Tolerance for ATP-Insensitive $K_{ATP}$ Channels in Transgenic Mice

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Abstract—To examine the role of sarcolemmal $K_{ATP}$ channels in cardiac function, we generated transgenic mice expressing GFP-tagged Kir6.2 subunits with reduced ATP sensitivity under control of the cardiac $\alpha$-myosin heavy chain promoter. Four founder mice were isolated, and both founders and progeny were all apparently normal and fertile. Electrocardiograms from conscious animals also appeared normal, although mean 24-hour heart rate was approximately 10% lower in transgenic animals compared with littermate controls. In excised membrane patches, $K_{ATP}$ channels were very insensitive to inhibitory ATP: mean $K_{1/2}$ ([ATP] causing half-maximal inhibition) was 2.7 mmol/L in high-expressing line 4 myocytes, compared with 51 $\mu$mol/L in littermate control myocytes. Counterintuitively, $K_{ATP}$ channel density was $\approx$4-fold lower in transgenic membrane patches than in control. This reduction of total $K_{ATP}$ conductance was confirmed in whole-cell voltage-clamp conditions, in which $K_{ATP}$ was activated by metabolic inhibition. $K_{ATP}$ conductance was not obvious after break-in of either control or transgenic myocytes, and there was no action potential shortening in transgenic myocytes. In marked contrast to the effects of expression of similar transgenes in pancreatic $\beta$-cells, these experiments demonstrate a profound tolerance for reduced ATP sensitivity of cardiac $K_{ATP}$ channels and highlight differential effects of channel activity in the electrical activity of the 2 tissues. (Circ. Res. 2001:89:E11-E13.)

Key Words: $K^+$ current ■ $K_{ATP}$ ■ transgenic ■ electrocardiogram

ATP-sensitive $K^+$ ($K_{ATP}$) channels are octameric complexes of 2 distinct subunits, a sulfonylurea receptor (SUR) and a pore-forming Kir6.2.2-17 SUR confers high-affinity block by sulfonylureas and stimulation by $K^+$ channel openers (PCOs) and MgADP.2,8-13 ATP inhibits channel activity through an interaction with the Kir6.2 subunit.3,14 –16 Reconstitution experiments indicate that essential properties of the cardiac $K_{ATP}$ channel are reiterated by coexpression of Kir6.2+$\alpha$SUR2A channels,2,17 and $K_{ATP}$ channels are completely absent in myocyte membranes of Kir6.2 knockout animals.18,19 although antisense oligonucleotide experiments confirm that SUR1 might be a constituent of neonatal ventricular $K_{ATP}$ channels.20 Complete metabolic inhibition, or anoxia, leads to opening of $K_{ATP}$ channels in the sarcolemmal membrane,21 and channel density is so high21,22 that maximal activation of $K_{ATP}$ can block cell-to-cell conductance and completely suppress action potential generation.23,24 Both experimental data25-28 and modeling25,29 indicate that activation of only 1% of the available $K_{ATP}$ conductance will be sufficient to shorten the action potential $\approx$50%. For normal cardiac function, therefore, it is critically important that $K_{ATP}$ channels remain substantially closed. Consistent with this notion, action potentials are unaltered in myocytes that completely lack $K_{ATP}$ channels.18 The major mechanism keeping the channels closed under normal conditions is expected to be the high ATP sensitivity: $K_{1/2}$ (cytoplasmic [ATP] causing half-maximal inhibition) is normally 25 to 100 $\mu$mol/L,22,30,31 whereas intracellular [ATP] is normally 4 to 10 mmol/L.32

