Ischemic Preconditioning in the Hippocampus of a Knockout Mouse Lacking SUR1-Based \(K_{\text{ATP}}\) Channels

Alvaro Muñoz, PhD; Mitsuhiro Nakazaki, MD, PhD; J. Clay Goodman, MD; Roberto Barrios, MD; Carlos G. Onetti, PhD; Joseph Bryan, PhD; Lydia Aguilar-Bryan, MD, PhD

**Background and Purpose**—ATP-sensitive K\(^{+}\) (\(K_{\text{ATP}}\)) channels have been implicated in the mechanism of neuronal ischemic preconditioning. To evaluate the role of neuronal/β-cell–type \(K_{\text{ATP}}\) channels, SUR1 null (Sur1KO) mice lacking (\(K_{\text{IR}}\)6.2/SUR1), \(K_{\text{ATP}}\) channels were subjected to a preconditioning protocol with the use of double carotid occlusion.

**Methods**—Wild-type C57BL/6 and Sur1KO mice were subjected to a double carotid block for 40 minutes with or without a 20-minute preconditioning block. After a 10-day reperfusion period, damage was assessed histologically in the hippocampal CA1, CA2, and CA3 areas and in the dentate gyrus. The neuroprotective effects of intracerebroventricular injections of diazoxide, which selectively affects mitochondria versus opening SUR1-type \(K_{\text{ATP}}\) channels, and 5-hydroxydecanoate, a selective blocker of mito\(K_{\text{ATP}}\) channels, were evaluated with the same protocol.

**Results**—Neurons in the CA1 region of both Sur1KO and wild-type animals subjected to a 20-minute ischemic insult were protected equally from neuronal damage produced by a subsequent 40-minute ischemic period. Pretreatment with diazoxide protected both Sur1KO and wild-type neurons, while 5-hydroxydecanoate augmented neurodegeneration in both strains of animals when administered before a 20-minute bout of ischemia.

**Conclusions**—SUR1-based \(K_{\text{ATP}}\) channels are not obligatory for neuronal preconditioning or augmentation of neurodegeneration by 5-hydroxydecanoate. *(Stroke. 2003;34:164-170.)*

**Key Words:** cerebral ischemia ■ decanoic acids ■ diazoxide ■ hippocampus ■ ischemic preconditioning ■ potassium channels ■ sulfonylurea receptors ■ mice

A TP-sensitive potassium (\(K_{\text{ATP}}\)) channels couple metabolism with membrane electrical activity in many tissues. \(K_{\text{ATP}}\) channels are heteromultimers composed of 4 inward rectifier potassium channel pore subunits (\(K_{\text{IR}}\)6.x) and 4 sulfonylurea receptors (SURs) that regulate channel activity.\(^{1,2}\) SUR1-type \(K_{\text{ATP}}\) channels have been identified and localized in brain,\(^{3-7}\) but other subunits are detected by polymerase chain reaction.\(^{8-11}\)

While a role for SUR1-type \(K_{\text{ATP}}\) channels in the regulation of insulin secretion from pancreatic β-cells is clear in outline,\(^{12,13}\) their role(s) in other processes is not well defined. Recent reports have shown that Sur2KO mice display increased insulin-induced glucose uptake into skeletal muscle,\(^{14}\) that \(K_{\text{IR}}\)6.2/SUR1 channels play a role in the control of firing in glucose-responsive neurons in the ventromedial hypothalamus,\(^{15}\) that \(K_{\text{IR}}\)6.2KO mice are extremely sensitive to induction of seizures by hypoxia,\(^{16}\) and that \(K_{\text{IR}}\)6.1KO mice die prematurely from ischemia and cardiac arrest due to vasospasm.\(^{17}\) Myocardium was the first tissue in which a brief anoxic episode was observed to protect, or precondition, against prolonged ischemia.\(^{18,19}\) Subsequently preconditioning was described in skeletal muscle, small intestine, and brain.\(^{20}\) In brain, cell surface \(K_{\text{ATP}}\) channels, primarily SUR1/\(K_{\text{IR}}\)6.2, are argued to be obligatory for neuronal preconditioning.\(^{21-25}\) Hypoxia induces a fall in [ATP]/[ADP] that can activate neuronal \(K_{\text{ATP}}\) channels\(^{26,27}\) and produce membrane hyperpolarization, which is suggested to decrease calcium overload by inactivating voltage-dependent calcium channels and reducing release of excitotoxic glutamate.\(^{21,26}\) In rats, intracerebroventricular administration of cromakalim, a \(K_{\text{ATP}}\) channel opener, reduced neuronal damage to a level comparable to that obtained by ischemic preconditioning, an effect prevented by glibenclamide, an inhibitor of SUR1/\(K_{\text{IR}}\)6.2-type \(K_{\text{ATP}}\) channels.\(^{23}\) The finding that diazoxide, long known to hyperpolarize β-cells and used clinically to treat hyperinsulinemic states, could exert a cardioprotective action at concentrations not expected to open plasma membrane channels\(^{28,29}\) questioned the role of surface \(K_{\text{ATP}}\) channels. Protection by diazoxide was antagonized by glibenclamide and 5-hydroxydecanoate (5-HD), originally identified as a low-
affinity blocker of surface K<sub>ATP</sub> channels<sup>30,31</sup> but later reported to act selectively on mitochondria<sup>28–33</sup> although this specificity is questioned.<sup>36</sup> A number of reports have studied the protective effects of diazoxide and their antagonism by 5-HD. Both sarcolemmal and mitochondrial K<sub>ATP</sub> channels are implicated in ischemic preconditioning<sup>33,34</sup> and in neuroprotection.<sup>37–39</sup> The importance of surface channels is controversial. Tanno et al.<sup>40</sup> for example, provided pharmacological evidence that both surface and mitochondrial channels play a role in cardioprotection, while others have concentrated on a role for mitochondria.<sup>34,35</sup>

