Regulation of action potential duration under acute heat stress by $I_{\text{K,ATP}}$ and $I_{\text{K1}}$ in fish cardiac myocytes

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Regulation of action potential duration under acute heat stress by $I_{\text{K,ATP}}$ and $I_{\text{K1}}$ in fish cardiac myocytes. Am J Physiol Regul Integr Comp Physiol 286: R405–R415, 2004. First published October 30, 2003; 10.1152/ajpregu.00500.2003.—The mechanism underlying temperature-dependent shortening of action potential (AP) duration was examined in the fish (Carassius carassius L.) heart ventricle. Acute temperature change from +5°C to +18°C (heat stress) shortened AP duration from 2.8 ± 0.3 to 1.3 ± 0.1 s in intact ventricles. In 56% (18 of 32) of enzymatically isolated myocytes, heat stress also induced reversible opening of ATP-sensitive K+ channels and increased their single-channel conductance from 37 ± 12 pS at 18°C to 51 ± 13 pS at 37°C ($Q_{10} = 1.38$) ($P < 0.01; n = 12$). The ATP-sensitive K+ channels of the crucian carp ventricle were characterized by very low affinity to ATP both at +8°C [concentration of Tris-ATP that produces half-maximal inhibition of the channel ($K_{1/2}^{\text{ATP}}$) = 1.35 mM] and +18°C ($K_{1/2}^{\text{ATP}}$ = 1.85 mM). Although acute heat stress induced ATP-sensitive K+ current ($I_{\text{K,ATP}}$) in patch-clamped myocytes, similar heat stress did not cause any glibenclamide (10 μM)-sensitive changes in AP duration in multicellular ventricular preparations. Examination of APs and K+ currents from the same myocytes by alternate recording under current-clamp and voltage-clamp modes revealed that changes in AP duration were closely correlated with temperature-specific changes in the voltage-dependent rectification of the background inward rectifier K+ current ($I_{\text{K1}}$). In ~15% of myocytes (4 out of 27), $I_{\text{K,ATP}}$-dependent shortening of AP followed the $I_{\text{K1}}$-induced AP shortening. Thus heat stress-induced shortening of AP duration in crucian carp ventricle is primarily dependent on $I_{\text{K1}}$. $I_{\text{K,ATP}}$ is induced only in response to prolonged temperature elevation or perhaps in the presence of additional stressors.

IN THEIR NATURAL ENVIRONMENT, ectothermic animals are often exposed to acute and chronic temperature changes, and different species show variable tolerance to high and low temperatures. Usually the animal’s tolerance range is widened by gradual and reversible acclimation processes, while the ability to tolerate acute temperature changes is an inherent genetic property of each species (33). Field observations of several fish species show that fish transcend large thermoclines to forage or escape predation, which exposes animals to acute temperature changes (8, 23). Under such instances of thermal stress, cellular myocardial plasticity is required to preserve adequate contractility, to avoid disturbances in electrical excitation, and to prevent metabolic imbalance under changing demand. In ectothermic animals, the duration of the cardiac action potential (AP) is sensitive to environmental temperature changes, which directly or indirectly affects the force and rate of cardiac contraction and thus cardiac energy demand (10, 11, 36). In the present work, we studied the effect of heat stress on cardiac AP in an eurythermic fish species, the crucian carp (Carassius carassius L.).

Initially, ATP-sensitive K+ channels were shown to provide cardiac protection under ischemic insults (for reviews see Refs. 13, 35, 41), while more recent studies indicate that in the cardiovascular system and other tissues, ATP-sensitive K+ channels contribute to general stress tolerance by providing protective feedback mechanisms (34, 42). Indeed, knockout of Kir6.2 protein in mice produces a phenotype of decreased tolerance to stress with reduced exercise capacity, inability to sustain cardiac performance, improper intracellular Ca2+ handling, and vulnerability to arrhythmias under vigorous catecholamine challenge (42). Recently we showed that the cardiac ATP-sensitive K+ current ($I_{\text{K,ATP}}$) is present in several ectothermic vertebrates, but it is much smaller than in mammals and difficult to activate even under complete metabolic inhibition (30). This suggests that $I_{\text{K,ATP}}$ is not crucial for adaptation to hypoxia or anoxia, and thus the physiological role of the ATP-sensitive K+ channels in ectotherms remains elusive. As it was suggested that ATP-sensitive K+ channels are active in myocardocytes of the cold-acclimated goldfish (Carassius auratus L.) and promote their temperature tolerance (12), we hypothesized that $I_{\text{K,ATP}}$ is activated by acute heat stress and thereby participates in temperature-dependent regulation of cardiac AP duration in fish myocytes. We examined the temperature-related changes in the properties of ATP-sensitive K+ channels and their contribution to regulation of AP duration under heat stress in the cold-acclimated crucian carp, a close relative of the goldfish. To evaluate the physiological significance of the $I_{\text{K,ATP}}$, it appeared necessary to examine also the contribution of the background inward rectifier current, $I_{\text{K1}}$, to the heat stress response.

