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Diverse phenotypes of outward currents in cells that have survived in the 5-day-infarcted heart

Wen Dun and Penelope A. Boyden

Department of Pharmacology, Center for Molecular Therapeutics, Columbia University, New York, New York

Abstract

We have shown reduced density and altered kinetics in slowly activating K^+ currents (I_{Ks}) in epicardial border zone (EBZ) cells (IZs) of the 5-day-infarcted canine heart (Jiang M, Cabo, C, Yao J-A, Boyden PA, and Tseng G-N. *Cardiovasc Res* 48: 34–43, 2000). β -Adrenergic stimulation with isoproterenol increases I_{Ks} in normal cells (NZs). In this study, we used a voltage-clamp protocol with an external solution to isolate I_{Ks} from contaminating currents to determine the effects of 1 μ M isoproterenol on I_{Ks} in IZs and NZs. Under our recording conditions, 10 μ M azimilide-sensitive currents were stimulated with isoproterenol to compare responsiveness of I_{Ks} to isoproterenol in the two cell groups. I_{Ks} tail density was reduced 67% in IZs (*group I*, $n = 26$) compared with NZs ($n = 24$, $P < 0.05$). Isoproterenol-stimulated azimilide-sensitive tail currents were increased 1.72 ± 0.2 -fold in NZs and 2.2 ± 0.3 -fold in IZs ($P > 0.05$). In 33% of IZs (*group II*, $n = 13$), native currents showed no tail currents; however, isoproterenol-stimulated azimilide-sensitive currents were voltage dependent, fast activating, and large in amplitude compared with *group I* IZs, similar to “lone” KCNQ1 currents. Using short clamp pulses, we also found an increase in sustained currents sensitive to tetraethylammonium chloride (TEA) and no change in C-9356-sensitive currents in IZs with little or no transient outward current. In some IZs where I_{Ks} is downregulated, the effect of isoproterenol on I_{Ks} was similar to that on I_{Ks} in NZs. In others, the existence of lone KCNQ1-type currents, which are sensitive to β -adrenergic stimulation, is consistent with our findings of an increased KCNQ1-to-KCNE1 mRNA ratio (Jiang et al.). Accompanying altered I_{Ks} in IZs are an enhanced TEA-sensitive current and a normal C-9356-sensitive current.

Keywords

epicardial border zone; delayed rectifier; tetraethylammonium; C-9356; isoproterenol

VOLTAGE-GATED K^+ channels are highly responsive to their environment, with many studies using models of acquired heart disease describing highly remodeled K^+ currents. Most studies have noted a significant downregulation of the transient outward current (I_{to}), the K^+ current important for early action potential repolarization. Fewer studies have reported on the density and function of the important components of the delayed rectifier currents [rapidly and slowly activating K^+ currents (I_{Kr} and I_{Ks})]. In the chronic atrioventricular block model, reduced I_{Ks} and coordinated downregulation of KCNQ1 and KCNE1 have been described (22,24). In a feline model of hypertrophy in the healed heart after myocardial infarction, I_{Kr}

is downregulated (28), whereas in canine cells from the left ventricular midwall injured by coronary microembolizations, I_{K_r} is increased and KCNE2 is decreased (11).

In a canine pacing-induced failing heart model, I_{K_s} is downregulated but I_{K_r} is not (16). E-4031-sensitive currents are increased in some subendocardial Purkinje cells that survive in the infarcted heart (21). Finally, in cells that have survived in the epicardial border zone (EBZ) of the 5-day-infarcted heart (IZs), we found that I_{K_r} and I_{K_s} were reduced in density but that mRNA changes in specific α - and β -subunits of these currents were not consistent with these changes in function (10). When the changes in delayed rectifier currents were incorporated into our computer model of IZs (4), we noted that I_{K_r} and I_{K_s} provide little current for repolarization of IZs. However, there are no data on β -adrenergic-stimulated I_{K_s} in IZs. Perhaps there is a change in other K^+ currents that do not normally contribute to canine repolarization. Thus, in this study, we sought to identify the nature and function of several K^+ currents in response to short and long voltage-clamp pulses in IZs of the 5-day-infarcted canine heart. Furthermore, we determined how I_{K_s} in IZs is affected by exposure to the β -adrenergic receptor agonist isoproterenol.

