Kir6.2 is required for adaptation to stress

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Reaction to stress requires feedback adaptation of cellular functions to secure a response without distress, but the molecular order of this process is only partially understood. Here, we report a previously unrecognized regulatory element in the general adaptation syndrome. Kir6.2, the ion-conducting subunit of the metabolically responsive ATP-sensitive potassium (K_{ATP}) channel, was mandatory for optimal adaptation capacity under stress. Genetic deletion of Kir6.2 disrupted K_{ATP} channel-dependent adjustment of membrane excitability and calcium handling, compromising the enhancement of cardiac performance driven by sympathetic stimulation, a key mediator of the adaptation response. In the absence of Kir6.2, vigorous sympathetic challenge caused arrhythmia and sudden death, preventable by calcium-channel blockade. Thus, this vital function identifies a physiological role for K_{ATP} channels in the heart.

Ion channels control the electrical potential across the cell membrane of all living organisms. The profile of channel expression within the cell is defined by evolution through natural selection (1, 2). Developed as channel/enzyme multimers, K_{ATP} channels combine properties of two different classes of protein to adjust rapidly and precisely membrane excitability according to the metabolic state of the cell (3–7). Identified in metabolically active tissues of a broad range of species, K_{ATP} channels were discovered originally in heart muscle where they are expressed in high density (8, 9). Functional cardiac K_{ATP} channels can be formed only through physical association of the pore-forming Kir6.2 subunit with the regulatory sulfonylurea receptor SUR2A (10–12). In this complex, which harbors an intrinsic ATPase activity, nucleotide interaction at SUR2A gates potassium permeation through Kir6.2, a property believed to be responsible for the fine metabolic modulation of membrane potential-dependent cellular functions (7, 13–16).

The physiological role of K_{ATP} channels as metabolic sensors has been understood best in the regulation of hormone secretion in pancreatic β-cells and more recently in the hypothalamus (17–21). In the heart, definition of the function of this protein complex thus far has been limited to acute protection against ischemic events (22). In fact, under ischemia, the opening of as few as 1% of K_{ATP} channels is sufficient to produce significant shortening of the cardiac action potential (23), manifested globally by ST-segment elevation on the electrocardiogram (24). Yet, beyond the impact in pathophysiology, a physiological role for the cardiac K_{ATP} channel that supports its maintenance in hearts of many species is lacking (25).

The general adaptation syndrome is a ubiquitous reaction vital for self-preservation under conditions of stress such as exertion or fear (26–28). Mediated by a catecholamine surge, this syndrome generates an alteration of physiologic and biochemical functions to sustain a superior level of bodily performance and allows confrontation or escape in response to threat by the so-called fight-or-flight response (26–28). Execution of this metabolically demanding program without distress relies on an adequate escalation of cardiac function coupled with adjustment of feedback mechanisms to preserve cellular homeostasis (26–29). Yet, components of this homeostatic mechanism in the general adaptation syndrome have remained elusive.

Here, we demonstrate that functional K_{ATP} channels are required for adaptation to stress. Knockout (KO) of Kir6.2 disrupted an electrical feedback mechanism responsible for cellular calcium handling, thereby impairing cardiac performance and inducing lethal arrhythmia under vigorous sympathetic stimulation. Thus, this study identifies a physiological role for K_{ATP} channels in maintaining cardiac cellular homeostasis in the adaptive reaction to stress.

Materials and Methods

Kir6.2 KO. Mice deficient in K_{ATP} channels were generated by targeted disruption of the *Kir6.2* gene as described (30). Kir6.2-KO mice were backcrossed for five generations to a C57BL/6 background and compared with aged-matched C57BL/6 WT controls.

Treadmill Stress. A two-track treadmill (Columbus Instruments, Columbus, OH) was used to study male WT and Kir6.2-KO mice simultaneously (8–12 weeks old). The exercise-stress protocol consisted of stepwise increases in either incline or velocity at 3-min intervals. A shock grid at the end of the treadmill delivered a mild painful stimulus to enforce running. Ten days before exercise stress, mice were acclimated daily for 45 min on a nonmoving treadmill followed by 15 min at a velocity of 3.5 m/min. Animal protocols were approved by the Institutional Animal Care and Use Committee at the Mayo Clinic.

***In Vivo* Hemodynamics.** Ventricular pressure measurements were obtained online with a 1.4-F micropressure catheter (SPR-671, Millar Instruments, Houston) positioned in the left ventricle in anesthetized (2.5% 2,2,2-tribromoethanol 0.18–0.15 ml/g of body weight), ventilated, open-chest mice before and after isoproterenol administration. Signals were digitized at 11.8 kHz, and left ventricular-developed pressure (LVDP) was calculated as the difference between maxima and minima of the pressure waveform. Applying an electrocardiogram-triggered fast-gradient echo cine sequence, magnetic resonance imaging (31) with a 7T scanner (Bruker, Billerica, MA) was performed on isofluorane (2%)-anesthetized mice through a short-axis slice of 1.0-mm thickness at the midpapillary muscle level. End-diastolic and end-systolic slice volumes obtained by using volumetric analysis (Analyze, Mayo Clinic, Rochester, MN) were used to calculate ejection fraction [EF = (end-diastolic volume − end-systolic volume)/end-diastolic volume].

