J. exp. Biol. 156, 139–151 (1991)
Printed in Great Britain

INTERACTIONS BETWEEN ION EXCHANGE AND METABOLISM IN ERYTHROCYTES OF THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

BY B. L. TUFTS* AND R. G. BOUTILIER

Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

Accepted 16 November 1990

Summary

Experiments were carried out to investigate the relationship between ion exchange and energy metabolism in rainbow trout erythrocytes *in vitro*. Under resting conditions, the sodium/potassium pump accounts for 20 % of the cellular energy budget. In the presence of the β-adrenergic agonist isoproterenol, however, this increases to 43 %. Inhibition of the sodium/potassium pump with ouabain results in greater increases in erythrocyte water content and sodium and chloride concentrations and a greater decrease in erythrocyte potassium concentration following stimulation by isoproterenol. Moreover, the decrease in erythrocyte NTP levels observed following adrenergic stimulation does not occur when the sodium/potassium pump is inhibited with ouabain. Inhibition of the sodium/potassium pump also abolishes the increase in oxygen consumption by the cells which normally takes place following adrenergic stimulation. Finally, depletion of erythrocyte NTP levels by the sodium ionophore monensin or by previous incubation with nitrogen does not result in a significant increase in oxygen consumption. Thus, catecholamines appear to be crucial for the metabolic–membrane coupling that occurs following adrenergic stimulation in rainbow trout erythrocytes.

Introduction

β-Adrenergic stimulation of sodium/proton exchange in the erythrocyte membrane of a number of fish species has been well documented and the ion exchange processes involved in the adrenergic regulation of erythrocyte pH and volume in fishes have been summarized in several recent reviews (Cossins, 1989; Hoffmann and Simonsen, 1989; Nikinmaa and Tufts, 1990). It has also been demonstrated that catecholamines affect the metabolism of fish erythrocytes. β-Adrenergic stimulation causes a decrease in the levels of NTP within salmonid erythrocytes

* Present address: Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Key words: erythrocytes, trout, ion exchange, metabolism, *Oncorhynchus mykiss*.

(Nikinmaa, 1983; Ferguson and Boutilier, 1988, 1989; Ferguson *et al.* 1989). The reason for this decrease, however, has not been clearly determined. Nikinmaa (1983) demonstrated that there is a decrease in pyruvate kinase activity in adrenergically stimulated rainbow trout erythrocytes; therefore, inhibition of glycolysis following adrenergic stimulation may contribute to the fall in NTP. In addition, there is evidence that the activity of the sodium/potassium ATPase is increased following β-adrenergic stimulation in nucleated erythrocytes (Bourne and Cossins, 1982; DeVries and Ellory, 1982; Borgese *et al.* 1987). Thus, an increased consumption of ATP by the sodium/potassium ATPase under these conditions may also contribute to the decrease in erythrocytic NTP levels. There is also a large increase in the rate of oxygen consumption by salmonid erythrocytes in the presence of elevated levels of catecholamines (Ferguson and Boutilier, 1988, 1989; Ferguson *et al.* 1989; Boutilier and Ferguson, 1989). These results suggest that adrenergic stimulation is associated with an increased ATP turnover *via* oxidative phosphorylation (Boutilier and Ferguson, 1989). Finally, since the degree of adrenergic pH regulation achieved is well correlated with the increase in oxygen consumption, there appears to be a tight coupling of the ionic and metabolic events under these conditions (Ferguson *et al.* 1989). A direct link between these ionic and metabolic events, however, has not been clearly demonstrated. Thus, the purpose of the present experiments was to examine the role of the sodium/potassium ATPase in the coupling of the ionic and metabolic events that are associated with adrenergic stimulation in rainbow trout erythrocytes. In addition, we have evaluated this coupling by manipulating sodium/potassium ATPase activity and erythrocyte NTP levels in the absence of catecholamines and by determining the impact on erythrocyte oxygen consumption.

Materials and methods

Animals

Freshwater rainbow trout *Oncorhynchus mykiss* (200–500 g; $N=30$) were obtained from Merlin Fish Farms and maintained in the aquarium facility at Dalhousie University. The fish were held in fiberglass tanks (1.5 m×1.5 m×1.0 m) supplied with dechlorinated tap water (10–15 °C) and were fed commercial fish pellets.

The trout were anesthetized in a buffered 3-aminobenzoic acid ethyl ester (MS-222, Sigma):freshwater mixture (1:10 000) and chronically cannulated in the dorsal aorta using the method of Smith and Bell (1964). Surgery was followed by overnight recovery in lightproof Perspex boxes. Prior to the experiments, blood was collected from several quietly resting cannulated animals and pooled in a chilled tonometer.