MATERIALS AND METHODS

Animals. Crucian carp (n = 54; body mass 69.2 ± 2.8 g) were caught from a local pond near Joensuu in eastern Finland in November and immediately brought into the lab where they were held in large tanks (1,000 liter) at constant +4°C temperature for at least 3 wk before experimentation. Aerated groundwater was delivered to the tanks at 0.5 l/min. Winter-acclimatized carp do not forage (32), and thus food was not provided. All experiments were conducted with the permission of the local committee for animal experimentation.

Isolation of cardiac myocytes. Ventricular myocytes were isolated using established protocols (39). The fish was stunned by a blow to the head and pithed. The whole heart was carefully excised and cannulated through the bulbus arteriosus. The heart was retrogradely per-
fused, using a hydrostatic pressure head of 50 cmH\textsubscript{2}O, first with Ca\textsuperscript{2+}-free saline for 8 min and then with proteolytic enzymes for 18 min. Both solutions were oxygenated with 100% O\textsubscript{2}. The Ca\textsuperscript{2+}-free saline contained (in mM) 100 NaCl, 10 KCl, 1.2 KH\textsubscript{2}PO\textsubscript{4}, 3 MgSO\textsubscript{4}, 50 taurine, 20 glucose, and 10 HEPES adjusted to pH 6.9 with KOH. For enzymatic digestion, 0.75 mg/ml collagenase (type 1A), 0.50 mg/ml trypsin (type IX), and 0.75 mg/ml fatty acid free albumin (all from Sigma, St.Louis, MO) were added to Ca\textsuperscript{2+}-free saline, which was recirculated using a peristaltic pump. After enzymatic digestion, the ventricle was separated, and single cells were liberated by agitation through the opening of a Pasteur pipette. Myocytes were stored at +4°C and used within 5 h from the isolation.

**Whole cell patch clamp.** For whole cell patch clamp, a small aliquot of cells was placed in the recording chamber (RCP-10T, Dagan, Minneapolis, MN; 0.5 ml). After the myocytes had settled, they were superfused at a constant 1.4 m/min flow of physiological saline, which contained (in mM) 150 NaCl, 5.4 KCl, 1.2 CaCl\textsubscript{2}, 1.2 MgCl\textsubscript{2}, 10 glucose, and 10 HEPES adjusted to pH 7.6 with NaOH. Tetrodosaline contained (in mM) 100 NaCl, 10 KCl, 1.2 KH\textsubscript{2}PO\textsubscript{4}, 3 MgSO\textsubscript{4}, and 10 glucose, and 10 HEPES adjusted to pH 7.2 with KOH. When filled with pipette solution, the mean resistance of the electrodes was 3.71 ± 0.12 M\textohm. Whole cell currents were recorded using an EPC-9 amplifier and Pulse v8.88 software (HEKA). The current-voltage (IV) relations were constructed by applying ramp pulses from +60 to −120 mV (170 mV/s) every 5 s from the holding potential of −80 mV. Temperature of the physiological saline was regulated to desired values using a commercial temperature controller (TC-10; Dagan) and was recorded with an accuracy of 0.1°C together with electrophysiological data on the hard disk of the computer. Giblenclampide (10 μM; Sigma) was added to the external solution to block I\textsubscript{KATP}.

APs of enzymatically isolated myocytes were recorded in current-clamp mode of the amplifier with patch pipettes filled with the following solution (in mM): 140 KCl, 5 Na\textsubscript{2}ATP, 1 MgCl\textsubscript{2}, 0.03 Tris-GTP, 10 HEPES adjusted to pH 7.2 with KOH. The external solution was the same as used for K\textsuperscript{+} currents but without TTX and nifedipine. APs were elicited by 2-ms depolarizing pulses (0.4–0.6 mV). The recording mode alternated every 5 s after first determining at −80 mV the inward current at similar voltage distance from the theoretical reversal potential of K\textsuperscript{+} (−78.9 mV at +8 and −81.7 mV at +18°C) with the following equation:

\[
R = [(−60 mV)/[E_k − (−60 mV − E_k)]
\]

where \(E_k\) is the theoretical reversal potential. Thus \(R = 1\) and \(R = 0\) correspond to the absence and maximal rectification, respectively.