Electrophysiology and Contractility. Excised hearts were perfused retrogradely with Krebs–Henseleit buffer (118 mM NaCl/4.7

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Abbreviations: KO, knockout; LVDP, left ventricular-developed pressure; LVP, left-ventricular pressure.

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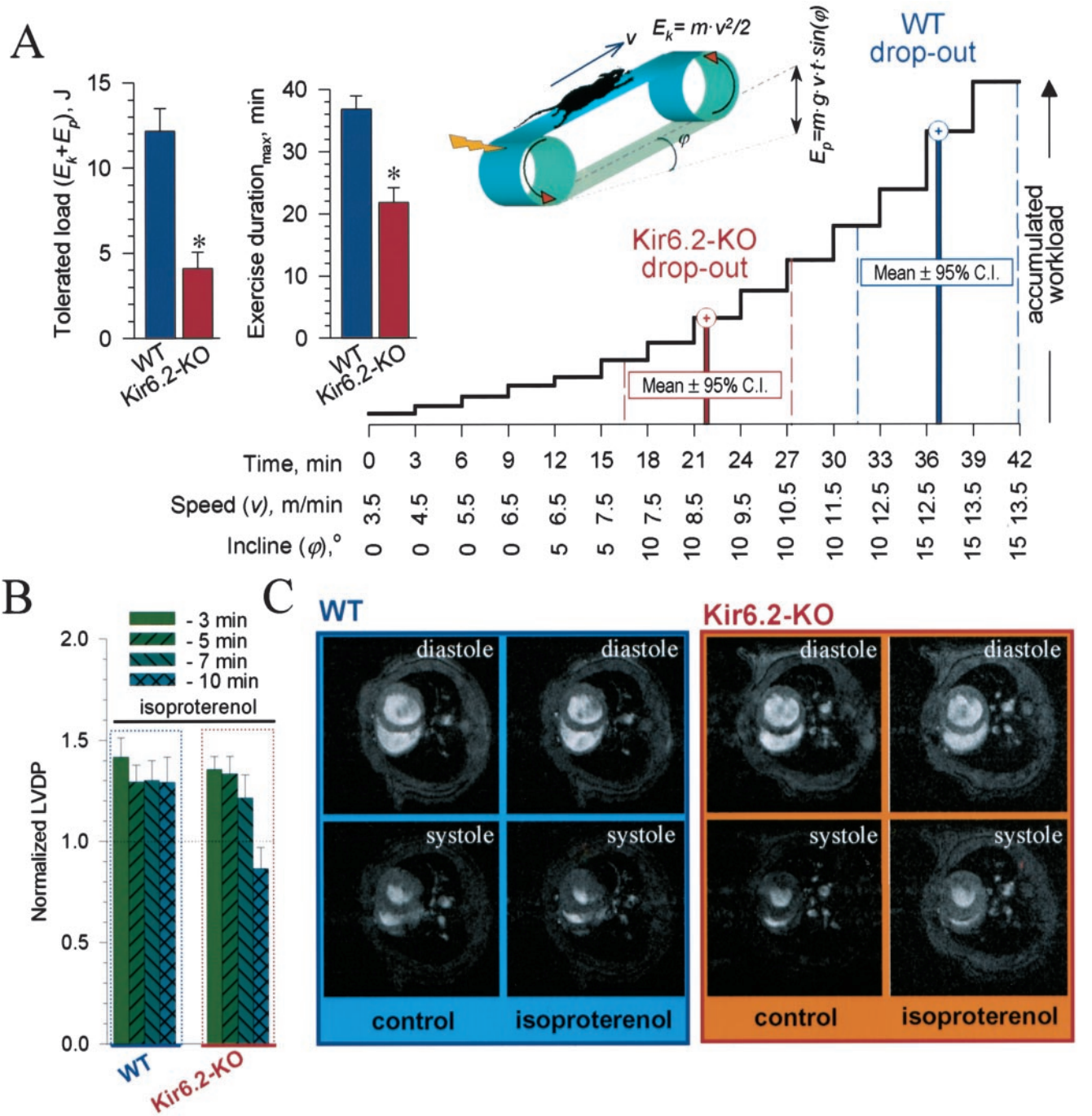