Significant reduction of ATP sensitivity is expected to cause action potential shortening at normal [ATP]. Consistent with this general prediction, transgenic expression of mutant $K_{ATP}$ channels with only $\approx$5-fold reduction of ATP sensitivity in pancreatic $\beta$-cells (which contain $K_{ATP}$ channels at similar density and with similar ATP sensitivity to cardiac myocytes) leads to profound neonatal diabetes and death within 1 week due to $K_{ATP}$-induced suppression of excitability and insulin secretion.33 In the present study, we generated transgenic mice that express ATP-insensitive Kir6.2 subunits in the myocardium. Strikingly, these mice tolerate expression of $K_{ATP}$ channels with $\approx$40-fold reduction in ATP sensitivity, without gross physiological or morphological defects. Patch-clamp analysis reveals the expected channel phenotype in sarcolemmal $K_{ATP}$ channels, with ATP sensitivity in the millimolar range in excised patches. However, contrary to predictions, channels are not active in intact cells, and with
the exception of a slight reduction in heart rate (<10%), there is no obvious difference in cardiac electrical activity. These results indicate that metabolic regulation of K<sub>ATP</sub> channels in the heart and pancreas may be very different, pointing to significant effects of cellular metabolism or sulfonylurea receptor subtype in this regulation.

Materials and Methods

Construction of Mutant Kir6.2 Constructs

The mutant Kir6.2[ΔN,K185Q]-GFP construct (see Figure 1 for details), tagged at the C-terminus with green fluorescent protein (GFP), was prepared by deletional mutagenesis using polymerase chain reaction (PCR). The product was subcloned into pCMV6b, and nucleotide sequences were verified by fluorescence-based cycle sequencing using AmpliTaq DNA polymerase. To confirm functional expression, mutant Kir6.2 subunits were coexpressed with SUR2A in COSm6 cells using transient transfection, as described previously.34

Generation of Transgenic Mice

All procedures complied with the standards for the care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996). DNA constructs were inserted downstream of the αMHC promoter in the transgenic plasmid pαMHC. The expression cassette was excised by enzyme digestion, purified, and microinjected into fertilized eggs of C57Bl6×CBA mice, according to standard techniques, in the Washington University Neuroscience Transgenic Facility. Transgenic mice were identified by PCR on mouse-tail DNA using GFP-specific oligonucleotide primers. Four Kir6.2[ΔN,K185Q]-GFP founder transgenic mice were identified and bred to homogeneity by multiple (6×3) back-crossing to C57Bl6 mates. Of the four lines that were generated, transgene expression was moderate in lines 1 through 3 and highest in line 4. Accordingly, analysis was concentrated on moderate-expressing line 2 and high-expressing line 4 animals, and all presented data are from these animals.

Northern Blot Analysis

Total cellular RNA was isolated by guanidinium extraction using the RNAzol B method (Tel-Test Inc). Total RNA from a single heart (15 mg) was denatured in formaldehyde and electrophoresed on a 1% agarose gel. After transfer to nylon membrane (Hybond-N, Amersham Pharmacia Biotech), hybridization was performed in Rapid-hyb buffer (Amersham Pharmacia Biotech) with <sup>32</sup>P-labeled SUR2A cDNA fragment (nucleotides +1 to 1800 relative to the translation start site) and the entire Kir6.2[ΔN,K185Q]-GFP DNA construct (1.8 kb) as probes. β-Actin expression was assessed and used to normalize for RNA loading. Membranes were washed repeatedly according to manufacturers recommendation (Amersham Pharmacia Biotech): 2×SSC, 0.1% (wt/vol) SDS, and 0.1×SSC, 0.1% (wt/vol) SDS.

Figure 1. Transgenic Kir6.2 channel constructs. A, Kir6.2 contains 2 transmembrane segments, M1 and M2, a pore-forming H5 segment, and cytoplasmic N' and C' termini. Transgenic mutations are indicated, GFP was fused to the C' terminus. B, Representative K<sup>+</sup> current from inside-out membrane patches from COSm6 cells expressing SUR2A, plus wild-type Kir6.2 (left) or Kir6.2[ΔN,K185Q]-GFP (right). C, Steady-state dependence of membrane current on [ATP], relative to current in zero ATP (I<sub>rel</sub>) for wild-type and mutant Kir6.2 coexpressed with SUR1 or SUR2A in COSm6 cells (from experiments as in B, n=3 to 8 patches). Lines correspond to least squares fits of the Hill equation (relative current = 100/(1 + ([ATP]/K<sub>1/2</sub>)<sup>H</sup>), with H = 1.3, and K<sub>1/2</sub> = 12 μmol/L (Kir6.2 + SUR1, •), 7 μmol/L (Kir6.2 + SUR2A, ○), 4200 μmol/L (Kir6.2[ΔN,K185Q]-GFP + SUR1, ◦), and 340 μmol/L (Kir6.2[ΔN,K185Q]-GFP + SUR2A, ⋄).
Solutions Used in Isolated Tissue Studies

