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SHORT COMMUNICATION

GLYCOLYTIC FUNCTION IN GOLDFISH HEPATOCYTES AT DIFFERENT TEMPERATURES: RELEVANCE FOR Na^+ PUMP ACTIVITY AND PROTEIN SYNTHESIS

GERHARD KRUMSCHNABEL, SABINE MALLE, PABLO J. SCHWARZBAUM AND WOLFGANG WIESER

Institut für Zoologie, der Universität Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria

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Cellular metabolism of ectotherms may respond to temperature changes with a shift in the choice of substrate preferentially catabolized for ATP production (Johnston and Dunn, 1987). This has been shown in acclimated fish, in which a temperature-dependent switch from carbohydrates as the main fuel for energy metabolism to other fuels, or *vice versa*, can occur. Such shifts help to indicate the relative importance of aerobic glycolysis as an ATP-supplying process. The preferred energy source at a given temperature depends upon the temperature range investigated, as well as on the species, on the tissue and upon nutritional factors. The adaptive significance of shifts between energy sources is not always clear. While in some cases ecological arguments may provide plausible explanations (e.g. seasonal adaptation to migration and reproductive requirements; Stone and Sidell, 1981; Moerland and Sidell, 1981), evidence is accumulating that structural or functional features inherent in the metabolic organisation of the cell may also play a role in determining the nature of such responses (Sidell, 1983; Sidell and Hazel, 1987). Of particular relevance in this respect is the interaction between diffusional flux and the rate of enzymatic activity in shaping specific temperature effects on cellular metabolism.

We studied this problem by subjecting isolated goldfish hepatocytes to acute temperature changes and measuring the rate of total and function-related metabolism both in the presence and in the absence of the glycolytic inhibitor iodoacetic acid (IAA). Using this approach, we hoped to learn to what extent the temperature relationships of different metabolic functions depended upon the site of ATP production and thus on the distribution of these sites within the cell.

Goldfish (*Carassius auratus* L.) were obtained from commercial suppliers and were kept in 100 l aquaria at 15 °C. The fish were killed and hepatocytes were isolated from freshly excised livers as described in detail elsewhere (Krumschnabel *et al.* 1994). The final cell pellet was resuspended in Hepes-buffered saline (in $mmol\,l^{-1}$: 135 NaCl, 3.8

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KCl, 1.3 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 10 $NaHCO_3$, 10 Hepes, pH 7.6 at 20 °C, including 2 % bovine serum albumin) and kept on ice. Thirty minutes prior to the measurement of oxygen consumption ($\dot{V}_{O_2}$), the cells were transferred to the desired temperature and preincubated in a shaking water bath in the presence or absence of 0.5 mmol l^{-1} iodoacetic acid (IAA) and with or without 10 mmol l^{-1} ethionine. $\dot{V}_{O_2}$ was determined in a Cyclobios Oxygraph chamber as described in detail in Schwarzbaum *et al.* (1992). When a steady rate of respiration had been reached, either ouabain (OB, an inhibitor of Na^+/K^+-ATPase; 1 mmol l^{-1} final concentration) or cycloheximide (CH, an inhibitor of protein synthesis; 15 mmol l^{-1} final concentration) was added to the cells from concentrated stock solutions. After the addition of OB or CH, respiration stabilized at a new, lower steady state within 5 min. The concentration of 15 mmol l^{-1} CH is high compared with values published in the mammalian literature. However, both a dose–response curve of $\dot{V}_{O_2}$ at different concentrations of CH made in preliminary experiments and the fact that, in rainbow trout hepatocytes, levels of CH as high as 30 mmol l^{-1} were required to inhibit the incorporation of labelled amino acids into protein (Pannevis and Houlihan, 1992) seem to provide justification for the use of such a high dose. Lactate was measured as described in Krumschnabel *et al.* (1994).