Experimental protocol

The protocol consisted of three series of experiments. In the first series, the collected blood was distributed in 4 ml samples to each of four humidified

intermittently rotating glass tonometers. Two of these tonometers received 100 μl of ouabain (final concentration, 10^{-4} mol l^{-1}) dissolved in saline. The remaining two tonometers served as controls and received only 100 μl of saline. The tonometers were then equilibrated for 1 h at 15 °C with a humidified 1 % CO_2:99 % air mixture supplied by gas-mixing pumps (Wöstoff, Bochum, FRG). Following the equilibration period, an 800 μl blood sample was removed from each of the tonometers with a 1 ml gas-tight Hamilton syringe. From this sample, 200 μl of blood was added to 200 μl of chilled perchloric acid and centrifuged in a 0.5 ml Eppendorf tube. Immediately after centrifugation, the supernatant was removed and frozen in liquid nitrogen for later analysis of nucleotide triphosphate (NTP) concentration. Two 25 μl samples of blood were also removed for the determination of hemoglobin (Hb) and the remaining sample was distributed evenly into two 0.5 ml Eppendorf tubes and centrifuged. The erythrocyte pellet from each of these tubes was saved for analysis of erythrocyte ion concentrations and erythrocyte water content. The plasma supernatant was discarded. At this point, a further 100 μl of saline was added to both a control and a ouabain tonometer. The other control and ouabain tonometers each received 100 μl of isoproterenol (final concentration 10^{-5} mol l^{-1}) dissolved in saline. 1 ml of blood was then removed from each tonometer into a 1 ml Hamilton syringe for the determination of erythrocyte oxygen consumption over a 3 h period (see below). The sampling procedure for whole-blood NTP, erythrocyte ion concentrations and erythrocyte water content was repeated 90 and 180 min after the addition of isoproterenol.

In the second series of experiments, two 7 ml samples of blood were equilibrated for 1 h at 10 °C with a humidified 1 % CO_2:99 % air mixture before the control samples were taken. After 1 h, a 1 ml sample was removed from each tonometer with a gas-tight Hamilton syringe to determine erythrocyte levels of sodium, NTP, Hb and erythrocyte water content as in the first series. At this point, each tonometer received either 20 μl of monensin (final concentration 5.0×10^{-6} mol l^{-1}) in dimethyl sulphoxide (DMSO) or 20 μl of DMSO alone as a control. The sampling procedure was then repeated after 1, 10 and 60 min. An additional 1 ml sample of blood was also removed with the 10 min sample for the determination of erythrocyte oxygen consumption.

In the final series of experiments, blood collected from cannulated fish was distributed in 3 ml samples to each of four tonometers. The blood was then equilibrated at 15 °C with a humidified 1 % CO_2:99 % air mixture for 1 h. At this time, using a 1 ml gas-tight Hamilton syringe, a blood sample (250 μl) was taken from each tonometer for the measurement of NTP and hemoglobin. The procedure for processing the blood sample for NTP and hemoglobin was similar to that in the first series of experiments. At this point, the gas flowing through two of the tonometers was changed to humidified 100 % N_2 to deplete the erythrocyte concentration of NTP. Another sample was then taken from each of the four tonometers after a further 2 h (with two of the blood pools equilibrating under these anoxic conditions). The two anoxic (NTP-depleted) tonometers were then returned to the previous 1 % CO_2:99 % air mixture. At this stage, 100 μl of saline

or isoproterenol (final concentration 10^{-5} mol l^{-1}) was added to both an NTP-depleted and a 1% CO_2:99% air (NTP-control) tonometer. Blood samples for NTP and hemoglobin were then taken 10, 90 and 180 min following this addition. Ten minutes after this addition, a 1 ml blood sample was also removed for the determination of erythrocyte oxygen consumption over a 3 h period.

Analyses

Erythrocyte water content was determined by weighing the wet cell pellet, drying it to a constant mass at 80 °C and reweighing it. The water content was then calculated using the formula:

$$\%H_2O = 100 - [(100 \times \text{dry mass})/\text{wet mass}].$$

The erythrocyte pellet was dissolved in 8% perchloric acid to extract the ions prior to analysis. Sodium and potassium concentrations were then measured with a Corning 410 flame photometer (Ciba Corning Diagnostics Corp., Canada). It should be noted that no correction was made for trapped extracellular fluid and our reported values for erythrocyte sodium concentration are, therefore, somewhat higher than the actual erythrocyte concentration. In similar studies, trapped extracellular fluid is invariably reported as a constant (Albers and Goetz, 1985; Borgese *et al.* 1986). Thus, application of this correction factor would not affect the large relative changes observed in this study. Chloride concentrations were measured with a Buchler–Cotlove chloride titrator (Buchler Instruments Inc., USA). A Gilford Response narrow-beam UV–VIS spectrophotometer was used for the determination of Hb and NTP concentrations. The blood hemoglobin content was determined in duplicate by adding 25 μl samples of whole blood to 5 ml of Drabkins reagent (Sigma) and measuring the absorbance at 540 nm. Coupled NAD/NADH enzymatic assays (Sigma) were utilized in the analysis of NTP and the absorbance was measured at 340 nm. Erythrocyte oxygen consumption was determined by incubating a 1 ml sample of whole blood in a gas-tight Hamilton syringe and measuring the oxygen content and hemoglobin concentration at 0, 90, and 180 min during the 3 h incubation. The rate of decrease in oxygen content was found to be linear over this period. Oxygen content was measured on 20–50 μl blood samples (dependent on hematocrit) using the Tucker method (Tucker, 1967). The significance of the results was assessed using a Student's paired *t*-test with $P<0.05$ accepted as significant.

