SUR2B, and SUR2A and SUR2C, specific primers were chosen on each side of the splicing site, thus amplifying 2 fragments of different size when both isoforms were expressed. Atrial cardiomyocytes, characterized by the expression of ANP (lane 4), expressed both Kir6.1 (lane 6) and Kir6.2 (lane 8), and SUR1A and SUR1B (lane 10). SUR2A is expressed, as shown by the 249-bp fragment amplified by the SUR2A-specific primers (lane 12), as well as the SUR2B (173-bp) isoform amplified with the SUR2A-2B primers (lane 14). The short splice variant SUR2C is not expressed by rat atrial cardiomyocytes, the SUR2A-2C primers only amplifying a 358-bp SUR2A fragment (lane 16). Positive controls were performed on expression vectors containing either the SUR2C (lane 18) or the SUR2A cDNA (lane 19), thus showing that the SUR2A-2C primers were effective. Negative controls without RT were obtained for all primer pairs (see lanes 1, 3, 5, 7, 9, 11, 13, and 15), indicating that the PCR products were not due to contamination with genomic DNA. This expression pattern was obtained from 3 different cell cultures, and similar results were found with whole atrial extracts (not shown).

### Discussion

The Neonatal Atrial Appendage K\textsubscript{ATP} Channel as a New Subtype

We have shown that neonatal atrial appendage cardiomyocytes display a cellular K\textsubscript{ATP} current that is activated by hypotonic stretch of the membrane and shows a high affinity for the sulfonylurea glyburide (IC\textsubscript{50}=1.2 mmol/L) as well as for the K\textsuperscript{+} channel opener cromakalim and diazoxide, with a higher affinity for diazoxide. The channel displays a flickering pattern activity within long-lasting bursts (134-ms mean duration), a unitary conductance of 58 pS in the presence of a high K\textsuperscript{+} level on the extracellular side of the membrane, and an IC\textsubscript{50} for ATP ≈0.1 mmol/L. Confirming the results obtained on whole-cell current, single activated K\textsubscript{ATP} channels were inhibited by glyburide in the nanomolar range. Neonatal atrial appendage cardiomyocytes displayed a wide expression pattern of K\textsubscript{ATP} channel subunits, including all Kir6.x and SUR isoforms except for the SUR2C subtype. Together, these results lead us to propose the neonatal atrial K\textsubscript{ATP} channel as a new pharmacological and functional subtype.

### Pharmacological Properties of Neonatal Atrial K\textsubscript{ATP} Current

As an attempt to determine which SUR subtype is involved in atrial K\textsubscript{ATP} channel function, we compared its pharmacological properties with data obtained by other groups on ventricular cardiomyocytes, pancreatic \(\beta\)-cells, and reconstructed K\textsubscript{ATP} currents (Table). The pancreatic K\textsubscript{ATP} current, and its corresponding functional subunit association SUR1A/Kir of 6.2, yields a high affinity for glyburide (mmol/L range) and for diazoxide but a low affinity for cromakalim, the latter being sometimes even described as ineffective. The high affinity for glyburide remains controversial, with IC\textsubscript{50} values varying from the nmol/L to the \(\mu\)mol/L range, depending on the experimental conditions and the metabolic state of the cell. However, the affinity of ventricular K\textsubscript{ATP} channels for glyburide remains controversial, with IC\textsubscript{50} values varying from the nmol/L to the \(\mu\)mol/L range, depending on the experimental conditions and the metabolic state of the cell.

The pharmacological properties of the neonatal atrial appendage K\textsubscript{ATP} current presented here appear to differ from both the pancreatic and ventricular subtypes, the sensitivity to diazoxide constituting a distinctive feature compared with ventricular K\textsubscript{ATP} channels. The high affinity for diazoxide and cromakalim should be interpreted by taking into account the experimental conditions. Indeed, the activity of these 2 channel openers depends on cytosolic ATP, involving a decrease in the channel sensitivity to ATP and possibly a phosphorylation. Thus, the affinities for openers we measured in the presence of 2 mmol/L internal ATP on