METHODS

Animal preparation

This investigation conforms to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* [DHHS Publication No. (NIH) 85-23, 1996], and all protocols were approved by the Institution's Animal Care and Use Committee.

Healthy male mongrel dogs (12–15 kg, 2–3 yr old) were used in these studies. Under isoflurane (30 mg/kg) anesthesia, myocardial infarction was produced by a two-step total occlusion of the left coronary artery using procedure described by Harris (9). At 5 days after occlusion, hearts ($n = 35$) were used to disperse cells from the EBZ (17). Epicardial slices from normal noninfarcted hearts were used for preparation of normal cells (NZs).

Myocyte preparation

Single Ca^{2+} -tolerant cells were dispersed using a modification of our previously described method (17). Slices were rinsed twice in a Ca^{2+} -free solution (in mM: 115 NaCl, 5 KCl, 35 sucrose, 10 dextrose, 10 HEPES, and 4 taurine, pH 6.95) to remove blood. The solution was triturated in 20 ml of enzyme-containing solution [collagenase type II (Worthington Biochemical) at 0.38 mg/ml and protease type XIV (Sigma Chemical) at 0.05 mg/ml] at 36–37°C for 20 min; then it was decanted and discarded. The second trituration without the protease was discarded after 30 min. The next six to seven triturations were each done for 15 min. Each time, the solution was centrifuged at 500 rpm for 3 min for collection of the supernatant and dispersed cells. Resuspension solution was changed every 30 min for solutions containing increasing concentrations of Ca^{2+} . With this procedure, the viable cell yield was ~30–40%. Only rod-shaped NZs with staircase ends, clear cross-striations, and surface membranes free from blebs were used. IZs had a ruffled appearance with somewhat irregular cross-striations. Additionally, small dark droplets were apparent in IZs and were used to identify myocytes that had survived in the EBZ (17).

Electrophysiological studies

An aliquot of cells was transferred onto a polylysine-coated glass coverslip placed at the bottom of a 0.5-ml tissue chamber that had been mounted on the stage of an inverted microscope (Diaphot, Nikon, Tokyo, Japan). Myocytes were continuously superfused (2–3 ml/min) with Tyrode solution containing (in mM) 140 NaCl, 5.4 KCl, 2.0 $MgCl_2$, 1.8 $CaCl_2$,

10 HEPES, and 10 dextrose (pH 7.4) at 30.0–31.0°C. Cell capacitance was 177 ± 6.2 and 208 ± 8.4 pF for NZs and IZs, respectively.

In the first set of studies, short clamp pulses were used to determine the nature of currents remaining in IZs in the absence of I_{to} . To measure the outward currents, patch pipettes with resistances of 1–1.5 M Ω when filled with the internal solution (in mM: 140 KCl, 1 MgCl₂, 10 EGTA, 5 MgATP, 5 creatine phosphate, 0.2 GTP, and 10 HEPES, with pH adjusted to 7.2 with KOH) were used. For recording, cells were superfused with an Na⁺-free solution (in mM: 144 *N*-methyl-D-glucamine chloride, 5.4 KCl, 1 MgCl₂, 2.5 CaCl₂, 0.5 CdCl₂, and 10 HEPES 10, pH 7.4) at 30–31°C. Na⁺ currents were suppressed with an Na⁺-free solution, and L-type Ca²⁺ current ($I_{Ca,L}$) was blocked with 0.5 mM Cd²⁺. Membrane currents associated with Na⁺/Ca²⁺ exchange were eliminated because of the absence of external Na⁺. Currents were elicited by 200-ms voltage steps to transmembrane potentials (V_t) of –50 and +60 mV from a holding potential of –60 mV at 0.1 Hz after a 10-ms prepulse to –90 mV. I_{200} was taken as the amplitude of the current at the end of the test pulse relative to zero-current level (7). Currents sensitive to tetraethylammonium chloride (TEA; 5 mM) or to a compound specific for K_v1.5 currents in canine atria, C-9356 (8), were determined in cells of each group and compared.