For the solutions, concentrations are in mmol/L, except as noted. Solution A: NaCl 140, KCl 5.8, KH2PO4 0.5, Na2HPO4 0.4, MgSO4 0.9, glucose 11.1, HEPES 10. Solution B: Solution A + CaCl2 0.01, collagenase (Worthington Biochemical Corp) 130 μg/mL; Tyrode: NaCl 137, KCl 5.4, MgCl2 0.5, NaH2PO4 0.16, NaHCO3 3, HEPES 5, Glucose 5, pH 7.3 (NaOH). KB: Glutamic acid 50, taurine 20, glucose 10, MgSO4 3, KCL 30, KH2PO4 30, HEPES 20, pH 7.3 (KOH). KINT: KCl 150, HEPES 5, EGTA 10, pH 7.3 (KOH).

Expression of KATP Channels in COSm6 Cells

COSm6 cells were plated at a density of approximately 2.5 × 10^5 cells per well (30-mm six-well dishes) and cultured in Dulbecco’s Modified Eagle Medium plus 10 mmol/L glucose (DMEM-HG), supplemented with fetal calf serum (FCS, 10%), penicillin (100 U/mL), and streptomycin (100 μg/mL). The following day, cells were transfected using FuGENE 6 transfection reagent (Roche, Diagnostics Corp), with 1 μg of each DNA, according to manufacturer’s recommendations, then incubated in DMEM-HG plus 10% FCS and penicillin/streptomycin.

Preparation of Isolated Cardiomyocytes

Ventricular myocytes were isolated from adult mice (6 weeks to 5 months) using standard procedures. Briefly, hearts were excised from anesthetized animals (Metofane; Schering-Plough Animal Health Corp, NJ; according to manufacture’s instructions) and perfused retrogradely through the aorta for 4 minutes with solution A. Hearts were then perfused at 37°C for 20 minutes with solution B filtered at 5 kHz. Off-line analysis was performed using ClampFit software. A value of P < 0.05 was considered significant.

Electrophysiology

GFP fluorescence was visualized in cells excited by high-power Hg lamp (Nikon Instruments). Inside-out patch-clamp and whole-cell voltage- and current-clamp experiments were performed at room temperature, as previously described.23 Standard bath (intracellular) and pipette (extracellular) solution used in patch-clamp experiments was KINT (additions are described in the text). All currents were measured at a membrane potential of −50 mV (pipette potential = +50 mV). For whole-cell voltage-clamp experiments, pipette tip resistance was 1 to 3 MΩ when filled with KINT solution. The recording chamber was grounded by an agar bridge and was perfused by modified Tyrode solution (see above). Cells were held at −80 mV, and 40-ms voltage ramps from −100 mV to +40 mV were given every 13 seconds. After the ramp, a 40-ms pulse to +40 mV was applied, followed by a 4-second ramp from −100 to +40 mV and two 40-ms step pulses to −100 mV and to −60 mV, for measurement of I_KATP. I_KATP was induced by application of NaCN (2 mmol/L) and 2-deoxyglucose (DOG, 10 mmol/L) in Tyrode solution and was estimated from the additional current at +40 mV. In current clamp mode, action potentials were stimulated with just suprathreshold square pulses of 1 to 2 ms at 2 kHz.

For generation of voltage ramps and data collection, Clampfit software was used to control voltage ramps and perform data collection. Data were filtered at 5 kHz. Off-line analysis was performed using Clampfit and Microsoft Excel programs. Data are presented as mean ± SE (standard error of the mean). Statistical analysis was performed using unpaired Student’s t test. A value of P < 0.05 was considered significant.

