J. Membrane Biol. 194, 119–127 (2003)
DOI: 10.1007/s00232-003-2032-x

The Journal of
Membrane
Biology

Effects of Chronic Hypoxia on Inward Rectifier K^+ Current (I_{K1}) in Ventricular Myocytes of Crucian Carp (*Carassius carassius*) Heart

V. Paajanen, M. Vornanen
University of Joensuu, Department of Biology, P.O. Box 111, 8010 Joensuu, Finland

Received: 10 December 2002/Revised: 14 April 2003

Abstract. Some ectothermic vertebrates show unusually good tolerance to oxygen shortage and it is therefore assumed that they might, as a defense mechanism, decrease number or activity of ion channels in order to reduce membrane leakage and thereby ATP-dependent ion pumping in hypoxia. Although several studies have provided indirect evidence in favor of this 'channel arrest' hypothesis, only few experiments have examined activity of ion channels directly from animals exposed to chronic hypoxia or anoxia in vivo. Here we compare the inwardly rectifying K^+ current (I_{K1}), a major leak and repolarizing K^+ pathway of the heart, in cardiac myocytes of normoxic and hypoxic crucian carp, using the whole-cell and cell-attached single-channel patch-clamp methods. Whole-cell conductance of I_{K1} was 0.5 ± 0.04 nS/pF in normoxic fish and did not change during the 4 weeks hypoxic (O_2 < 0.4 mg/l; 2.68 mmHg) period, meanwhile the activity of Na^+/K^+ATPase decreased 33%. Single-channel conductance of the I_{K1} was 20.5 ± 0.8 pS in control fish and 21.4 ± 1.1pS in hypoxic fish, and the open probability of the channel was 0.80 ± 0.03 and 0.74 ± 0.04 ($P > 0.05$) in control and hypoxic fish, respectively. Open and closed times also had identical distributions in normoxic and hypoxic animals. These results suggest that the density and activity of the inward rectifier K^+ channel is not modified by chronic hypoxia in ventricular myocytes of the crucian carp heart. It is concluded that instead of channel arrest, the hypoxic fish cardiac myocytes obtain energy savings through 'action potential arrest' due to hypoxic bradycardia.

Key words: Channel arrest — Fish heart — Patch clamp — Hypoxic adaptation — Inward rectifier potassium channels

Correspondence to: V. Paajanen; email: vpaajane@cc.joensuu.fi

Introduction

Ion gradients across the plasma membrane are vital for the function of nerve and muscle cells by allowing the generation of negative resting membrane potential (RMP) and action potentials (AP) by ion currents through Na^+-, K^+- and Ca^{2+}-specific channels. Maintenance of ion gradients is an active process, which may consume 50–60% of the normoxic ATP production in excitable cells (Schramm, Klieber & Daut, 1994; Rolfe & Brown, 1997; Boutilier, 2001). A major part of this is due to ATP-driven transfer of Na^+ and K^+ across the cell membrane by the Na^+/K^+ATPase. Although vital for excitability, the large energy cost of ion pumping poses a problem to animal cells when they are exposed to hypoxic or anoxic conditions, because reduced active ion pumping may be unable to counterbalance passive ion leakage down the electrochemical gradient in hypoxic cells. Indeed, brain and heart cells of the mammals are damaged by short periods of anoxia/hypoxia, as energy insufficiency collapses ion gradients and RMP (for reviews *see* Pierce & Gzubryt, 1995; Ducceschi et al., 1996; Kristián & Siesjö, 1996; Lipton, 1999). In contrast to endotherms, some ectothermic vertebrates are able to tolerate chronic hypoxia or anoxia, and therefore these species must have physiological mechanisms that prevent or delay the hypoxic damage. As anaerobic metabolism cannot maintain high ATP production for long periods, chronic hypoxia tolerance must be based on reduced ATP demand. Specifically, it has been postulated that the cells of these species will reduce the density and/or activity of ion channels, which would limit dissipative ion leakage through channels and allow the maintenance of ion gradients and RMP with lower ATP production (Hochachka, 1986).

The channel-arrest hypothesis has been tested in neurons and hepatocytes of hypoxia-tolerant turtles

(Doll, Hochachka & Reiner, 1991; Perez-Pinzon et al., 1992; Buck & Hochachka, 1993; Buck & Bickler, 1998), but never in heart muscle, an organ that has also high energy demand and is continuously active. RMP of cardiac myocytes is maintained by the inwardly rectifying K^+ current (I_{K1}) that allows continuous K^+ efflux and clamps the RMP close to the reversal potential of potassium ions (Sakmann & Trube, 1984a; Roden et al., 2002). Thus, unlike Na^+ and Ca^{2+} channels, which are only transiently opened during action potentials, I_{K1} forms a continuous K^+ leakage pathway in the sarcolemma (SL) of resting cardiac myocytes and sets demands on the Na^+/K^+ATPase even in diastole. In addition, I_{K1} contributes to phase-3 repolarization of the cardiac AP (for reviews *see* Barry & Nerbonne, 1996; Lopatin & Nicholls, 2001; Roden et al., 2002), which further increases K^+ leakage in contracting myocytes through the same channels. Due to the continuous activity of I_{K1} throughout the cardiac cycle and its direct functional connection to the activity of Na^+/K^+ATPase, one of the main energy-consuming processes of the cardiac myocyte, the inwardly rectifying K^+ channel could be a potential target for channel arrest in hypoxic heart. Therefore, the predictions of the channel arrest hypothesis, that (i) whole-cell current densities, and/or (ii) single-channel conductance/open probability of the cation channels are lower in hypoxic than normoxic animals, were tested on the cardiac I_{K1} of the crucian carp (*Carassius carassius* L.), one of the most anoxia-tolerant vertebrates (Blazka, 1958).