At 15 °C, about 75 % of total aerobic ATP production can be assigned to two specific functions, namely Na^+/K^+-ATPase activity and protein synthesis (Table 1). The latter accounted for about 50 % of total oxygen consumption ($\dot{V}_{O_2tot}$), compared with a value of 80 % reported for rainbow trout hepatocytes (Pannevis and Houlihan, 1992). However, the trout hepatocytes had been incubated in a medium containing amino acids, which presumably stimulated protein synthesis, as was the case with rabbit reticulocytes (Siems *et al.* 1986). Exposure of the goldfish hepatocytes to IAA for 30 min led to a significant reduction in $\dot{V}_{O_2tot}$ and ouabain-sensitive oxygen consumption ($\dot{V}_{O_2OBS}$), whereas

Table 1. *Oxygen consumption of goldfish hepatocytes at 8, 15 and 25 °C in controls and in cells treated with iodoacetic acid*

	$\dot{V}_{O_2tot}$	$\dot{V}_{O_2CHS}$	$\dot{V}_{O_2OBS}$
8 °C	0.046±0.013 (5)	0.026±0.013	0.007±0.006
+IAA	0.037±0.012 (4)	0.019±0.011	0.010±0.003 (3)
15 °C	0.126±0.019 (8)	0.065±0.015	0.030±0.011
+IAA	0.092±0.004 (3)*	0.053±0.006	0.018±0.001*
+IAA+P/M[a]	0.122±0.050 (5)	–	0.023±0.012
25 °C	0.291±0.051 (9)	0.157±0.044	0.061±0.015
+IAA	0.215±0.042 (9)*	0.126±0.048	0.034±0.023*
+IAA+P/M	0.317±0.068 (4)	0.144±0.038	0.019±0.006*

Values are given in nmol O_2 mg^{-1} fresh mass min^{-1}. Results are expressed as mean ± S.D. with the number of experiments given in parentheses.

$\dot{V}_{O_2tot}$, $\dot{V}_{O_2CHS}$ and $\dot{V}_{O_2OBS}$ are total, cycloheximide-sensitive and ouabain-sensitive oxygen consumption, respectively.

IAA, iodoacetic acid; P/M, 2.5 mmol l^{-1} pyruvate and 2.5 mmol l^{-1} malate were added to the IAA-treated cells.

[a]Data taken from Krumschnabel *et al.* (1994).

*Significantly different from the respective controls, $P<0.05$ (Student's *t*-test).

cycloheximide-sensitive oxygen consumption ($\dot{V}_{O_2CHS}$) was not affected (Table 1). The reduction of $\dot{V}_{O_2}$ by IAA appears to have been due to substrate limitation since, in a previous study, we were able to reverse the consequences of IAA inhibition by the addition of pyruvate and malate (Table 1; Krumschnabel *et al.* 1994). This effect is also in agreement with other studies in which an IAA-induced decrease in cellular ATP concentration (Ikehara *et al.* 1984) or the suppression of specific functions (Winkler, 1981; Tsukuda and Osada, 1992) could be prevented or diminished by the addition of adequate substrate. A qualitatively similar result to that seen at 15 °C was obtained when the cells were maintained and measured at 25 °C. Again, $\dot{V}_{O_2tot}$ and $\dot{V}_{O_2OBS}$ were significantly suppressed in the presence of IAA, while no significant effect was exerted on $\dot{V}_{O_2CHS}$. However, unlike the effect at 15 °C, supplementing the hepatocytes with pyruvate and malate did not restore Na^+/K^+-ATPase activity to control levels. In contrast, when the experimental temperature was lowered to 8 °C, inhibition of glycolysis resulted in only a very slight change in all three components of aerobic metabolism studied (Table 1). By monitoring lactate production during the period of IAA inhibition, we examined the possibility, discussed by Stone and Sidell (1981), that this lack of effect could have been due to a decreased permeability of the cells to IAA at low temperature. At both 8 °C and 15 °C, cellular lactate content decreased slightly during 30 min of incubation with IAA (data not shown), whereas in untreated controls lactate accumulated at a rate of $0.057\pm0.029\,nmol\,mg^{-1}$ fresh mass min^{-1} (±S.D. N=8). We conclude that the permeability of the hepatocytes to IAA was unaffected by temperature in the range investigated.

