Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part C 142 (2006) 205 – 211
CBP
www.elsevier.com/locate/cbpc

Potassium transmembrane fluxes in anoxic hepatocytes from goldfish (*Carassius auratus* L.) ☆

P.N. Mut [b], M.V. Espelt [a,1], G. Krumschnabel [c], P.J. Schwarzbaum [a,*]

[a] *Instituto de Química y Fisicoquímica Biológicas (Facultad de Farmacia y Bioquímica), Universidad de Buenos Aires, C1113AAD Buenos Aires, Argentina*
[b] *Laboratorio de Biomembranas, Cátedra de Fisiología, (Facultad de Medicina), Universidad de Buenos Aires, C1121ABG Buenos Aires, Argentina*
[c] *Institut für Zoologie and Center of Molecular Biosciences, Leopold Franzens Universität Innsbruck, A-6020, Austria*

Received 27 May 2005; received in revised form 26 September 2005; accepted 1 October 2005
Available online 17 November 2005

Abstract

Despite the fact that anoxic goldfish hepatocytes can maintain the transmembrane gradients of Na^+, H^+ and Ca^{2+}, cyanide (CN) intoxication leads to a rapid breakdown of K^+ homeostasis. In this study, [$^{86}Rb^+$] K^+ fluxes across the plasma membrane of goldfish hepatocytes were studied in order to identify the possible causes of this imbalance. Four minutes of cyanide incubation induced an acute and stable 61% decrease of K^+ influx (mostly driven by Na,K-ATPase activity), whereas K^+ efflux increased by 24.3%, this imbalance yielding a net K^+ efflux of 0.279±0.024 nmol 10^{-6} cells^{-1} min^{-1}. This uncoupling was not observed when glycolytic ATP production was inhibited with iodoacetic acid. Although the CN-induced decrease of K^+ influx was fully reversible upon washout of the inhibitor, it could not be prevented by any of the following treatments: (1) addition of 2% bovine serum albumin, which binds extracellular fatty acids known to activate specific K^+ channels; (2) addition of ascorbate, which acts as a radical scavenger; (3) inclusion of 5 mM glucose as an extracellular carbon source; and (4) removal of medium oxygen (obtained by nitrogen bubbling). Regarding the elevation of K^+ efflux in the presence of CN, neither ATP-dependent K^+ channels nor the KCl cotransporter appeared to be activated, whereas $BaCl_2$, an inhibitor of voltage-gated K^+ channels, decreased K^+ efflux of CN-intoxicated cells to control levels. In summary, these results indicate that, in goldfish hepatocytes, the CN-induced K^+ imbalance results from acute Na,K-ATPase inhibition together with the activation of voltage-dependent K^+ channels, the latter probably resulting from transient membrane depolarization.

Keywords: Anoxia; Liver; Cyprinids; Fish; Sodium pump; Hepatocytes; Ion homeostasis

1. Introduction

Most vertebrate cells have a low tolerance to anoxia and, therefore, have a continuous need for energy derived from oxidative metabolism. When oxygen is absent, loss of key metabolic functions occurs, putting viability at risk. One of the main consequences of anoxia is a rapid fall in ATP levels and a breakdown of the steady-state distribution of diffusible ions. Particularly the maintenance of cytosolic K^+ is assumed to be of critical importance for cell survival under energy-limited conditions (Lutz and Nilsson, 1997; Krumschnabel et al., 1998; Hochachka and Lutz, 2001; Espelt et al., 2003). Thus, in hepatocytes from rat and trout (two anoxia intolerant species), intoxication with cyanide (a form of chemical anoxia) led to a rapid decrease of [ATP], membrane depolarization, an increase in cytosolic Ca^{2+} and, importantly, a decrease of K^+ influx causing decoupling of K^+ transmembrane fluxes (Kawanishi et al., 1991; Krumschnabel et al., 1997, 1999). Exceptions to this cellular intolerance to anoxia of vertebrate cells include liver cells from the painted freshwater turtle and goldfish (Buck, 2004). Turtle hepatocytes have been shown to maintain the cytosolic concentrations of ATP and K^+ and the membrane potential for many hours of anoxia (Buck et al., 1993). In comparison, in goldfish hepatocytes cyanide causes a decrease

☆ This paper is part of a special issue of CBP dedicated to "The Face of Latin American Comparative Biochemistry and Physiology" organized by Marcelo Hermes-Lima (Brazil) and co-edited by Carlos Navas (Brazil), Tania Zenteno-Savín (Mexico) and the editors of CBP. This issue is in honour of Cicero Lima and the late Peter W. Hochachka, teacher, friend and devoted supporter of Latin American science.

* Corresponding author.
E-mail address: pablos@qb.ffyb.uba.ar (P.J. Schwarzbaum).
[1] Present Address: Research Division, Anesthesiology Department, Vanderbilt University, Nashville, TN, USA.

