Inhibition of Succinate Dehydrogenase by Diazoxide Is Independent of the ATP-Sensitive Potassium Channel Subunit Sulfonylurea Type 1 Receptor

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BACKGROUND: Diazoxide maintains myocyte volume and contractility during stress via an unknown mechanism. The mechanism of action may involve an undefined (genotype unknown) mitochondrial ATP-sensitive potassium channel and is dependent on the ATP-sensitive potassium channel subunit sulfonylurea type 1 receptor (SUR1). The ATP-sensitive potassium channel openers have been shown to inhibit succinate dehydrogenase (SDH) and a gene for a portion of SDH has been found in the SUR intron. Diazoxide may be cardioprotective via inhibition of SDH, which can form part of an ATP-sensitive potassium channel or share its genetic material. This study investigated the role of inhibition of SDH by diazoxide and its relationship to the SUR1 subunit.

STUDY DESIGN: Mitochondria were isolated from wild-type and SUR1 knockout mice. Succinate dehydrogenase activity was measured by spectrophotometric analysis of 2,6-dichloroindophenol reduction for 20 minutes as the relative change in absorbance over time. Mitochondria were treated with succinate (20 mM), succinate + 1% dimethylsulfoxide, succinate + malonate (8 mM) (competitive inhibitor of SDH), or succinate + diazoxide (100 μM).

RESULTS: Both malonate and diazoxide inhibit SDH activity in mitochondria of wild-type mice and in mice lacking the SUR1 subunit ($p < 0.05$ vs control).

CONCLUSIONS: The ability of DZX to inhibit SDH persists even after deletion of the SUR1 gene. Therefore, the enzyme complex SDH is not dependent on the SUR1 gene. The inhibition of SDH by DZX can play a role in the cardioprotection afforded by DZX; however, this role is independent of the ATP-sensitive potassium channel subunit SUR1. (J Am Coll Surg 2013;216: 1144–1149. © 2013 by the American College of Surgeons)

The cardioprotective mechanism of action of mitochondrial ATP-sensitive potassium (mK_{ATP}) channel opener, diazoxide (DZX), remains elusive. We and others have demonstrated the cardioprotective properties of DZX.[1-5] In an isolated myocyte model of myocardial stunning, DZX maintained myocyte volume and contractility during exposure to stress in 3 different species.[6-10]

Diazoxide is generally believed to be more selective for a purported mK_{ATP} channel.[1] The K_{ATP} channels are composed of a potassium inward rectifier channel forming subunit (Kir) and a sulfonylurea regulatory (SUR) subunit.[11] There are 2 proposed types of cardiac K_{ATP} channels: a sarcolemmal K_{ATP} (sK_{ATP}) and a purported mK_{ATP} channel. The sK_{ATP} channel is composed of SUR2A and Kir 6.2 subunits in mouse ventricle and SUR1 and Kir 6.1 subunits in mouse atria.[12] However, both SUR1 and SUR2A subunits have been identified in mouse heart[12] and in neonatal rat ventricular tissue.[13]

Unlike sK_{ATP} channel, the mK_{ATP} channel has not been cloned and its genetic material is undefined. In addition, measuring ion flux across a mitochondrial membrane to confirm mK_{ATP} channel activity is not

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Abbreviations and Acronyms

DZX = diazoxide
Kir = potassium inward rectifying subunit
mK_{ATP} = mitochondrial ATP-sensitive potassium
SDH = succinate dehydrogenase
sK_{ATP} = sarcolemmal ATP-sensitive potassium
SUR1 = sulfonylurea type 1 receptor
WT = wild-type

feasible. Therefore, investigation of the mechanism of action of DZX requires indirect methods.