Materials and Methods

Animals

Winter-acclimatized crucian carp ($N = 68$; body mass 31.6 ± 1.8 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 (1000 l) at constant 4°C temperature. Aerated groundwater was delivered to the tanks at 0.5 l/min. Winter-acclimatized carp do not forage (Penttinen & Holopainen, 1992), and therefore no fodder was provided. After 8 weeks of habituation to laboratory conditions at constant photo period (9 hour light 15 hour dark) the fish were separated into a normoxic and a hypoxic group. Normoxic animals were maintained at constant oxygen pressure of 10.6 ± 0.2 mg/l O_2 (77.84 ± 0.15 mmHg) at 4°C for the whole 4-week test period. Hypoxic animals were maintained for 4–28 days under severely reduced oxygen tension at the same temperature as the normoxic fish. For hypoxic exposure, one carp each was put into water-filled Erlenmayer bottles (2 l), and oxygen was driven out by N_2-gassing until O_2 tension was less than 0.39 mg/l (2.86 mm Hg) (Cellox 325 with WTW Multiline P4, Germany). The bottle was quickly sealed with a rubber stopper and placed on the bottom of the tank. After selected periods of hypoxia, the bottles were opened, oxygen tension measured and the animals used for the experiments. 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 (Vornanen & Tuomennoro, 1999). Fish were stunned by a sharp blow to the head and killed by decapitation. The whole heart was carefully excised and a cannula was inserted through bulbus arteriosus into the ventricle. The heart was perfused retrogradely, using a hydrostatic pressure head of 80 cm H_2O, first with Ca^{2+}-free saline for 15 minutes and then with proteolytic enzymes for 22 minutes. The Ca^{2+}-free saline contained (in mM): 100 NaCl, 10 KCl, 1.2 KH_2PO_4, 3 $MgSO_4$, 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^{2+}-free saline, which was recirculated using a peristaltic pump. For the normoxic animals, both solutions were oxygenated with 100% O_2, while myocyte isolation from hypoxic animals occurred without exogenous O_2. After enzymatic digestion, the ventricle was separated and diced with scissors into small pieces. Single cells were liberated by agitation of the tissue samples through the opening of a Pasteur pipette. Myocytes were stored in closed 20-ml glass bottles at 4°C and used within 5 hours from the isolation. No changes in the amplitude of the whole-cell current ($r^2 = 0.05$) or single-channel kinetics were evident during the 5-hour storage.

Whole-Cell Recording of the I_{K1}

For whole-cell patch clamp, a small aliquot of cells was taken into the recording chamber (RCP-10T, Dagan, Minneapolis, MI, 0.5 ml). After the myocytes had settled, they were superfused at a constant 1.4 ml/min flow of physiological saline that contained (in mM): 150 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose and 10 HEPES adjusted to pH 7.6 with NaOH. Temperature of the solution was regulated to +5 ± 0.1°C (TC-10 controller; Dagan). Tetrodotoxin citrate (0.5 μM; Tocris Cookson, UK) and nifedipine (10 μM; Sigma) were added to the saline to prevent Na^+ (I_{Na}) and L-type Ca^{2+} currents ($I_{Ca\text{-}L}$), respectively. A complete block of Na^+ channels is obtained with 0.5 μM TTX since the I_{Na} of teleost cardiac myocytes is TTX-sensitive (K_d about 10 nM; Vornanen, unpublished results). Patch pipettes were pulled from borosilicate glass (Garner, Claremont CA) using a two-stage vertical puller (L/M-3P-A, List-Medical, Darmstadt, Germany), and filled with K^+-based electrode solution that contained (in mM): 140 KCl, 1 $MgCl_2$, 5 EGTA, 4 MgATP and 10 HEPES adjusted to pH 7.2 with KOH. When filled with pipette solution, the resistance of the electrodes was 3.10 ± 0.04 MΩ. Whole-cell currents were recorded using an appropriate amplifier (EPC-9, HEKA, Germany) and commercial software (Pulse v8.58, HEKA, Germany). After achieving a gigaohm seal, pipette capacitance was compensated, and after getting access to whole-cell configuration, membrane capacitance was measured by integrating the capacitive current induced by small hyperpolarizing steps. No leak or series resistance compensation was used. The mean series resistance (10.9 ± 0.3 MΩ) caused a voltage error of 4.5 ± 0.2 mV at −120 mV ($V_m = V_p - R_s I$) and 10.8 ± 0.7% underestimation of the real conductance of I_{K1} (*compare* Ishihara et al., 2002). The error was similar in hypoxic and normoxic myocytes and was not corrected. The current-voltage (I/V) relations were constructed by applying 1000-ms square pulses from −120 to +60 mV in 10-mV increments every 10 s from the holding potential of −80 mV.

Single-Channel Recording

The kinetics of the I_{K1} was measured from normoxic and 20-day hypoxic carp. Cell-attached recordings of single-channel currents

were conducted using the same K^+-based solution in the pipette and in the bath. It contained (in mM): 134 KCl, 1.8 $CaCl_2$, 2 $MgCl_2$, 10 glucose and 10 HEPES adjusted to pH 7.6 with KOH ($[K^+]$ = 141 mM). Pipettes were pulled from borosilicate glass (Garner, Claremont, CA), using a two-stage vertical puller (PP-83, Narishige, Tokyo, Japan), and were fire-polished on a microforge (MF-83, Narishige, Tokyo, Japan). The immersed part of the pipette shaft was coated with Sylgard (WPI, UK). The mean resistance of the pipettes was 9.0 ± 2.1 MΩ. Single-channel conductance was constructed 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. Open probability (P_o) and open-time distribution were constructed by applying −100 mV pulses for 20 s. Currents were sampled at 2 kHz and low-pass filtered at 1 kHz. Data were analyzed using TAC and TACFIT (Bruxton) and SigmaPlot 6.0 (SPSS) software. Single-channel current amplitudes and P_o were measured by constructing amplitude histograms and fitting Gaussian distributions to them with the Log-likelihood method (TAC). P_o, 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 the Log-likelihood method on log(event times) (TACFIT). The number of closed states was judged by a statistical approach, as described by Colquhoun and Sigworth (1995).

