The mitochondrial bioenergetic phenotype for protection from cardiac ischemia in SUR2 mutant mice

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Aggarwal NT, Pravdic D, McNally EM, Bosnjak ZJ, Shi N, Makielski JC. The mitochondrial bioenergetic phenotype for protection from cardiac ischemia in SUR2 mutant mice. Am J Physiol Heart Circ Physiol 299: H1884–H1890, 2010. First published October 8, 2010; doi:10.1152/ajpheart.00363.2010.—The sulfonylurea receptor-2 (SUR2) is a subunit of ATP-sensitive potassium channels (KATP) in heart. Mice with the SUR2 gene disrupted (SUR2m) are constitutively protected from ischemia-reperfusion (I/R) cardiac injury. This was surprising because KATP, either sarcolemmal or mitochondrial or both, are thought to be important for cardioprotection. We hypothesized that SUR2m mice have an altered mitochondrial phenotype that protects against I/R. Mitochondrial membrane potential (ΔΨm), tolerance to Ca2+ load, and reactive oxygen species (ROS) generation were studied by fluorescence-based assays, and volumetric changes in response to K+ were measured by light scattering in isolated mitochondria. For resting SUR2m mitochondria compared with wild type, the ΔΨm was less polarized (46.1 ± 0.4 vs. 51.9 ± 0.6%), tolerance to Ca2+ loading was increased (163 ± 2 vs. 116 ± 2 μM), and ROS generation was enhanced with complex I (8.5 ± 1.2 vs. 4.9 ± 0.2 arbitrary fluorescence units (afu)/s) or complex II (351 ± 51.3 vs. 166 ± 36.2 afu/s) substrates. SUR2m mitochondria had greater swelling in K+ medium (30.2 ± 3.1%) compared with wild type (14.5 ± 0.6%), indicating greater K+ influx. Additionally, ΔΨm decreased and swelling increased in the absence of ATP in SUR2m, but the sensitivity to ATP was less compared with wild type. When the mitochondria were subjected to hypoxia-reoxygenation, the decrease in respiration rates and respiratory control index was less in SUR2m. ΔΨm maintenance in the SUR2m intact myocytes was also more tolerant to metabolic inhibition. In conclusion, the cardioprotection observed in the SUR2m mice is associated with a protected mitochondrial phenotype resulting from enhanced K+ conductance that partially dissipated ΔΨm. These results have implications for possible SUR2 participation in mitochondrial KATP.

ATP-SENSITIVE POTASSIUM CHANNELS (KATP), either in the sarcolemma or the mitochondrial membranes, or both, are implicated in the cardiac protection against ischemia-reperfusion injury (I/R) (9, 19). KATP in the sarcolemma are formed from the pore-forming inwardly rectifying K+ channel (Kir) subunits and regulatory sulfonylurea receptor subunits (SUR) (15). KATP current in the sarcolemma (sarcKATP) of ventricular myocytes are thought to arise predominantly from a combination of Kir6.2 and SUR2A (25), but the existence and constitution of KATP channels in mitochondria remains controversial (21).

A SUR2 mutant mouse (SUR2m) designed to disrupt SUR2 was made by targeting exons 14 to 18 that encode the first nucleotide-binding domain (5). These SUR2m mice are hypertensive, arrhythmic, and exhibit coronary vasospasm and sudden cardiac death. They also lack pinacidil-, diazoxide-, and glybenclamide (Gly)-sensitive sarcKATP currents in the cardiac, smooth, and skeletal muscle (4, 5, 16). Because of the importance of KATP in cardioprotection, disruption of KATP might be expected to abrogate protection. Nonetheless, SUR2m mice actually had increased protection against both acute adrenergic stress and I/R compared with wild-type (WT) mice (26). Ischemic preconditioning (IPC) did not provide any further protection against I/R in these mice (28), suggesting that SUR2m mice were constitutively protected against I/R.

