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Original article

Mice lacking sulfonylurea receptor 2 (SUR2) ATP-sensitive potassium channels are resistant to acute cardiovascular stress

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Abstract

Adenosine triphosphate-sensitive potassium (K_{ATP}) channels are thought to mediate the stress response by sensing intracellular ATP concentration. Cardiomyocyte K_{ATP} channels are composed of the pore-forming Kir6.2 subunit and the regulatory sulfonylurea receptor 2 (SUR2). We studied the response to acute isoproterenol in SUR2 null mice as a model of acute adrenergic stress and found that the episodic coronary vasospasm observed at baseline in SUR2 null mice was alleviated. Similar results were observed following administration of a nitric oxide donor consistent with a vasodilatory role. Langendorff-perfused hearts were subjected to global ischemia, and hearts from SUR2 null mice exhibited significantly reduced infarct size (54±4 versus 30±3%) and improved cardiac function compared to control mice. SUR2 null mice have hypertension and develop cardiac hypertrophy. However, despite longstanding hypertension, fibrosis was absent in SUR2 null mice. SUR2 null mice were administered nifedipine to block baseline coronary vasospasm, and hearts from nifedipine-treated SUR2 null mice exhibited increased infarct size compared to untreated SUR2 null mice (42±3% versus 54±3%). We conclude that conventional sarcolemmal cardiomyocyte K_{ATP} channels containing full-length SUR2 are not required for mediating the response to acute cardiovascular stress.

Keywords: Sulfonylurea receptor; K_{ATP} channel; Vasospasm; Myocardial infarction; Cardiac stress

1. Introduction

Found in nearly all tissues and cell types, adenosine triphosphate-sensitive potassium channels (K_{ATP}) serve as important sensors of cellular energetics [1,2]. K_{ATP} channels sense intracellular concentrations of ATP and ADP and thereby regulate membrane potential [3]. K_{ATP} channels are protein complexes composed of four Kir6.x (Kir6.1 or Kir6.2) and four sulfonylurea receptor (SUR1 or SUR2) proteins. In addition to the pore-forming Kir6.x and regulatory SUR subunits, multiple accessory proteins including adenylate kinase, creatine kinase, lactate dehydrogenase, and glyceraldehydes-3-phosphate dehydrogenase among others have been reported [4–6]. Distinct combinations of K_{ATP} subunits generate tissue and cell-type-specific K_{ATP} channels with unique regulatory properties [1]. In addition to varying subunit constitution, alternative spicing occurs at 3′ end of the *SUR2* gene results in two additional variants, SUR2A and SUR2B, that have differing carboxy-termini [7–9]. Cardiomyocyte K_{ATP} channels are composed of the Kir6.2 and SUR2A subunits [10,11], while K_{ATP} channels in the vascular smooth muscle are composed of the Kir6.1 and SUR2B subunits [1,9,12].

Cardiovascular K_{ATP} channels have important protective roles particularly during periods of increased stress [2]. Mice generated to lack the gene encoding Kir 6.2 (*KCNJ11*) are

Abbreviations: K_{ATP} channel, adenosine triphosphate-sensitive potassium channels; SUR, Sulfonylurea receptor; ECG, electrocardiogram; LVDP, left ventricular developed pressure.

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viable and lack K_{ATP} channel activity in cardiomyocytes. Kir6.2 null animals were shown to have an impaired cardiac response to both acute and chronic stress [13–16]. Kir6.2 null mice were unable to maintain an elevated cardiac output following isoproterenol challenge and when subjected to treadmill stress tests exhibited a survival disadvantage [13]. Isoproterenol challenge in Kir6.2 null mice also induced repolarization arrhythmias leading to ventricular arrhythmias and sudden death [13]. Exposing Kir6.2 null mice to chronic stress, either by swimming or volume overload, revealed an impaired cardiac response similar to acute stress studies [14,15]. Following 4 weeks of chronic exercise, Kir6.2 mice exhibited reduced survival, cardiac hypertrophy and reduced cardiac output [14]. Hypertension resulting from salt-induced volume overload also caused a similar adverse phenotype of impaired cardiac function, cardiac hypertrophy with fibrosis, and reduced survival [15]. Treatment of Kir6.2 null mice with verapamil, a calcium channel antagonist, ameliorated the adverse effects of stress suggesting that improper calcium handling by cardiomyocytes is an important contributor to cardiac pathology in stress. Finally, Kir6.2 null mice do not exhibit ischemic preconditioning, the phenomenon where brief episodes of ischemia paradoxically result in protection against later, more severe ischemic insults [17,18]. Both Kir6.2 null and control mice exhibited similar infarct size following ischemia, although no reduction of infarct size was observed following a preconditioning stimulus in Kir6.2 null mice [17].