Fig. 1. (*A*) Under the treadmill exercise-stress test, with stepwise escalating velocity and incline, K_{ATP} channel-deficient (Kir6.2-KO) mice demonstrated significantly lower exercise tolerance compared with the WT mice. This manifested in lower tolerated workload and a shorter endured time of exercise stress. Workload was defined as a sum of kinetic ($E_k = m \cdot v^2/2$) and potential ($E_p = m \cdot g \cdot v \cdot t \cdot \sin\varphi$) energy of the mice on the treadmill, where m is animal mass, v is running velocity, g is acceleration due to gravity, t is elapsed time at a given protocol level, and φ is the angle of incline. The point of drop-out was defined by failure to sustain performance at a given workload level. (*B*) Change in LVDP [LVDP = LVP_{max} − left-ventricular pressure $(LVP)_{min}$] relative to baseline in response to the β-sympathomimetic isoproterenol (5 μg/kg, i.p.) measured in anesthetized WT and Kir6.2-KO mice. (*C*) End-diastolic (*Upper*) and end-systolic (*Lower*) magnetic resonance images in a midventricular slice acquired at rest (*Left*) and after isoproterenol injection (5 μg/kg, i.p.; *Right*) in intact, anesthetized, WT and Kir6.2-KO mice. Note that Kir6.2-KO shows a larger residual end-systolic volume than WT in the presence of isoproterenol.

mM KCl/1.2 mM $MgSO_4$/1.2 mM KH_2PO_4/0.5 mM Na-EDTA/25 mM $NaHCO_3$/2.5 mM $CaCl_2$/11 mM glucose, pH = 7.4, bubbled with 95%O_2/5% CO_2 at 37°C) and paced with a pacing catheter (NuMed, Hopkinton, NY) at 100-ms cycle length (A310 Accupulser, World Precision Instruments, Sarasota, FL). A monophasic action-potential probe (EPTechnologies, San Jose, CA) was placed on the left-ventricular epicardium, and amplified signals (IsoDam, World Precision Instruments) were acquired at 11.8 kHz. A fluid-filled balloon attached to a pressure transducer (Harvard Apparatus) was placed in the left ventricle, and diastolic pressure was set to 10 mmHg (1 mmHg = 133 Pa). Signals were acquired at 1 kHz.