Electrocardiograms Recorded in Conscious Mice

Radiofrequency transmitters (TA10E-A-F20 or TA10ETA-F20) were implanted in mice anesthetized with ketamine/xylazine. Telemetry was performed in the Mouse Physiology Core of the Center for Cardiovascular Research at Washington University School of Medicine 2 to 4 days after transmitter implantation. Each mouse, individually caged, was placed on top of a separate receiver (RPC-1). Lead II ECG recordings were acquired (1 kHz) with commercially available software (Dataquest A.R.T. Gold version 2.0 Acquisition Software, Data Sciences International, running on a dedicated Compaq Deskpro 2000 computer) according to the following schedule: 240 seconds every 30 minutes for 24 hours. Heart rates (determined from R–R intervals) were obtained from the Dataquest Analysis software. Premature ventricular contractions (PVC) and instances of AV block were manually determined from the entire data stream by a blinded observer.

Results

Creation of ATP-Insensitive Kir6.2 Transgenes

Truncation of the N-terminal 30 amino acids of Kir6.2 (ΔN) results in recombinant KATP channels with approximately 10-fold lower ATP sensitivity than normal and reduced high affinity sulfonylurea sensitivity, without alteration of single channel current.34,35 This deletion, combined with a point mutation (K185Q), results in a further ~30-fold reduction of ATP sensitivity when coexpressed with SUR1.34 For the present study, the double mutant (Kir6.2[ΔN,K185Q]) was fused at the C-terminus to green fluorescent protein (GFP) (Figure 1A), allowing fluorescence detection. In COSm6 cells, Kir6.2[ΔN,K185Q]-GFP + SUR2A channels were ~50-fold less sensitive to inhibition by ATP than wild-type Kir6.2 + SUR2A channels (Figures 1B and 1C; Kd = 340 μmol/L and 7 μmol/L, respectively). To specifically study the effects of manipulation of KATP channels in cardiac myocytes, we generated transgenic mice expressing the Kir6.2[ΔN,K185Q] transgene under α-myosin heavy chain (αMHC) promoter control. Four founder mouse lines were generated, generating transgenic lines 1 to 4.

Electrocardiograms Are Essentially Normal in ATP-Insensitive KATP Channels

That mice expressing the Kir6.2[ΔN,K185Q]-GFP transgene are viable was an initial surprise because, as discussed in the next section, the transgene is expressed at high levels, and transgene KATP channels are very insensitive to ATP. Isolated transgenic hearts appeared grossly normal, with no hypertrophy. We assessed the phenotype of transgenic hearts using radiofrequency telemetry in conscious mice (Figure 2). Of 12 matched littermate animals that were implanted, 5 transgenic and 3 wild-type animals were excluded because of death before completion of the study or questionable PCR results. Thus, data from a total of 7 transgenic and 9 littermate controls are presented. There were no obvious abnormalities in the ECG waveforms in transgenic animals (Figure 2A). In both control and transgenic hearts, there was a typical ~20% variability of mean heart rate (Figure 2B); however, a significantly (P < 0.01) lower heart rate (~10%) was observed in both line 2 and line 4 (see Materials and Methods) transgenic animals (Figure 2C). In all recordings, there were a total of 31 ventricular ectopic events in 6/9 nontransgenic animals (at least 3 events each in 4 animals) but only 12 events in 4/7 transgenic animals (only 2 animals with >1 event). In addition to reduced ectopy in transgenic animals, there was a greater incidence of atrioventricular block (148 missed beats in 5/7 animals and at least 5 missed beats in 4
animals) by comparison with nontransgenic littermates (5 missed beats in 2/9 animals and only 1 animal with 1 missed beat). Although the number of events in each case is small, these data suggest a reduced ventricular excitability and tendency to missed excitation in the transgenic animals.