Results

The effects of ouabain and/or isoproterenol on the erythrocyte water content and ion concentrations are illustrated in Fig. 1. By itself, ouabain did not cause any significant changes in the erythrocyte water content (Fig. 1A). As demonstrated in previous studies, addition of isoproterenol resulted in a large increase in the erythrocyte water content after 3 h. In the presence of ouabain plus isoproterenol, however, the erythrocyte water content was significantly greater than that in cells

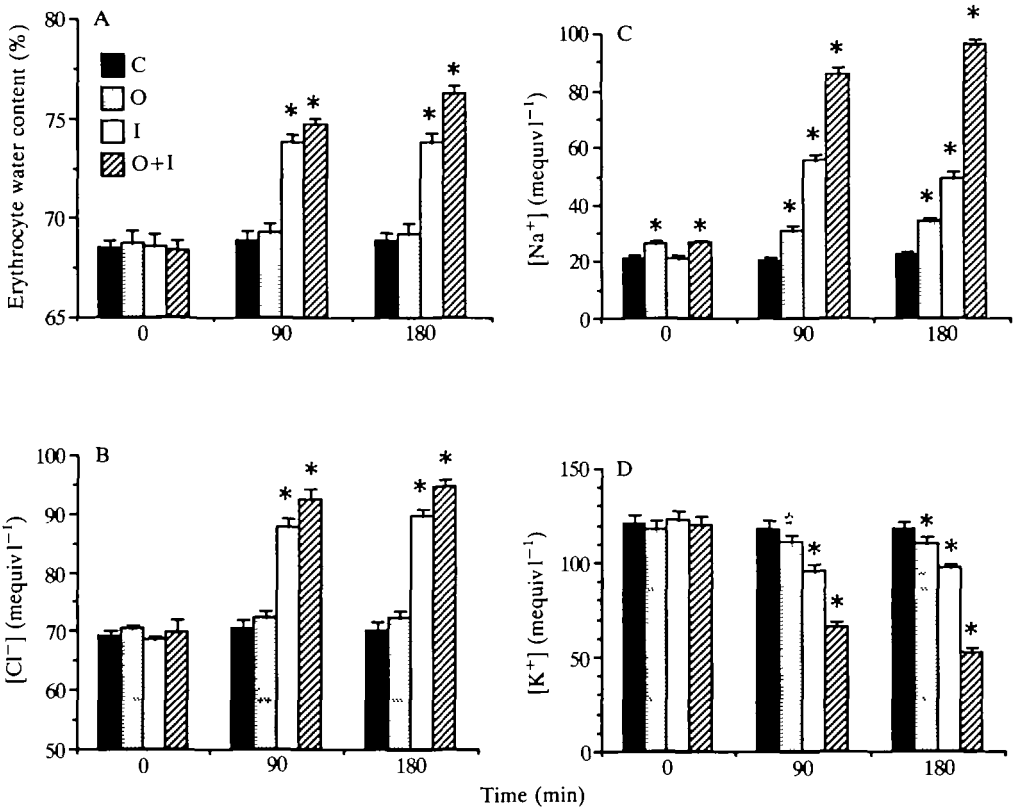

Fig. 1. (A) Water content and concentration (mequiv l^{-1} cell water) of (B) chloride, (C) sodium and (D) potassium in rainbow trout erythrocytes prior to (time 0) and 90 and 180 min following treatment with either isoproterenol (10^{-5} mol l^{-1}) or saline (control). C, saline control; O, ouabain (10^{-4} mol l^{-1}); I, isoproterenol; I+O, isoproterenol plus ouabain. All values are means+S.E. (N=6 individual experiments). Asterisks denote significant difference from control at each time.

with only isoproterenol added. Similarly, the isoproterenol-induced change in erythrocyte chloride concentration was also significantly greater in the presence of ouabain (Fig. 1B).