Mitochondria play important roles in cardioprotection through changes in the transmembrane potential (11) and calcium homeostasis, which may involve potassium cycling (8), although this concept has been disputed (17). A favorable mitochondrial bioenergetic phenotype for myocardial protection resulting from preconditioning has been defined as 1) a partially dissipated mitochondrial membrane potential (ΔΨm), 2) preservation of respiration and ATP production, 3) resistance to Ca2+ uptake, and 4) generation of protective reactive oxygen species (ROS) (18). Long forms of SUR2 are disrupted in the SUR2m mouse, but a smaller 55-kDa splice variant targeted to mitochondria survives the disruption, and this variant in heterologous systems formed a relatively ATP-insensitive K+ conductance (28). We hypothesized that such a conductance might favorably affect the bioenergetic phenotype of the mitochondria from SUR2m mice to provide cardiac protection against I/R.

MATERIALS AND METHODS

SUR2m mice. Studies were conducted in accordance with guidelines set forth by the University of Wisconsin, Madison, the Animal Welfare Act regulations, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The studies were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin. Male FVB WT or SUR2m mice (n = 40 each) aged 8–12 wk were used. SUR2m mice were previously generated by targeted disruption of exons 14–18 encoding the first nucleotide binding fold (5). Detailed methods are available in Supplemental Fig. S1 (Supplemental data for this article may be found on the American Journal of Physiology: Heart and Circulatory Physiology website).

Isolation of cardiomyocytes and mitochondria. Cardiac ventricular cell mitochondria were isolated by homogenization and differential centrifugation as described previously (18). Protein concentration was
assessed, and mitochondria were used within 4 h. For all measurements, 0.5 mg mitochondrial protein/ml was used. Ventricular myocytes were isolated by enzymatic dissociation with 0.2 mg/ml Liberase blendzyme (Roche Diagnostic) as reported previously (27) with some modifications.

\[ \Delta V_m \text{ and tolerance to Ca}^{2+} \text{ upload.} \]  

\[ \Delta V_m \]  was monitored spectrophotometrically (\( \lambda_{\text{ex}} 503 \text{ nm, } \lambda_{\text{em}} 527 \text{ nm} \)) using rhodamine 123 (5 nM) as described (22) in the presence or absence of ATP (200 \( \mu M \)) and oligomycin (5 \( \mu g/ml \)). Diazoxide (0.1 mM), Gly (20 \( \mu M \)), and carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP; 1 \( \mu M \)) were added when indicated. \( \Delta V_m \) was expressed as the percentage of rhodamine 123 fluorescence relative to the fluorescence after FCCP. Tolerance to \( \text{Ca}^{2+} \) loading was assessed in the presence of ATP (200 \( \mu M \)) by adding \( \text{Ca}^{2+} \) in 20-\( \mu M \) increments and determining the cumulative amount of \( \text{Ca}^{2+} \) required to cause at least 5\% depolarization within 4 s. Mitochondria were suspended in EGTA-free buffer.

**Measurement of \( K^+ \) influx by mitochondrial swelling.** Mitochondrial swelling was determined as change in mitochondria volume. As mitochondria volume increases the light scattering decreases. A change in mitochondria volumes was measured as change in light (\( \lambda; 540 \text{ nm} \)) scattering, in response to \( K^+ \) (Fig. 1A). Mitochondrial swelling was determined as change in mitochondria volume. As mitochondria volume increased when mitochondria were moved from low-\( K^+ \) (5 mM) to a high-\( K^+ \) (140 mM) buffer with or without ATP. ATP or vehicle was added at time 0. In some experiments, valinomycin (Val; 2 nM) was added to induce the maximal volume (\( V_{\text{max}} \)) at the times indicated. In the presence of ATP, the swelling was expressed as percentage change in volume (\( V \)) just before addition of Val normalized to the \( V_{\text{max}} \). The effect of ATP on swelling was determined as the percentage difference in volume at 200 s (\( V_{\text{200}} \)) in the presence of ATP from the \( V_{\text{200}} \) in the absence of ATP.

**Mitochondrial \( H_2O_2 \) release rate.** Rates of \( H_2O_2 \) release were measured spectrophotometrically (\( \lambda_{\text{ex}} 530 \text{ nm, } \lambda_{\text{em}} 583 \text{ nm} \)) using Amplex red (12.5 \( \mu M \)) in the presence of 0.1 units/ml horseradish peroxidase (3) with complex I substrates (pyruvate and malate, 5 mM each) or complex II substrate succinate (10 mM). \( H_2O_2 \) production was blocked by FCCP (1 \( \mu M \)). Rates of \( H_2O_2 \) were expressed as the slope of the curves (\( \mu M \)/s) after addition of substrates.