High Expression of Kir6.2-GFP Transgene in Cardiac Myocytes

Of the four transgenic lines, myocytes from line 4 had the highest GFP fluorescence levels. In confocal slices, a cross-striated fluorescence pattern was visible (Figure 3A), consistent with a sarcomeric distribution. Given the uncertainty regarding the potential molecular substrate of the mitochondrial KATP channel, we co-stained cells with Mito Tracker Red. Longitudinal arrays typical of interfibrillar mitochondria (Figure 3A, center panel) showed no obvious overlap with GFP fluorescence (Figure 3A, right panel), supporting other indications that Kir6.2 is not a component of the mitochondrial KATP channel. 37

Variable Fluorescence Levels Correlate With Channel Phenotype

As shown in Figures 4A and 4B, $K_{ATP}$ channel activities in myocytes from control littermate animals have characteristic high ATP sensitivity, with typical $K_{1/2}$ of 10 to 100 μmol/L. $K_{ATP}$ channels in transgenic myocytes had considerably reduced ATP sensitivity, with $K_{1/2}$ up to 2–orders-of-magnitude higher in the highest expressing line 4 myocytes (Figure 4B). A greater variability of ATP sensitivity was seen in line 2 myocytes, which corresponded with striking cell-to-cell variability of transgene expression, indicated by GFP fluorescence variability (Figure 3B). Pooled data from wild-type, line 2, and line 4 myocytes gave mean $K_{1/2}$ of 51±6 μmol/L, 514±5 μmol/L, and 2727±12 μmol/L, respectively, n = 21, 30, 23 patches). Because $K_{ATP}$ channels are tetramers of Kir6.2 subunits, 6,38 channel phenotypes will lie on a spectrum between that of pure wild-type and pure mutant. 39,40 Figure 4C illustrates the range of ATP sensitivities observed in individual patches, plotted against the current density in each patch, and reveals a surprising feature: there is a negative correlation between $K_{1/2}$ and current density. Transgenic overexpression in lines 2 and 4 leads to a counterintuitive reduction in the total number of active channels.

Northern blots of isolated whole heart mRNA were used to determine levels of mRNA expression for the relevant channel subunits. These indicate very high levels of Kir6.2[K185Q]-GFP transcript relative to wild-type native Kir6.2 transcript (Figure 3C) but no change in SUR2A transcript levels (Figure 3C). There is no evidence for a reduction of either Kir6.2 or SUR2A mRNA, arguing against reduced SUR2A levels as a potential mechanism for the reduced total $K_{ATP}$ conductance in the transgenic myocytes.
The most striking physiological feature of the transgenic myocytes is that channels remain substantially closed in the intact cell (Figure 4A), even though sensitivity to ATP inhibition is reduced to millimolar levels. Indeed, significant on-cell activity was not observed in any patches, although the presence of inward rectifier (I_{K1}) and voltage-gated (Kv) channel activity on-cell make it difficult to exclude a very low K_{ATP} conductance.

K_{ATP} Conductance in Transgenic Mice Is Lower Than in Control Myocytes

Whole-cell voltage-clamp experiments were performed on line 2 myocytes that fluoresced green and on wild-type mice. I_{KATP} was induced by 2 mmol/L cyanide plus 10 mmol/L 2-deoxyglucose. Figure 5 shows the time course of current at +40 mV for typical control (top) and line 2 (bottom) myocytes. Immediately after membrane rupture, only small currents were detected, and in the control cell, massive I_{KATP}...
developed with a distinct delay after metabolic inhibition. In the transgenic cell, a marked increase in IK ATP also occurred some time after initiation of metabolic inhibition, but a gradual increase in outward current was evident soon after breaking in to the whole-cell mode. Similar "creep" of the outward current was observed in all transgenic cells but not in control cells (Figure 6). No significant difference was found for the time to half-maximal IK ATP after starting metabolic inhibition, nor for the time to rigor contracture: IK ATP appeared after 70±10 seconds (n=8) and 82±7 seconds (n=10) and rigor contracture appeared after 273±70 seconds (n=8) and 249±22 seconds (n=11) in transgenic and in control cells, respectively. However, the maximum K ATP current was significantly smaller in transgenic myocytes (transgenic: 10±2 nA, n=11; control 37±4 nA, n=13), and in experiments where Rs and Cm were reliably measured, a corresponding difference in conductance, normalized to membrane capacity, was found (control: 7.9±2.15, n=5; transgenic 2.0±0.8, n=7). These data again indicate that transgenic expression of Kir6.2[ΔN,K185Q]-GFP subunits did not lead to an elevated maximum IK ATP. In contrast, the maximum KATP current was actually reduced, further confirming that transgenic myocytes exhibit a lower K ATP density than wild-type control myocytes.

