J. exp. Biol. 147, 169–188 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

REGULATION OF HEPATIC GLUCONEOGENESIS AND GLYCOGENOLYSIS BY CATECHOLAMINES IN RAINBOW TROUT DURING ENVIRONMENTAL HYPOXIA

BY PATRICIA A. WRIGHT*, STEVE F. PERRY AND THOMAS W. MOON

Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

Accepted 28 June 1989

Summary

This study tests the hypothesis that catecholamines regulate glucose availability during hypoxia in the rainbow trout by activating glycogen phosphorylase (GPase) while inhibiting pyruvate kinase (PK) in the liver. The net result would be an increase in liver glycogenolysis and a reduction of glycolysis and/or enhancement of gluconeogenesis. We used the criteria of Stalmans & Hers (1975) and report much lower resting percent GPase *a* (active) values (20–30%) than those previously published. Dorsal aortic injections of epinephrine or norepinephrine increased plasma glucose (16–46%), had no effect on liver or muscle glycogen levels, decreased the activity of PK, and increased total and percent GPase *a* activities. Pre-treatment with the β-adrenoreceptor antagonist propranolol eliminated these effects. During moderate hypoxia, plasma glucose remained unchanged, while lactate levels increased fourfold. When fish were pre-treated with propranolol, hypoxia depressed plasma glucose levels (−26%), total and percent GPase *a*, and increased PK activity, suggesting that hypoxia mediated the dephosphorylation of these enzymes. We conclude that catecholamines stimulate hepatic β-adrenoreceptors during hypoxia and sustain plasma glucose levels by nullifying the deleterious effects of hypoxia on metabolic function. The specific metabolic consequences of these catecholamine-mediated effects are an increase in the activity of the active form of GPase and a reduction in PK activity, which suggests an activation of glycogenolysis and an inhibition of glycolysis and/or activation of gluconeogenesis, respectively.

Introduction

When trout encounter environmental hypoxia they respond initially by lowering metabolic rate, then by increasing anaerobic metabolism (Boutilier *et al.* 1988).

* Present address: Laboratory of Kidney and Electrolyte Metabolism, National Institutes of Health, Building 10, Room 6N307, Bethesda, MD 20892, USA.

Key words: glucose, hypoxia, catecholamines, trout, liver.

Some tissues are glucose-dependent (e.g. nervous tissue), but have little reserve capacity and, for this reason, it is important that plasma glucose levels are regulated. Moreover, rapid increases in glucose availability are often required to meet the increased metabolic demands of tissues. It is not surprising, therefore, that catecholamine levels, well known as mediators of rapid physiological change, increase markedly in arterial blood during exposure to low environmental oxygen (Butler *et al.* 1979; Tetens & Christensen, 1987; Fievet *et al.* 1987; Boutilier *et al.* 1988). The purpose of this study was to determine whether catecholamines play a role in the regulation of glucose metabolism *in vivo* during short-term moderate hypoxia.

Catecholamines exert hyperglycemic effects in fish (e.g. Thorpe & Ince, 1974; deRoos & deRoos, 1978; Morata *et al.* 1982; Ottolenghi *et al.* 1984, 1985, 1986; Janssens & Lowrey, 1987; Mommsen *et al.* 1988), which are due, in part, to activation of glycogenolysis (Nakano & Tomlinson, 1967; Birnbaum *et al.* 1976; Rush & Umminger, 1978; Ristori & Laurent, 1985; Mommsen *et al.* 1988). Epinephrine also activates gluconeogenesis, but its relative importance is not clear. Mommsen *et al.* (1988) reported that glycogenolysis accounted for 97% of glucose production in trout hepatocytes with or without epinephrine. Ottolenghi *et al.* (1985), however, concluded that in isolated, perfused catfish livers, glycogenolysis dominated at high doses of epinephrine, but gluconeogenesis was the dominant source of glucose at low doses.

The role of catecholamines in glucose metabolism has often been studied *in vitro*, but less frequently in intact fish. One of the difficulties with using hepatocytes *in vitro* is that freshly isolated cells are in negative glycogen balance and rates are unlikely to reflect those found *in situ* (Mommsen *et al.* 1988). In this study we use intact fish to eliminate this *in vitro* preparation artifact.