**Effect of metabolic inhibition on myocytes.** \( \Delta V_m \) was determined with tetramethylrhodamine ester (TMRE, 25 nM, \( \lambda_{\text{ex}} 543 \text{ nm and } \lambda_{\text{em}} 594 \text{ nm} \)) as described (18). Myocytes were perfused with normal Tyrode for 5 min and then with a Tyrode in which glucose was substituted with deoxyglucose (20 mM) and NaCN (0.5 mM) (2-DG/CN Tyrode) to cause metabolic inhibition for 15 min. TMRE fluorescence was measured to determine \( \Delta V_m \) and 2,4-dinitrophenol (DNP, 0.1 mM) was added at the end to completely depolarize the mitochondria.

**Statistical analysis.** Data were expressed as means \( \pm \) SE. Two groups of data were compared by nonparametric unpaired Student’s \( t \)-test, and more than two groups were analyzed by one-way ANOVA. Groups of data were compared by nonparametric unpaired Student’s \( t \)-test, and more than two groups were analyzed by one-way ANOVA.

**RESULTS**

\[ \Delta V_m \text{ in isolated mitochondria.} \]  

\[ \Delta V_m \]  was measured in the presence or absence of 200 \( \mu M \) ATP; mitochondria were treated with oligomycin and supplemented with a metabolic substrate (Fig. 1). Representative traces show that \( \Delta V_m \) of SUR2m mice were less polarized than WT with ATP present (Fig. 1A), but the \( \Delta V_m \) of SUR2m tended to be more polarized than WT with ATP absent (Fig. 1B). Summary data (Fig. 1C) show the significant differences. In the absence of ATP, the...
change in $\Delta \Psi_m$ of WT was greater ($\sim$13%) than the change in SUR2m mice ($\sim$5%), suggesting that, although $\Delta \Psi_m$ in SUR2m was sensitive to ATP, $\Delta \Psi_m$ was more sensitive to ATP in WT. Furthermore, in the presence of ATP, diazoxide decreased the $\Delta \Psi_m$ of WT but did not affect the $\Delta \Psi_m$ of SUR2m (Fig. 1A). In the presence of ATP, Gly (20 $\mu$M) increased the $\Delta \Psi_m$ in WT but not in SUR2m mitochondria (Supplemental Fig. S1). These results on $\Delta \Psi_m$ are consistent with a diazoxide-, Gly-, and ATP-sensitive $K^+$ conductance in WT mitochondria that has been disrupted in SUR2m.

$K^+$ influx in the mitochondria and matrix swelling. K$^+$ influx in the matrix causes a partial depolarization, increased ion exchange, and increased water intake in mitochondria, causing swelling. This volumetric change in response to increased extramitochondrial K$^+$ has been a standard method to assess K$^+$ influx in mitochondria (7). Volume changes, in the presence of ATP, in mitochondria from SUR2m and WT mice are shown in Fig. 2A. Val, a K$^+$ ionophore, was added at the end to induce maximum volume to allow normalization. Swelling was expressed as the percentage of volume (V) just before adding valinomycin (Val; 2 mM) scattered due to mitochondria (0.5 mg protein) volume was measured as the light (A; 540 nM) scattered due to mitochondria in K$^+$ buffer. The volume increased (i.e., light scattering was decreased) when mitochondria previously in 5 mM K$^+$ were suspended in a 140 mM K$^+$ respiration buffer supplemented with pyruvate and malate (5 mM each) and ATP (200 $\mu$M). The volume (V) just before adding valinomycin (Val; 2 mM) is expressed as the percentage of maximum volume ($V_{max}$) induced by Val. A representative experiment (A) and summary data (B) show that swelling (change in volume) was significantly greater for SUR2m in the presence of ATP. The effect of absence of ATP on mitochondria volume is shown in presence of ATP. The rate of swelling was determined, as rate constant ($k$), from the one-phase exponential decay curves shown in Fig. 2C. In the presence of ATP, the “$k$” of WT mitochondria [0.002 ± 0.002 arbitrary units (AU)/s] tended to be less than that of SUR2m mitochondria (0.010 ± 0.003 AU/s); however, the data did not reach a significant difference.