In order to explore further the coupling of Na^+/K^+-ATPase and glycolysis, another series of experiments was performed at 15 °C in the presence of the adenosyl trapping agent ethionine (Farber, 1971; Aw and Jones, 1982, 1985). Ethionine allows the conversion of ATP to the poorly metabolizable product *S*-adenosylethionine, while leaving the ATP/ADP ratio largely unaffected (Aw and Jones, 1982). As Fig. 1 demonstrates, incubation with ethionine at 15 °C had no influence on $\dot{V}_{O_2tot}$, while $\dot{V}_{O_2CHS}$ was significantly reduced and $\dot{V}_{O_2OBS}$ showed a 30 % decrease, close to the values obtained in IAA-treated cells. At 25 °C, the effect of ethionine on Na^+/K^+-ATPase activity was the same as that at 15 °C. This result seems to indicate that the reduction of the rate of ATP *consumption* ($\dot{V}_{O_2CHS}$, $\dot{V}_{O_2OBS}$) was not due to a reduction of the rate of ATP *production* ($\dot{V}_{O_2tot}$), but must have been due to the limitation of ATP availability caused by the ethionine treatment.

Table 2 summarises the efects of temperature on metabolic functions in terms of Q_{10} values. As is typical for many metabolic processes, the Q_{10} values were higher in the lower temperature range than in the upper range (Wieser, 1973). The Q_{10} value of 8 for $\dot{V}_{O_2OBS}$ was high between 8 and 15 °C, but dropped to 2.3 when glycolysis was inhibited. In the range 15–25 °C, Q_{10} values between 2.3 and 2.4 were found for $\dot{V}_{O_2tot}$ and $\dot{V}_{O_2CHS}$, but lower values were obtained for $\dot{V}_{O_2OBS}$.

Whereas at 15 °C the inhibitory effect of IAA on total and ouabain-sensitive $\dot{V}_{O_2}$ was reversed by the addition of pyruvate and malate, at 25 °C the reduction persisted for $\dot{V}_{O_2OBS}$ despite the addition of these substrates. These differential effects of temperature on energy allocation might be explained as follows. In the hepatocytes of various species,

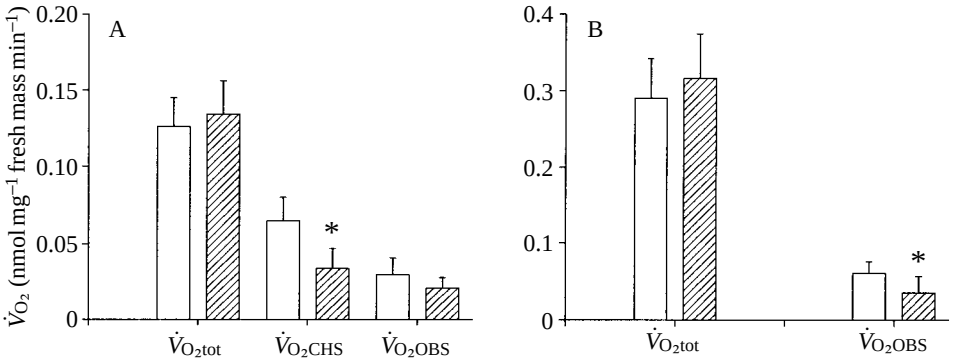

Fig 1. Oxygen consumption of goldfish hepatocytes at 15 ˚C (A) and 25 ˚C (B) in control (open columns) and ethionine-treated (hatched columns) cells. Values are means + S.D. of 8–9 preparations for controls and 3–5 preparations for ethionine-treated cells. *Significantly different from the respective controls, $P<0.05$ (Student's *t*-test). $\dot{V}_{O_2OBS}$, ouabain-sensitive $\dot{V}_{O_2}$; $\dot{V}_{O_2CHS}$, cycloheximide-sensitive $\dot{V}_{O_2}$; $\dot{V}_{O_2tot}$, total $\dot{V}_{O_2}$.