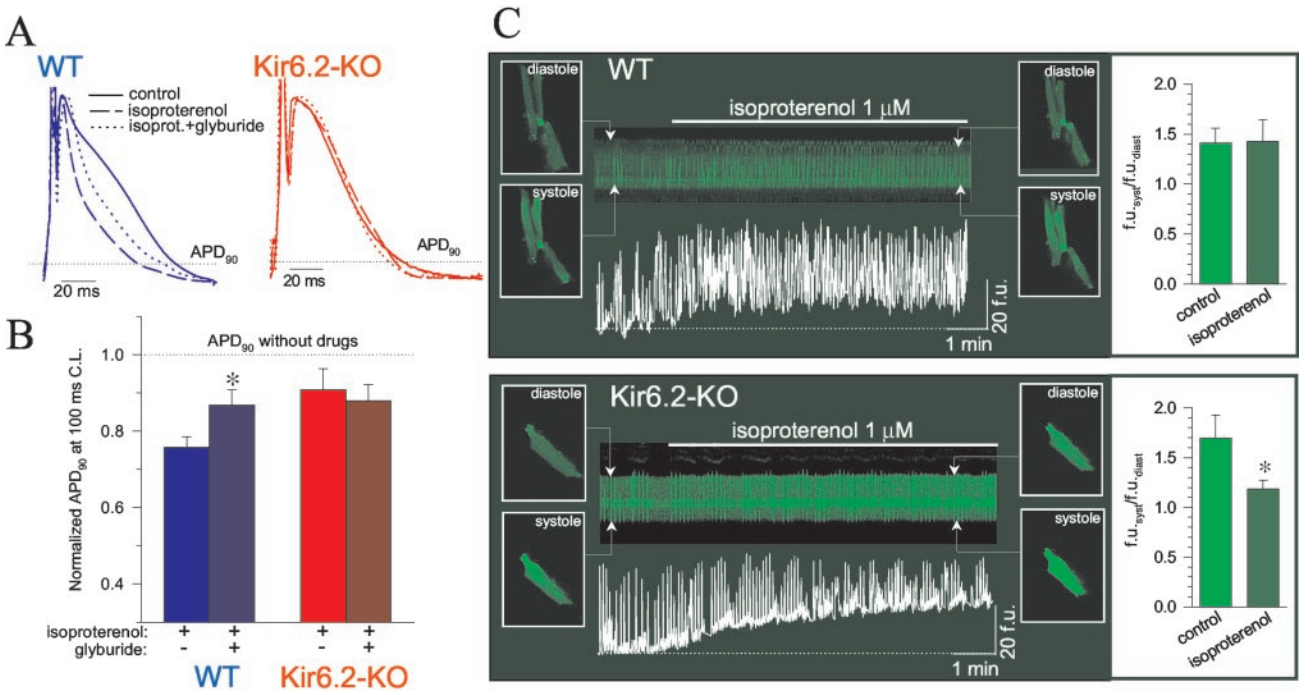

Fig. 2. Distorted control of action potential duration associated with Ca^{2+} overload in K_{ATP} channel-deficient heart under β-adrenergic stimulation. (*A*) Representative monophasic action potential tracings in hearts paced at 600 per min (cycle length, C.L. = 100 ms). In WT but not in Kir6.2-KO there was marked shortening at 90% repolarization (APD_{90}) in response to a 5-min long exposure to isoproterenol (500 nM), an effect partially reversed by glyburide (10 μM), a K_{ATP} channel blocker. (*B*) Average changes of APD_{90} for WT and Kir6.2-KO hearts perfused with 500 nM isoproterenol and in the additional presence of glyburide (10 μM) are presented normalized to respective baselines. In WT, isoproterenol induced a significant reduction of APD_{90} ($P < 0.05$), with a significant glyburide-sensitive component (*, $P < 0.05$), whereas in Kir6.2-KO, the isoproterenol-induced shortening of APD_{90} was modest ($P < 0.05$) and was not sensitive to glyburide ($P > 0.05$). (*C*) Cardiomyocytes loaded with the Ca^{2+}-sensitive probe, Fluo3-AM (3 μM), were paced at a frequency of 1 Hz. In WT, isoproterenol induced an increase in Ca^{2+} transients with a preserved systolic/diastolic Ca^{2+} ratio. In the Kir6.2-KO, excessive Ca^{2+} accumulation observed in diastole after isoproterenol challenge resulted in a decrease of the systolic/diastolic ratio. (*Insets*) Cardiomyocytes in systole and diastole, with disrupted Ca^{2+} handling in the Kir6.2-KO, manifested in excessive Ca^{2+}-induced fluorescence in particular during diastole compared with the WT. Green traces represent Ca^{2+}-induced fluorescence levels as a function of time obtained in a transverse plane of single cells. White traces are deconvolutions of corresponding fluorescent frames. Bar graphs are average values for WT ($n = 5$) and Kir6.2-KO ($n = 5$).

Calcium Imaging. Two-dimensional confocal images (Zeiss LSM 510 Axiovert) of cardiomyocytes, isolated with collagenase type 4 (2,200 units/100 ml) and loaded with the Ca^{2+}-fluorescent probe Fluo3-AM (Molecular Probes), were acquired by scanning 256 × 256 pixels per image every 328 ms with the 488-nm line of an argon/krypton laser. Images were deconvoluted and analyzed by using METAMORPH software (Visitron Universal Imaging, Downingtown, PA).

Telemetry. Telemetry devices (Data Sciences International, St. Paul, MN) were implanted in the peritoneum, and leads were tunneled s.c. in a lead II configuration under isofluorane anesthesia. Mice recovered for ≥2 weeks before experiments. Electrocardiogram signals were acquired at 2 kHz. The QT interval was defined as the time from start of the Q wave to the end of the T wave of the electrogram. Corrected for heart rate, the QT interval (QT_c) was calculated by using the Mitchell transformation [$QT_c = QT/(RR/100)^{0.5}$], where *RR* (in ms) is the interval between previous and current R wave (32).

Nucleotide Content. Adenine nucleotide levels were determined in 0.6 M perchloric acid/1 mM EDTA extracts from liquid N_2 freeze-clamped hearts (33). Extracts were neutralized with 2 M K_2HCO_3, and nucleotides were profiled by high-performance chromatography (HP 1100, Hewlett–Packard) with a MonoQ HR5/5 column (Amersham Pharmacia). Nucleotides were eluted with a linear gradient of triethylammonium bicarbonate buffer (34).

Statistical Analysis. Values are expressed as mean ± SD in the text and as mean ± SE in the figures. Comparisons within groups were made by using the paired Student's *t* test and between groups by using analysis of variance (ANOVA).

Results and Discussion

Deletion of Kir6.2 Compromises Exertional Capacity. Physical stress such as exercise is a natural trigger of the general adaptation syndrome (28). Under the exercise-stress test, mice in which the ion-conducting Kir6.2 subunit was deleted genetically to disrupt the K_{ATP} channel (Kir6.2-KO) performed at a significantly reduced level than age- and gender-matched WT controls (Fig. 1*A*). The tolerated workload, a parameter that incorporates time of effort with speed and incline of the treadmill, was more than 3-fold lower in Kir6.2-KO ($n = 10$) compared with the genetically intact animals ($n = 10$, $P < 0.05$; Fig. 1*A*). Thus, functional K_{ATP} channels are required to secure an optimal stress-adaptation capacity of the organism.