In the absence of ATP, the $k$ increased in WT mitochondria by 17.5 times, whereas in SUR2m mitochondria it increased by only 0.2 times. Maximum swelling in WT mitochondria, in the absence of ATP, reached saturation levels within 100 s in contrast to the SUR2m mitochondria, which swelled more slowly. The data suggest that mitochondrial swelling depended on an ATP-induced increased K$^+$ flux more strongly in WT than in SUR2m mitochondria.

Sensitivity of $\Delta \Psi_m$ to Ca$^{2+}$ load. Mitochondrial depolarization decreases the electrochemical gradient for the uptake of Ca$^{2+}$ in the matrix and dissipates the capacity of mitochondria to load Ca$^{2+}$ in the matrix (13). To test resistance to Ca$^{2+}$ loading, mitochondria from SUR2m and WT mice were challenged with 20-$\mu$M pulses of Ca$^{2+}$ concentrations until the occurrence of dissipation of $\Delta \Psi_m$ (as described in MATERIALS AND METHODS), as a measure of tolerance to mitochondrial Ca$^{2+}$ uptake (Fig. 3). Representative traces (Fig. 3A) and the summary data (Fig. 3B) show that, in mitochondria from WT mice, the Ca$^{2+}$ concentration required to cause depolarization was 47 $\mu$M lower than the concentration required for the SUR2m mice. These data show that tolerance to Ca$^{2+}$ overload was greater in the SUR2m mitochondria, consistent with a protected phenotype.

ROS generation in the mitochondria. Increase in signaling ROS is associated with the protection against I/R injury by
activating protective pathways (17). To estimate ROS generation, we measured production of H2O2 in the presence of ATP when the mitochondria were supplemented with complex I substrate (Fig. 4A) or complex II substrate (Fig. 4B). The rate of ROS generation reported as change in au per second (Fig. 4, C and D) was significantly greater for SUR2m mitochondria compared with WT for both complex I substrates (Fig. 4C) and for complex II substrate (Fig. 4D) (P < 0.05). Thus, at resting state, SUR2m mitochondria demonstrated increased basal production of ROS, which could serve in protective signaling.

**Mitochondrial respiration and hypoxia-reoxygenation.** The less-polarized ΔΨm of SUR2m mitochondria, increased resistance to Ca2+ overload, and increased ROS generation observed in SUR2m were consistent with the protective mitochondrial phenotype demonstrated in other models of protection (2, 6, 18, 24). To test resistance to hypoxia, changes in the mitochondria respiration rates from normoxia to posthypoxiareoxygenation were determined. O2 consumption was measured at state 2, 3, and 4 as an index of respiration rate and is summarized in Table 1. The respiration control index (RCI) was determined as a ratio of state 3 and 4 respiration rates to estimate coupling in these mitochondria. The prehypoxic RCI for WT and SUR2m mitochondria respiring with succinate, or pyruvate and malate, were >2.5, indicating coupled mitochondria. The respiration rates and RCI of the freshly isolated SUR2m mitochondria, respiring on succinate or pyruvate and malate, were greater at normoxia after isolation than the WT mitochondria (P = not significant). This may represent a protection conferred by SUR2m mitochondria after the stress of the isolation procedure. After hypoxia-reoxygenation, RCI was reduced in both WT and SUR2m (Table 1), but the decrease was greater in the WT mitochondria compared with SUR2m. Individually, the decrease in state 3 and 4 respiration rates due to hypoxia-reoxygenation in WT mitochondria was greater than the decrease in SUR2m mitochondria. Together these data suggest that the respiration was better preserved in SUR2m mitochondria after hypoxia-reoxygenation compared with WT mitochondria.

ΔΨm in intact myocytes and protection against metabolic inhibition. Cytosolic components of myocytes may modulate the functions of mitochondria; therefore, we measured mitochondrial ΔΨm in intact myocytes. To evaluate the effect of metabolic inhibition, the TMRE-loaded myocytes were perfused with 2-DG/CN Tyrode. Representative images of TRME fluorescence at 5 min (with normal Tyrode), at 10 and 18 min with 2-DG/CN-Tyrode, and at 20 min when DNP was added to completely depolarize the mitochondria are shown in Fig. 5A. ΔΨm was measured as the percentage change in fluorescence from the baseline, and summary data are presented in Fig. 5B. In WT or SUR2m myocytes, TMRE fluorescence decreased with time when perfused with the 2-DG/CN-Tyrode. However, after 18 min, the decrease in TMRE fluorescence was greater in the WT compared with the SUR2m myocytes (Fig. 5B). DNP addition completely dissipated the mitochondria ΔΨm. These data show a strong preservation of cardiac mitochondrial function after a strong metabolic challenge as shown by preserved ΔΨm in the SUR2m mice myocyte.