### Pharmacological Properties of K\textsubscript{ATP} Channels

<table>
<thead>
<tr>
<th>K\textsubscript{ATP} Current</th>
<th>Diazoxide EC\textsubscript{50}</th>
<th>Cromakalim EC\textsubscript{50}</th>
<th>Glyburide IC\textsubscript{50}</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial cardiomyocytes</td>
<td>~1 nmol/L</td>
<td>~10 nmol/L</td>
<td>1.2 nmol/L</td>
<td>Results, present study</td>
</tr>
<tr>
<td>Pancreatic (\beta)-cells</td>
<td>20 to 100 (\mu)mol/L</td>
<td>No activation at 100 (\mu)mol/L</td>
<td>0.1 to 30 nmol/L</td>
<td>6, 7, 11, 30, 31, 32, 43</td>
</tr>
<tr>
<td>Ventricular cardiomyocytes</td>
<td>No effect or inhibition</td>
<td>10 to 300 (\mu)mol/L activates</td>
<td>6 to 10 nmol/L</td>
<td>6, 11, 35, 37, 39, 41</td>
</tr>
<tr>
<td>SUR1A-Kir6.2</td>
<td>60 (\mu)mol/L</td>
<td>No effect (500 (\mu)mol/L)</td>
<td>0.1 to 6 (\mu)mol/L</td>
<td>34, 36, 38, 40</td>
</tr>
<tr>
<td>SUR2A-Kir6.2</td>
<td>No effect (300 (\mu)mol/L)</td>
<td>30 (\mu)mol/L activates</td>
<td>30 to 350 nmol/L</td>
<td>18, 33, 49</td>
</tr>
<tr>
<td>SUR2B-Kir6.2</td>
<td>58 (\mu)mol/L</td>
<td>...</td>
<td>1 (\mu)mol/L inhibits</td>
<td>11, 13, 19, 42</td>
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whole-cell current are expected to be higher than the affinities on intact cells, in which the cytosolic ATP level is thought to be higher.

The most recently cloned SUR2 subtype, SUR2B, considered to be the smooth muscle subtype, confers sensitivity to diazoxide in reconstituted K\textsubscript{ATP} channels.\textsuperscript{16,19,42} Further pharmacological characterization of the SUR2B subtype is needed to speculate whether it could be involved in the neonatal atrial appendage K\textsubscript{ATP} channel formation.

**Single Atrial Appendage K\textsubscript{ATP} Channel Properties:**

**Burst Kinetics and Conductance**

Neonatal atrial K\textsubscript{ATP} channels display characteristic features shared by all K\textsubscript{ATP} channels: weak inward rectification, flickering activity within bursts, fast rundown after excision of the membrane patch, and refreshment on washout of cytosolic ATP. However, burst kinetics, unitary conductance, and ATP sensitivity have been shown to depend on the subtypes constituting the channel.\textsuperscript{11,18,45} The pancreatic K\textsubscript{ATP} channel has been described as opening in bursts that are shorter than those of the ventricular channel: \(\approx 20\) to 40 ms burst duration for the pancreatic channel versus \(212\) ms for the ventricular one. In contrast, the intraburst kinetics were similar for both types, with a mean open time of \(1\) to \(4\) ms and a mean closed time of \(0.2\) to \(0.6\) ms.\textsuperscript{6,33,34,45–47} The atrial K\textsubscript{ATP} channel has a mean open time of \(1.4\) ms and a mean closed time of \(0.7\) ms within bursts of \(133.8\pm20.4\) ms (\(n=15\)) duration. This latter value, although closer to what has been reported for the ventricular K\textsubscript{ATP} channel, is significantly lower (\(P<0.05, 2\)-tailed \(t\) test) than the ventricular burst duration reported by Alekseev et al.\textsuperscript{45} 212.5\pm23.6 (\(n=7\)).

The sensitivity of the neonatal atrial appendage K\textsubscript{ATP} channel to ATP, with an IC\textsubscript{50} \(\approx 100\) \(\mu\text{mol/L}\), is closer to the 20 to 100 \(\mu\text{mol/L}\) value reported for the ventricular channel\textsuperscript{6,45,48} than to the high-affinity 10 to 30 \(\mu\text{mol/L}\) value reported for the pancreatic channel.\textsuperscript{6,18} However, in terms of channel conductance, the atrial appendage K\textsubscript{ATP} channel differs from the 70- to 90-pS ventricular channel by its lower conductance of 58 pS, a value similar to what has been reported for the pancreatic channel. Recently, Babenko et al.\textsuperscript{49} showed virtually identical functional and pharmacological properties for the human ventricular K\textsubscript{ATP} channel and the SUR2A/Kir6.2 reexpressed channel, a finding that further supports the notion that the atrial appendage K\textsubscript{ATP} channel does differ from the ventricular K\textsubscript{ATP} subtype.

The neonatal atrial appendage K\textsubscript{ATP} channel appears to differ not only from the adult ventricular K\textsubscript{ATP} channel, but also from the neonatal ventricular one. The latter shares the following properties with the adult ventricular channel: a unitary conductance of 75 pS, an IC\textsubscript{50} for ATP of 4.8 \(\mu\text{mol/L}\), openings in bursts with a flickering pattern, and similar open and close time duration. This channel also shows a very low sensitivity to diazoxide, which could constitute a difference compared with adult channel properties. On neonatal ventricular myocytes, 0.5 \(\text{mmol/L}\) diazoxide induces a small, whole-cell K\textsubscript{ATP} current of 5 pA/pF in 50% of the cells, representing 17% of the maximal current induced by pinacidil.\textsuperscript{50} This effect of diazoxide is 1 million times lower than for neonatal atrial appendage K\textsubscript{ATP} channels. However, the high sensitivity of the neonatal atrial appendage K\textsubscript{ATP} channel to diazoxide that was observed in whole-cell recordings remains to be verified by single-channel analysis, given that the macroscopic current may contain channels not presented in the unitary recordings.

