Genetic Disruption of Kir6.2, the Pore-Forming Subunit of ATP-Sensitive K+ Channel, Predisposes to Catecholamine-Induced Ventricular Dysrhythmia

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Metabolic-sensing ATP-sensitive K+ channels (KATP channels) adjust membrane excitability to match cellular energetic demand. In the heart, KATP channel activity has been linked to homeostatic shortening of the action potential under stress, yet the requirement of channel function in securing cardiac electrical stability is only partially understood. Here, upon catecholamine challenge, disruption of KATP channels, by genetic deletion of the pore-forming Kir6.2 subunit, produced defective cardiac action potential shortening, predisposing the myocardium to early afterdepolarizations. This deficit in repolarization reserve, demonstrated in Kir6.2-knockout hearts, translated into a high risk for induction of triggered activity and ventricular dysrhythmia. Thus, intact KATP channel function is mandatory for adequate repolarization under sympathetic stress providing electrical tolerance against triggered arrhythmia. Diabetes 53 (Suppl. 3):S165–S168, 2004

RESEARCH DESIGN AND METHODS

Kir6.2-knockout mice. Mice deficient in Kir6.2 channels were generated by targeted disruption of the KCNJ11 gene, which encodes the pore-forming Kir6.2 subunit of the channel complex (12). Kir6.2-knockout mice were backcrossed for five generations into a C57BL/6 background. This investigation was approved by the Mayo Clinic Institutional Animal Care and Use Committee.

In situ aortic cannulation and Langendorff perfusion. Mice were anaesthetized with intraperitoneal injection of 2,2,2-tribromoethanol (0.375 mg/g body wt; Sigma), intubated, and ventilated, and the aortic root was cannulated in situ (10). Perfusion was sustained ex vivo on a Langendorff system, at 90 cm H2O with 37°C-prewarmed and 100% O2-bubbled Tyrode solution (in mmol/l: NaCl 137, KCl 5.4, CaCl2 2, MgCl2 1, HEPES 10, and glucose 10, pH 7.4 with NaOH). After a 10-min equilibration, KCl was reduced to 2.7 mmol/l, and MgCl2, to 0.5 mmol/l, with the atrioventricular node cauterized to allow ventricular pacing (13). Coronary flow was monitored with a T106 blood flow meter (Transonic Systems).

Electrogram and monophase action potential recordings. Orthogonal electrogram signals were simultaneously recorded using four silver-silver chloride electrodes surrounding the perfused heart in a simulated “Einthoven” configuration, and signals were amplified by an electrocardiographic amplifier (Gould Electronics). A catheter (NuMed) was placed in the left ventricular endocardium to pace the heart at twice diastolic threshold intensity with 2-ms pulse width and 100-ms cycle length using a pulse generator (A310 Accupuls; World Precision Instruments). Monophase action potentials were continuously recorded from the left ventricle by a probe (EP Technologies) positioned on the epicardial surface, and amplified signals (IsoDam; World

From the 1Division of Cardiovascular Diseases, Department of Medicine, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, Rochester, Minnesota; and the 2Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine. K, KATP; Kir6.2, and compared with the wild type. In KATP channel knockout hearts, sympathomimetic challenge unmasked an inadequate repolarization reserve predisposing to abnormal action potentials with afterdepolarizations and inducing ventricular dysrhythmia. Hence, KATP channels are required for electrical adaptation that protects against triggered arrhythmia within the adrenergically stressed myocardium.

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Whole-cell patch clamp recording from isolated cardiomyocytes. Cardiomyocytes were enzymatically dissociated from the ventricular myocardium (10). Action potentials were recorded at 30 ± 1°C from current-clamped isolated cells paced at 1 Hz, and were superfused with Tyrode solution (pH 7.2 adjusted with KOH) using the whole-cell patch clamp technique with 5–10 mol/l pipettes containing (in mmol/l) KCl 120, MgCl2 1, Na2ATP 5, HEPES 10, EGTA 0.5, and CaCl2 0.01 (14).

Statistics. Comparisons were made using the Student’s t test. A significance level of 0.05 was preselected. Data are reported as means ± SE.

RESULTS AND DISCUSSION

Whereas at baseline the action potentials were similar, the metabolic challenge of adrenergic stimulation induced distinct outcomes depending on the presence of functional KATP channels, with significant shortening of the action potential duration observed in wild-type hearts but not in age- and sex-matched counterparts lacking the Kir6.2 pore-forming channel subunit (Kir6.2-KO) (Fig. 1A and B).