Impaired Adaptive Cardiac Response in the Kir6.2-KO. Fundamental to general adaptation to stress is augmentation of cardiac performance driven by the sympathetic system in support of the body's escalated metabolic requirement (27, 29). *In vivo*, direct ventricular pressure measurements revealed that under adrenergic stress, a lack of functional K_{ATP} channels compromised the ability of the heart to sustain performance (Fig. 1*B*). LVDP was similar between Kir6.2-KO ($n = 5$) and WT ($n = 5$) at baseline (114 ± 20 and 110 ± 20 mmHg, respectively, $P > 0.05$). However,

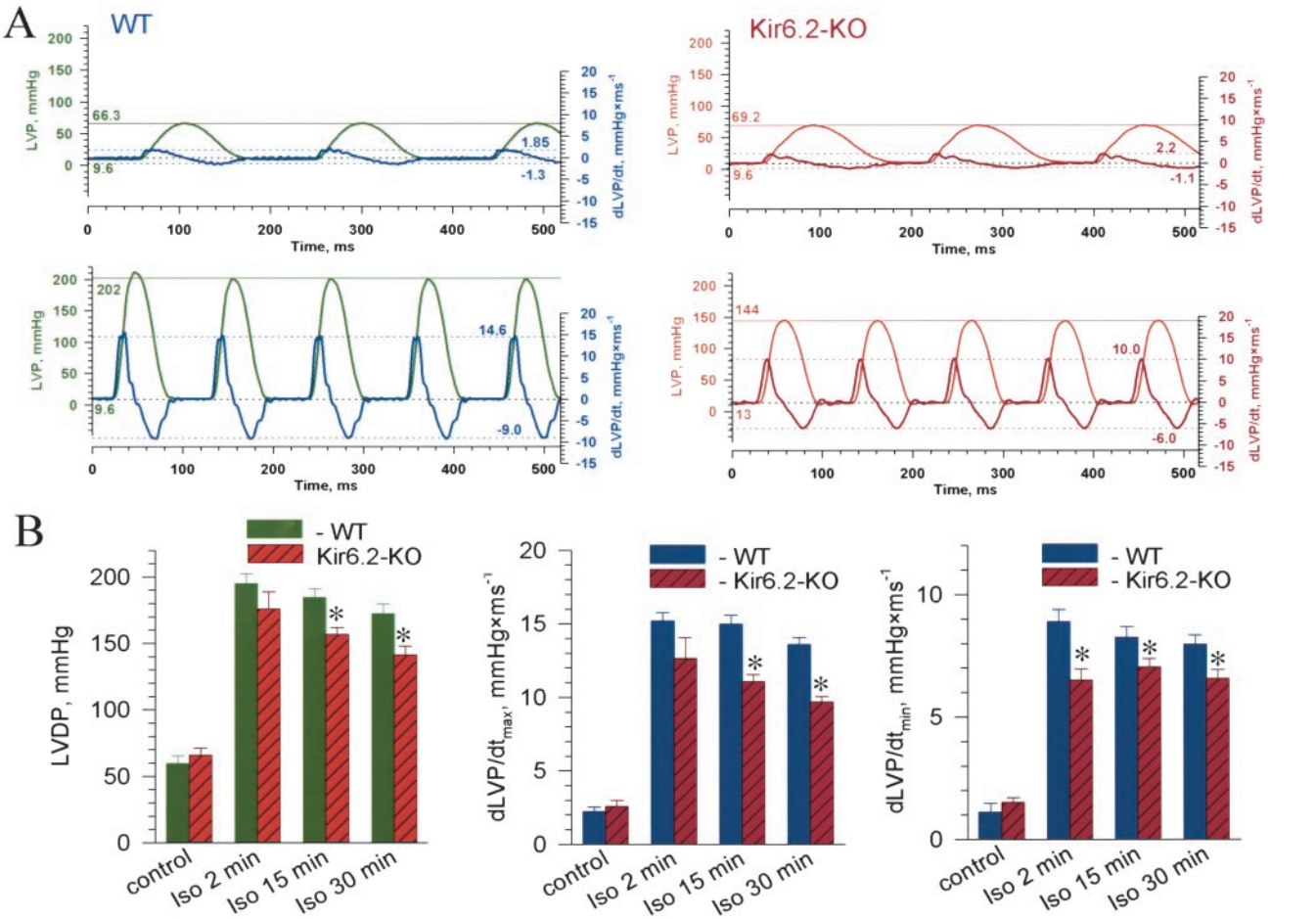

Fig. 3. Aberrant contractile mechanics in K_{ATP} channel-deficient hearts during sympathetic stimulation. (*A*) LVP (*Left*, *y* scale) and corresponding first derivative (dLVP/dt, *Right*, *y* scale) in WT and Kir6.2-KO isolated and retrogradely perfused hearts before (*Upper*) and 15 min after (*Lower*) application of isoproterenol (500 nM). (*B*) Kir6.2-KO hearts were unable to maintain LVDP at the same level as WT hearts under β-adrenoreceptor stimulation. Although not significantly different at baseline, at 15 min and beyond of isoproterenol perfusion, LVDP, dLVP/dt$_{max}$, and dLVP/dt$_{min}$ were significantly lower in Kir6.2-KO than WT ($P < 0.05$).