There seems to be no difference between atrial appendage and ventricular cardiomyocytes with respect to density of the K\textsubscript{ATP} channels on the plasma membrane. Assuming a mean maximal cellular K\textsubscript{ATP} current of 1000 pA at +50 mV, a unitary inward conductance of the atrial appendage K\textsubscript{ATP} channel of 11 pS in the presence of 5 \(\text{mmol/L}\) extracellular K\textsuperscript{+},\textsuperscript{46} and a half-reduced outward conductance of 5.5 pS (inward rectification), the density of atrial K\textsubscript{ATP} channels can be estimated to be 1500 channels per cell, a value similar to the 2000 to 3000 channels per cell reported for ventricular myocytes.\textsuperscript{48}
atrial appendage cardiomyocytes to determine which \( K_{\text{ATP}} \) subunits were expressed. Our results show a wide expression pattern, with the 2 Kir6.x isoforms, Kir6.1 and Kir6.2, and 4 SUR isoforms, SUR1A, SUR1B, SUR2A and SUR2B. SUR2C was not expressed in rat neonatal atrial myocytes, thus confirming the results of Chutkow et al,\(^{17}\) who reported that the SUR2C isoform was not expressed in rat tissues. The expression of Kir6.2 and Kir6.1 was already reported in rat heart,\(^{12,15}\) SUR1A expression was reported in rat heart,\(^{12}\) to a lower degree than SUR2A,\(^{17,18}\) whereas SUR2A and SUR2B have also been shown to be both expressed in atrium of mouse heart.\(^{19}\) The tissue distribution pattern of SUR1B, recently cloned from a rat pancreatic cell line (GenBank accession No. AF039595), is not yet described, and its expression by rat atrium could constitute a characteristic feature. The inclusion of dexamethasone and T3 in the culture medium is not responsible for this expression pattern, because in their absence the same subunits were expressed, and the same sensitivity to glyburide and diazoxide was observed in the electrophysiological recordings (not shown).

The results do not allow a conclusion on the neonatal atrial \( K_{\text{ATP}} \) channel composition, but they support the view that atrial \( K_{\text{ATP}} \) channels are a heteromultimeric association of several SUR subtypes showing mixed pharmacological and functional properties with regard to other known \( K_{\text{ATP}} \) channel types. For example, a mixture of SUR1A and SUR2B could explain several properties of the neonatal atrial \( K_{\text{ATP}} \) channel, as follows: conductance, diazoxide, and glyburide sensitivity of the SUR1A type, and bursts kinetics, ATP, and cromakalin sensitivity of the SUR2A type. However, the contribution of SUR1B and the possibility of a new uncloned SUR subtype must also be taken into account.

Possible Physiological Functions of the Atrial Appendage \( K_{\text{ATP}} \) Channels

The physiological role of cardiac \( K_{\text{ATP}} \) channels in ischemic preconditioning and hypoxia-triggered events has been well documented,\(^{5,7,11,35,40}\) but atrial \( K_{\text{ATP}} \) channels also appear to be involved in cardiac secretion. Opening of atrial \( K_{\text{ATP}} \) channels, either pharmacologically (diazoxide or pinacidil) or metabolically (2-deoxyglucose), abolishes the stretch-stimulated ANP secretion in isolated heart and cultured atrial appendage cardiomyocytes (Reference 4 and J.H. Jiao et al, unpublished data, 1995–1999). It is tempting to draw analogies with \( K_{\text{ATP}} \) channel–triggered secretion of insulin by pancreatic \( \beta \)-cells. However, \( K_{\text{ATP}} \) channels, normally open in resting pancreatic \( \beta \)-cells, are closed in cardiac myocytes,\(^{7,44}\) in which they could only modulate stimulated ANP release. The links between membrane stretch, \( K_{\text{ATP}} \) channel activation, and ANP release are still unknown. Our results confirm van Wagoner’s\(^{25}\) finding that stretch opens atrial \( K_{\text{ATP}} \) channels. Other potent stimulators of ANP release, such as endothelin and hypoxia,\(^{2,3,4,27}\) are known to open cardiac \( K_{\text{ATP}} \) channels.\(^{6,51,52}\) This suggests that activation of \( K_{\text{ATP}} \) channels could be a common mechanism for feedback inhibition of stimulated ANP release.

Acknowledgments

This study was supported by the Swiss National Science Foundation (Grant 31-49798.96) and the following foundations: Société Aca-démique de Genève, Horten, de Reuter, Sandoz, and the Roche Research Foundation. We thank William A. Chutkow for providing the expression vectors containing SUR2A and SUR2C cDNA, Uta Schmidt for helpful discussion, and Dr Rui de Sousa for numerous suggestions on the manuscript.

References


A Novel KATp Current in Cultured Neonatal Rat Atrial Appendage Cardiomyocytes
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Circ Res. 1999;85:707-715
doi: 10.1161/01.RES.85.8.707

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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