**Single-channel patch clamp.** ATP sensitivity and single-channel kinetics of the I\textsubscript{KATP} were examined with cell-attached and inside-out configurations of the patch clamp and analyzed using TAC, TACFIT (Bruxton), and SigmaPlot 6.0 (SPSS software) as described previously (31). Pipettes were pulled from borosilicate glass (Garner, Claremont, CA) using a two-stage vertical puller (PP-83, Narishige, Tokyo), coated with Sylgard (WPI) and fire-polished on a microforge (MF-83, Narishige). The mean resistance of the pipettes was 12.3 ± 0.2 M\textohm when filled with the K-based solution (in mM): 134 KCl, 1.8 CaCl\textsubscript{2}, 2 MgCl\textsubscript{2}, 10 glucose, and 10 HEPES adjusted to pH 7.6 with KOH (K\textsuperscript{+} = 141 mM). The same solution was used as the bath solution. With flow rate of 1.4 ml/min, the bath solution was completely changed in <5 s.

To measure the ATP sensitivity of the I\textsubscript{KATP} in inside-out patches, the internal saline was modified by omitting CaCl\textsubscript{2} and adding 0.1–10 mM Tris-ATP from stock solutions. The addition of Tris-ATP resulted in 2.4% dilution of the internal saline. ATP solutions were stored frozen at −20°C and used within 8 h from defrosting. Inside-out patches were drawn out in ATP-free saline and, if ATP-sensitive K\textsuperscript{+} channel(s) were detected, it was allowed to stabilize for 3 min. Solution was then changed to an ATP-containing solution, and after 30–90 s, channel activity was recorded for 20 s. Finally, the solution flow was switched back to ATP-free saline. The recording was accepted for analysis only if the ATP-dependent inhibition of the I\textsubscript{KATP} was reversible. One to three ATP concentrations could be tested with one inside-out patch. Relative open-state probability (NP\textsubscript{o}) was used as a current variable when constructing concentration-response curves for the I\textsubscript{KATP}. The plots were fitted with Hill’s equation

\[
1/[1 + ([ATP]/K_{1/2})^H]
\]

where [ATP] is the concentrations of Tris-ATP in the bath, K\textsubscript{1/2} is the concentration of Tris-ATP that produces half-maximal inhibition of the channel, and H is the Hill coefficient (steepness of the curve).

Single-channel kinetics of the I\textsubscript{KATP} were measured at +8 and +18°C. Open-state probabilities (P\textsubscript{o}) and open-time distributions were obtained from 20-s recordings at −100 mV elicited at 30-s intervals. For P\textsubscript{o} determinations, the currents were sampled at 4 kHz and low-pass filtered at 2 kHz, while 10-KHz sampling rate was used for measuring rapid single-channel kinetics. P\textsubscript{o} was estimated by constructing amplitude histograms, which were fitted with Gaussian distributions using the log-likelihood method (TAC). In P\textsubscript{o} experiments, only membrane patches having either one or two channels (mean 1.2 ± 0.4) were accepted for analyses. Open-time and closed-time analyses were performed on patches, which had only a single open-current level. Open and closed times were detected with time-course fitting, and probability density functions (pdf) were analyzed from idealized data with Log-likelihood method on log (event times) (TACFIT). The kinetic model was assumed to be similar to mammals with three different closed states (2).

Single-channel conductance was determined in inside-out patches by applying 5-s square pulses from −120 to +80 mV in 20-mV increments every 10 s from the holding potential of 0 mV. The single-channel conductance was calculated at negative voltages (−120 to 0 mV) where the IV relation was linear. The slope conductance was first determined at +8°C, and then temperature was quickly changed from +8 to +18°C to obtain conductance at higher temperature for calculating the Q\textsubscript{10} value. Single-channel conductance of the I\textsubscript{K1} was obtained from cell-attached patches as by-product of the I\textsubscript{KATP} recordings before patch excision.

**Microelectrode recordings.** The whole heart (35 mg in wet weight) was excised, and the ventricle was cut in two parts to allow free access of oxygenated (O\textsubscript{2}) solution to the tissue. The heart was fixed with insect pins on Sylgard-coated bottom of the 15-ml recording chamber, which was filled with physiological saline (in mM): 150 NaCl, 3 KCl, 1.2 MgSO\textsubscript{4}, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 1.8 CaCl\textsubscript{2}, 10 HEPES, and 10 glucose adjusted to pH 7.6 with NaOH. Heart was allowed to equilibrate at +4°C for ~1 h to reach a stable heart rate before increasing temperature. Ventricular APs were recorded with sharp microelectrodes filled with 3 M KCl (6–30 M\textohm). Analog signals were amplified by a high-impedance amplifier (KS-700, WPI) and digitized (Digidata-1200 AD/DA board Axon Instruments) before storing on the computer with the aid of Axotape (Axon) acquisition software. Recordings were made from spontaneously beating heart or from ventricles paced at constant rate of 0.5 Hz ±19°C (24).