A different set of conditions was used to measure current elicited by long clamp pulses, i.e., I_{Ks} . Pipettes with resistances of 1–1.5 M Ω when filled with an internal solution containing (in mM) 125 K-aspartate, 20 KCl, 1 MgCl₂, 5 HEPES, 5 K-ATP, and 10 EGTA, with pH adjusted to 7.3 with KOH, were used. After formation of the gigaohm seal, the stray capacitance was electronically nulled, the cell membrane under the pipette tip was ruptured, and then, by a brief increase in suction, the whole cell recording configuration was formed. A period of 5 min was allowed for intracellular dialysis to begin before the solution was switched to the external recording solution (132 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1.2 mM MgSO₄, 20 mM HEPES, 11.1 mM dextrose, 5 μ M nisoldipine, 1 μ M dofetilide, and 100 μ M DIDS, with pH adjusted to 7.3 with NaOH). Currents were elicited using a series of 5-s pulses to various test potentials from holding potentials of –50 mV, followed by repolarization to –20 mV for recording of tail current. Data collection was begun 10 min after membrane rupture and completed within 30 min to minimize “run down” of I_{Ks} (10).

In some experiments, currents elicited in NZs and IZs were determined and compared as currents blocked by 10 μ M azimilide. Azimilide has been shown to inhibit I_{Ks} via a binding site on the KCNQ1 protein (3). At this concentration and under our recording conditions, azimilide has little or no effect on other currents (27).

Data acquisition and analysis

Clamp protocol generation and data acquisition were controlled by Clampex (version 8) via an analog-to-digital converter (Digidata 1200, Axon Instruments). Transmembrane currents were obtained using Axopatch 200 amplifiers (Axon Instruments), low-pass filtered at 2 kHz, digitized at 0.1 kHz, and sampled online to a computer hard disk. Clampfit of pClamp (version 8.1) was used for amplitude measurement and curve fitting.

Drugs

Azimilide, dofetilide, and nisoldipine were first prepared as stock solutions and then added to the test solution as required. DIDS (Sigma) was prepared daily as a 10 mM stock solution, dissolved in water, and added to the test solution for a final concentration of 100 μ M. DIDS-containing solutions were protected from light to prevent degradation. Isoproterenol (Sigma) stock solution was made on the day of the experiment (1 mg/ml) and added to the test solution for a final concentration of 1 μ M. TEA (Sigma) was dissolved in double-distilled

water for stock solution. C-9356 was a gift from Cardiome Pharma (Vancouver, BC, Canada). The stock solution of C-9356 (20 mM) was made in DMSO; final bath concentration of DMSO was 0.05%.

Statistics

Values are means $\pm$ SE. Statistical analysis was performed using SigmaStat Student's *t*-test and paired *t*-test. $P < 0.05$ was significant.

RESULTS

Effect of TEA on I_{200}

The time course of the effects of 5 mM TEA in NZs and IZs is shown in Fig. 1A. TEA inhibits outward currents of NZs and IZs. A typical current-voltage (*I-V*) curve of I_{200} in the absence and presence of TEA and the TEA-sensitive current is plotted in Fig. 1B. We found smaller TEA-sensitive I_{200} in NZs: 0.06 ± 0.02 pA/pF in NZs ($n = 10$) vs. 0.32 ± 0.07 pA/pF in IZs ($n = 12$) at +40 mV ($P < 0.05$). Percent inhibition of I_{200} by TEA was significantly larger at +40 mV in IZs than in NZs: $22.1 \pm 3.9\%$ vs. $4.5 \pm 1.7\%$ ($P < 0.05$; Fig. 1C). Thus, in IZs with reduced or absent I_{to} (17), there is a TEA-sensitive increase in plateau outward current.