A second problem addressed by this study relates to methodological differences in the measurement of glycogen phosphorylase (GPase) activity. Wosilait & Sutherland (1956) first separated the two forms of GPase (*b*, inactive; *a*, active) by measuring activities in the absence and in the presence of AMP, respectively. Unless caffeine is present, however, the activity of rat liver GPase *b* is significantly stimulated by AMP (Stalmans & Hers, 1975). This may explain the consistently high liver resting percent GPase *a* $[= a/(a+b)]$ values reported in the literature (90–95%, Ottolenghi *et al.* 1985, 1986; 83%, Perry *et al.* 1988; 72%, Mommsen *et al.* 1988). Such high percent GPase *a* values suggest that GPase is almost completely activated at rest, a situation which affords little possibility of regulatory control. In the present study, we rectified this technical problem by applying the criteria of Stalmans & Hers (1975) to GPase activities.

We have analyzed the individual effects of epinephrine, norepinephrine and hypoxia (with/without β-adrenoreceptor blockade) on tissue glucose and glycogen levels, and on the kinetic behavior of two key enzymes involved at critical points in glucose metabolism, glycogen phosphorylase and pyruvate kinase. PK was measured because of its key role in regulating metabolic flux through glycolysis and gluconeogenesis (Pilkis *et al.* 1988).

Materials and methods

Animals

Rainbow trout (*Salmo gairdneri*) of both sexes, weighing 150–250 g, were obtained from Thistle Springs Trout Farm (Ashton, Ontario) and maintained as reported previously (Perry & Vermette, 1987) at seasonal temperatures (15–18°C). Preliminary PK kinetic properties were determined on resting intact fish, held in individual, opaque, Perspex chambers. Fish were killed by a sharp blow to the head, the liver was immediately removed, and frozen between aluminum blocks cooled in liquid N_2. Tissue samples were stored at −70°C until analysis.

In all other experiments, trout were anesthetized, surgically fitted with a dorsal aortic (DA) catheter (Soivio *et al.* 1972), and left to recover for 48 h in individual Perspex chambers. Three series of experiments were performed (series I, II and III) and in each series there were four separate groups of fish (Table 1). Experiments were performed over the summer season when ambient water temperature increased from 15°C to a peak of 18°C. Fish in series I were held at 15°C, series II at 18°C and series III at 16°C.

Experimental protocol

In each series, fish were pre-injected with either heparinized, physiological saline (Wolf, 1963) or propranolol, which was followed 1 h later by the experimen-

Table 1. *Experimental design of series I, II and III*

	Experiment	Pre-injection	Experimental injection/ treatment
Series I			
Epinephrine	Saline control	Saline	Saline
	Epinephrine	Saline	Epinephrine
	Propranolol control	Propranolol	Saline
	Propranolol + epinephrine	Propranolol	Epinephrine
Series II			
Norepinephrine	Saline control	Saline	Saline
	Norepinephrine	Saline	Norepinephrine
	Propranolol control	Propranolol	Saline
	Propranolol + norepinephrine	Propranolol	Norepinephrine
Series III			
Hypoxia	Saline control	Saline	Normal water
	Hypoxia	Saline	Hypoxic water
	Propranolol	Propranolol	Normal water
	Propranolol + hypoxia	Propranolol	Hypoxic water

tal treatment of saline, epinephrine or norepinephrine injection, or exposure to external hypoxia (Table 1). Propranolol was dissolved in heparinized saline immediately before use, adjusted to pH 7·8, and injected over a 30 s period into the DA catheter. The volume injected was 0·2 ml at a dose of 2 mg kg^{-1} body mass and was followed by an infusion of 0·3 ml of saline to flush the catheter. The calculated circulating level of propranolol was approximately 5×10^{-5} mol l^{-1}, about three orders of magnitude greater than peak catecholamine levels (see Fig. 2). Epinephrine and norepinephrine solutions (bitartrate salts; pH 7·8) were prepared immediately prior to infusion. The volume injected was 0·2 ml at an appropriate dose to achieve a circulating epinephrine/norepinephrine concentration of 5×10^{-8} mol l^{-1}, followed by a 0·3 ml saline bolus to flush the catheter.