Na^+/K^+ATPase Activity

Na^+/K^+ATPase activities of normoxic and 3-week hypoxic carp were measured in crude cardiac homogenates, as described earlier (Aho & Vornanen, 1997). Briefly, ventricles from two fish were pooled, minced with scissors and then homogenized in five volumes of ice-cold TES-buffered medium (in mM): 250 sucrose, 20 TES, 1 dithiothreitol, 1 EGTA, pH 7.6). The homogenate was further diluted to 1:50 (w:v) using TES buffer and centrifuged at 1000 × *g* for 5 min (4°C) to sediment unbroken tissue. Na^+/K^+ATPase activity of the supernatant fraction was determined at 37°C by incubating the samples in Tris buffer (in mM: 50 Tris-HCl, 4 $MgCl_2$, 4 Tris-ATP, 20 KCl and 100 NaCl, pH 7.5) in the presence and absence of 1 mM ouabain. The ATPase activity was determined from the amount of liberated inorganic phosphate (Atkinson, Gatenby & Lowe, 1973). The ouabain-inhibitable portion of the total ATPase activity was considered to represent Na^+/K^+ATPase activity. Homogenate protein was determined with the method of Lowry et al. (1951).

Statistics

Data are given as means ± SEM. Correlation between the duration of hypoxic period and the size of whole-cell currents were analyzed with Spearman rank order correlation. Single-channel results and Na^+/K^+ATPase activities were tested with Student's *t*-test for unpaired samples. $P < 0.05$ were considered statistically significant.

Results

Extent of Hypoxia and Hypoxia-Tolerance of the Fish

At the beginning of the hypoxic period, O_2 tension of water was less than 0.39 mg/l (2.68 mmHg) and dropped down to 0.27 ± 0.01 mg/l (1.98 ± 0.07 mmHg) within the first 4 days of hypoxia and remained at that level for the whole 4-week period. As the amount of oxygen extracted from water is able to support aerobic metabolism of 20 g carp for about half an hour (Blazka, 1958), the fish have been largely dependent on anaerobic metabolism and thus were truly hypoxic or even anoxic.

Crucian carp survived the 4-week period of deep hypoxia at 4°C without any mortality. Hypoxic fish maintained balanced upright position, but avoided active movements. Immediately after the opening of the bottle, hypoxic carp were unresponsive to the touch of the oxygen sensor, but started active movements when they were taken out of the water.

Amplitude of the Whole-Cell I_{K1}

Current density at −120 mV and whole-cell conductance between −120 and −90 mV were measured at four-day intervals from fish maintained under hypoxia from 4 to 28 days, and compared to the values of the normoxic fish. Figure 1 shows representative whole-cell recordings of the I_{K1} from normoxic and hypoxic fish. The reversal potential, extrapolated from linear currents between −120 and −90 mV to zero current level, varied from −70.4 ± 0.8 to −74.7 ± 0.7 mV (including 4.2 mV junction-potential correction) and was not affected by hypoxic exposure. These values are not far from the theoretical Nernstian equilibrium potential for K^+ (−78.3 mV) at 5°C. At strongly negative voltages (−120 mV), a small, time-dependent inactivation of the I_{K1} was evident in both normoxic and hypoxic myocytes (Fig. 1). The density and conductance of the I_{K1} in normoxic fish was 24 ± 4 pA/pF and 0.51 ± 0.04 nS/pF, respectively, and these values did not differ from the respective values of the hypoxic fish (Fig. 1; $P > 0.05$ Spearman correlation). These findings indicate that hypoxia did not have any effect on the whole-cell I_{K1} of the crucian carp ventricular myocytes.

Single-Channel Activity and Kinetics

As intracellular perfusion of myocytes in whole-cell configuration modifies the native intracellular milieu and may interfere with the normal cellular signaling by pH_i, second messengers and covalent modification (Ruppersberg, 2000), hypoxic downregulation of I_{K1} might not be seen in these recordings. Therefore, cell-attached single-channel recordings were also conducted. Single-channel recordings were collected from 7 hypoxic (20 days in duration) and 11 normoxic carp. In the majority of patches, the dominating single-channel conductance was a 21 pS channel with relatively infrequent changes between open and closed states (Fig. 2). In addition to the 21-pS channel, in a few patches two smaller currents with con-