Effect of the $K_v1.5$ blocker C-9356 on I_{200}

Because TEA inhibited I_{200} in IZs (see *Effect of TEA on I_{200}*), we considered whether other components that may not be expected on the basis of normal electrophysiology might contribute to I_{200} , for example, the atrium-specific current I_{Kur} , which presumably is due to $K_v1.5$. Thus we determined and compared the effects of a drug that blocks $K_v1.5$ selectively (at 10 μ M), with minor or no effects on $K_v3.1$, $K_v2.1$, or $K_v4.2$ currents (8). We used 10 μ M C-9356 in our study of currents in NZs and IZs in the absence of TEA. Figure 2A shows the time course of the effect of C-9356 as it equilibrates in an NZ and an IZ. Note the effect on I_{200} in each cell type. Average *I-V* plots of the effect of C-9356 on I_{200} are shown in Fig. 2, B and C, for cells that showed a response. The percent inhibition by C-9356 at +40 mV did not differ between the two cell groups ($25.2 \pm 2.9\%$ and $22.8 \pm 4.7\%$ in NZ and IZ, respectively, $P > 0.05$). Thus there is no change in C-9356-sensitive currents in the greatly remodeled IZ.

Effects of isoproterenol on I_{Ks}

Increased TEA-sensitive sustained currents in IZs with short clamp pulses could be due to I_{Ks} that is altered (10), because KCNQ1 channels are TEA sensitive (14). Therefore, in this set of studies, we elicited currents with long clamp pulses in IZs and NZs in the absence or presence of β -adrenergic stimulation. Recording conditions (see METHODS) were designed to eliminate contaminating currents that could occur as we tested the effects of 1 μ M isoproterenol in both cell types. Figure 3A shows the currents elicited in the absence and presence of isoproterenol and the corresponding azimilide-sensitive currents for a typical NZ. Depolarization pulses (to +10 and +60 mV) elicited time-dependent outward currents that continued to increase during the 5-s pulses. Subsequent repolarization was accompanied by slowly decaying tail currents. Isoproterenol produced a marked increase in I_{Ks} tail density (from 0.38 ± 0.06 to 0.64 ± 0.08 pA/pF at +60 mV, $P < 0.05$), representing an average 1.7 ± 0.2 -fold effect in NZs ($n = 15$; Fig. 3B). Average *I-V* plots of time-dependent azimilide-sensitive currents during test pulses in NZs are shown in Fig. 3C. Under these conditions, isoproterenol-stimulated azimilide-sensitive currents represent iso-proterenol-enhanced I_{Ks} , because other azimilide-sensitive currents are blocked (e.g., I_{Kr} by 1 μ M dofetilide).

In IZs, we observed two contrasting phenotypes of isoproterenol-stimulated azimilide-sensitive currents (Fig. 4): *group I* and *group II* IZs. In *group I*, I_{Ks} is downregulated, and isoproterenol affected I_{Ks} by producing a slowly activating current. This response was observed in 67% of IZs tested. In *group II*, isoproterenol-stimulated azimilide-sensitive currents were voltage dependent, fast activating, large in amplitude, and showed no apparent tail currents. In 33% of IZs tested, we observed the *group II* phenotype. In 4 of 33 (12%) NZs tested, we saw a small current that was similar to this phenotype.

In *group II* IZs, where I_{Ks} is downregulated in the absence of drug (Fig. 5A), the effect of isoproterenol on I_{Ks} was similar to that in NZs; i.e., there was no difference in the “fold” effect ($P > 0.05$; Fig. 5B). Azimilide-sensitive currents in *group II* IZs were not different from those in NZs at short (300-ms) intervals (Fig. 5C) but were significantly decreased in IZs at longer intervals (Fig. 5C, *inset*). In *group II* IZs (Fig. 6), currents in the absence of isoproterenol were small (Fig. 6A), and azimilide-sensitive currents were time independent and time dependent (Fig. 6B). Sensitive currents at 300 and 5,000 ms were strikingly increased over NZs and *group I* IZs (Figs. 6C and Fig. 7A). Finally, the activation of azimilide-sensitive currents was rapid in *group II* IZs compared with *group I* IZs and NZs ($P < 0.05$; Fig. 7B).