Blocking the sodium/potassium pump with ouabain results in an increase in erythrocyte sodium concentration which was significant at all three sample times (Fig. 1C). From these data, the sodium leak across the erythrocyte membrane can be estimated to be 2.5 mmol h^{-1}. Inhibition of the pump also caused a decrease in the erythrocyte potassium concentration which was significant after 90 and 180 min. The potassium leak across the erythrocyte membrane (2.8 mmol h^{-1}) was roughly equivalent to the inward sodium leak. Addition of isoproterenol exacerbated these differences. In cells not treated with ouabain, 3 h after the addition of isoproterenol the erythrocyte sodium concentration had increased to 49.2±5.3 mequiv l^{-1}. In the ouabain+isoproterenol-treated cells, however, the

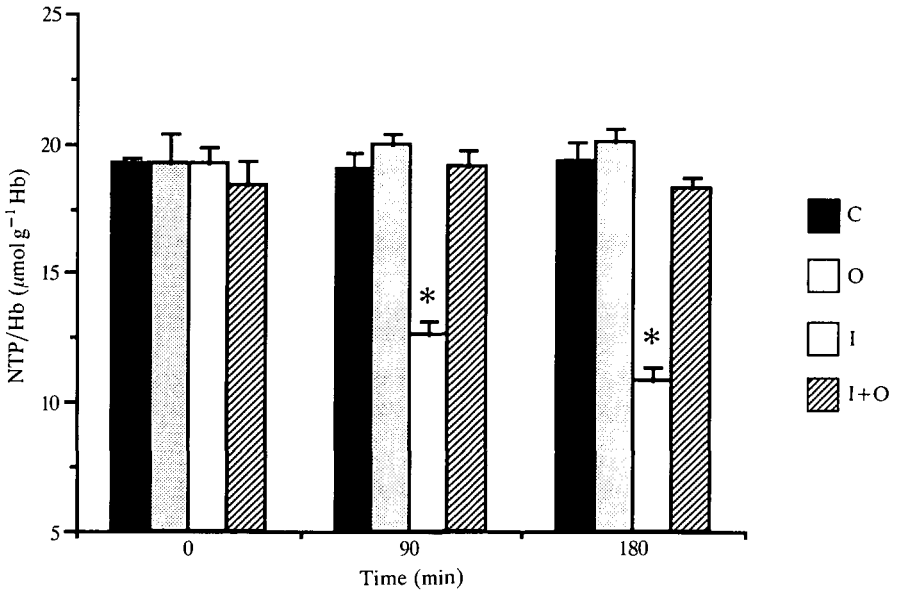

Fig. 2. The NTP/Hb concentration in rainbow trout erythrocytes prior to (time 0) and 90 and 180 min following treatment with either isoproterenol (10^{-5} mol l^{-1}) or saline (control). C, saline control; O, ouabain (10^{-4} mol l^{-1}), I, isoproterenol; I+O, isoproterenol plus ouabain. All values are means+S.E. (N=6 individual experiments). Asterisks denote significant difference from control at each time.

erythrocyte sodium concentration reached 95.9±5.1 mequiv l^{-1} by the 3 h sample. The erythrocyte potassium concentration decreased significantly after 3 h of incubation in the presence of isoproterenol. Again, in the presence of ouabain, this difference was magnified.

Incubation of rainbow trout erythrocytes for 3 h in the presence of ouabain did not cause any significant change in the erythrocyte NTP/Hb ratio (Fig. 2). Addition of isoproterenol, however, caused the NTP/Hb ratio to fall by 43 % after 3 h. In the presence of ouabain, this isoproterenol-induced decrease in the erythrocyte NTP level was eliminated and the NTP/Hb ratio did not change significantly from the control value.

The presence of ouabain alone caused a significant decrease in the erythrocyte oxygen consumption (Fig. 3). The oxygen consumption of erythrocytes under control conditions was 85.9±5.5 nmoles g^{-1} Hb h^{-1}, whereas that of ouabain-treated cells was 68.7±4.5 nmol g^{-1} Hb h^{-1}. Thus, the oxygen requirements of the sodium/potassium pump appear to be about 20 % of the resting metabolic rate in rainbow trout erythrocytes. Addition of isoproterenol resulted in a significant increase (40 %) in the oxygen consumption of untreated cells. In the presence of ouabain, however, the addition of isoproterenol did not significantly alter the rate of oxygen consumption.

Addition of the sodium ionophore monensin to rainbow trout erythrocytes

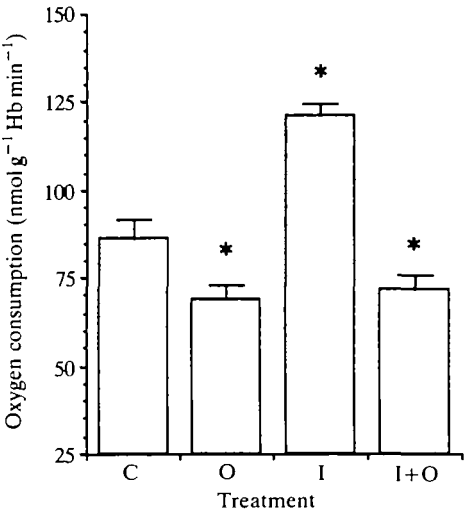

Fig. 3. Oxygen consumption of rainbow trout erythrocytes under different conditions at 15°C. C, saline control; O, ouabain (10^{-4} mol l^{-1}), I, isoproterenol (10^{-5} mol l^{-1}), I+O, isoproterenol plus ouabain. All values are means+S.E. (N=6 individual experiments). Asterisks denote significant difference from control.