**Statistics.** Data are given as means ± SE. Student’s t-test for unpaired or paired samples or one-way ANOVA and Student-Newman-Keuls post hoc test were used to compare the mean values. P < 0.05 was considered statistically significant.
RESULTS

Effect of temperature on cardiac APs. Microelectrode recordings of ventricular APs and associated contractions at +4°C and +18°C are shown in Fig. 1. Acute temperature change shortened AP duration from 2.8 ± 0.3 s at +4°C to 1.3 ± 0.1 s at +18°C (P < 0.05 ANOVA, n = 4).

Induction of whole cell I_{K,ATP} by acute heat stress. In crucian carp ventricular myocytes, acute heat stress induced a glibenclamide-sensitive (10 μM) outward current, suggesting that ATP-sensitive K⁺ channels were activated (Fig. 2A). This current was quantified at 0 mV where other ionic currents are negligible. As seen in Fig. 2A, no I_{K,ATP} was present at +8°C, but the current appeared when temperature had risen to ~+12°C and then increased with increasing temperature. Cooling to +8°C practically abolished the current, and rewarming activated it again. It should be noted that the currents in Fig. 2 are corrected with the temperature coefficient (Q_{10} = 1.38) of the single-channel conductance and therefore reflect the number of activated channels. In the majority of myocytes (n = 18), the heat-induced I_{K,ATP} was relatively small (G = 21.2 ± 3.2 pS/pF at +8°C) and reversible. In some myocytes (n = 4), the current was larger (100.4 ± 26.4 pS/pF at +8°C; n = 4; P < 0.001) and irreversible, and in the rest of the cells (n = 10) heat stress did not induce a glibenclamide-sensitive current at all. These experiments were all conducted with 4 mM ATP in the pipette solution, which suggests that in the majority of myocytes (56%), high cytosolic ATP concentration does not prevent the opening of ATP-sensitive K⁺ channels under heat stress. This could occur if heat directly activated the channels or if channel ATP sensitivity was so close to the physiological ATP concentration that they were activated by local subsarcolemmal ATP depletion. Internal perfusion of the cells may also affect channel activity. To clarify these possibilities, ATP sensitivity and single-channel properties of the I_{K,ATP} were examined.

Effects of temperature on I_{K,ATP} in cell-attached patches. In cell-attached patches, the cell interior is not perfused, and ion currents can be measured in more native internal surrounding. Even under these conditions, in 33% of the myocytes (7 of 21) acute heat stress (from +4°C to between +13 and +19°C) induced the opening of the ATP-sensitive K⁺ channels (Fig. 3). The current was recognized as I_{K,ATP} by its conductance and single-channel kinetics, which were similar to inside-out patches (see below). The induction of I_{K,ATP} in cell-attached patches suggests that the opening of the channels is not solely due to internal perfusion of the myocytes but could be related to heat stress.

ATP sensitivity. Usually I_{K,ATP} appeared immediately when the inside-out patch was drawn out. However, in 53% of the cases current rundown appeared during the first 3 min of perfusion with ATP-free solution, and these patches were discarded. In the rest of patches, application of ATP-containing solution affected the P_o of the channels within a few seconds in a reversible and repeatable manner. Dose-response curves indicate that the ATP sensitivity of the I_{K,ATP} in crucian carp ventricular myocytes is very low both at +8 (K_{1/2} = 1.35 mM) and +18°C (1.85 mM) (Fig. 4). Although there was only a small difference in ATP affinity between experimental temperatures, the slope of the curve was shallower at higher temperature (H = 3.83 and 1.99 at +8°C and at +18°C, respectively). According to the dose-response curve, the P_o of the channels at 4 mM intracellular ATP would be 0.01 and 0.15 at +8°C and at +18°C, respectively.

Single-channel conductance and kinetics in inside-out patches. Single-channel properties of the I_{K,ATP} were examined at +8 and +18°C to see whether the temperature effect could be explained by temperature-dependent changes in conductance or kinetics of the channels.

At negative voltages (−120–0 mV), the conductance of the I_{K,ATP} (inward current) was linear, whereas at more positive voltages the outward current rectified inwardly (Fig. 4C). The rectification was at least partially Mg²⁺ dependent as it was diminished in Mg²⁺-free bath solution (not shown). The conductance of the I_{K,ATP} was slightly increased (Q_{10} = 1.38) with increasing temperature and was 37.8 ± 1.3 pS at +8°C and 52.0 ± 0.9 pS at +18°C. In the absence of ATP, the P_o was high both at +8°C (0.73 ± 0.01) and at +18°C (0.71 ± 0.02) (P = 0.72; n = 11).