DISCUSSION

We have demonstrated that I_{200} is more sensitive to TEA in IZs than in NZs, although I_{200} in NZs and IZs shows a similar response to the $K_v1.5$ blocker C-9356. The effects of β -adrenergic stimulation on K^+ current were similar in NZs and IZs; the exception is a new phenotype of adrenergic-mediated currents, the *group II* IZs.

Our previous reports of the remodeling of ion channels in cells that survive in the 5-day-infarcted heart (IZs) showed that I_{to} is absent (17), Ca^{2+} current is reduced (1), and K^+ current is altered (10). In the latter study (10), we found altered kinetics as well as amplitude in K^+ currents in IZs. Because of these changes, action potential repolarization in IZs is critically dependent on remaining currents. The nature of these currents is not totally known. In this study, we hypothesized that there may be a change in other K^+ currents that do not normally contribute to canine repolarization. We tested for the existence of I_{Kur} or a rapidly activating outward current similar to the current that is key to atrial cell repolarization. $K_v1.5$ protein is thought to underlie this current in canine atrial cells (8). We previously showed pronounced structural remodeling (lateralization) of $K_v1.5$ protein in cells that survive in the EBZ (19). However, in the present study, we report no difference in C-9356-sensitive currents between NZs and IZs. In contrast to a previous report (8), we found small C-9356-sensitive currents in normal canine ventricular cells, suggesting that this current may contribute to ventricular repolarization under normal conditions.

To our surprise, we show an upregulation of TEA-sensitive currents in IZs. The molecular nature of this upregulated TEA-sensitive current is unknown, but, on the basis of its TEA sensitivity, this current most probably corresponds to an up-regulated $K_v2.1$ current, which in mouse ventricle underlies $I_{to,slow2}$ (25).

I_{Ks} . We considered that an upregulated TEA-sensitive current could be due to an altered I_{Ks} (10), because TEA blocks slowly activating K^+ channels (14). However, when clamp studies were designed to isolate and study I_{Ks} in NZs and IZs, we found no difference in currents (in the presence of isoproterenol) at the end of short intervals in most IZs (Fig. 5C). We found that in IZs where I_{Ks} was reduced, isoproterenol affected the current as it did in NZs (Figs. 3B and 5B). In a previous study, we showed that the responsiveness of $I_{Ca,L}$ to β -adrenergic stimulation was reduced in these 5-day-infarcted myocytes (2). In contrast to those results,

we report here that the fold effect of β -adrenergic stimulation on I_{Ks} did not differ between NZs and IZs, although I_{Ks} was smaller in *group I* IZs. Our knowledge that $I_{Ca,L}$ and K^+ current are differentially regulated by cAMP-dependent protein kinase cascade in the newborn canine epicardial cell (5) and under special cases of atrial fibrillation (6) suggests preferential paths of activation of different ion channels by specific and different pools of cAMP in canine cells.

In a large group of IZs (*group II*), we found a “different” type of azimilide-sensitive current in the presence of isoproterenol (Fig. 6). Although single-cell PCR was not completed in each of these *group II* IZs, our previous mRNA studies showed that mRNA of KCNQ1 returns to its control level, whereas that of KCNE1 remains reduced in the EBZ of the 5-day-infarcted heart (10). If these changes in mRNA lead to corresponding changes in subunit protein levels, we might expect an effect on kinetics of K^+ current in cells surviving in the infarcted heart, because KCNE1 subunits are decreased relative to KCNQ1 subunits. Studies designed to determine the molecular basis for PKA regulation of I_{Ks} have shown that expression of KCNE1 is required with KCNQ1, and the KCNQ1 NH₂-terminal residue is critical for this action (13,15). Recently, it has been observed that lone KCNQ1 (Kv-LQT1) can be a target for cAMP (26), suggesting that adrenergic stimulation may modulate this K^+ current at two (or more) sites: KCNE1 and KCNQ1. We suggest that because KCNQ1 mRNA is not different in cells from EBZ of the 5-day-infarcted heart and KCNE1 is markedly reduced, IZs have ionic currents with a structural component that is solely KCNQ1 proteins. Such “lone” KCNQ1 currents would resemble those of *group II* cells in Fig. 6. Interestingly, during development of the canine epicardial cell, similar-appearing K^+ currents change phenotype as KCNE1 is expressed and I_{Ks} increases (12). Alternatively, *group II* IZs have a K^+ current complex that is formed by KCNQ1 aligned with another member of the KCNE family, such as KCNE3. Recently, KCNE3 has been shown to convert the slow voltage-dependent activation of K^+ current to a voltage-dependent rapidly activating current (18,20,23).