To assess whether SUR1-based K<sub>ATP</sub> channels have a role in ischemic preconditioning, we evaluated the effects of ischemia on SUR1 null (Sur1KO) mice lacking functional K<sub>ATP</sub> channels.<sup>41</sup> Ischemic damage and delayed preconditioning were induced by occlusion of the carotid arteries in sedated Sur1KO and wild-type (C57BL/6) mice. Diazoxide and 5-HD administered by intracerebroventricular injection before occlusion were used to assess their acute neuroprotective effects in the absence of SUR1. The effects of these insults were assessed in the hippocampus, particularly in CA1 pyramidal neurons, sensitive indicators of ischemic damage.

**Materials and Methods**

**Materials**

Diazoxide and 5-HD were purchased from Sigma Chemical Company.

**Mice**

The generation of Sur1KO mice was described previously.<sup>41</sup> Sur1KO and control C57BL/6 wild-type mice were on a 12-hour dark/light cycle and fed standard mouse chow. All treatment protocols were approved by the Animal Welfare Committee of Baylor College of Medicine.

**Ischemic Insults**

A 2-vessel occlusion model<sup>42</sup> was used to study ischemic insult and preconditioning in 12- to 16-week-old adult male C57BL/6 and Sur1KO mice. Mice were anesthetized by intraperitoneal injection of 70 mg/kg pentobarbital 15 minutes before surgery. A ventromedial-cervical incision exposed both common carotid arteries, which were isolated from the vagus nerve and adjacent veins. The common carotid arteries were occluded with 30-g pressure microaneurysm clips for times selected to induce reproducible hippocampal damage. Rectal temperature was maintained at normal values with the use of a heat lamp and thermal blanket. In the sham group, the carotid arteries were isolated, and the incision was left open for 20 minutes. A second group was subjected to a 20-minute occlusion of both carotid arteries (20-minute carotid artery occlusion group). A third group was subjected to a more prolonged ischemic event by occluding both arteries for 40 minutes (40-minute carotid artery occlusion group). Ischemic preconditioning was induced by a 20-minute occlusion, followed by recovery for 3 days before a second 40-minute occlusion (preconditioning group). Propylactylatic care was taken to avoid infections after surgery. After 10 days of reperfusion, mice were killed for brain fixation.