Single-channel kinetics were recorded at the highest feasible sampling rate for allowing good resolution of short events and were done at −100 mV where the current amplitude was relatively large. In general, high temperature increased the transition of the channel between different states. Single-channel open times, burst lengths, and interburst closed times were 28, 37, and 33% shorter, respectively, at +18°C than at +8°C (Fig. 5). Single-channel closed times were well fitted with a double-exponential equation. High temperature increased the relative frequency of the short closings at the expense of long closings and shortened the duration of the long closed states (τ_{c2}) by 44%.

Is AP duration of intact ventricle regulated by the I_{K,ATP}? As heat stress is able to induce the opening of ATP-sensitive K⁺ channels, the contribution of I_{K,ATP} to temperature-dependent shortening of ventricular APs was studied in intact ventricles with microelectrodes. If I_{K,ATP} contributes to the shortening of AP at higher temperatures, we would expect the effect to be reversed by glibenclamide, a specific blocker of the ATP-sensitive K⁺ channels as previously demonstrated in goldfish.
heart (12). This was not the case for crucian carp (Fig. 6). Although APs were dramatically shortened by increasing the temperature from \(4^\circ\)C to \(18^\circ\)C, \(10^{-6}\)M glibenclamide had no effect on AP duration. Similarly, no effect was seen with \(10^{-6}\)M glibenclamide and after 1.5 h of incubation. This strongly suggests that heat stress alone is unable to trigger the opening of ATP-sensitive K\(^+\) channels in intact ventricles.

How is AP duration regulated in carp myocytes? To further investigate the regulation of AP duration under heat stress, we alternately recorded APs and K\(^+\) currents from the same myocyte. The recording mode was switched every fifth second between current clamp and voltage clamp while temperature was increased. It is clear that the duration of AP was closely correlated with changes in the Ba\(^{2+}\)-sensitive and glibenclamide-insensitive outward current between \(-70\) and \(-20\) mV. Thus the background inward rectifier K current \(I_{K1}\) is a strong modifier of the AP duration in carp cardiac myocytes (Fig. 7A). Indeed, at \(+4^\circ\)C the outward \(I_{K1}\) was very small, but as temperature increased the overshoot in voltage range between \(-70\) and \(-20\) mV also increased (Fig. 7B). It should be noted that there was a severalfold increase in the outward current, while the inward current was enhanced according to Q\(_{10}\) (1.56) of the single-channel conductance of the \(I_{K1}\) (Fig. 7D). This was confirmed by comparing the effect of temperature on inward current and outward current at similar distance from \(E_K\), which indicated dramatic relief of rectification at high temperatures \((P < 0.001)\) (Fig. 7A). This indicates that temperature does not affect \(I_{K1}\) equally at all voltages but specifically modifies the rectification properties of the \(I_{K1}\). The change in rectification was seen in all 27 myocytes in which \(I_{K1}\) and AP were alternately measured.

At single-channel level, two different kinetic modes of the \(I_{K1}\) (called slow and fast \(I_{K1}\) from here onward) were distinguished. The conductances of the two \(I_{K1}\) modes were identical...
Fig. 3. Representative cell-attached recordings of single-channel ATP-sensitive K⁺ current (I<sub>K,ATP</sub>) at −100 mV and open probabilities (obtained as a number of events in 20-s sample) indicating a heat stress-induced opening of the channel. Short horizontal lines indicate closed channel level.

Fig. 4. ATP sensitivity and single-channel conductance of the I<sub>K,ATP</sub> in inside-out patches of crucian carp ventricular myocytes at +8 and +18°C. A: original recordings showing decreases in the open-state probability of the channels by 2 mM Tris-ATP at +8 and +18°C. Short horizontal lines indicate the closed channel level. [ATP]<sub>i</sub>, intracellular ATP concentration. B: concentration-response curves for ATP-dependent changes in open-state probability of the I<sub>K,ATP</sub>. Each point represents a mean (±SE) from 3–7 patches. NP<sub>o</sub>, relative open-state probability; K<sub>1/2</sub>, the concentration of Tris-ATP that produces half-maximal inhibition of the channel; H, the Hill coefficient (steepness of the curve). C: single-channel current-voltage relationship of the I<sub>K,ATP</sub> at +8 and +18°C. The slope conductance (G) was calculated for the linear portion of the curve between −120 and 0 mV. Each point represents a mean (±SE) from 4 to 28 patches.
and clearly smaller than the conductance of the \( I_{K_{\text{ATP}}} \). The mean open times of the slow \( I_{K_{\text{1}}} \) were three to four times longer than those of the fast \( I_{K_{\text{1}}} \) (Fig. 7D). Heat stress from +8 to +18°C increased the conductance of the slow and fast \( I_{K_{\text{1}}} \) with \( Q_{10} \) of 1.56 and reduced the mean open times to about one-half of the initial value. At +8°C, the fast \( I_{K_{\text{1}}} \) was present in 3 of 18 patches, while at +18°C it occurred in 6 of 17 patches, suggesting an increased tendency of the \( I_{K_{\text{1}}} \) to be in the fast mode at high temperatures.