In summary, for some IZs where I_{Ks} is downregulated, isoproterenol affects I_{Ks} as it affects I_{Ks} in NZs. In other IZs, lone KCNQ1-type currents, which are sensitive to β -adrenergic stimulation, exist and are consistent with our findings of an increased KCNQ1-to-KCNE1 mRNA ratio determined in our previous work (10). Accompanying these altered I_{Ks} in IZs are an enhanced TEA-sensitive current and a normal C-9356-sensitive current. Heterogeneity in IZ K^+ current phenotypes induced by isoproterenol will contribute to diverse adrenergic responses of EBZ fibers.

Acknowledgments

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Fig. 1.

Effects of tetraethylammonium chloride (TEA, 5 mM) on currents elicited by 200-ms voltage steps from -50 to +60 mV at 0.1 Hz after a 10-ms prepulse to -90 mV (I_{200}) in normal cells (NZs) and surviving cells in the epicardial border zone of a 5-day-infarcted heart (IZs). *A*: typical recording of the time course of the effect of 5 mM TEA on outward currents elicited with a holding potential of -60 mV and a transmembrane potential (V_t) of +40 mV for 200 ms in NZs and IZs. NZ records have been enlarged/truncated to emphasize the effect on sustained currents. Dotted line, zero-current level. *B*: typical current-voltage (I - V) plot of I_{200} before TEA (Predrug), with 5 mM TEA, and TEA-sensitive currents in an IZ. I_{200} was measured as the amplitude of the current at the end of the test pulse relative to zero-current level. *C*: percent inhibition of I_{200} by TEA at +40 mV in NZs ($n = 12$) and IZs ($n = 10$). * $P < 0.05$ vs. NZs.

Fig. 2.

Effects of C-9356 (10 μ M) on I_{200} in NZs and IZs. *A*: typical recording of the time course of the effect of 10 μ M C-9356 on outward currents in an NZ and an IZ. See Fig. 1 legend for protocol. NZ records have been enlarged/truncated to emphasize the effect on sustained currents. Dotted line, zero-current level. *B*: effect of C-9356 on I - V relation of I_{200} in NZs. C-9356 (10 μ M) significantly inhibited I_{200} in NZs. * P < 0.05 vs. control. *C*: effect of C-9356 on I - V relation of I_{200} in IZs. Similar to its effect on NZs, C-9356 significantly inhibited I_{200} in IZs. * P < 0.05 vs. control.

Fig. 3.

Effects of isoproterenol (1 μ M, Iso) stimulation on slowly activating K^+ current (I_{Ks}) in NZs. **A**: representative traces of I_{Ks} in control (Predrug) and with isoproterenol (1 μ M) and typical azimilide (AZ)-sensitive currents. I_{Ks} was isolated in the presence of nisoldipine (2 μ M), dofetilide (1 μ M), and DIDS (100 μ M) to block Ca^{2+} current, rapidly activating K^+ current (I_{Kr}), and Cl^- current, respectively. Currents were elicited from a holding potential of -50 mV to V_t of $+10$ and $+60$ mV for 5 s, followed by repolarization to -20 mV for recording of tail currents (*inset*). Time interval was 30 s. Dotted lines, zero-current level. I_{Ks} tail currents were measured as peak tail currents (arrow) upon repolarization on the basis of zero-current level. $\circ$, AZ-sensitive current at 300 ms of activation (in pA/pF). **B**: in NZs ($n = 15$), I_{Ks} tail density was significantly increased with isoproterenol stimulation. $*P < 0.05$ vs. control. At $+60$ mV, the effect was 1.7 ± 0.2 -fold. **C**: average I - V relation of time-dependent AZ-sensitive currents ($n = 9$) at 300 ms of activation in the presence of 1 μ M isoproterenol.