including fish (Blair *et al.* 1990; Braunbeck and Storch, 1992) and mammals (Pfaller *et al.* 1968; Hochachka, 1988), the mitochondria are more or less evenly distributed in the cell rather than being located close to the plasma membrane, as in some other cell types (Rossier *et al.* 1987). Protein synthesis takes place at the ribosomes, which are distributed throughout the cytosol. Thus, when steady-state conditions prevailing at a given temperature are disrupted by blocking glycolytic flux, energy-consuming processes located in the cell membrane are at a disadvantage compared with cytosolic functions in the vicinity of the mitochondria, which remain the sole source of ATP under these conditions. A similar effect was postulated by Aw and Jones (1985), who compared the effects of varied ATP concentrations on Na^+/K^+-ATPase and on a cytoplasmic ATPase in rat liver cells. A simplified analysis of radial diffusion of ATP from mitochondria showed that, when ATP supply is limited, ATPases remote from mitochondria may experience a more dramatic decrease in ATP concentration than ATPases in the proximity of mitochondria. In contrast, when ATP concentration is lowered throughout the cell by the addition of ethionine, both Na^+ pump activity and protein sythesis are equally affected, irrespective of their cellular location (Fig. 1).

Upon the addition of adequate substrate to IAA-treated cells, oxidative phosphorylation is stimulated and ATP supply is again sufficient to sustain the normal

Table 2. *Q_{10} values of the means of total (tot), cycloheximide-sensitive (CHS) and ouabain-sensitive (OBS) oxygen consumption ($\dot{V}_{O_2}$) of goldfish hepatocytes, with or without inhibition by iodoacetic acid (IAA)*

	$\dot{V}_{O_2tot}$		$\dot{V}_{O_2CHS}$		$\dot{V}_{O_2OBS}$	
	–IAA	+IAA	–IAA	+IAA	–IAA	+IAA
8–15 °C	4.22	3.69	3.70	4.34	8.00	2.32
15–25 °C	2.31	2.34	2.41	2.38	2.03	1.89

levels of activity of the Na^+ pump. At high temperatures, however, the increase in metabolic activities may outstrip the potential for ATP flux by diffusion across the cytosol. A Q_{10} of about 1 was found for diffusion of the ATP analogue 5′-adenylyl-imidodiphosphate (AMP-PNP) in cytosolic extracts of muscle tissue of white perch (Sidell and Hazel, 1987).

When hepatocytes were transferred from high to low temperatures, Na^+/K^+-ATPase activity decreased with an apparent Q_{10} of 8, while $\dot{V}_{O_2tot}$ was far less affected in this range (Table 2). Accordingly, diffusional delivery of ATP to the membranes should be able to keep pace with the relatively lower energy requirement of the Na^+ pump. In fact, under these conditions, Na^+/K^+-ATPase activity was unaffected by the presence of IAA (Table 1). This relationship is expected to hold for acute temperature changes only. In the long term, adaptive processes in the membranes (homeoviscous adaptation, increased pump density; see Cossins *et al.* 1994) tend to offset the acute effects of temperature. In consequence, cellular metabolism will again face the problem of having to match ATP requirement at the cell membrane with ATP supply. Under these conditions, the best strategy would appear to be the support of membrane function by a combination of oxidative phosphorylation and glycolysis, thus maintaining a short diffusion distance between ATP-producing and ATP-consuming sites. A functionally similar strategy is believed to be followed in muscle cells of cold-acclimated fish, where diffusion distances are shortened by an increase in mitochondrial density (Tyler and Sidell, 1984; Johnston and Dunn, 1987; Londraville and Sidell, 1990).

In summary, our experiments lend support to the following two hypotheses. First, at high temperatures the shift from other substrates to carbohydrates may be necessary because the ATP produced by substrate phosphorylation by glycolysis is required for fuelling the Na^+ pump and other peripheral ATP-consuming processes when these proceed at high rates. Second, low Q_{10} values may sometimes be due to the activity of an ATP-consuming function being diffusion-limited at the higher temperature.

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