In series I and II, an initial blood sample (0·8–1·0 ml) was collected 30 min after the pre-injection and a final blood sample (0·8–1·0 ml) was collected 15 min after the experimental injection (Table 1). The initial blood sample was taken for control measurements, and compared to blood samples taken 15 min after the experimental injection.

In series III, the degree and duration of hypoxia were chosen to induce a marked increase in circulating catecholamines within a minimum time (Boutilier *et al.* 1988). External hypoxia was achieved by gassing the inflowing water with 100 % N_2 in a counter-current gas-exchange column. By carefully adjusting the water and gas flow rates through the column, the oxygen tension of the inflowing water ($Pw_{O_2} = 7{\cdot}1 \pm 0{\cdot}4$ kPa, S.E.) was kept constant for a 30 min experimental period.

In all series, fish were killed by a sharp blow to the head immediately after the final blood sample had been withdrawn through the DA catheter. The liver was excised and frozen between aluminum blocks cooled in liquid N_2. White muscle samples (0·5–1·0 g) were then excised and quickly frozen in the same manner. Tissue samples were stored at −70°C until analysed (<24 h for glucose and glycogen, <4 days for liver enzyme analyses).

Analytical procedures

Blood was analysed for pH (pHe), red cell pH (pHi), plasma glucose and catecholamine levels. In series III, additional measurements were made of whole-blood oxygen tension (Pa_{O_2}), content (Ca_{O_2}) and plasma lactate ([lactate]). pHe, Pa_{O_2} and Ca_{O_2} were determined as described by Vermette & Perry (1988). Plasma was separated by centrifugation (2 min at 13 000 *g*), acidified (0·6 mol l^{-1} $HClO_4$), and stored (−20°C) for later determination of plasma glucose levels. Plasma samples (100–200 μl) were also stored for subsequent determination of catecholamines and [lactate] (series III only) at −70°C. Plasma glucose and lactate levels were measured enzymatically according to Bergmeyer (1974). Epinephrine and norepinephrine levels were measured by HPLC and electronic detection, as described in detail by Woodward (1982).

Liver and muscle glucose and glycogen concentrations were determined in t[illegible] following manner. Frozen tissue samples were weighed, deproteinized in iced

$HClO_4$ (0·6 mol l^{-1}, 1:4 dilution), and homogenized. A sample of neutralized supernatant was hydrolyzed with amyloglucosidase (in acetate buffer, pH 4·6–4·8) and glucose was determined as above. Glycogen (in μmol glucosyl units g^{-1} tissue) was determined as the difference in glucose concentrations in the hydrolyzed and nonhydrolyzed samples.

The activities of GPase and PK were determined spectrophotometrically by recording the change in absorbance of NAD(P) at 340 nm ($E_{mu} = 6{\cdot}22$). Liver tissue was sonicated (Koutes MicroUltrasonic cell disrupter) in a phosphorylation–dephosphorylation 'stopping buffer' (5:1 w/v; 50 mmol l^{-1} imidazole, 15 mmol l^{-1} β-mercaptoethanol, 100 mmol l^{-1} KF, 5 mmol l^{-1} EDTA, 5 mmol l^{-1} EGTA, pH 7·5, at room temperature; after Stalmans & Hers, 1975), layered onto a 3 ml Sephadex (G-25) column equilibrated with stopping buffer, and centrifuged at low speeds according to Christopherson (1983) to remove small metabolites. PK activity was determined in 50 mmol l^{-1} imidazole buffer (pH 7·5) containing 10 mmol l^{-1} $MgCl_2$, 2·5 mmol l^{-1} ADP, 100 mmol l^{-1} KCl, 0·12 mmol l^{-1} NADH, excess lactate dehydrogenase (10 units) and either 0·2, 0·4 or 5·0 mmol l^{-1} phosphoenolpyruvate (PEP, omitted for control). GPase activity was determined in 50 mmol l^{-1} phosphate buffer (pH 7·0) containing 0·4 mmol l^{-1} $NADP^+$, 15 mmol l^{-1} $MgSO_4$, 0·2 mmol l^{-1} glucose-1,6-bisphosphate, 2 mmol l^{-1} AMP, excess glucose-6-phosphate dehydrogenase and phosphoglycerate mutase (each 1 unit), 2 mg ml^{-1} dialyzed glycogen (omitted for control) and either 0·25 mmol l^{-1} EDTA or 10 mmol l^{-1} EDTA and 10 mmol l^{-1} caffeine. Caffeine inactivates the inactive or *b* form of GPase and therefore provides a direct measure of the active or *a* form of the enzyme. GPase *a* activity is defined as that in the presence of caffeine and total GPase as activity without caffeine (Stalmans & Hers, 1975).