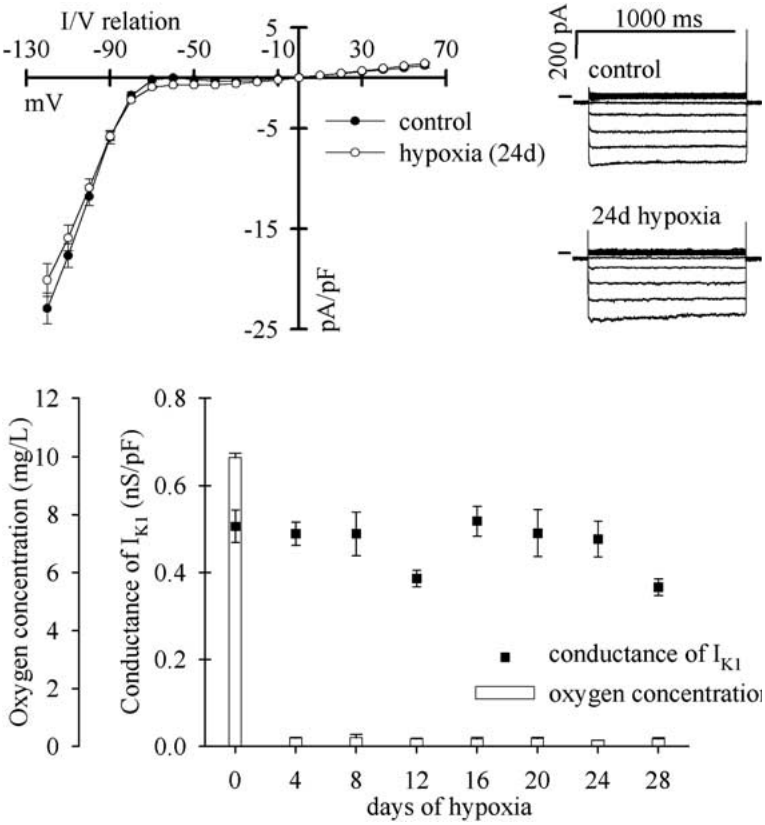

Fig. 1. Comparison of the whole-cell I_{K1} from hypoxic and normoxic carp. (*Top left*) Current-voltage relationships and representative current tracings (*top right*) or normoxic and 24-day hypoxic carp at +5°C. Currents were elicited by 1000-ms voltage pulses between −120 and +60 mV in 20-mV increments from the holding potential of −80 mV. (*Bottom*) Whole-cell conductance of the I_{K1} in carp maintained either at normoxia or 4 to 28 days under severe hypoxia. The results are means ± SEM of 8–16 myocytes from 2 fish for each point.

ductance of 0.3 and 0.7 times the 21 pS channel were detected (Fig. 3). These currents were regarded as subconductance states of the 21 pS channel because in some recordings these conductances appeared as a direct transition between fully open and fully closed states of the 21-pS conductance. Openings of these subconductance states lasted up to 300 ms and occurred less than once in a minute. In a few cases (n = 5), at least one small (13.7 ± 1.18 pS) inwardly rectifying current was also detected. The sublevels of the 21-pS channel and the 14-pS conductance were equally infrequent in normoxic and hypoxic fish. Besides these inwardly rectifying currents a larger (> 40 pS) nonrectifying and flickering current, most likely representing an ATP-sensitive K^+ channel activity (I_{KATP}), was noticed in a few recordings. I_{KATP} occurred more frequently in hypoxic than normoxic myocytes. The few patches having I_{KATP} and the other rare currents were excluded from the quantitative analysis of single-channel conductance. This analysis indicated that the single-channel conductance of the I_{K1} of the normoxic carp (20.5 ± 0.8 pS, n = 17) does not differ from the value of 20-day hypoxic carp (21.5 ± 1.1 pS, n = 11) ($P > 0.05$) (Fig. 2). The reversal potential of the I_{K1} in cell-attached recordings was −23.2 ± 2.8 mV and −16.7 ± 2.7 mV in normoxic and hypoxic fish, respectively ($P > 0.05$).

The P_o of the I_{K1} was analyzed from patches having only one active channel and was equally high in normoxic (0.80 ± 0.03) and in hypoxic (0.74 ± 0.04) myocytes ($P > 0.05$; n = 18 for both groups). The probability of getting a membrane patch with a single I_{K1} channel using 9 MΩ pipettes was similar (~0.6) in normoxic and hypoxic fish.

Open- and closed-time distributions were calculated from three successive 20-s clamps to −100 mV in patches with only one active I_{K1} channel and lacking the subconductance states. This analysis required long recordings due to relatively infrequent changes between open and closed states, typical for the I_{K1}, and were therefore done only at −100 mV. The mean open time (τ_o), estimated by assuming a monoexponential distribution, did not differ between normoxic (680 ± 60 ms) and hypoxic (800 ± 120 ms) fish ($P > 0.05$; n = 8 for both groups). The closed-time distribution was wide in both groups and the longest measured continuous closed states lasted for about 10 seconds. The closed-time distribution was best fitted by assuming 4 different closed states. The closed times tended to be longer in hypoxic than normoxic fish, but the differences were not statistically significant (Table 1). Probability density functions had similar shape in normoxic and hypoxic fish, further indicating that hypoxia does not change the relative frequency to be in a particular closed state.