Previously, the cardioprotection afforded by DZX was localized to a non-sK_{ATP} channel location, as DZX failed to generate a potassium current via the sK_{ATP} channel and by the evidence that DZX provides no cardioprotective benefit to mouse myocytes lacking the SUR1 subunit.[14]

Interestingly, DZX is also a known inhibitor of the mitochondrial enzyme complex II, succinate dehydrogenase (SDH), which is a component of the electron transport chain.[15-17] Succinate dehydrogenase inhibition by DZX has been shown to decrease reactive oxygen species generation, decrease ATP breakdown, and preserve ATP concentration during stress, and has been proposed to be a non-K_{ATP} channel mechanism of cardioprotection.[16,17] Malonate and 3-nitropropionic acid, both inhibitors of SDH, are also cardioprotective, mimic ischemic preconditioning, and decrease oxygen radical production.[18-20]

The 2 proposed cardioprotective mechanisms of DZX (K_{ATP} channel opening and SDH inhibition) might be associated or linked.[20-22] Four specific mitochondrial proteins (mitochondria ATP-binding cassette 1, phosphate carrier, adenine nucleotide translocator, ATP synthase) have been identified that associate with SDH.[22] This multiprotein complex was capable of generating a potassium current and potassium influx on exposure to DZX. This potassium current was diminished in the presence of ATP and 5-hydroxydecanoate, both mK_{ATP} channel inhibitors; but not with HMR-1098, an sK_{ATP} channel inhibitor. Malonate, a competitive inhibitor of SDH, has also been shown to generate a potassium current leading to mitochondrial matrix swelling (a proposed consequence of mK_{ATP} channel activity) and is inhibited by ATP and 5-hydroxydecanoate.[20] In addition, a genetic link between a K_{ATP} channel and SDH has been proposed.[21] A gene encoding an anchoring protein (CII-3) of the SDH enzyme has been identified in an intron of the SUR1 gene.

These proposed associations between the SDH enzyme complex and SUR1 taken together with the knowledge that SUR1 is required for DZX cardioprotection and the suggestion that the inhibition of SDH underlies the cardioprotection afforded by DZX,[3,16,17] led to the hypothesis for the current study. We hypothesized that the genetic deletion of the SUR1 would result in the loss of SDH activity.

METHODS

All animal procedures were approved by the Animal Studies Committee at Washington University School of Medicine and all animals received humane care in compliance with the National Institutes of Health's *Guide to Care and Use of Laboratory Animals*.[23]

Mitochondrial succinate dehydrogenase activity

Mitochondria were isolated from hearts of wild-type (WT) C57BL/6 mice and SUR1(−/−) mice. SUR1(−/−) mice were created by removal of the 1-kbp gene segment containing both promoter and exon 1 sequences of SUR1 gene by remediated recombination.[24] SUR1(−/−) mice were originally generated on a 129Sv background and then backcrossed >6× onto C57BL/6. Genotype was confirmed by polymerase chain reaction.[24]

Mice (either sex, 6 to 15 weeks old, average weight 25 g) were anesthetized with 3% Avertin (0.3 g 2,2,2 tribromoethanol, 1.86 μL 2-methyl-2-butanol, and 9.841 mL sterile water) intraperitoneally and rapid cardiectomy was performed. Ventricular tissue was rapidly minced and homogenized with a 7-mL Dounce homogenizer containing cold buffer (10 mM/L *N*-[2-hydroxyethyl]piperazine-*N*-[4-butanesulfonic acid]), 1 mM/L ethylenediamine tetraacetic acid potassium, and 250 mM/L sucrose, adjusted to a pH of 7.1 with 20% potassium hydroxide. The homogenate was transferred to microcentrifuge tubes and centrifuged at 900*g* for 10 minutes at 4°C. Supernatant was then centrifuged at 5,000*g* for 15 minutes. Supernatant was discarded and 300 μL homogenization buffer was added to each pellet. A Bradford protein assay (Thermo Scientific) was used to determine and normalize total protein per pellet. Mitochondria were stored in a −20°C freezer and thawed on ice just before use and kept on ice throughout each assay. All other solutions were kept at room temperature.

Mitochondria, at a concentration of 1.8 μg, were exposed to one of the following solutions (in a 1-mL reaction): 20 mM succinate (control) (Sigma) (n = 11 WT, n = 8 SUR1[−/−]), succinate + 1% dimethylsulfoxide (Sigma) (n = 10 WT, n = 8 SUR1[−/−]), succinate + 8 mM malonate (competitive inhibitor of SDH) (Sigma) (n = 11 WT, n = 8 SUR1[−/−]), and succinate + 100 μM DZX (K_{ATP} channel opener,