After a 10-min perfusion with the sympathomimetic isoproterenol (1 μmol/l), monophasic action potential duration at 90% repolarization (APD90) shortened from 82 ± 2 to 74 ± 2 ms in wild-type hearts (P < 0.01, n = 6; Fig. 1A). In contrast, APD90 remained at 79 ± 3 and 80 ± 3 ms before and following isoproterenol treatment, respectively, in Kir6.2-KO hearts (n = 6) (Fig. 1B). This deficit in repolarization led to distorted action potential profiles with characteristic phase 3 early afterdepolarizations manifested as distinct humps in hearts lacking functional KATP channels (Fig. 1B and C). In all Kir6.2-KO hearts (n = 8), adrenergic challenge induced early afterdepolarizations, which occurred in 97 ± 2% of the action potentials examined (Fig. 1D). This is in contrast to the action potential profile of the wild type (n = 6) that maintained a smooth repolarization contour following isoproterenol challenge (Fig. 1A) without significant afterdepolarizations (1 ± 1%; P < 0.01 vs. Kir6.2-KO) (Fig. 1D).

FIG. 1. Isoproterenol challenge induced action potential shortening (APD90) in wild-type (WT) (A) but not Kir6.2-KO (B) hearts, which developed early afterdepolarizations (EAD) (C). D: Incidence of EAD in the initial 50 action potentials after 5 min of isoproterenol infusion.
Abnormal electrical response of Kir6.2-KO hearts under adrenergic challenge was not associated with an isoproterenol-induced deficit in coronary perfusion (Fig. 2A). In fact, abnormal electrical activity during repolarization observed at the whole-heart level was reproduced at the single-cell level using action potential recording in isoproterenol-stressed current-clamped Kir6.2-KO cardiomyocytes (Fig. 2B).

Afterdepolarizations in isoproterenol-challenged Kir6.2-KO hearts translated into increased electrical vulnerability (Fig. 3). In the absence of functional KATP channels, afterdepolarizations induced triggered activity, disrupting regular rhythm and manifesting as premature ventricular complexes on the electrogram (Fig. 3A). On average, isoproterenol-induced afterdepolarizations complicated by triggered activity were observed in six of eight Kir6.2-KO (75%) compared with one of six wild-type (16%) hearts, translating into a 16-fold higher risk (P < 0.05) of developing premature ventricular complexes (Fig. 3B).

Hence, absence of KATP channels produces a deficit in the repolarization reserve leading to a pronounced susceptibility of Kir6.2-KO hearts to isoproterenol-induced ventricular dysrhythmia. Thus, sarcolemmal KATP channels provide for membrane electrical stability that reduces the risk for arrhythmia under hyperadrenergic conditions.

Imposed catecholamine stress is a well-established precipitator of triggered activity and arrhythmia (15,16). Analogous to hearts with genetic and/or environmental compromise of repolarizing currents, as observed in congenital or acquired long QT syndrome (17), here isoproterenol challenge was proarrhythmic in the KATP channel-deficient myocardium provoking afterdepolarizations and triggered activity. This is in line with pharmacological studies that demonstrate that KATP channel activation with potassium channel openers prevents—whereas channel blockade with sulfonylurea drugs enhances—afterdepolarizations and triggered activity (18–20). In fact, in a recent randomized clinical trial in patients with type 2 diabetes, the sulfonylurea glyburide, but not the alternative oral hypoglycemic agent metformin, caused an increase in QT prolongation with QT dispersion on the electrocardiogram (21). Moreover, mutations in the cardiac sulfonylurea receptor inducing deficits in KATP channel function have been identified in patients with cardiomyopathy and ventricular arrhythmia (8).

Suppression of KATP channel activity, whether by genetic deletion of channel subunits or through use of channel antagonists, predisposes to inadequate calcium handling and intracellular calcium overload (5,9). In turn, excessive cytosolic calcium acts as a trigger for early depolarizations (22,23). Conversely, in the intact heart, where KATP channel opening is promoted by β-adrenergic-mediated phosphorylation of channel proteins (24,25) or subsarcolemmal ATP depletion (26), shortening of cardiac

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**FIG. 2.** A: Similar coronary flow in the absence and presence of isoproterenol (1 μmol/l) in wild-type (WT) and Kir6.2-KO hearts. B: Isoproterenol-induced abnormal repolarization with early afterdepolarization in an isolated current-clamped Kir6.2-KO cardiomyocyte.

**FIG. 3.** A: Kir6.2-KO hearts with early afterdepolarizations (EAD) demonstrated triggered activity on monophasic action potentials (MAP) and premature ventricular complexes (PVC) on electrograms (EG). For comparison, an EG is shown from wild-type hearts that are not prone to EAD-triggered activity/PVC. B: Incidence of triggered activity with accompanying PVC in the initial 50 action potentials following 5 min of isoproterenol infusion.
action potentials would balance catecholamine-induced increase in calcium influx protecting against triggered arrhythmia.

In summary, the present study underscores the homeostatic requirement for functional K<sub>ATP</sub> channels in the adaptation of cardiac repolarization under adrenergic stress. In this regard, a deficit in K<sub>ATP</sub> channel function is here identified as a previously unrecognized risk factor for the development of catecholamine-induced afterdepolarizations and triggered arrhythmia.

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