**Intracerebroventricular Injections**

Diazoxide or 5-HD was injected 15 minutes before occlusion of the common carotid arteries. Intracerebroventricular injections were administered into the right cerebral ventricle of mice anesthetized with pentobarbital (80 mg/kg IP). A cranial incision exposed the bregma fissure and left the bone free of connective tissue. A micro-drill was used to reduce bone thickness; then 4 μL of solution was injected (1 μL/30 s) via a precisely positioned 10-μL Hamilton syringe with the use of the following coordinates: anterior/posterior, −0.15; medial/lateral, −0.10; dorsal/ventral, +0.25 with respect to the bregma fissure (right ventricle according to Franklin and Paxinos<sup>43</sup>). Drug dilutions and control intracerebroventricular injections were in artificial cerebrospinal fluid (aCSF) (see below). Diazoxide was injected at 0.1 mmol/L; 5-HD was injected at either 0.25 or 2.5 mmol/L. Solutions were sterilized with the use of a 0.22-μm filter before injection. After injection the needle was carefully withdrawn, and the skin flaps were stapled. After 15 minutes to allow the drugs to diffuse, the common carotid arteries were isolated as described. Control animals were treated but not occluded; the experimental group was occluded for 20 minutes. To determine the neurological effects of 5-HD alone, mice were injected with 2.5 mmol/L 5-HD and subjected to a sham operation. Mice injected with diazoxide were subjected to 40-minute occlusion. To monitor extracranial temperature, a thermal probe was fixed on the skull during the ischemic period, and temperature was maintained with the use of a heating lamp and thermal blanket. After surgery, mice were placed in a postoperative cage and kept warm and undisturbed for a minimum of 2 hours of observation. After 10 days of reperfusion, mice were killed for brain fixation.

**Brain Fixation**

Mice were euthanized with pentobarbital (120 mg/kg IP). The heart was exposed, an incision was made in the right ventricle, and a hypodermic syringe was used to perfuse with PBS followed by 20 mL of neutralized 10% formalin in PBS. The whole brain was removed, weighed, and postfixed at room temperature in formalin solution for at least 24 hours. A 3-μm-thick block of tissue containing the hippocampus was embedded in paraffin. Four 4-μm sections were cut and stained with conventional hematoxylin-eosin.

**Neuropathological Evaluation**

Ischemic damage and neuroprotection were evaluated by scoring dead versus intact neurons, on the basis of nuclear morphology, in the hippocampal CA1, CA2, and CA3 and the granular and polymorphic layers of the dentate gyrus in both hemispheres. The percentage of intact neurons, defined as those not showing chromatin condensation or dark blue nuclear staining, was determined by scoring all neurons in 1 to 3 randomly chosen fields. A total of 100 neurons were evaluated blindly by 2 investigators for each hippocampal region. The scoring procedure was repeated on the same region in a second brain section, and the 2 percentages were averaged. Representative images were obtained with a DC290 digital camera (Kodak) mounted on a Zeiss EL-Einsatz microscope.

**Hypoxic Conditions**

Mice were placed in a small chamber (volume approximately 1 120 000 mm<sup>3</sup>) with inlet and outlet ports. The inlet port was connected to a gas cylinder containing a 5% O<sub>2</sub>/95% N<sub>2</sub> mixture. Gas exchange was initiated by opening a valve and perfusing the chamber at a flow rate of approximately 1 750 000 mm<sup>3</sup>/s. Complete exchange was accomplished in <3 seconds.

**Solutions**

aCSF solution contained the following (in mmol/L): 147 NaCl, 4 KCl, 1.2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, and 10 HEPES; the pH was adjusted to 7.4 with NaOH. A 200-mmol/L stock solution of diazoxide was prepared in 0.4 mol/L NaOH. A small volume was diluted in aCSF to 0.1 mmol/L final concentration, and the pH was adjusted before intracerebroventricular injection. A 500-mmol/L stock solution of 5-HD was prepared in sterile water and diluted in aCSF to either 0.25 or 2.5 mmol/L. Drugs were freshly diluted before intracerebroventricular injection.

**Data Analysis**

Brain and body weights were analyzed with the use of a 1-tailed Student’s t test. ANOVA was performed between groups and strains with the use of the Tukey posttest when necessary. All data are presented as mean±SD. Graphics as well as statistical analysis were...
performed with the use of GraphPad Prism, version 3.00 (GraphPad Software).

**Results**

After a sham operation, Sur1KO mice showed no differences in survival or body or brain weights compared with wild-type mice (Table 1). Rectal and extracranial temperatures were maintained to prevent hypothermic effects on neuroprotection.44 Occlusion of the common carotid arteries for 20 minutes produced no appreciable neurological damage; 40 minutes of ischemia induced severe damage in both Sur1KO and wild-type animals. Survival rates were comparable between groups. The effect of prolonged ischemia was reversed in both groups (10/10) by a prior 20-minute ischemic insult (Table 1).