Inward rectifier current (I K1, steady state current at −120 mV after break-in) was not significantly different between control and transgenic myocytes, but the steady-state outward current at +40 mV was smaller in transgenic (4.4±0.6, n=20) than in control cells (7±1 nA, n=14). This may reflect a reduction in the noninactivating component of voltage-gated K+ conductances. Although detailed analysis of voltage-gated K currents will be necessary for full description of time-dependent currents, a reduction of I K could compensate for a threshold activation of KATP in transgenic myocytes.

**Action Potentials Are Not Shortened in Transgenic Myocytes**

Action potential duration (APD) is typically very sensitive to KATP channel activation. We recorded action potentials in current clamp mode from control and transgenic myocytes (Figure 7A). The zero current potential was variable (but more negative in transgenic myocytes, Figure 7C), and in all cases, resting potential was adjusted to −80 mV by continuous holding current. The holding current was not significantly different between control and transgenic myocytes (0.81±0.19 nA, cf. 0.77±0.20 nA). Action potential peak amplitude was not significantly altered in transgenic myocytes (Figure 7D) and, consistent with minimal activation of KATP, APD was not shortened. APD showed more variability and on average was longer (whether averaged with respect to time, Figure 7B, or with respect to fractional repolarization, Figure 7E) in transgenic myocytes.

**Discussion**

Tolerance for Reduced Sarcolemmal KATP Channel ATP Sensitivity

A paradigm for understanding the role of KATP channels in control of cellular excitability is that metabolic inhibition leads to reduced cytoplasmic [ATP]/[ADP] ratio, which then leads to opening of KATP channels, action potential shortening, and cessation of electrical activity.13 Reduced ATP sensitivity is thus predicted to lead to channel opening at higher [ATP]/[ADP], perhaps even in normal metabolic conditions. In transgenic mice expressing β-cell KATP channels with as little as a 5-fold reduction of ATP sensitivity, KATP channels are active enough to abolish insulin secretion, causing a profound neonatal diabetes and death within 1 week.33 In the present study, we generated transgenic mice expressing mutant KATP channels with ≈40-fold reduction of ATP
sensitivity in cardiac myocyte membranes. There is high-level expression of the transgene in ventricular myocytes (Figure 3), with no evidence for localization in mitochondria, consistent with previous indications that Kir6.2 is a major constituent of the sarcolemmal but not mitochondrial, KATP channels.2,17–20,37,42 Strikingly, these animals tolerate these transgenes without significant problems, even though the expected phenotype is present in sarcolemmal KATP channels, with ATP sensitivity in the millimolar range in the highest expressing myocytes (Figure 4).

Paradoxical Reduction in K<sub>ATP</sub> Density in Transgenic Animals
A second striking feature of the present data are the marked reduction of K<sub>ATP</sub> current density in transgenic myocytes. The negative correlation between K<sub>i,2</sub> (reflecting transgene expression levels) and channel density is counter to the naïve expectation that the total number of channel forming subunits might be increased. Two possible explanations seem reasonable. Because the channel is normally formed as an octamer of 2 subunits (Kir6.x and SURx),6,7,38 either native Kir6.2, or SURx subunits could be actively downregulated by the cell in response to the presence of ATP-insensitive channels. The massive level of Kir6.2 transcript in transgenic animals makes it unlikely that native Kir6.2 subunits will be rate limiting for channel expression. Similarly, normal levels of SUR2A transcript (Figure 3C) argues against the likelihood that SUR2A levels will be reduced.