1532-0456/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2005.10.002

of intracellular ATP concentration to 50% of control values, but the intracellular concentrations of Ca^{2+}, H^+ and Na^+ are kept almost unaltered (Krumschnabel et al., 1997, 1998, 2001; Espelt et al., 2003). This is achieved by reallocating metabolic energy from acutely dispensable functions to essential ones (Krumschnabel and Wieser, 1994), preventing the rise of cytosolic Ca^{2+} by a net extrusion of the cation (Krumschnabel et al., 1997) and secreting protons which, together with a high cytosolic buffer capacity, allows cytosolic pH to be maintained (Krumschnabel et al., 2001).

Contrary to our expectations, and irrespective of the above-described metabolic strategies developed by goldfish hepatocytes in maintaining ionic homeostasis, we recently observed that in these cells, chemical anoxia induced an acute breakdown of K^+ homeostasis (Espelt et al., 2003). While the mechanisms implicated have not been studied so far, this may in principle be the consequence of a reduction of K^+ influx across the plasma membrane, an increase of K^+ efflux, or both.

Thus, in order to elucidate this question, we conducted a study on K^+ homeostasis in goldfish hepatocytes when these cells are subjected to different forms of energy limitation, with particular focus on the effect of CN intoxication.

2. Materials and methods

2.1. Chemicals

Collagenase (Type VIII), fatty acid-free bovine serum albumin, *N*-ethylmaleimide, ascorbic acid, glucose, glibenclamide and ouabain, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium dithionite was purchased from Merck (Buenos Aires, Argentina). [$^{86}Rb^+$] RbCl (4 mCi mg^{-1}; approx. 40 mCi mL^{-1}) was from NEN Life Science Products (Boston, MA, USA). All other reagents were of analytical grade.

2.2. Animals

Goldfish *Carassius auratus* L. (10–30 g) were obtained commercially from local dealers in Buenos Aires. They were kept in 200-L tanks at 20 °C. Fish were acclimated for at least 2 weeks before being used.

2.3. Isolation of hepatocytes

Fish were killed by a blow on the head and transection of the spinal cord. The small size of goldfish, together with the diffuse distribution of hepatic tissues in cyprinids, prevented the use of the perfusion techniques for the isolation of hepatocytes. Therefore, goldfish hepatocytes were isolated by incubating fragments of liver tissue with a collagenase medium as described before (Schwarzbaum et al., 1992; Krumschnabel et al., 1994). Incubation medium consisted of (in mM) 10 HEPES, 135 NaCl, 3.8 KCl, 1.3 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 10 $NaHCO_3$, pH=7.6 at 20 °C; osmolarity 289 mOsmol L^{-1}. For comparative reasons, one series of experiments was conducted with trout hepatocytes, which were isolated and maintained as described previously (Krumschnabel et al., 1996).

The viability of isolated hepatocytes (> 95%) was routinely assessed by Trypan blue exclusion (Tennant, 1964) and was maintained throughout the experiments.

2.4. Treatments applied to the goldfish hepatocytes suspension

Except otherwise stated, cells from goldfish were incubated at 20 °C (at pH 7.6) under the following treatments:

2.4.1. Chemical anoxia (CN)

Cells were incubated in the presence of 2 mM NaCN. Cyanide is commonly used to simulate anoxia since it is a strong inhibitor of cytochrome *c* oxidase, thereby blocking the reduction of oxygen (final electronic acceptor) to water during oxidative phosphorylation.

2.4.2. Physiological anoxia (N_2)

To create true, physiological anoxia, hepatocytes were incubated in saline containing 2 mM sodium dithionite, the medium being in addition bubbled with a mixture of $99N_2$:$1CO_2$ for 30 min before the onset of the experiment (Jian and Haddad, 1994).

2.4.3. Anoxic chemical anoxia (CN-N_2)

In this case, cells were incubated with CN in saline treated as described for physiological anoxia.

2.4.4. Glycolytic blockage

Cells were incubated in medium containing 0.5 mM iodoacetic acid (IAA), an inhibitor of glycolysis at the glyceraldehyde-3-phosphate dehydrogenase stage (Dawson et al., 1993; Krumschnabel et al., 1994).

2.4.5. Blockage of mitochondrial and glycolytic ATP production

In order to simultaneously block the two main pathways of ATP production in goldfish, hepatocyte cells were incubated with 2 mM CN plus 0.5 mM IAA (CN-IAA) (Dorigatti et al., 1997).

2.5. Other treatments

One millimolar ouabain (Ob) was used to inhibit Na,K-ATPase activity, while 2% (w/v) of fatty acid-free bovine serum albumin (BSA) was used to bind potentially liberated extracellular fatty acids that may activate specific K^+ channels (Zou et al., 2001). Incubation of cells in the presence of CN under normoxia can lead to the generation of reactive oxygen species (Gores et al., 1989; Dawson et al., 1993). In order to scavenge these radicals, in some experiments, cells were incubated in the presence of 1 mM ascorbate, a well-known antioxidant.

For measurements of K^+ efflux in Cl^--free medium (denoted as Cl^--free K^+ efflux), goldfish cells were isolated