We now studied stress response in mice lacking SUR2, the partner protein of Kir6.2 in cardiomyocytes. SUR2 (*ABCC9*) mutant mice were generated by genetic disruption of the exons 12–16 that encode the first nucleotide binding fold 1 domain [19]. Full-length SUR2 is normally expressed in cardiomyocytes, smooth muscle, skeletal muscle and endothelial cells. SUR2 null mice lack conventional, full-length SUR2 and glibenclamide-sensitive sarcolemmal K_{ATP} channel activity as measured by whole-cell patch clamp techniques in isolated cardiomyocytes and vascular smooth muscle cells [19,20]. SUR2 null mice exhibit an unusual phenotype of repetitive coronary artery spasm seen as ST segment elevation on radiofrequency telemetric monitoring. Episodes of vascular spasm may cluster and lead to arrhythmias and sudden death [20]. Coronary vasospasm in SUR2 null mice can be reduced by administering nifedipine, a calcium channel antagonist. In addition, SUR2 null animals exhibit hypertension [20]. These cardiovascular phenotypes likely result from loss of K_{ATP} channels from multiple tissues including cardiac, skeletal, and smooth muscle. In contrast to the stress intolerance seen in Kir6.2 null mice, we found that SUR2 null mice exhibit cardioprotection against sympathetic stress at baseline. Additionally, SUR2 mice have smaller myocardial infarcts compared to controls. Treatment with nifedipine to suppress vascular spasm abrogates ischemic cardioprotection implicating coronary artery spasm as a potential mediator of cardioprotection. Importantly, cardioprotection in SUR2 null mice occurs independent of sarcolemmal cardiomyocyte K_{ATP} channels.

2. Methods

2.1. Animals

SUR2 null mice were previously generated by targeted disruption of exons 12–16 encoding the nucleotide binding fold 1 as described previously [19]. Heterozygous SUR2 null mice were bred onto the FVB mouse substrain for more than 5 generations. Heterozygous mutant mice were interbred to generate homozygous null SUR2 animals. Male SUR2 null mice aged 12–18 weeks were used for experimental studies. Age-matched male wild-type FVB mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, Indiana, USA) as controls. Animals were housed, treated, and handled in accordance with guidelines set forth by the University of Chicago's Institutional Animal Care and Use Committee, the Animal Welfare Act regulations, and the NIH Guide for the Care and Use of Laboratory Animals.

2.2. ECG telemetry and data collection

Continuous ambulatory ECG recordings were obtained from SUR2 null and control mice using PhysioTel Implants (Model TA10EA-F20, Data Sciences International, St. Paul, Minnesota, USA) [20]. To implant monitors, isoflurane anesthesia was delivered at 3% during anesthesia induction and at 0.5% for maintenance via nose cone. Monitors were then surgically implanted subcutaneously on the dorsum and ECG leads tunneled subcutaneously into a Lead II configuration and sutured into place using 5/0 monofilament suture. Mice were allowed to recover for 24 h before baseline ECG data were collected.

Isoproterenol dose was determined following dosage experiments using 1, 5, and 10 μg/g and examining the sustained increase in heart rate under ECG telemetry. The 5 μg/g dose was chosen for consistently inducing tachycardia for more than 60 min. Baseline heart rate was established as the heart rate immediately prior to drug administration. The maximum heart rate and increase in heart rate (ΔHR) following injection were determined, and the length of time while the heart rate was greater than 1/2 ΔHR was defined as the tachycardic period. For experiments, monitors were implanted followed by a baseline recording period of 24 h. Then injections of isoproterenol (5 μg/g body mass delivered by intraperitoneal injection) or diethylenetriamine/nitric oxide adduct (DETA/NO, 120 μg/g body mass delivered by intraperitoneal injection) in phosphate-buffered saline were given. ECG data were collected throughout the experiment. The incidence and duration of each episode of coronary vasospasm during the screening periods were documented and reported as seconds of ST segment elevation per hour of screened ECG data.

2.3. Langendorff experiments

Mice were heparinized (0.5 U/g body mass, intraperitoneal injection) 30 min prior to surgical explant, and then anesthetized with inhaled 3% isoflurane. Hearts were rapidly excised and