**DISCUSSION**

Mitochondria from SUR2m mice have a favorable bioenergetic phenotype for protection. Results from this study give insight into the mechanisms by which SUR2m mice unexpectedly showed a constitutively protected myocardial phenotype. Compared with WT mice, mitochondria from SUR2m hearts were slightly depolarized at rest (Fig. 1), had greater volume in the presence of ATP (Fig. 2), were more resistant to Ca2+ overload (Fig. 3), and had a basal level of mildly increased ROS production (Fig. 4), all consistent with a favorable bioenergetic phenotype for protection (18). In addition, mild ROS increase activates protective signaling in the mitochondria, although this is debatable in the literature (18). In addition, the function of SUR2m mitochondria, as assessed by RCI, recovered better after hypoxia reperfusion compared with the WT mitochondria (Table 1). The prehypoxic RCI of the SUR2m mitochondria was better compared with the RCI of the WT mitochondria, and, individually, the prehypoxic respiration rates were also faster in SUR2m mitochondria, consistent with the partially depolarized state of these mitochondria to start with. This suggests that the mitochondria from the SUR2m mice were protected from the stress of the isolation procedure.
procedure. Moreover, the posthypoxic state 3 and state 4 respiration recovered better in the SUR2m mitochondria compared with the WT mitochondria (Table 1), indicating that more WT mitochondria were uncoupled and underwent hypoxia- and reoxygenation-induced injury. The change observed following anoxia-reoxygenation was mainly due to a decrease in state 3 respiration, whereas state 4 was unaltered. This suggests that the major point of the damage might be the respiratory chain, whereas inner membrane integrity was not different between the two groups. The protective phenotype of SUR2m mitochondria was also seen in the intact myocytes where the $\Psi_m$ of SUR2m myocytes were maintained longer than in the WT myocyte after metabolic inhibition (Fig. 5). All of these observations are consistent with a constitutive protective bioenergetic mitochondrial phenotype in SUR2m mice.

Is there a diazoxide- and WT-insensitive mitochondrial K$_{ATP}$ in cells lacking full-length SUR2?

The slightly dissipated $\Psi_m$ of the SUR2m at rest suggests a net change in the balance of the K$^+$ conductance that sets $\Psi_m$ (21). Although we cannot exclude a decrease in H$^+$ conductance or gradient as the mechanism for this effect, the $\Psi_m$ data (Fig. 1) and the volume data (Fig. 2) are more consistent with an increased K$^+$ influx (10, 14) in the SUR2m mitochondria. Moreover, $\Psi_m$ of SUR2m mitochondria was more depolarized in the absence of ATP compared with the $\Psi_m$ in the presence of ATP (Fig. 1), indicating that SUR2m $\Psi_m$ had some sensitivity to ATP.

Table 1. Respiration rates in mitochondria at normoxia or after hypoxia-reoxygenation