although the WT maintained the response to β-adrenergic stimulation by isoproterenol at 30% above baseline, the LVDP of mice lacking K_{ATP} channels gradually declined to 14% below baseline at 10 min post-isoproterenol ($P < 0.05$; Fig. 1*B*). Furthermore, *in vivo* magnetic resonance microimaging showed a differential response of ejection fraction to isoproterenol by 11 min, with WT increasing by 4 ± 2% (from a baseline of 65 ± 5%, $n = 3$) and the Kir6.2-KO decreasing by 11 ± 2% (from a baseline of 72 ± 4%, $n = 3$) in 3 of 4 anesthetized mice (Fig. 1*C*). Thus, K_{ATP} channels are essential in securing cardiac function in the animal under sympathetic stimulation, a central mediator of the general adaptation syndrome.

Defective Control of Cardiac Membrane Excitability Disrupts Ca^{2+} Handling in the Kir6.2-KO. The adrenergic-induced increment in cardiac work imposes a significant demand on cardiac metabolic resources, mostly due to energy-consuming Ca^{2+} handling (35, 36). To prevent cellular Ca^{2+} overload and associated energy depletion, increased Ca^{2+} influx is normally balanced by a compensatory increase in outward ion currents (37, 38). Such a protective feedback mechanism is found here to be defective in the K_{ATP} channel-deficient myocardium, which in contrast to WT displayed less shortening of the action potential after β-adrenoreceptor stimulation at a fixed heart rate (Fig. 2*A*). On average, the monophasic action potential duration at 90% repolarization (APD_{90}), which was not significantly different between groups at baseline, was decreased by 15 ± 4 ms in the WT ($n = 5$) versus 5 ± 4 ms in the Kir6.2-KO ($n = 8$) heart ($P < 0.05$; Fig. 2*B*), indicating a K_{ATP} channel-dependent component in isoproterenol-mediated action potential shortening (39). Indeed, shortening of the action potential duration was reversed after K_{ATP}-channel blockade in WT (by 7 ± 5 ms; $P < 0.05$) but not in Kir6.2-KO hearts (Fig. 2 *A* and *B*). Accordingly, although β-adrenergic stimulation did not perturb the systolic/diastolic Ca^{2+} ratio in WT cardiomyocytes, in Kir6.2-KO cardiac cells catecholamine challenge produced diastolic Ca^{2+} loading with reduction of the systolic/diastolic Ca^{2+} ratio ($P < 0.05$; Fig. 2*C*). Thus, under sympathetic stress, a lack of Kir6.2 disrupts K_{ATP} channel-dependent control of action potential duration responsible for maintenance of cellular Ca^{2+} handling.

Depleted Functional Reserve of Kir6.2-Deficient Heart Muscle. Consistent with Ca^{2+} overload, impeded contraction and relaxation were observed in the K_{ATP} channel-deficient isolated heart (Fig. 3*A*). Parallel to defects found in the whole animal (Fig. 1 *B* and *C*), LVDP decreased significantly in Kir6.2-KO (176 ± 13 to 157 ± 5 mmHg, $n = 8$) but not WT hearts (195 ± 7 to 185 ± 6 mmHg, $n = 8$) by 15 min into β-adrenergic stimulation (Fig. 3*B*). Simultaneously, rates of contraction (dLVP/dt$_{max}$) and relaxation (dLVP/dt$_{min}$) were significantly reduced in Kir6.2-KO (11 ± 1 and 7.0 ± 0.3 mmHg/ms, respectively) compared with WT (15 ± 1 and 8.3 ± 0.4 mmHg/ms, respectively) hearts. Thus,