Cyclic AMP was measured in liver tissue using a cyclic-[^{3}H]AMP kit (Amersham Corp.). Tissues were homogenized in 0·7 % perchloric acid containing EDTA, neutralized and analyzed as directed.

Results are given as means ± 1 standard error (S.E.) of the mean. Initial and final blood sample values were compared using a Student's paired *t*-test ($P < 0{\cdot}05$). Comparisons between groups of fish were performed using a Student's unpaired *t*-test ($P < 0{\cdot}05$).

Results

Pyruvate kinase kinetics

The substrate-dependence of pyruvate kinase is shown in Fig. 1A. The substrate concentration for half-maximal saturation ($S_{0{\cdot}5}$) for the enzyme with respect to phosphenolpyruvate (PEP) was 0·60 mmol l^{-1} (Fig. 1B) and V_m at 15°C was 16·1 μmol g^{-1} min^{-1} (Fig. 1A). The Hill constant (n_H), calculated from the slope of the line in Fig. 1B, was 1·80. An n_H value greater than 1 indicates positive cooperativity in the binding between ligand and regulatory enzyme. L-Alanine (2 mmol l^{-1}) did not significantly modify the kinetic properties of PK with respect

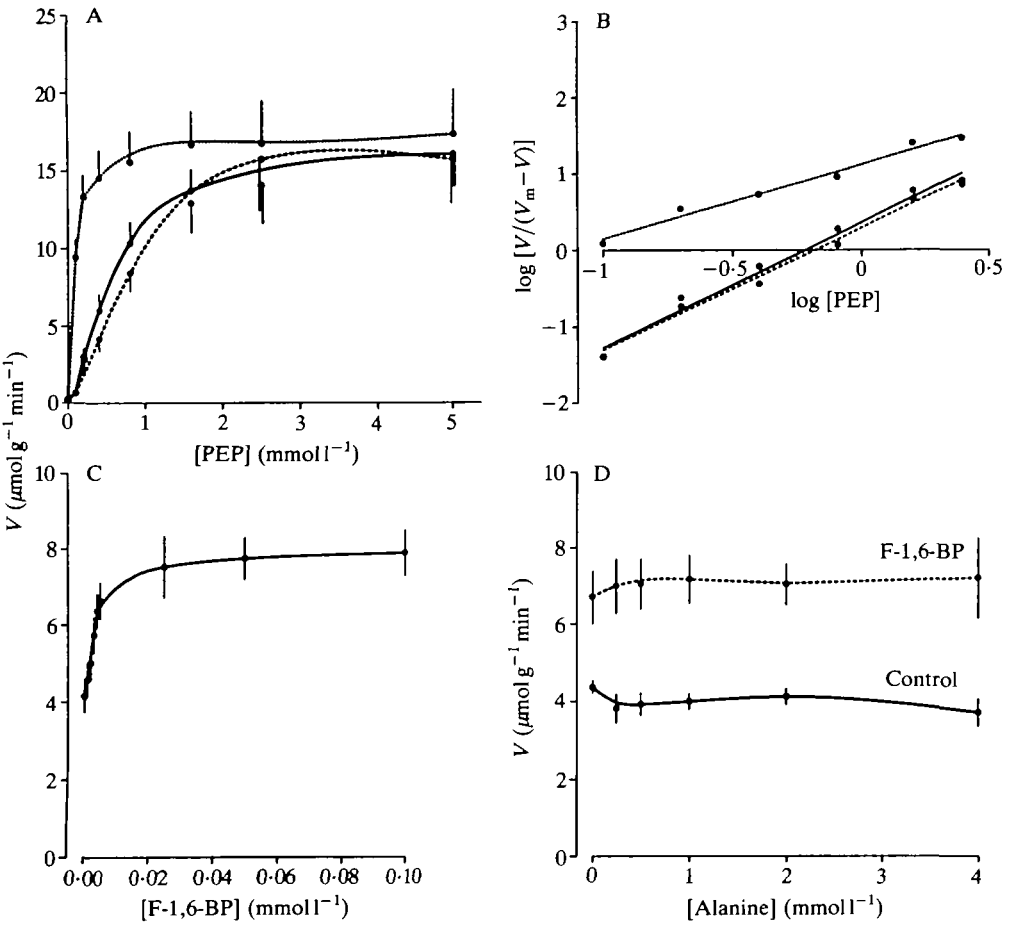

Fig. 1. (A) Phosphoenolpyruvate (PEP) saturation curves of pyruvate kinase in whole liver from intact trout (15°C) with no modulators (solid line, $N = 5$), with the addition of 0·1 mmol l^{-1} fructose-1,6-bisphosphate (F-1,6-BP) (dotted line, $N = 5$) or 2 mmol l^{-1} alanine (dashed