**Sur1KO Mice Exhibit Ischemic Preconditioning**

Neuronal survival after an ischemic insult was scored as the percentage of intact neurons. Neuronal morphology in the CA1 region was unaltered in animals subjected to sham surgery or 20 minutes of ischemia. Figure 1 shows representative images from the CA1 of wild-type (Figure 1a and 1b) and Sur1KO mice (Figure 1f and 1g). After 20 minutes of ischemia, neuronal survival rates were approximately 100% for the control groups and 90.0 ± 9.5% versus 89.7 ± 4.8% (P = 0.05) for wild-type and Sur1KO mice, respectively. Forty minutes of ischemia followed by 10 days of reperfusion reduced neuronal survival in both wild-type (Figure 1c) and Sur1KO mice (Figure 1h): 38.8 ± 18.9% for wild-type versus 32.4 ± 15.4% for Sur1KO animals (Figure 1e and 1j). The

**TABLE 1. General Parameters**

<table>
<thead>
<tr>
<th>Survival*</th>
<th>Wild Type (C57BL/6)</th>
<th>Sur1KO</th>
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<tr>
<td>Sham</td>
<td>100.0% (8/8)</td>
<td>100.0% (8/8)</td>
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<tr>
<td>20’ CAO</td>
<td>100.0% (8/8)</td>
<td>100.0% (7/7)</td>
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<tr>
<td>40’ CAO</td>
<td>58.3% (7/12)</td>
<td>60.0% (9/15)</td>
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<tr>
<td>Precon</td>
<td>100.0% (10/10)</td>
<td>100.0% (10/10)</td>
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<tr>
<th>Temperature, °C</th>
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<tr>
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<td>20’</td>
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<td>40’</td>
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<table>
<thead>
<tr>
<th>Body weight, g</th>
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<tr>
<td>27.40 ± 3.03 (n=50)§</td>
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<table>
<thead>
<tr>
<th>Brain weight, g</th>
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<td>0.419 ± 0.022 (n=22)</td>
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*The data are expressed as the percentage of animals surviving after 10 days. The number in parentheses indicates the number of animals used in the experiment. Values indicate †extracranial and ‡rectal temperatures. There were no statistically significant differences in temperature or body weight as determined by 1-way ANOVA between strains and groups.

§P = 0.241 by 1-tailed Student’s t test; **P = 0.499 by 1-tailed Student’s t test. Values are expressed as mean ± SD; n = number of observations. CAO indicates carotid artery occlusion; Precon, ischemic preconditioning.

Sur1KO Mice Exhibit Ischemic Preconditioning

Neuronal survival after an ischemic insult was scored as the percentage of intact neurons. Neuronal morphology in the CA1 region was unaltered in animals subjected to sham surgery or 20 minutes of ischemia. Figure 1 shows representative images from the CA1 of wild-type (Figure 1a and 1b) and Sur1KO mice (Figure 1f and 1g). After 20 minutes of ischemia, neuronal survival rates were approximately 100% for the control groups and 90.0 ± 9.5% versus 89.7 ± 4.8% (P > 0.05) for wild-type and Sur1KO mice, respectively. Forty minutes of ischemia followed by 10 days of reperfusion reduced neuronal survival in both wild-type (Figure 1c) and Sur1KO mice (Figure 1h): 38.8 ± 18.9% for wild-type versus 32.4 ± 15.4% for Sur1KO animals (Figure 1e and 1j). The

Figure 1. K_{ATP} channels are not required for neuronal ischemic preconditioning. Representative micrographs are shown of neurons in the CA1 of wild-type, C57BL/6 mice after sham operation (a), 20 minutes of carotid artery occlusion (CAO) (b), 40 minutes of CAO (c), or a 20-minute preconditioning ischemic bout (Precon.) followed 3 days later by 40 minutes of CAO (d). Panels e, f, g, h, and i are corresponding micrographs from Sur1KO mice treated in the same manner. Panels e and j summarize the results from wild-type and Sur1KO mice. *P < 0.001, comparison vs all groups and between strains; **P > 0.05, comparison of 40-minute CAO for wild-type mice vs 40-minute CAO for Sur1KO mice. Numbers in parentheses indicate number of animals used. Values are mean ± SD. Bars = 0.030 mm.
40-minute values are not significantly different from each other but are significantly less than the control and 20-minute occlusion values ($P<0.001$).

A prior ischemic attack affected neuronal survival in wild-type and Sur1KO animals subjected to 40 minutes of occlusion. Preconditioning is clearly seen in the CA1 in both wild-type (Figure 1d) and Sur1KO (Figure 1i) animals. A 20-minute ischemic event 3 days before a 40-minute attack increased neuronal survival (91.2 ± 6.8% for wild-type and 95.4 ± 6.7% for Sur1KO animals; Figure 1e and 1j).