In addition to the \( I_{K_{\text{1}}} \), a \( \text{Ba}^{2+} \)-insensitive outward current was always present at voltages more positive than +20 mV. This current was identified as \( \text{Na}^{+}/\text{Ca}^{2+} \) exchange current because it was insensitive to replacement of \( \text{Cs}^{+} \) for \( \text{K}^{+} \) in both pipette and bath solutions but was completely blocked with 10 mM NiCl\(_2\) (Fig. 7C). It should be also noted that crucian carp ventricle myocytes do not have E-4031-sensitive delayed rectifier channels (\( I_{K_{r}} \)).

In ~15% of cells (4 of 27), heat stress reversibly induced \( I_{K_{\text{ATP}}} \) in the presence of 5 mM intracellular ATP concentration.

Fig. 5. Temperature-dependent changes in single-channel kinetics of ATP-sensitive \( K^{+} \) channels. A: representative recordings of single-channel activity at +8 and +18°C. Sampling and filtering frequencies were 10 and 5 kHz, respectively. Short horizontal lines indicate the closed-channel level. B: frequency distributions and mean durations of single channel open (\( \tau_{o} \)) and closed times (fast closings \( \tau_{c1} \) and slow closings \( \tau_{c2} \)), interburst closed times (\( \tau_{c} \)), and burst lengths (\( \tau_{b} \)) of the single ATP-sensitive \( K^{+} \) channels at +8 and +18°C. Sampling frequency was 10 kHz, and the data were low-pass filtered with 5 kHz for intraburst events. Ten-millisecond duration was used as a criterion to separate individual bursts. Due to the contamination by the slow component of the closed times (\( \tau_{c2} \)), the frequency distributions of the interburst closed times were fitted with a double-exponential equation with the value of \( \tau_{c2} \) as the fast component. Open-state probability (right) was recorded with 4- to 10-kHz sampling rate.
The present results show that acute heat stress can induce the opening of ATP-sensitive K⁺ channels in enzymatically isolated myocytes of the crucian carp heart, but this mechanism does not play a significant role in the regulation of AP duration in intact ventricular muscle. Shortening of AP duration under heat stress occurs mainly as a consequence of temperature-specific changes in the rectification of the background inward rectifier current the \( I_{K1} \).

Effect of temperature on the \( I_{K,ATP} \). The ATP-sensitive K⁺ channels remained closed at low experimental temperatures, but in 56% of the patch-clamped myocytes, heat stress induced the opening of the channels in the presence of 4 mM ATP in the pipette. The reversibility of the response on cooling suggests that it was specific for temperature change and not a general stress response or due to myocyte fatigue. The temperature-dependent increase in the \( I_{K,ATP} \) was almost solely due to opening of new channels because the \( Q_{10} \) of the single-channel conductance was relatively small (1.38) and similar to that of the mammalian cardiac myocytes (1.3) (14). High temperatures slightly increased flickering rate of the channels between open and closed states as single-channel open times, closed times (\( T_c \)), burst lengths, and interburst closed times were reduced by 28–44% at +18°C compared with +8°C. In mammalian myocytes, little changes in the kinetics of the \( I_{K,ATP} \) were found above +25°C (14). The difference between mammalian and fish cardiac myocytes may be attributed to lower experimental temperatures of the present work, which allowed better resolution of the small changes in fast single-channel events.

To our knowledge, heat-induced opening of ATP-sensitive K⁺ channels has not been previously documented. Quite in contrast, it has been indicated that the mammalian Kir6.2/SUR2A is activated by transient cooling from 32–35°C to +5°C (43). The cooling-induced channel activity is probably due to the reduction of ATPase activity of the SUR2A, which allows long-lived binding of MgADP to the nucleotide-binding domain and thus effective relief of ATP inhibition. We are not able to provide mechanistic explanation for heat-induced opening of the channel in fish cardiac myocytes, but it is likely that the nucleotide-binding domains of the fish heart SUR are significantly different from their mammalian counterparts as is also suggested by differences in ATP-binding affinity of the channels.