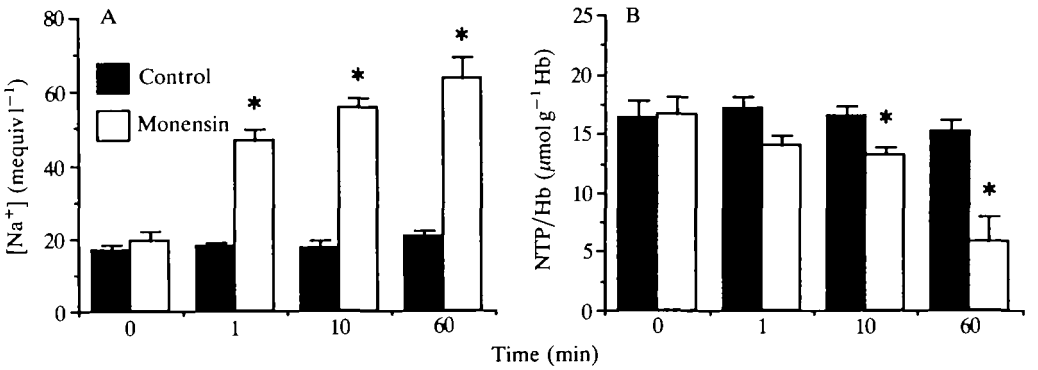

Fig. 4. Concentration of (A) sodium (mequiv l^{-1} cell water) and (B) NTP/Hb in rainbow trout erythrocytes prior to (time 0) and 1, 10 and 60 min following addition of saline (control) or monensin (5×10^{-6} mol l^{-1}). All values are means+S.E. (N=5 individual experiments). Asterisks denote significant difference from saline control at each time.

caused a large and rapid increase in the erythrocyte sodium concentration (Fig. 4A) similar to that caused by isoproterenol. This increase in erythrocyte sodium concentration in the presence of monensin was also associated with a significant decrease in the erythrocyte concentration of NTP (Fig. 4B); the levels of NTP had decreased to 35% of the control value by the 60 min sample. There was no significant change, however, in the oxygen consumption of the erythrocytes

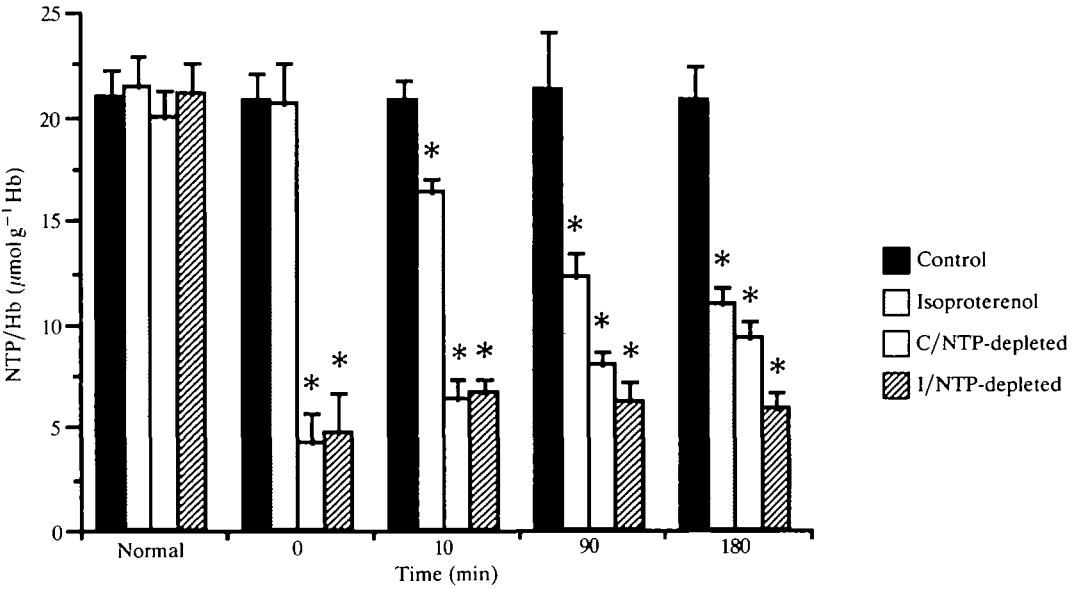

Fig. 5. Concentration of NTP/Hb in rainbow trout erythrocytes under normal conditions, following 2 h of incubation in either 1 % CO_2:99 % air or N_2 (time 0), and 10, 90 and 180 min following restoration of 1 % CO_2:99 % air in all tonometers and addition of either saline (control) or isoproterenol (10^{-5} mol l^{-1}). C/NTP-depleted, saline control of NTP-depleted cells; I/NTP-depleted, isoproterenol treatment of NTP-depleted cells. All values are means+S.E. (N=6 individual experiments). Asterisks denote significant difference from saline control at each time.

incubated with monensin. Under control conditions, the oxygen consumption was 109.6±8.3 nmol g^{-1} Hb min^{-1} (N=5) and in the presence of monensin, it was 111.3±7.5 nmol g^{-1} Hb min^{-1} (N=5).