Fig. 4.

Two types of AZ-sensitive currents (I_{Ks}) were observed in the presence of isoproterenol in IZs. Currents were elicited from a holding potential of -50 mV to a V_t of -10 to $+60$ mV for 5 s, followed by repolarization to -20 mV (*inset*). Two types of phenotypic responses were observed in IZs: *group I* (*Grp I*, 26 of 39 IZs) and *group II* (*Grp II*, 13 of 39 IZs).

Fig. 5.

Effects of isoproterenol on I_{Ks} in *group IIZs*. **A**: representative traces of I_{Ks} in control (Predrug) and with isoproterenol (1 μ M) and AZ-sensitive currents. See Fig. 3 legend for recording conditions and protocols. Dotted lines, zero-current level; arrow, I_{Ks} tail currents measured as peak tail currents; ●, AZ-sensitive current at 300 ms (in pA/pF); ▲, AZ-sensitive current at 5,000 ms at +60 mV (in pA/pF). **B**: in *group IIZs* ($n = 19$), although I_{Ks} tail density was reduced vs. NZ values, it was significantly increased with isoproterenol. * $P < 0.05$ vs. control. At +60 mV, effect was 2.2 ± 0.3 -fold, which was not different from NZs ($P > 0.05$). **C**: average I - V relation of time-dependent AZ-sensitive currents at 300 ms in the presence of 1 μ M isoproterenol in *group IIZs* ($n = 14$). *Inset*: maximal currents at 5,000 ms at +60 mV were reduced in *group IIZs*. * $P < 0.05$ vs. NZs.

Fig. 6.

Effects of isoproterenol on I_{Ks} in *group II* IZs. **A**: representative traces of K^+ current (I_K) in control (Predrug) and with isoproterenol and AZ-sensitive currents. See Fig. 3 legend for recording conditions and protocols. Note marked difference in the genotype of this isoproterenol-stimulated AZ-sensitive current. **B**: average I - V relations of AZ-sensitive time-independent currents in *group II* IZs ($n = 13$). For comparison, time-dependent component is plotted (Δ). $\blacktriangle$, AZ-sensitive current at 5,000 ms at +60 mV (in pA/pF); $\blacksquare$, AZ-sensitive current at 300 ms (in pA/pF). **C**: average I - V relation of AZ-sensitive currents at 300 ms of activation in *group II* IZs ($n = 13$) and I - V relations of NZs and *group I* IZs. Currents were significantly larger in *group II* IZs than in NZs and *group I* IZs. * $P < 0.05$ vs. NZs. $\dagger P < 0.05$ vs. *group I* IZs.

Fig. 7.

Effects of isoproterenol on activation of I_{Ks} in NZs, *group IIZs*, and *group IIIZs*. *A*: summary of AZ-sensitive current density at the end of the depolarizing pulse (+60 mV). In NZs and *group IIZs*, there was only a time-dependent component. In *group IIIZs*, there was a large time-independent component. Note similarity of time-dependent current in *group IIIZs* and NZs. Time-dependent current was smaller in *group IIZs* than in NZs and *group IIIZs*. $^+P < 0.05$ vs. NZs. $^{\dagger}P < 0.05$ vs. *group IIIZs*. *B*: activation time constants (τ) at +60 mV for NZs, *group IIZs*, and *group IIIZs*. Currents during depolarization pulses at +60 mV were fit with a single-exponential function. NZ and *group IIZ* currents activated more slowly than *group IIIZ* currents. $^*P < 0.05$ vs. NZ. $^{\dagger}P < 0.05$ vs. *group IIZ*.