Neuronal damage in the hippocampus (CA1, CA2, CA3, granular, and polymorphic dentate gyrus cell layers) after treatment is summarized in Table 2. Neurons in the control and 20-minute occlusion groups appeared normal in all regions; 40 minutes of occlusion produced extensive neuronal damage in all of the regions tested without significant differences between Sur1KO and wild-type animals. The surviving CA1 neurons in both groups were reduced to approximately 30% after 40 minutes of ischemia, confirming that the CA1 is a sensitive indicator of ischemic damage.

**5-HD Produces Neuronal Damage While Diazoxide Provides Neuroprotection in Sur1KO Mice**

5-HD (0.25 mmol/L) markedly reduced the survival of CA1 neurons when injected into the right cerebral ventricle 15 minutes before a mild ischemic insult (20-minute preconditioning occlusion), which had no effect in the absence of 5-HD. The effect was most marked in the right hemisphere (25.4 ± 10.4% for wild-type versus 20.1 ± 11.8% for Sur1KO animals). Consistent with drug diffusion, damage was apparent in the left hemisphere (72.8 ± 7.6% versus 81.1 ± 18.0% for wild-type versus Sur1KO animals; compare Figure 2c, 2d, 2j, 2k). Neuronal survival, after aCSF injection and 20 minutes of occlusion, was the same as in sham controls (Figure 2g and 2n).

Diazoxide (0.1 mmol/L) injected 15 minutes before a severe ischemic attack markedly improved the survival of wild-type and Sur1KO CA1 neurons (Figure 1c versus Figure 2e and 2f and Figure 1h versus Figure 2i and 2m). Surviving CA1 neurons in the diazoxide-treated hemispheres were >90% in both wild-type and Sur1KO animals versus <40% for untreated animals (Figure 2g and 2n).

**5-HD Alone Does Not Affect Neuronal Viability**

5-HD potentiated the damage induced by mild ischemia (Figure 2). To determine whether 5-HD produced damage without ischemia, right ventricles were injected with 2 drug concentrations. Figure 3 shows representative images from right and left Sur1KO CA1 regions injected with 0.25 mmol/L 5-HD followed 15 minutes later by occlusion of the carotid arteries for 20 minutes. The majority of the CA1 neurons in the right hemisphere are dead or dying (Figure 3a), whereas neurons in the left hemisphere (Figure 3b) are not significantly damaged. Injection of 10-fold more 5-HD without ischemia had little effect (Figure 3c and 3d). A similar result was obtained with wild-type mice, indicating that 5-HD in the absence of ischemia had little effect.

**Sur1KO Mice Display Increased Sensitivity to Hypoxia**

Yamada et al. have shown that unsedated K$_atp$6.2KO mice have increased sensitivity to hypoxia-induced seizures. Under similar conditions, Sur1KO animals exhibit a hypersensitivity to induced seizure. Only 2 of 10 Sur1KO animals tested survived. The average time of death after sudden seizure for 8 animals was estimated to be 35 seconds. All the wild-type mice survived >2.5 minutes of hypoxia.

**Discussion**

Sur1KO mice lacking the high-affinity sulfonylurea receptor and SUR1-based K$_atp$ channels can precondition CA1 neurons as effectively as wild-type mice. Sur1KO and wild-type animals are both damaged by cerebral ischemia in the absence of preconditioning and are both insensitive to prolonged ischemia after a preconditioning episode or pretreatment with diazoxide. In the absence of ischemic stress, the Sur1KO and wild-type mice were unaffected by a high dose of 5-HD, confirming earlier reports. However, 5-HD augmented the effect of a mild ischemic insult, producing equivalent neural damage in both Sur1KO and wild-type animals. The usual treatment paradigm uses 5-HD to block the protective effect(s) of diazoxide. The basis for this K$_atp$ channel-independent augmentation of ischemic damage is unclear but is consistent with the report that 5-HD is an open channel blocker and that mitoK$_atp$ channels open during a
preconditioning episode. Like their K\(_{\text{ATP}}\)6.2KO counterparts, Sur1KO mice are hypersensitive to hypoxia, indicating that there is no major upregulation of compensating ion channels.