Incubation of rainbow trout erythrocytes for 2 h with nitrogen caused a 79 % decrease in the NTP/Hb ratio (i.e. control *vs* zero time; Fig. 5). Further incubation of these NTP-depleted cells for 3 h in a 1 % CO_2:99 % air atmosphere resulted in a significant increase in the NTP/Hb ratio, but not to the control level. Over the 3 h period when oxygen was once again available to the cells the NTP/Hb ratio increased. This 3 h value was approximately 50 % of the control value. Changes in the NTP/Hb ratio caused by isoproterenol were different when NTP-depleted (previously anoxic) cells were compared with cells that had been incubated entirely in the presence of the 1 % CO_2:99 % air mixture. Cells incubated in the 1 % CO_2:99 % air mixture showed a significant decrease in the NTP/Hb ratio within the first 10 min following the addition of isoproterenol; after 180 min, the NTP/Hb ratio had fallen to 50 % of the original control values. In contrast, when the NTP-depleted cells were re-oxygenated and stimulated with isoproterenol, their NTP/Hb ratio initially increased. However, 3 h after the

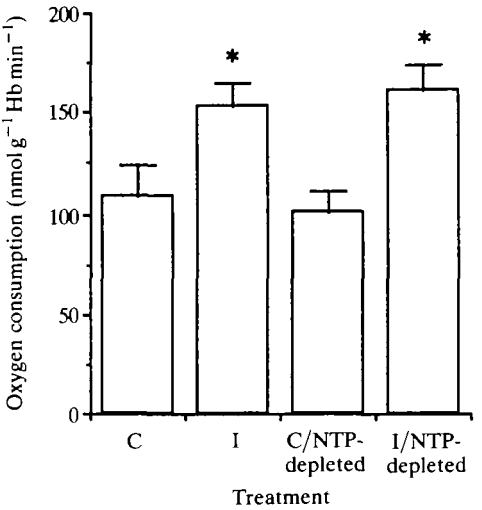

Fig. 6. Oxygen consumption of rainbow trout erythrocytes. C, saline control; I, isoproterenol; C/NTP-depleted, saline control of NTP-depleted cells; I/NTP-depleted isoproterenol treatment of NTP-depleted cells. All values are means+S.E. (N=6 individual experiments). Asterisks denote significant difference from saline control.

addition of isoproterenol, these values fell to levels that were not significantly different from the values immediately after anoxia (i.e. zero time).

Despite the large decrease in erythrocyte NTP levels, 2 h of anoxia had no significant effect on the oxygen consumption of the erythrocytes (Fig. 6). Moreover, upon addition of isoproterenol, the magnitude of the increase in red blood cell oxygen consumption was the same for both control and NTP-depleted cells.

Discussion

Adrenergic stimulation of rainbow trout erythrocytes in the presence of ouabain is associated with a larger increase in the erythrocyte concentration of sodium and a larger decrease in the erythrocyte concentration of potassium compared with cells with a functional sodium/potassium ATPase (Borgese *et al.* 1987; Fig. 1). In contrast to the experiments by Borgese *et al.* (1987), however, we find that the increase in cell water is significantly greater in the ouabain-treated cells (Fig. 1). A probable explanation of these differences is that the experiments of Borgese *et al.* (1987) were carried out in the presence of nitrogen, whereas the present experiments were conducted under aerobic conditions. Salmonid erythrocytes derive over 90 % of their required ATP from aerobic respiration and incubation of these cells under anaerobic conditions causes a pronounced fall in NTP levels (Ferguson and Boutilier, 1988; Ferguson *et al.* 1989; Fig. 5). Under anaerobic

conditions, therefore, it is not surprising that Borgese *et al.* (1987) reported an insignificant effect of ouabain on the adrenergic swelling response since the sodium/potassium ATPase may have already been partially inhibited, even in the absence of ouabain, by the low levels of ATP.

Adrenergic stimulation of salmonid erythrocytes *in vitro* results in a marked decrease in NTP levels (Nikinmaa, 1983; Ferguson and Boutilier, 1988, 1989; Ferguson *et al.* 1989). In the present study, erythrocyte NTP concentration had fallen by approximately 50 % 3 h after the addition of isoproterenol (Fig. 2). A decrease in pyruvate kinase activity following adrenergic stimulation has been demonstrated in both nucleated and non-nucleated erythrocytes (Mairbaurl and Humpeler, 1981; Nikinmaa, 1983). These results suggest that inhibition of pyruvate kinase activity and, therefore, glycolytic flux, may contribute to the decrease in NTP in adrenergically stimulated trout erythrocytes. In the present study, however, erythrocytes incubated in the presence of ouabain showed no significant changes in their levels of NTP following adrenergic stimulation (Fig. 2). According to our results, therefore, the increased activity of the sodium/potassium ATPase is entirely responsible for the drop in NTP levels in adrenergically stimulated salmonid erythrocytes.