line, $N = 6$). (B) Hill plots of data in A. (C) F-1,6-BP saturation curve of pyruvate kinase at 0·8 mmol l^{-1} PEP (10°C, $N = 4$). (D) Alanine saturation curves of pyruvate kinase at 0·8 mmol l^{-1} PEP with no modulator (control, solid line) and with 0·1 mmol l^{-1} F-1,6-BP (dashed line) (10°C, $N = 3$). Bars indicate ±1 S.E.M.

to PEP ($S_{0.5} = 0{\cdot}66$ mmol l^{-1}, $V_m = 15{\cdot}7$ μmol g^{-1} min^{-1}, $n_H = 1{\cdot}84$; Fig. 1A,B); this effect was independent of alanine concentration or the presence of the activator fructose-1,6-bisphosphate (F-1,6-BP) (Fig. 1D). In contrast, F-1,6-BP (0·1 mmol l^{-1}) had a strong activating effect, decreasing $S_{0.5}$ from 0·53 to 0·07 mmol l^{-1} (Fig. 1A,B). This stimulation of PK by F-1,6-BP occurs at low activator concentrations (Fig. 1C), with a K_a value of 0·60 μmol l^{-1}. F-1,6-BP decreased the n_H value from 1·80 to 0·99, indicating an absence of cooperativi between ligand (PEP) and enzyme.

It is not possible to determine the $S_{0.5}$ for GPase with respect to glycogen because of the presence of unknown, but substantial, levels of endogenous tissue glycogen.

Plasma catecholamine levels

It is clear that plasma catecholamine levels increased in response to epinephrine and norepinephrine injections and hypoxia (Fig. 2). Because of the large variability in the magnitude of the responses, however, the changes were generally not