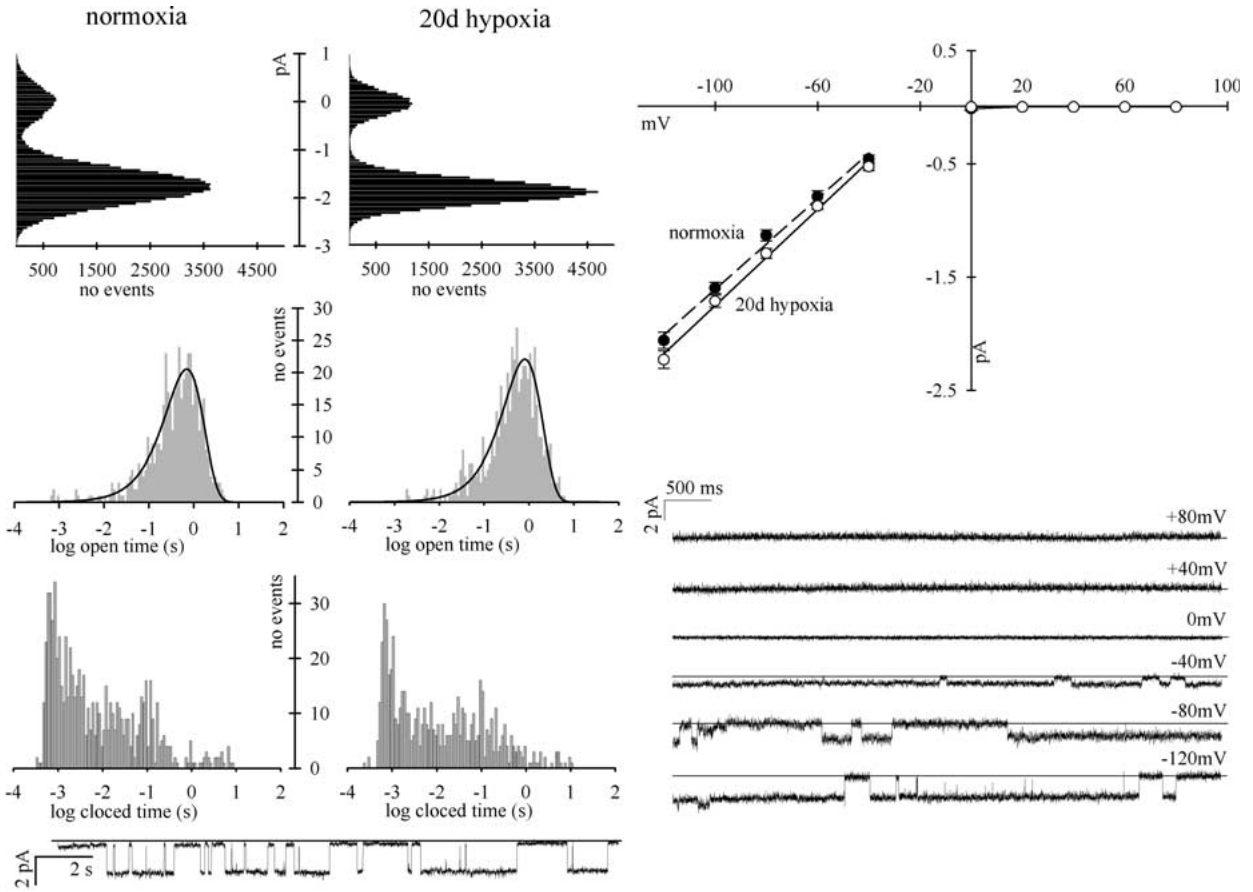

Fig. 2. Single-channel properties of the I_{K1} of normoxic and hypoxic carp at +5°C. (*Left*) Single-channel open probability (*top*), open-time distribution (*middle*), and closed-time distribution together with a representative current tracing (*bottom*) at −100 mV. (*Right*) Current-voltage relationships of normoxic and 20-day hypoxic carp (*top*) together with representative current tracing at different voltages (*bottom*). The results are means ± SEM, 8–18 myocytes.

Na^+/K^+ATPase Activity

The activity of ouabain-sensitive ATPase in crude cardiac homogenates was 39.4 ± 4.03 and 26.5 ± 3.73 mol/mg/min in normoxic and hypoxic carp ($P < 0.05$), respectively, indicating a 33% reduction in Na^+-pump activity after 3-week hypoxia (Fig. 4).

Discussion

Absence of Channel Arrest in the Activity of the I_{K1} in Carp Cardiac Myocytes

The present study indicates that, in ventricular myocytes of the crucian carp heart, exposure of fish to chronic hypoxia does not change the amplitude or kinetics of the I_{K1} on either the whole-cell or single-channel level. This finding is in contradiction with the predictions of the channel-arrest hypothesis (Hochachka, 1986) that in hypoxia-tolerant animals passive ion fluxes through cell membrane are suppressed by reduction in number or properties of ion channels. Whether this is a genuine property of hypoxic fish cardiac myocytes or due to the selection of experimental conditions, species, tissue or ion channel, is discussed below.

Crucian carp is perhaps the most anoxia-tolerant fish species and one of the most anoxia-tolerant vertebrates, as indicated by the experiments of Blazka (1958) almost fifty years ago.

The anoxia/hypoxia tolerance of crucian carp and its close relative, goldfish (*Carassius auratus*), is based on metabolic downregulation and efficient anaerobic metabolism (Johnston & Bernard, 1983; Van Waversveld, Addink & van den Thillart, 1989). Furthermore, the anoxia tolerance of crucian carp varies seasonally, as indicated by much better anoxia tolerance of the winter-acclimatized fish in comparison to carp caught in summer (Piironen & Holopainen, 1986). Acclimatization to seasonal changes in oxygen availability is therefore an endogenous and physiologically regulated process associated with prominent changes in biochemical composition of tissue cells (Vornanen, 1994a, 1994b). Therefore, we tried to imitate the natural seasonality of crucian carp by

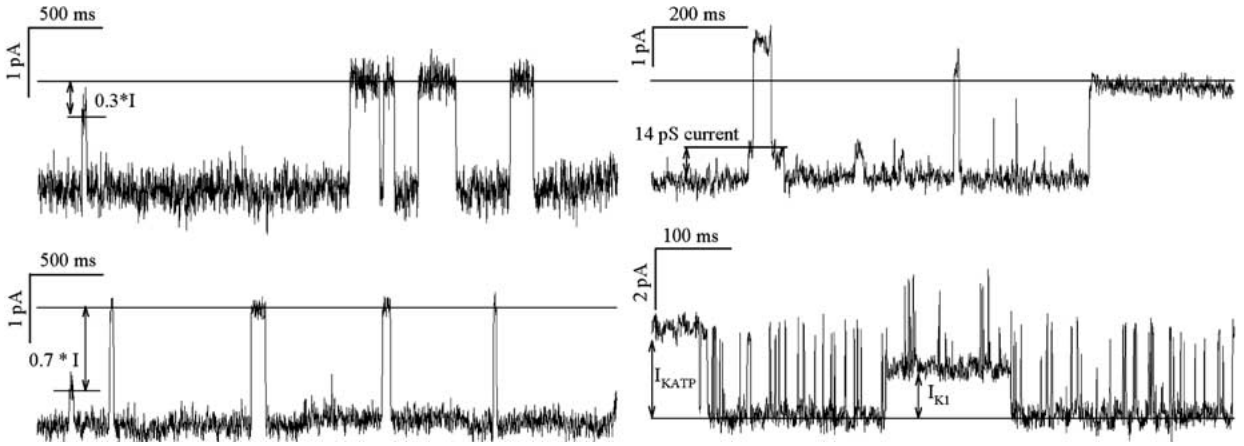

Fig. 3. Single-channel recordings showing subconductance states of the 21-pS I_{K1} channel (at −100 mV) (*left*), the small 14-pS inward rectifier conductance (at −120 mV) (*top right*) and I_{KATP} (at −80 mV) (*bottom right*). Note the different scale bars.