The sodium/potassium pump accounts for approximately 20 % of the total energy consumption of rainbow trout erythrocytes (Fig. 3). Thus, under resting conditions, the energy allocation to the sodium/potassium pump is roughly similar to that in rabbit reticulocytes (Rapoport, 1985). In adrenergically stimulated salmonid erythrocytes, however, the oxygen consumption may actually double (Ferguson and Boutilier, 1988). In the present study, adrenergic stimulation was associated with a 40 % increase in oxygen consumption which could be entirely inhibited by ouabain (Fig. 3). This indicates that, during adrenergic stimulation, the energy requirements of the sodium/potassium pump increase to approximately 43 % of the cellular energy budget. Similarly, when rat cortical synaptosomes respond to ionic stress, the energy requirements of the sodium/potassium ATPase also increase from 10 % to 30 % (Kalman, 1984). Similar calculations on erythrocytes of Atlantic salmon (Ferguson and Boutilier, 1988) indicate that the requirements of the sodium/potassium ATPase may reach as much as 50 % of the energy flow through adrenergically stimulated salmonid erythrocytes. Our data indicate that the increased consumption of NTP associated with the increased activity of the sodium/potassium ATPase following adrenergic stimulation is a prerequisite for the large increase in oxygen consumption in salmonid erythrocytes. In the absence of any change in NTP consumption (i.e. when ouabain is present), there is no increase in erythrocyte oxygen consumption (Figs 2, 3).

The ionophore monensin causes sodium/proton exchange across membranes and incubation of rainbow trout erythrocytes in the presence of this ionophore results in ionic events that are similar to those produced by catecholamines (Borgese *et al.* 1986). In addition, monensin causes a drop in erythrocyte NTP levels, presumably due to an increased consumption of NTP by the sodium/potassium pump following the large influx of sodium (Fig. 4). Monensin does not,

however, stimulate an increase in oxygen consumption by rainbow trout erythrocytes, despite the apparent increase in NTP consumption (see Results). Thus, although an increase in NTP consumption by the sodium/potassium ATPase does contribute to the increase in oxygen consumption observed in these cells following adrenergic stimulation, these data suggest that catecholamines may also contribute to the increase in oxygen consumption *via* a stimulatory effect on the pathways of aerobic metabolism in these cells. It is important to consider, however, that monensin may have an inhibitory effect on the metabolic pathways in these cells which could limit the potential scope of aerobic metabolism. The fact that we observed no significant decrease in oxygen consumption in the presence of monensin argues against this possibility, but further investigation into the effects of monensin on the aerobic metabolism of these cells is clearly warranted.

Equilibration of rainbow trout erythrocytes for 2 h with nitrogen resulted in a 79 % decrease in NTP levels (Fig. 5). In the absence of isoproterenol, erythrocyte NTP levels slowly increased but, 3 h after the restoration of an oxygenated atmosphere, these levels were still less than 50 % of those in control cells. In addition, we were unable to detect any increase in the rate of oxygen consumption by these cells (Fig. 6). Thus, it would appear that even extremely low levels of NTP (and presumably high levels of ADP and inorganic phosphate) do not, in themselves, stimulate an increase in aerobic metabolism that is comparable to that observed after adrenergic stimulation. The fact that there is a slow increase in the NTP concentration of NTP-depleted cells when oxygen is restored in the absence of isoproterenol does indicate that there may be some level of respiratory control in these cells, but we could not detect any associated increase in oxygen consumption (Figs 5, 6). Thus, the expected increase in oxygen consumption in these cells, if it did occur, must have been extremely small. In contrast, both the NTP control and the NTP-depleted cells showed significant increases in oxygen consumption in the presence of isoproterenol (Fig. 6). These increases were similar to that observed in the first series of experiments and were not significantly different from each other. Thus, there is clearly no limitation to increased aerobic metabolism in these NTP-depleted cells. These results also suggest that the increased activity of the sodium/potassium ATPase may not be the only factor contributing to the documented increase in the aerobic metabolism of these cells after adrenergic stimulation. There is an increase in cyclic AMP concentration in fish erythrocytes following adrenergic stimulation and the adrenergically mediated ion movements are cyclic-AMP-dependent (Mahe *et al.* 1985). In other tissues, cyclic AMP activates protein kinases, which subsequently influence a number of cellular activities, including synchronous, but independent, regulation of various enzymes (Cohen, 1982, 1985; Barnes, 1986). Our results would suggest that catecholamines may also regulate pathways that produce aerobic energy in rainbow trout erythrocytes, possibly *via* cyclic-AMP-dependent changes in enzyme function. The elucidation of these mechanisms may provide an interesting area for further investigation.