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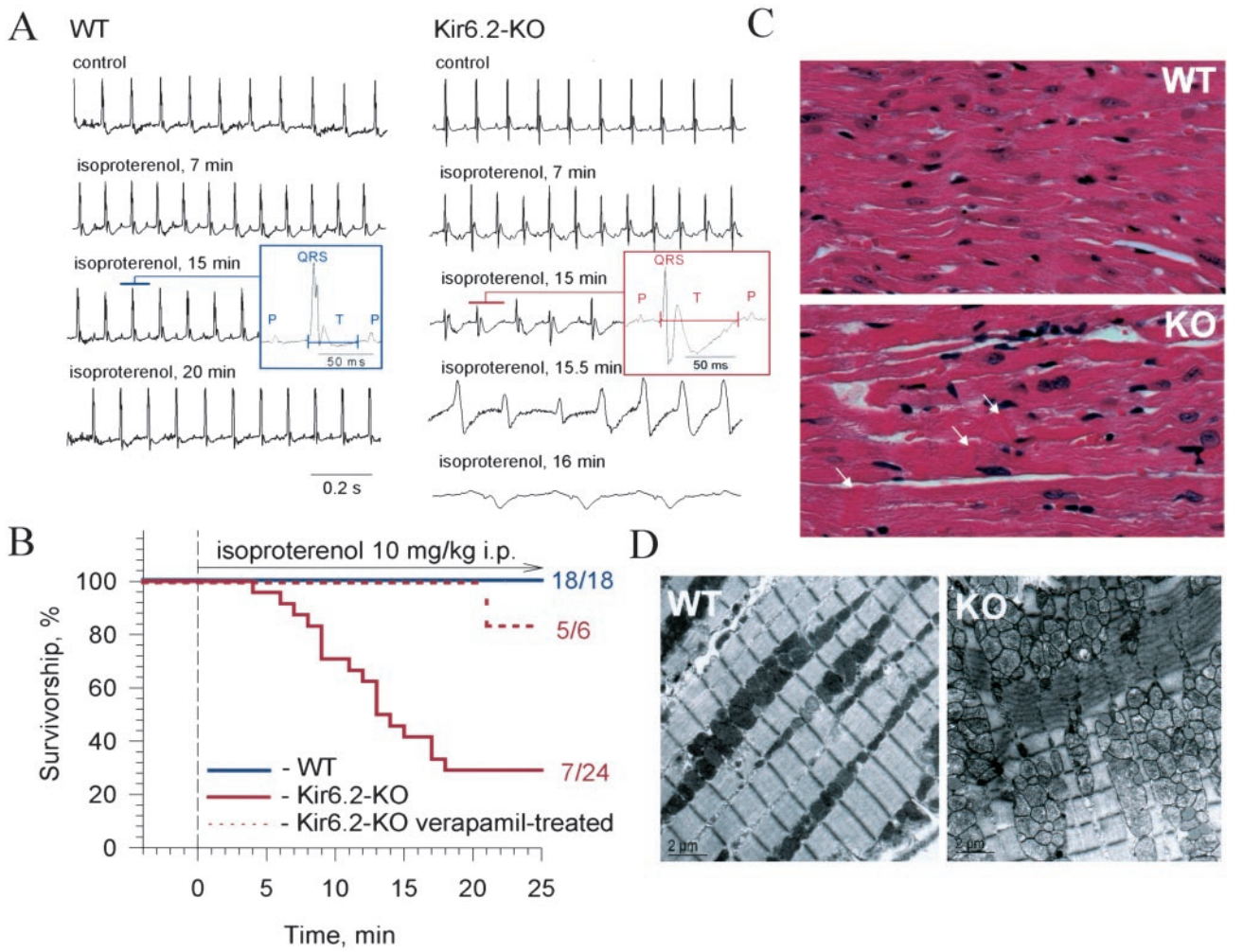

Fig. 4. K_{ATP} channels are required for cardiac electrical stability and survival under sympathetic challenge. (*A*) Telemetric recordings of cardiac electrograms in chronically instrumented mice show maintained sinus rhythm in the WT in contrast to aberrant repolarization with development of ventricular arrhythmia in the Kir6.2-KO after isoproterenol (5 mg/kg, i.p.) stress. Note that in Kir6.2-KO the third trace (15 min) shows 1:1 atrio-ventricular conduction with a prolonged QT interval, the fourth trace (15.5 min) ventricular arrhythmia, and the fifth trace (16 min) agonal rhythm. (*Insets*) Representative cardiac cycles from third tracings with waveforms indicated by letters,and measured QT interval by brackets. (*B*) Survival plots after isoproterenol challenge (10 mg/kg, i.p.) reveal no mortality in the WT ($n = 18$) versus a 73% mortality in the Kir6.2-KO ($n = 24$). The calcium channel blocker, verapamil (3 μg/kg), reduced mortality to 17% in Kir6.2-KO mice ($n = 6$). (*C*) Photomicrographs of sectioned, hematoxylin/eosin-stained, left-ventricular myocardium from 5 to 10 mg/kg, i.p., isoproterenol-challenged (15–45 min) mice. Contraction bands (*arrows*) are observed in Kir6.2-KO but not WT hearts. (*D*) Corresponding electron microscopy (×10,000) shows a band of shortened sarcomeres with typical mitochondrial swelling in KO but not WT.

in the heart the K_{ATP} channel is an essential component of the adaptive homeostatic mechanism responsible for maintenance of enhanced cardiac performance.