Table 1. Distribution of closed-time duration with 4 closed states

	τ_{c1}		τ_{c2}		τ_{c3}		τ_{c4}	
	ms	%	ms	%	ms	%	s	%
Normoxia	1.4	50	11.3	18	91.3	26	2.7	5
Hypoxia	1.4	45	20.4	23	174	26	3.1	5

exposing whole fish to chronic hypoxia instead of acute poisoning of the aerobic metabolism of cardiac myocytes. Carp were collected in October when they are physiologically acclimatized to winter anoxia after having gathered large amounts of glycogen in liver, heart and muscles, and being equipped with adequate metabolic machinery for the exploitation of the large carbohydrate stores in anaerobic metabolism (Hyvärinen, Holopainen & Piironen, 1985). It might be assumed that the winter-acclimatized carp had a reduced level of I_{K1} prior to the actual experiments, and therefore no further depression was seen during hypoxic exposure. This is not, however, the case, but quite in contrast, in the cold-acclimated and normoxic carp the density of the I_{K1} is higher than in warm-acclimated fish (Paajanen & Vornanen, 2002). As for the acute anoxia, the block of aerobic metabolism induces I_{KATP} rather than depresses I_{K1} in crucian carp cardiac myocytes (Paajanen & Vornanen, 2002).

Alternatively, the absence of the I_{K1} depression in crucian carp could be due to insufficient extent or duration of hypoxia. This is also very unlikely since the critical O_2 tension needed to support aerobic metabolism in crucian carp at 5°C is about 2.5 mg/l (1.84 mmHg) (Blazka, 1958), while in our experiments O_2 tension at the beginning of hypoxic exposure was only 0.39 mg/l (2.86 mmHg). Carp were able to extract oxygen from the hypoxic water down to the level of 0.27 mg/l (1.98 mmHg), and the 0.24 mg O_2 extracted from the 2-l flask is able to produce less than 1 cal energy in aerobic metabolism. This is sufficient for a 20-g crucian carp for only about half an hour (Blazka, 1958). Therefore, the fish must have been completely dependent on anaerobic metabolism within few hours from the onset of hypoxic exposure.

The I_{K1} was selected as the target current, as it is responsible for RMP and therefore excitability of the cardiac myocytes. It must allow K^+ leakage during both rest and activity and it is functionally connected to the ATP-consuming activity of sarcolemmal Na^+/K^+-ATPase. Thus, the activity of inwardly rectifying K^+ channels is a continuous energetic load to cardiac myocytes and thus a likely target for channel arrest. Although crucian carp cardiac myocytes have ATP-sensitive K^+ channels (Paajanen & Vornanen, 2002), they are not persistently activated under chronic hypoxia, as shown by the absence of outward current at 0 mV (Fig. 1). Indeed, it is relatively difficult to induce I_{KATP} in fish cardiac myocytes by metabolic inhibition (Paajanen & Vornanen, 2002), and therefore I_{K1} is the dominating K^+ current in these cells and a putative target for hypoxic modulation.

Properties of the I_{K1} in Crucian Carp Myocytes

The I_{K1} of crucian carp ventricle myocytes, in both normoxic and hypoxic fish, had a single-channel conductance of about 21 pS at 5°C. We are not aware of any previous results about the magnitude of I_{K1} in any other animal at such low temperature, which complicates the comparison with other species. The

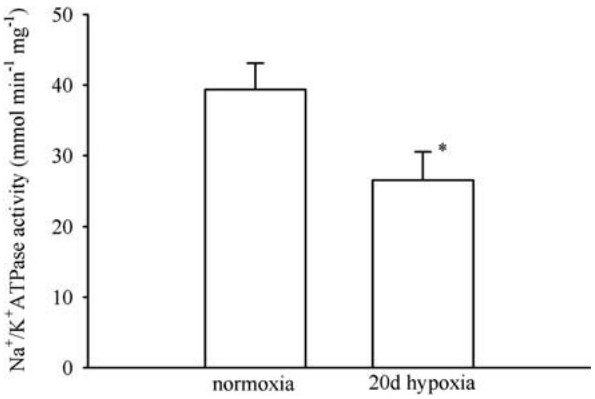

Fig. 4. The activity of ouabain-sensitive ATPase from crude cardiac homogenates of normoxic and 20-day hypoxic carp. The results are means ± SEM of 8 fish for both groups. *, Significantly ($P < 0.05$) different from the value of normoxic fish.

Q_{10} of the crucian carp I_{K1} between 5° and 17°C was 1.56 (*data not shown*), giving a conductance of about 41 pS at 20°C, which is in the range measured for the different forms of the mammalian I_{K1} at room temperature (20–49 pS) (Sakmann & Trube, 1984a; Matsuda & Stanfield, 1989; Masuda & Sperelakis, 1993; Xie, Takano & Noma, 1997; *see* also Lopatin & Nicholls, 2001). This value is also very similar to the 38-pS conductance of ventricular myocytes from warm-acclimated crucian carp at 17°C (Paajanen & Vornanen, *unpublished data*).

The P_o of the carp I_{K1} was relatively high (0.74–0.80) in both control and hypoxic fish. Although similar P_o values have been measured in some mammals (Xie, Takano & Noma, 1997), usually the P_o of the mammalian cardiac myocytes at −100 mV is somewhat lower than measured here for crucian carp ventricular myocytes (Masuda & Sperelakis, 1993; Matsuda & Stanfield, 1989; Sakmann & Trube, 1984b; Josephson & Brown, 1986). The mean open time of the carp I_{K1} is 5 to 10 times longer in comparison to mammalian cardiac myocytes ($\tau_o < 70$–200 ms) (Sakmann & Trube, 1984b; Hume & Uehara, 1985; Xie, Takano & Noma, 1997) or cloned IRK1 (Sabirov, Okada & Oiki, 1997). The difference might be due to low experimental temperature of the present study, because Mg^{2+} block of I_{K1} slows down with decreasing temperature (Ishihara et al., 1989). Future research should examine the temperature dependence of the I_{K1} kinetics and molecular composition of the fish cardiac channel and their significance for cardiac function in ectotherms.