In summary, the present study indicates that the energy requirements of the

sodium/potassium ATPase in the trout erythrocyte are approximately doubled after adrenergic stimulation. Furthermore, the decrease in erythrocyte NTP levels observed following adrenergic stimulation appears to be entirely due to increased consumption of ATP by the sodium/potassium pump, and this increased consumption of ATP contributes to the increase in oxygen consumption that is observed in adrenergically stimulated salmonid erythrocytes. The present results also suggest that catecholamines may have a stimulatory effect on the aerobic pathways within these cells and, therefore, may be crucial for the metabolic–membrane coupling observed in this and other studies (see Ferguson and Boutilier, 1988, 1989; Ferguson *et al.* 1989).

This work was supported by an NSERC operating grant to R.G.B. and an NSERC postdoctoral fellowship to B.L.T.

References

ALBERS, C. AND GOETZ, K. G. (1985). H^+ and Cl^- ion equilibrium across the red cell membrane in the carp. *Respir. Physiol.* **61**, 209–219.

BARNES, D. M. (1986). How cells respond to signals. *Science* **234**, 286–288.

BORGESE, G., GARCIA-ROMEU, F. AND MOTAIS, R. (1986). Catecholamine-induced transport systems in trout erythrocyte. *J. gen. Physiol.* **87**, 551–566.

BORGESE, F., GARCIA-ROMEU, F. AND MOTAIS, R. (1987). Ion movements and volume changes induced by catecholamines in erythrocytes of rainbow trout: effect of pH. *J. Physiol., Lond.* **382**, 145–157.

BOURNE, P. K. AND COSSINS, A. R. (1982). On the instability of K^+ influx in erythrocytes of the rainbow trout, *Salmo gairdneri*, and the role of catecholamine hormones in maintaining *in vivo* influx activity. *J. exp. Biol.* **101**, 93–104.

BOUTILIER, R. G. AND FERGUSON, R. A. (1989). Nucleated red cell function: metabolism and pH regulation. *Can J. Zool.* **67**, 2986–2993.

COHEN, P. (1982). The role of protein phosphorylation in neural and hormonal control of cellular activity. *Nature* **296**, 613–620.

COHEN, P. (1985). The role of protein phosphorylation in the hormonal control of enzyme activity. *Eur. J. Biochem.* **151**, 439–448.

COSSINS, A. (1989). Intracellular pH regulation by fish red cells. *Nature* **340**, 20–21.

DEVRIES, A. L. AND ELLORY, J. C. (1982). The effects of stress on ion transport in fish erythrocytes. *J. Physiol., Lond.* **324**, 51P.

FERGUSON, R. A. AND BOUTILIER, R. G. (1988). Metabolic energy production during adrenergic pH regulation in red cells of the Atlantic salmon, *Salmo salar*. *Respir. Physiol.* **74**, 65–76.

FERGUSON, R. A. AND BOUTILIER, R. G. (1989). Metabolic–membrane coupling in red blood cells of trout: the effects of anoxia and adrenergic stimulation. *J. exp. Biol.* **143**, 149–164.

FERGUSON, R. A., TUFTS, B. L. AND BOUTILIER, R. G. (1989). Energy metabolism in trout red cells: consequences of adrenergic stimulation *in vivo* and *in vitro*. *J. exp. Biol.* **143**, 133–147.

HOFFMANN, E. K. AND SIMONSEN, O. (1989). Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.* **69**, 315–382.

KALMAN, M. (1984). The ouabain-sensitive component of synaptosomal respiration. *Expl Brain Res.* **56**, 539–542.

MAHE, Y., GARCIA-ROMEU, F. AND MOTAIS, R. (1985). Inhibition by amiloride of both adenylate cyclase activity and the Na^+/H^+ antiporter in fish erythrocytes. *Eur. J. Pharmac.* **116**, 199–206.

MAIRBAURL, H. AND HUMPELER, E. (1981). *In vitro* influences of adrenaline on erythrocyte metabolism and oxygen affinity of hemoglobin. In *The Red Cell:* Fifth Ann Arbor Conference (ed. G. J. Brewer), pp. 311–319. New York: Alan R. Liss.

NIKINMAA, M. (1983). Adrenergic regulation of haemoglobin oxygen affinity in rainbow trout red cells. *J. comp. Physiol.* **152**, 67–72.

NIKINMAA, M. AND TUFTS, B. L. (1990). Regulation of acid and ion transfer across the membrane of nucleated erythrocytes. *Can. J. Zool.* **67**, 3039–3045.

RAPOPORT, S. M. (1985). Mechanisms of the maturation of the reticulocyte. In *Circulation Respiration and Metabolism. Current Comparative Approaches* (ed. R. Gilles), pp. 333–342. Berlin: Springer Verlag.

SMITH, L. S. AND BELL, G. R. (1964). A technique for prolonged blood sampling in free swimming salmon. *J. Fish Res. Bd Can.* **21**, 711–717.

TUCKER, V. A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. appl. Physiol.* **23**, 410–414.