We have demonstrated previously that there is no upregulation of SUR2 receptors or compensatory, metabolically regulated K\(^+\)/H\(^+\) channels in Sur1KO \(\beta\)-cells.\(^{41}\)

While K\(_{\text{ATP}}\) channels in \(\beta\)-cells are involved in the regulation of glucose-induced insulin release, the functions of neuronal K\(_{\text{ATP}}\) channels are less clear. They are considered to participate in plasma membrane hyperpolarization during ischemia or hypoxia, thus serving to reduce calcium overload and cell death.\(^{21,26,46}\) Using a protocol similar to that employed here, others\(^{22,23}\) have argued that K\(_{\text{ATP}}\) channels are "essential" for neuronal preconditioning. Zawar et al\(^{9}\) used single cell reverse transcription–polymerase chain reaction to show that the expression of SUR1 mRNA in CA1 pyramidal neurons is lower than in interneurons and glial cells and suggested that the relative paucity of K\(_{\text{ATP}}\) channels in CA1 neurons might account for their higher sensitivity to metabolic stress. Our results, in sedated animals in which neurons are presumably hyperpolarized as a consequence of potentiation of GABA receptor Cl\(^-\) channel activity by pentobarbi-

Figure 2. Sur1KO neurons are sensitive to diazoxide (Dzx) and 5-HD. Representative micrographs are shown of the CA1 from right and left hemispheres after right ventricle injections, as follows: wild-type mice: a, b, sham injection, 20-minute carotid artery occlusion (CAO) (sh-20'); c, d, 0.25 mmol/L 5-HD, 20-minute CAO (5HD-20'); e, f, 0.1 mmol/L diazoxide, 40-minute CAO (Dzx-40'); Sur1KO mice: h, i, sham injection, 20-minute CAO (sh-20'); j, k, 0.25 mmol/L 5-HD, 20-minute CAO (5HD-20'); l, m, 0.1 mmol/L diazoxide, 40-minute CAO (Dzx-40'). Panels g and n summarize the results; light bars indicate right ventricle; dark bars, left ventricle. Only the 5-HD groups show a significant right-left hemisphere difference (\(P<0.001\)). Numbers in parentheses indicate number of animals used. Values are means±SD. Bars=0.03 mm.

Figure 3. 5-HD markedly potentiates the effect of mild ischemia. Panels a and b show representative micrographs from the CA1 of a SUR1KO mouse after injection of 5-HD (0.25 mmol/L) into the right ventricle followed by occlusion of both carotid arteries for 20 minutes. Neuronal damage is evident in the right hemisphere. Panels c and d demonstrate the minimal effect of a 10-fold higher concentration of 5-HD without carotid occlusion. Similar results were observed in 2 other Sur1KO mice and in wild-type mice (data not shown). Bars=0.03 mm.
tal, clearly indicate that SUR1-type K\textsubscript{ATP} channels are not required for delayed preconditioning after a brief ischemic bout or for the acute neuroprotective effect of diazoxide administered before a severe ischemic attack.

Our results are consistent with the prevailing idea that neuronal preconditioning, like cardiac preconditioning,\textsuperscript{34,35,47} is mediated by mitoK\textsubscript{ATP} channels sensitive to 5-HD\textsuperscript{28,45,48} and diazoxide.\textsuperscript{28} Bajgar et al\textsuperscript{49} have reported that brain mitochondria have 6- to 7-fold higher concentrations of mitoK\textsubscript{ATP} channels with pharmacological properties equivalent to those found in heart and liver. However, 5-HD–sensitive channels may be required to preserve acute neuronal viability without a role in preconditioning. We have not established that diazoxide and 5-HD have mitoK\textsubscript{ATP} as a common target, but our results show that SUR1 plays no role in mitoK\textsubscript{ATP} channel function. While our results indicate that the biochemical mechanism(s) of preconditioning do not require SUR1-type K\textsubscript{ATP} channels, they do not eliminate an active role for these channels during periods of brief ischemia or hypoxia in unsedated animals. Brief hypoxia rapidly induces lethal seizures in K\textsubscript{ir6.2KO} and Sur1KO mice that preclude the development of preconditioning.

Conclusion

The data indicate that the biochemical mechanism(s) of preconditioning does not require SUR1-type K\textsubscript{ATP} channels. The results do not eliminate the possibility that K\textsubscript{ATP} channels play an active role during periods of brief ischemia. In unsedated knockout animals, brief periods of hypoxia are lethal, and therefore preconditioning does not proceed.

Acknowledgments

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References


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