Uncoupling of Na^+/K^+ATPase Activity from Channel Arrest

Although the I_{K1} was not downregulated in hypoxic carp hearts, the activity of SL Na^+/K^+ATPase was reduced by one third, indicating apparent uncoupling of K^+ leakage from Na^+-pump activity. This suggests that while hypoxic carp may reduce energy consumption in cation uptake, this does not necessarily require the arrest of K^+ channels. Thus the channel-arrest hypothesis in this form does not seem to be valid for the I_{K1} of the crucian carp cardiac myocytes and needs modification.

A possible, and likely, explanation for this uncoupling is the reflex bradycardia of fish in acute hypoxia (Randall & Shelton, 1963; Randall, 1966), also expressed by crucian carp (Vornanen & Tuomennoro, 1999). Indeed, oxygen limitation in hypoxia- and anoxia-tolerant vertebrates reduces cardiac power output severalfold, mainly by hypoxic bradycardia (Hicks & Farrell, 2000; Stecyk & Farrell, 2002). Hypoxic depression of heart rate, mediated by the activation of the parasympathetic nervous system, reduces the number of cardiac cycles per time and thereby the demand on depolarizing and repolarizing currents in the SL, and thus active cation uptake. Therefore, it seems that hypoxic fish heart achieves energy savings with 'action potential arrest', which is analogous to the 'spike arrest' of hypoxic turtle neurons. In turtle brain, reduced firing rate of neurons is achieved by several different mechanisms, including induction of I_{KATP}, increase in adenosine concentration and inactivation of NMDA receptors (Pék & Lutz, 1997; Buck & Bickler, 1998; Pék-Scott & Lutz, 1998). Although physiologically significant, the reduced Na^+-pump activity of the hypoxic heart may, however, produce relatively small energy savings in comparison to those achieved at the myofibrillar level by reduced myosin-ATPase cycling (Vornanen, 1994b).

Together, these findings suggest that in excitable cells of hypoxia-tolerant vertebrates, balance between energy production and energy consumption is attained by reducing the firing frequency of action potentials rather than by changing the number or kinetics of ion channels. It seems that similar to the function of the SL Na^+/K^+ATPase, whose activity is dynamically regulated by intracellular Na^+ load (Despa et al., 2002), the ion leakage through membrane can be adjusted by activity-dependent recruitment of ion channels.

Conclusions

The present findings indicate that, in principle, there are several possible mechanisms by which K^+ leakage through the I_{K1} could be reduced under chronic hypoxia. The number of 21-pS channels could be decreased or they could be replaced by the smaller 14-pS channels, the high P_o of the I_{K1} could be reduced, or low-subconductance states could be recruited instead of the fully-open state. Also the closed-state distribution could change to favor longer mean closed times. However, none of these alternatives

realized in the hypoxic ventricular myocytes of the crucian carp heart, and no channel arrest for the I_{K1} could be demonstrated. Future studies should address whether this holds also for other channels, other tissues and other hypoxia-tolerant species.

This study was supported by the Academy of Finland (project #53481). We thank laboratory technician Anita Kervinen for measuring Na^+/K^+ATPase activities.

References

Aho, E., Vornanen, M. 1997. Seasonality of ATPase activities in crucian carp (*Carassius carassius* L.) heart. *Fish Physiol. Biochem.* **16:**355–364

Atkinson, A., Gatenby, A.D., Lowe, A.G. 1973. The determination of inorganic orthophosphate in biological systems. *Biochim. Biophys. Acta* **320:**195–204

Barry, D.M., Nerbonne, J.M. 1996. Myocardial potassium channels: Electrophysiological and molecular diversity. *Annu. Rev. Physiol.* **58:**363–394

Boutilier, R.G. 2001. Mechanisms of cell survival in hypoxia and hypothermia. *J. Exp. Biol.* **204:**3171–3181

Blazka, P. 1958. The anaerobic metabolism of fish. *Physiol. Zool.* **31:**117–128

Buck, L.T., Bickler, P.E. 1998. Adenosine and anoxia reduce N-ethyl-D-aspartate receptor open probability in turtle cerebrocortex. *J. Exp. Biol.* **201:**289–297

Buck, L.T., Hochachka, P.W. 1993. Anoxia suppression of Na^+-K^+-ATPase and constant membrane potential in hepatocytes; support for channel arrest. *Am. J. Physiol.* **265:**R1020–R1025

Colquhoun, D., Sigworth, F.J. 1995. Fitting and statistical analysis of single-channel recordings. *In:* Single Channel Recordings. 2nd ed. B. Sakmann & E. Neher, editor. pp 483–587. Plenum Press, New York

Despa, S., Islam, M.A., Pogwizd, S.M., Bers, D.M. 2002. Intracellular $[Na^+]$ and Na^+ pump rate in rat and rabbit ventricular myocytes. *J. Physiol.* **539:**133–143

Doll, C.J., Hochachka, P.W., Reiner, P.B. 1991. Channel arrest: implications from membrane resistance in turtle neurons. *Am. J. Physiol.* **261:**R1321–R1324

Ducceschi, V., Di Micco, G., Sarubbi, B., Russo, B., Santangelo, L., Iacono, A. 1996. Ionic mechanisms of ischemia-related ventricular arrhythmias. *Clin. Cardiol.* **19:**325–331

Hicks, J.M.T., Farrell, A.P. 2000. The cardiovascular responses of the red-eared slider (*Trachemys scripta*) acclimated to either 22 or 5°C. I. Effects of anoxia exposure on *in vivo* cardiac performance. *J. Exp. Biol.* **203:**2765–3744

Hochachka, P.W. 1986. Defence strategies against hypoxia and hypothermia. Science **231:**234–241

Hume, J.R., Uehara, A. 1985. Ionic basis of the different action potential configurations of single guinea-pig atrial and ventricular myocytes. *J. Physiol.* **368:**525–544

Hyvärinen, H., Holopainen, I.J., Piironen, J. 1985. Anaerobic wintering of crucian carp (*Carassius carassius* L.). I. Annual dynamics of glycogen reserves in nature. *Comp. Biochem. Physiol.* **82A:**797–803