In vivo, \( I_{K,ATP} \) is regulated by the ATP sensitivity of individual channels, which are modulated by intracellular signaling systems (17, 22, 40) and control channel opening under different stresses (42). The ATP affinity of the channel in crucian carp ventricular myocytes is strikingly low with the \( K_{1/2} \) value of 1.35–1.85 mM. Under comparable experimental conditions, the ATP sensitivity of the mammalian channels is one to two orders of magnitude higher (16, 28). Both amino and carboxy terminal ends of the Kir6.2 and two binding domains of the SUR2A are needed to coordinate the ATP sensitivity of the mammalian ATP-sensitive K⁺ channels (3, 18, 26, 37). Furthermore, protein kinases, creatine phosphate, guanosine diphosphate, and especially PIP₂ are known to lower the ATP affinity of the mammalian ATP-sensitive K⁺ channels to submillimolar or even millimolar range (4, 15, 28). The molecular counterpart of the fish ATP-sensitive K⁺ channels is not yet known, and the intracellular regulation of ATP sensitivity by second messengers has not yet been studied in fish. Therefore, the cause of the unusually low ATP sensitivity of the fish \( I_{K,ATP} \) remains open.

A 10-degree rise in temperature shifted \( K_{1/2} \) by 0.5 mM to the right and changed Hill’s coefficient from 1.99 to 3.83, which might account for the temperature-sensitive induction of the \( I_{K,ATP} \). Unfortunately, these differences could not be resolved statistically as only one to three ATP concentrations could be tested in one inside-out patch, and each dose-response curve had to be constructed from large number of patches. The values derived from the dose-response curves suggest, however, that at an intracellular ATP concentration of 4 mM a 10-degree rise in temperature would increase the open-state probability of the channel from 0.01 to 0.15. If the maximum conductance of the \( I_{K,ATP} \) (160 pS/pF) (30) under complete metabolic inhibition is due to the opening of all the channels, a 10-degree increase in temperature would account for a rise in conductance from 1.6 to 24 pS/pF. The latter value is not far from the reversible conductance (21.2 pS/pF) change induced by heat stress in the present study. This could be caused by temperature-related changes in intracellular pH (from 7.55 at +8°C to 7.44 at +18°C), which is known to affect channel ATP sensitivity (9, 38) and activation (40). Alternatively, the temperature-induced opening of ATP-sensitive K⁺ channels might be an indirect consequence of temperature rise. It is conceivable that in the diffusion-restricted subsarcolemmal

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**DISCUSSION**

(Fig. 8). In these myocytes, the induction of the \( I_{K,ATP} \) occurred with some delay from the temperature change and was preceded by \( I_{K1} \)-dependent shortening of APs. However, the late changes in AP duration were closely correlated with changes in the amplitude of the \( I_{K,ATP} \) at 0 mV. The maximal conductance of the \( I_{K,ATP} \) in these myocytes was 130 ± 55 pS/pF at 8°C.

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**REGULATION OF ACTION POTENTIAL DURATION UNDER HEAT STRESS**

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space, a small local depletion of ATP could occur when temperature-dependent increase in ATP consumption, e.g., by the sodium pump, will exceed the rate of ATP production and diffusion (1). The low ATP affinity of the channel may make it sensitive to such local ATP depletions.

Effect of temperature on the $I_{K1}$. At the single-channel level, the temperature dependence of the $I_{K1}$ ($Q_{10} = 1.56$) was stronger than that of the $I_{K,ATP}$ ($Q_{10} = 1.38$) (see also Ref. 31). Whole cell experiments suggest, however, that temperature does not change the $I_{K1}$ equally at all membrane potentials but specifically affects the negative slope conductance at voltage range between $-70$ and $-20$ mV, suggesting that the inward rectification is modulated by high temperatures. To the best of our knowledge, this is the first study to provide evidence that rectification of the $I_{K1}$ is modulated by acute temperature changes. The almost complete reversibility of the changes in $I_{K1}$ on cooling suggests that the response was specific for temperature and not caused by washout of the intracellular signal molecules or regulators (21). The inward rectification of the $I_{K1}$ is due to voltage-dependent block of the channel by intracellular polyamines (spermine and spermidine) and/or Mg$^{2+}$ (19, 25). In this scheme, the relief of rectification at high temperatures could be due to either reduced affinity of the channel to these cations or changes in the intracellular concentrations of the cations. The biosynthesis and degradation rates of polyamines are probably accelerated at high temperatures, but their net effect on the level of free polyamine concentration is difficult to predict. On the other hand, the rectification effect

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Fig. 7. Alternate recording of APs and $K^+$ currents at 5-s intervals in enzymatically isolated ventricular myocytes of the crucian carp heart indicating late opening of ATP-sensitive $K^+$ channels under heat stress. A: representative recordings of APs and membrane currents from the same myocyte at $+18^\circ$C just before and after the induction of the $I_{K,ATP}$. B: time-dependent changes in AP duration and the conductance of the outward current between $-40$ and $0$ mV, and their sensitivity to 10 $\mu$M glibenclamide.