Detailed studies on recombinant K<sub>ATP</sub> channel expression demonstrate a requirement for appropriate 1:1 stoichiometry of SURx and Kir6.x subunits.6,38 Expression of Kir6.2 subunits in excess of that necessary to produce the requisite stoichiometry leads to reduced overall level of functional channels.6,38 The major consequence of mismatched stoichiometry appears to be loss of surface expression due to retention in the endoplasmic reticulum.43 This seems the most likely explanation for the paradoxical reduction of K<sub>ATP</sub> conductance in transgenic myocytes, but further experiments with restored SUR2A levels will be required to further test this possibility.

Metabolic Dependence of K<sub>ATP</sub> Channel Activity
The most surprising finding is that such ATP-insensitive channels are not significantly active in intact cells, and there are no gross defects in cardiac electrical activity (Figure 2). Many studies have demonstrated that a fall of intracellular
ATP is likely to be a major determinant of K\textsubscript{ATP} channel opening when metabolism is inhibited\textsuperscript{22,23,44}. Quantitative models of cardiac action potentials, incorporating experimentally measured K\textsubscript{ATP} properties, indicate that relatively small decreases of [ATP] within the millimolar range, coupled with an increase of [ADP] under such conditions, are likely to result in marked action potential shortening\textsuperscript{25–28} if ATP sensitivity is reduced by as little as 4-fold (not shown). The use of a hyperpolarizing holding current will cause a variable reduction of the APD, precluding detailed analysis of action potential durations.

Figure 6. Slow creep of outward current in transgenic myocytes after attainment of whole-cell configuration. Current at +40 mV is indicated for (A) wild-type control cells (n=9) and (B) line 2 transgenic cells (n=8), after ‘break-in’ to whole-cell configuration at time 0. There is no time-dependent change of WT current; the outward current increases slowly and approximately linearly for line 2 TG myocytes (slope=0.009 nA/s).

Figure 7. Action potentials in control and transgenic myocytes. A, Representative action potentials recorded from wild-type (WT) and line 4 transgenic (Line 4) myocytes stimulated at 1 Hz. B, Averaged (with regards to time after stimulus) action potentials from 8 control (○) and 7 line 4 transgenic (○) myocytes. C through E, Mean (±SEM) zero current potential after establishment of whole-cell configuration (C), peak action potential amplitude (D), and action potential duration (APD) at 30%, 50%, and 90% repolarization (E), from experiments as in A. Although mean APD was longer in transgenic myocytes, this lengthening was not significant (P>0.2).
potential waveforms. However, because the holding current was similar for both wild-type (WT) and transgenic (TG) myocytes, the artifactual reduction may be similar in both cases, and the main point still holds: there is no significant AP shortening in the TG, and channels are still substantially closed in intact cells even with \( \sim \)40-fold reduction of ATP sensitivity.

Nevertheless, the slow creep of outward current after onset of whole-cell voltage-clamp suggests that channels may be just on the threshold of opening in transgenic myocytes (Figure 6). The patch record in Figure 4A (right panel) shows even less activity on-cell than in the presence of 5 mmol/L ATP after excision. Because control intracellular [ATP] is unlikely to be much higher than 5 mmol/L,46 and MgADP would be expected to reduce the inhibitory effects of ATP, these observations suggest an additional mechanism keeping the channels closed. This idea is further supported by the surprising observation that the time to massive activation of these observations suggest an additional mechanism keeping the channels closed. This idea is further supported by the surprising observation that the time to massive activation of channels, which might previously have been ascribed to a sudden drop of [ATP], is also not significantly reduced in transgenic myocytes (Figure 5). What such a mechanism is remains speculation, but it is intriguing to note that differential nucleotide hydrolysis rates for different SUR isoforms may play a significant role in controlling physiological activation.45

Together with the dramatic intolerance for introduction of similar transgenes into pancreatic \( \beta \)-cells,33 these results highlight the significant but as yet unexplained differences in the physiological regulation of pancreatic and cardiac K\(_{\text{ATP}}\) channels, which lead to dynamic activity of the former, but not the latter, in normal physiology.46

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**References**


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