Ishihara, K., Mitsuiye, T., Noma, A., Takano, M. 1989. The Mg^{2+} block and intrinsic gating underlying inward rectification of the K^+ current in guinea-pig cardiac myocytes. *J. Physiol.* **419:**297–320

Ishihara, K., Yan, D.-H., Yamamoto, S., Ehara, T. 2002. Inward rectifier K^+ current under physiological cytoplasmic conditions in guinea-pig cardiac ventricular cells. *J. Physiol.* **540:**831–841

Johnston, I.A., Bernard, L.M. 1983. Utilization of the ethanol pathway in carp following exposure to anoxia. *J. Exp. Biol.* **104:**73–78

Josephson, I.R., Brown, A.M. 1986. Inwardly rectifying single-channel and whole cell K^+ currents in rat ventricular myocytes. *J. Membrane Biol.* **94:**19–35

Kristián, T., Siesjö, B.K. 1996. Calcium-related damage in ischemia. Life Sciences **59:**357–367

Lipton, P. 1999. Ischemic cell death in brain neurons. *Physiol. Rev.* **79:**1431–1568

Lopatin, A.N., Nicholls, D.G. 2001. Inward rectifiers in the heart: an update on I_{K1}. *J. Mol. Cell. Cardiol.* **33:**625–638

Lowry, O.H., Rosenborough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275

Masuda, H., Sperelakis, N. 1993. Inwardly rectifying potassium current in rat fetal and neonatal ventricular cardiomyocytes. *Am. J. Physiol.* **265:**H1107–H1111

Matsuda, H., Stanfield, P.R. 1989. Single inwardly rectifying potassium channels in cultured muscle cells from rat and mouse. *J. Physiol.* **414:**111–124

Paajanen, V., Vornanen, M. 2002. The induction of an ATP-sensitive K^+ current in cardiac myocytes of air- and water-breathing vertebrates. *Pfluegers Arch.* **444:**760–770

Penttinen, O.-P., Holopainen, I.J. 1992. Seasonal feeding activity and ontogenetic dietary shifts in crucian carp, *Carassius carassius*. *Environ. Biol. Fishes* **33:**215–221

Perez-Pinzon, M.A., Chan, C.Y., Rosenthal, M., Sick, TJ. 1992. Membrane and synaptic activity during anoxia in the isolated turtle cerebellum. *Am. J. Physiol.* **263:**R1057–R1063

Pék, M., Lutz, P.L. 1997. Role for adenosine in channel arrest in the anoxic turtle brain. *J. Exp. Biol.* **200:**1913–1917

Pék-Scott, M., Lutz, P.L. 1998. ATP-sensitive K^+ channel activation provides transient protection to the anoxic turtle brain. *Am. J. Physiol.* **275:**R2023–R2027

Pierce, G.N., Gzubryt, M.P. 1995. The contribution of ionic imbalance to ischemia/reperfusion-induced injury. *J. Mol. Cell. Cardiol.* **27:**56–63

Piironen, J., Holopainen, I.J. 1986. A note on seasonality in anoxia tolerance of crucian carp (*Carassius carassius* L.) in the laboratory. *Ann. Zool. Fenn.* **23:**335–338

Randall, D.J. 1966. The nervous control of cardiac activity in the tench (*Tinca tinca*) and the goldfish (*Carassius auratus*). *Physiol. Zool.* **39:**185–192

Randall, D.J., Shelton, G. 1963. The effects of changes in environmental gas concentrations on the breathing and heart rate of a teleost fish. *Comp. Biochem. Physiol.* **9:**229–239

Roden, D.M., Balser, J.R., George, A.L.Jr, Anderson, M.E. 2002. Cardiac ion channels. *Annu. Rev. Physiol.* 431–475

Rolfe, D.F.S., Brown, G.C. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* **77:**731–758

Ruppersberg, J.P. 2000. Intracellular regulation of inward rectifier K^+ channels. *Pfluegers Arch.* **441:**1–11

Sabirov, R.Z., Okada, Y., Oiki, S. 1997. Two-sided action of protons on an inward rectifier K^+ channel (IRK1). Pfluegers Arch. **433:**428–434

Sakmann, B., Trube, G. 1984a. Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *J. Physiol.* **347:**641–657

Sakmann, B., Trube, G. 1984b. Voltage dependent inactivation of inward-rectifying single channel currents in the guinea-pig heart cell membrane. *J. Physiol.* **347:**659–683

Schramm, M., Klieber, H.-G., Daut, J. 1994. The energy expenditure of actomyocyosin-ATPase, Ca^{2+}-ATPase and Na^+,K^+-ATPase in guinea pig cardiac ventricular myocytes. *J. Physiol.* **481:**647–662

Stecyk, J.A.W., Farrel, A.P. 2002. Cardiorespiratory responses of the common carp (*Cyprinus carpio*) to severe hypoxia at three acclimation temperatures. *J. Exp. Biol.* **205:**759–768

Van Waversveld, J., Addink, A., van den Thillart, G. 1989. Simultaneous direct and indirect calorimetry on normoxic and anoxic goldfish. *J. Exp. Biol.* **142:**325–335

Vornanen, M. 1994a. Seasonal adaptation of crucian carp (*Carassius carassius* L.) heart: glycogen stores and lactate dehydrogenase activity. *Can. J. Zool.* **72:**433–442

Vornanen, M. 1994b. Seasonal and temperature-induced changes in myosin heavy chain composition of the crucian carp hearts. *Am. J. Physiol.* **267:**R1567–R1573

Vornanen, M., Tuomennoro, J. 1999. Effects of acute anoxia on heart function in crucian carp: importance of cholinergic and purinergic control. *Am. J. Physiol.* **277:**R464–R475

Xie, L.-H., Takano, M., Noma, A. 1997. Development of inwardly rectifying K^+ channel family in rat ventricular myocytes. *Am. J. Physiol.* **272:**H1741–H1750