Kir6.2 Required for Survival Under Vigorous Adrenergic Stress. At isoproterenol concentrations used in experimental models of extreme β-adrenergic stress (40), Kir6.2-KO animals were unable to withstand the sustained and metabolically demanding increase in heart rate and contractility (Fig. 4*A*). Deletion of Kir6.2 resulted in aberrant myocardial repolarization, defined as prolongation of the QT_c interval on the electrocardiogram (from 55 ± 3 to 64 ± 4 ms in Kir6.2-KO, $n = 3$; $P < 0.05$), and inconsistent atrio-ventricular conduction, seen as variable PR intervals, immediately before development of ventricular arrhythmia (Fig. 4*A*). Indeed, inappropriate repolarization favors ventricular ectopy and reentry, precipitating fatal arrhythmia (38, 41). There was no prolongation of repolarization or ventricular arrhythmia in the WT ($n = 3$; Fig. 4*A*). Thus, intact K_{ATP} channels contribute to the maintenance of myocardial electrical stability under stress. In fact, sudden death occurred in over 70% of K_{ATP} channel-deficient animals but in none of the WT mice (Fig. 4*B*). Myocardial contraction bands (Fig. 4 *C* and *D*), a pathological finding associated with catecholamine-induced Ca^{2+} overload (42), were present in Kir6.2-KO mice [12 ± 3 bands/10 high-powered (×40) fields; $n = 4$] but were essentially absent in the WT mice (0.8 ± 0.5/10 high-powered fields, $n = 4$; $P < 0.05$). Calcium-channel blockade prevented or delayed death in Kir6.2-KO animals (Fig. 4*B*) and maintained normal tissue structure.

Thus, deletion of Kir6.2, the pore-forming core of the K_{ATP} channel complex, generated a maladaptive phenotype with increased vulnerability under stress manifested by aberrant regulation of cardiac membrane excitability, inadequate calcium handling, and ultimately ventricular arrhythmia and death. In diabetic patients, increased cardiovascular mortality (43, 44) and reduced exercise capacity (45) have been reported with sulfonylureas, and cardiovascular risk remains a therapeutic concern with these conventional K_{ATP} channel blockers (46).

Table 1. Cardiac adenine nucleotide content at baseline and after stress

	Baseline, $n = 4/6$					Exercise, $n = 6/8$					Isoproterenol, $n = 5/5$				
	ATP	ADP	AMP	Total	ATP/ADP	ATP	ADP	AMP	Total	ATP/ADP	ATP	ADP	AMP	Total	ATP/ADP
WT	32.7 ± 4.3	6.7 ± 2.4	2.6 ± 2.0	42.0 ± 2.1	5.7 ± 3.2	28.2 ± 2.9	7.2 ± 1.3	2.4 ± 1.3	37.8 ± 3.4	4.0 ± 0.9	28.0 ± 7.4	6.2 ± 2.8	1.9 ± 0.9	36.2 ± 9.3	5.0 ± 1.7
Kir6.2-KO	31.0 ± 10.2	5.3 ± 2.6	2.1 ± 2.2	38.5 ± 9.6	7.3 ± 3.8	26.6 ± 2.0	7.0 ± 1.4	2.1 ± 0.5	35.7 ± 6.3	3.9 ± 0.9	27.0 ± 10.2	5.4 ± 1.3	4.3 ± 4.0	36.6 ± 7.8	5.1 ± 2.9

Exercise stress was conducted using the treadmill protocol and upon drop-out hearts excised and freeze-clamped from isoflurane-anesthetized mice. Isoproterenol stress was induced in isolated hearts that were freeze-clamped at 15 min of isoproterenol (500 nM) perfusion. Total is the sum of ATP, ADP, and AMP. Concentrations are expressed as nmol/mg protein. Data are mean ± SD, and *n* represents the number of WT/Kir6.2-KO hearts.

The mechanism underlying K_{ATP} channel response to physiological stress is yet to be established. Here, under exercise and isoproterenol challenge, no major shift in bulk adenine nucleotide content was captured (Table 1), suggesting K_{ATP} channel gating may have been influenced by β-adrenergic-induced cAMP-dependent phosphorylation of channel proteins (47, 48) or by depletion of ATP in the subsarcolemmal compartment as a consequence of adenylate cyclase activation (39). In fact, in the compartmentalized cardiac cell, nucleotide-dependent K_{ATP} channel gating is highly responsive to metabolic fluctuations in the channel microenvironment (49–51). Moreover, channel gating can be modulated by the sarcolemmal phospholipid, phosphatidylinositol 4,5-bisphosphate (5, 52), proposed to increase under augmented cardiac workload (53).

In summary, K_{ATP} channels in the heart are demonstrated to serve the physiological role of homeostatic control in adaptation to stress, allowing execution of this fundamental response without distress. This homeostatic function of cardiac K_{ATP} channels provides a new perspective on molecular events underlying the general adaptation syndrome.

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