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Fig. 8. Alternate recording of APs and $K^+$ currents at 5-s intervals in enzymatically isolated ventricular myocytes of the crucian carp heart indicating late opening of ATP-sensitive $K^+$ channels under heat stress. A: representative recordings of APs and membrane currents from the same myocyte at $+18^\circ$C just before and after the induction of the $I_{K,ATP}$. B: time-dependent changes in AP duration and the conductance of the outward current between $-40$ and $0$ mV, and their sensitivity to 10 $\mu$M glibenclamide.
of spermine on some inward rectifier channels is reduced by decreases in intracellular pH (5), and therefore a temperature-related decrease in intracellular pH could explain the disproportionately large outward current at high temperatures. The interesting and physiologically important mechanisms underlying these temperature-dependent changes in rectification of the \( I_{K1} \) clearly warrant further study.

Relative significance of \( I_{K1} \) and \( I_{K,ATP} \) in the regulation of AP duration. In the goldfish, which is a very close relative to crucian carp, the temperature-dependent shortening of cardiac AP duration was strongly antagonized by 5 \( \mu \)M glibenclamide (12). In the current study, glibenclamide had no effect on ventricular AP duration in crucian carp, and therefore we were forced to abandon the hypothesis that the opening of \( I_{K,ATP} \) causes AP shortening under acute heat stress. As the effectiveness of glibenclamide as a blocker of the \( I_{K,ATP} \) is compromised under stress (6, 7), we sometimes exposed hearts to 100 \( \mu \)M glibenclamide for > 1 h, but without any effect on AP duration. Furthermore, the magnitude of temperature-induced change in AP in multicellular preparations was similar as the duration. Furthermore, the ability of the \( I_{K1} \) to control the resting membrane potential of cardiac myocytes was always preceded by a long delay from the onset of the heat stress. Furthermore, this effect was always preceded by an \( I_{K1} \)-mediated shortening of the AP. It seems that in crucian carp ventricular myocytes under normoxic conditions, the temperature-dependent regulation of AP duration occurs mainly through the background inward rectifier channels, while the ATP-sensitive \( K^+ \) channels come into play later and only under more severe stresses. Once induced, \( I_{K,ATP} \) practically abolished AP plateau. On the other hand, the significance of the \( I_{K,ATP} \) might have been underestimated because the intact ventricles in vitro were neither under normal work load nor exposed to epinephrine as is the case in physiological situations. Under these physiological stresses, the \( I_{K,ATP} \) may play a more significant role in the regulation of AP duration (42). Indeed, the low AP affinity of the crucian carp ATP-sensitive \( K^+ \) channels is expected to cause AP shortening at near normal ATP concentrations.

It was a rather unexpected finding that \( I_{K1} \) had such a dramatic effect on AP duration in fish ventricular myocytes, as \( I_{K1} \) mainly determines the rate of terminal repolarization and controls the resting membrane potential of cardiac myocytes (20) but it is generally thought that the channels are closed during the plateau of the AP. The ability of the \( I_{K1} \) to control AP duration in crucian carp ventricular myocytes cannot be due to its amplitude, which is not particularly large, but rather may be related to the high membrane resistance of the ectothermic animals. The AP plateau is maintained by a balance of depolarizing and repolarizing currents. Accordingly, the relatively small \( I_{Ca} \) and \( I_{Na} \) of the crucian carp ventricular myocytes compared with mammalian cardiac myocytes (39; Haverinen and Vornanen, unpublished observations) are offset by small changes in the conductance of the background inward rectifier (30). This is in line with the finding that overexpression of the Kir2.1 protein dramatically shortens AP duration in guinea pig ventricular myocytes (27). Considering that the maximum conductance of the \( I_{K,ATP} \) in crucian carp myocytes is <20% of the amplitude of the current in mammalian cardiac myocytes (30), it is not surprising that AP duration was shortened ~50% by activating only 1–2% of ATP-sensitive \( K^+ \) channels (Fig. 7), a value similar to that found in mammals (29). Thus the present findings indicate that relatively small changes in \( I_{K1} \) and \( I_{K,ATP} \) are able to regulate AP duration in crucian carp ventricular myocytes.

To conclude, the present results shows that 1) although the \( I_{K,ATP} \) can be induced by acute heat stress in crucian carp ventricular myocytes, it contributes little to temperature-dependent changes in AP duration under normoxic conditions; and 2) the background inward rectifier \( K^+ \) channels regulate AP duration by temperature-sensitive changes in the rectification properties of the channels. The latter mechanism is activated by minute temperature changes and is therefore likely to play significant physiological role whenever the body temperature of the fish changes.

GRANTS

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REFERENCES