<table>
<thead>
<tr>
<th>Respiration state</th>
<th>Oxygen consumption, nmol · min$^{-1}$ · mg protein$^{-1}$</th>
<th>Wild type</th>
<th>SUR2 mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Respiring on succinate ($N, n = 12–16, 9–12$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State2</td>
<td>31.1 ± 1.2</td>
<td>34.7 ± 1.7</td>
<td>37.8 ± 2.6†</td>
</tr>
<tr>
<td>State3</td>
<td>107.0 ± 5.8</td>
<td>67.2 ± 3.5***</td>
<td>123.2 ± 8.2†</td>
</tr>
<tr>
<td>State4</td>
<td>34.2 ± 2.0</td>
<td>31.5 ± 1.4</td>
<td>35.9 ± 2.7†</td>
</tr>
<tr>
<td>RCI</td>
<td>3.2 ± 0.1</td>
<td>2.1 ± 0.1**</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Respiring on pyruvate and malate ($N, n = 7–9, 4–6$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State2</td>
<td>9 ± 0.7</td>
<td>7.4 ± 1.2</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td>State3</td>
<td>77.7 ± 5.6</td>
<td>63.1 ± 6.5</td>
<td>76.0 ± 4.1</td>
</tr>
<tr>
<td>State4</td>
<td>13.9 ± 0.1</td>
<td>19.3 ± 3.7*</td>
<td>11.9 ± 0.0</td>
</tr>
<tr>
<td>RCI</td>
<td>5.5 ± 0.8</td>
<td>3.0 ± 0.9**</td>
<td>6.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. $N$, no. of experiments; $n$, no. of mice; SUR2, sulfonylurea receptor-2; RCI, respiratory control index. ***$P < 0.0001$, ** or ††$P < 0.001$, and * or †$P < 0.05$, compared with the corresponding prehypoxic value (asterisks) or compared with the corresponding value in the wild type (daggers).
ATP synthase was inhibited with oligomycin; therefore, this \( \Delta \Psi_{m} \) sensitivity to ATP was not due to ATP synthase-induced ion flux but was more likely caused by a membrane conductance. Moreover, swelling experiments indicated an ATP-sensitive \( K^{+} \) flux in the SUR2m mitochondria. However, diazoxide did not change the \( \Delta \Psi_{m} \) in SUR2m mitochondria, suggesting that the diazoxide-sensitive SUR2 elements in these mitochondria were absent. Taken together, the results showed that, in SUR2m mitochondria, both \( \Delta \Psi_{m} \) (Fig. 1) and volume (Fig. 2) are influenced by \( K^{+} \) influx, sensitive to ATP (but less so than WT), and are insensitive to diazoxide.

A diazoxide- and Gly-sensitive mitochondrial \( K_{ATP} \) (mito\( K_{ATP} \)) has been thought to be important for cardioprotection (20); if it is absent from SUR2m then how can this importance be reconciled? Our recent studies showed that, in the SUR2m mouse, the full-length SUR2 is absent, but short-form variants of SUR2 in various sizes (28, 55, and 66 kDa) persist and may account for a low-density Gly-insensitive sarc\( K_{ATP} \) found in SUR2m cardiomyocytes (23). A 55-kDa SUR2 variant unique to the mitochondrial fraction was cloned, and, when mitochondrial targeting sequences were removed, it was coexpressed with Kir6.x pores and produced a Gly-insensitive sarc\( K_{ATP} \) in a heterologous expression system (23) with an ATP sensitivity much less than conventional \( K_{ATP} \). The shortened structure of this short SUR2 form lacks transmembrane segments 1–6 and nucleotide-binding domain 1, which are reported to confer diazoxide sensitivity (1). We speculate that the short-form variants, and the 55-kDa form in particular, retained in the SUR2m mitochondria form a type of mito\( K_{ATP} \) that is both insensitive to diazoxide and is sensitive to ATP but with a decreased sensitivity compared with WT. These mito\( K_{ATP} \) are responsible for the ATP-sensitive \( \Delta \Psi_{m} \) and swelling observed in the SUR2m mitochondria. Moreover, at physiological concentrations of ATP, these mito\( K_{ATP} \) channels in SUR2m would remain open and cause a persistent potassium leak that underlies the protective phenotype of a partially dissipated \( \Delta \Psi_{m} \) and subsequent effects on volume, mitochondrial Ca\(^{2+} \) homeostasis, and ROS signaling.

Implications for mito\( K_{ATP} \) and mechanisms of protection. Studies on mitochondrial function have indicated several players that may be involved in cardioprotection (21) and that must be considered in interpreting the results of this study. These include the flux of anions through an inner membrane anion channel, transport of nucleotides through adenine nucleotide transporters, anion and metabolite flux by voltage-dependent anion channels, and energy transfer via phosphates through the creatine/kinase system. It is unlikely that such mechanisms are perturbed in the SUR2m mice because SUR2 has not been shown to directly interact with these mechanisms, but this possibility cannot be excluded. mito\( K_{ATP} \) conductances were not measured directly in this study, and the relative role of the different SUR2 variants, if any, remains to be studied. However, these results support the idea that one of the SUR2 variants, perhaps the long-form SUR2 present in WT but lacking in SUR2m, contributes and is necessary for many diazoxide-sensitive effects previously implicated as mito\( K_{ATP} \).
possible relevance to clinically relevant protection mechanisms remains to be determined.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