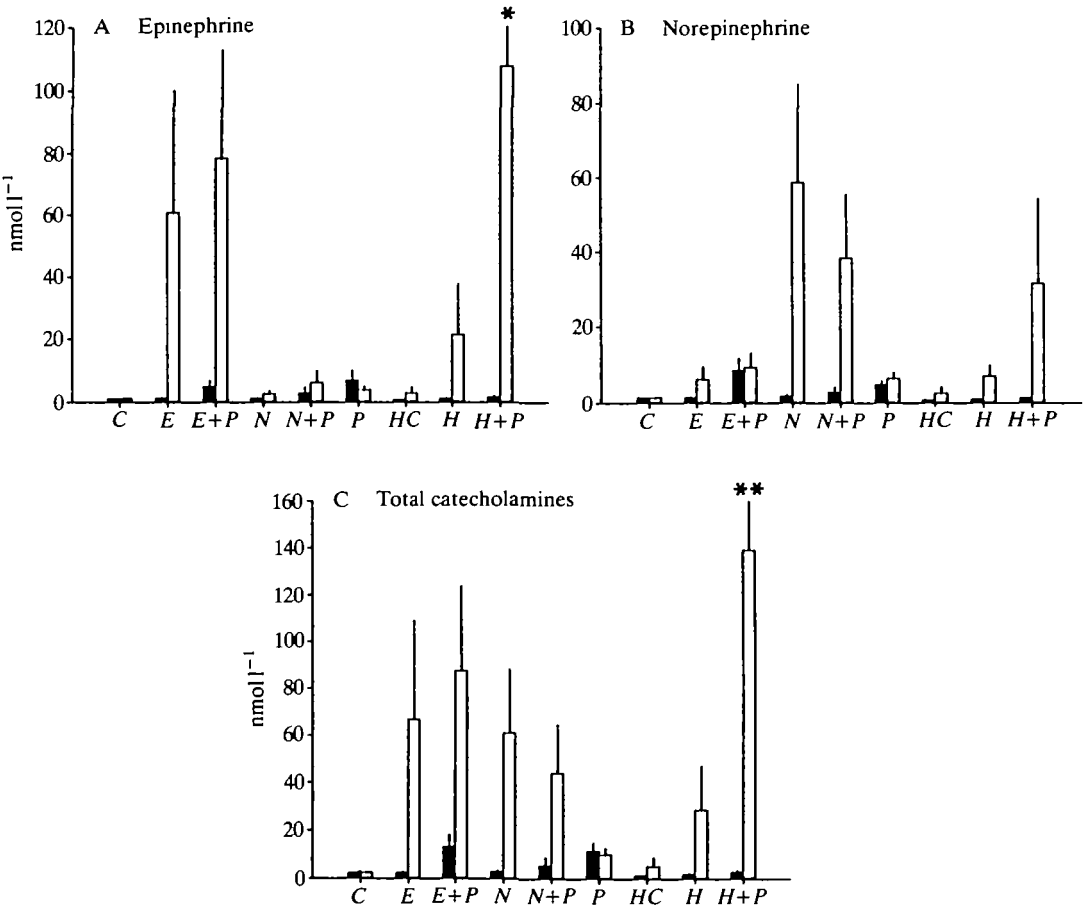

Fig. 2. Plasma epinephrine (A), norepinephrine (B) and total catecholamines (A+B = C) in nmol l^{-1} for control (*C*) series I and II, $N = 11$; epinephrine (*E*), $N = 6$; epinephrine + propranolol (*E+P*), $N = 7$; norepinephrine (*N*), $N = 6$; norepinephrine + propranolol (*N+P*), $N = 6$; propranolol series I and II (*P*), $N = 12$; control series III (*HC*), $N = 6$; hypoxia (*H*), $N = 8$; and hypoxia + propranolol (*H+P*), $N = 6$. Initial levels in each group of fish are shown by the filled bars and final levels, following the experimental treatment, by the open bars. Bars indicate ±1 S.E.M. (* S.E. = 201 and ** S.E. = 255).

significant. Control catecholamine levels were approximately 1 nmol l^{-1}, except for fish pre-treated with propranolol, where initial levels were two- to sevenfold higher (Fig. 2A, 2B). With epinephrine and norepinephrine injections, the final plasma concentration was 60 nmol l^{-1} in both cases. Hypoxia elevated epinephrine (22 nmol l^{-1}, Fig. 2A) and, to a smaller extent, norepinephrine levels (7 nmol l^{-1}, Fig. 2B). Pre-treatment with propranolol prior to hypoxia resulted in a considerably larger total catecholamine release to the plasma (140 nmol l^{-1}, Fig. 2C).

Series I: epinephrine

Epinephrine elevated plasma glucose levels (46%) relative to initial values (Table 2A). Liver glucose also increased compared with control values, but this was not significant. There were no changes in muscle glucose levels and, although muscle glycogen levels decreased, high variability masked any significant trends. Pre-injection with propranolol eliminated the increase in glucose following epinephrine treatment, implying that the epinephrine effect was mediated by β-receptors. Propranolol alone had no significant effect on glucose, but liver and

Table 2. *(A) Plasma glucose, liver and muscle glucose and glycogen and (B) blood pHe and pHi data for series I epinephrine experiments*

	Plasma glucose		Liver		Muscle	
	Initial	Final	Glucose	Glycogen	Glucose	Glycogen
A						
Saline control ($N=6$)	3·32 ± 0·60	3·20 ± 0·48	2·49 ± 0·23	47·31 ± 14·98	0·93 ± 0·15	12·72 ± 3·42
Epinephrine ($N=6$)	2·72 ± 0·28	3·97 ± 0·23*	4·40 ± 1·57	42·42 ± 25·03	0·81 ± 0·04	7·90 ± 1·11
Propranolol control ($N=8$)	2·94 ± 0·30	3·09 ± 0·20	2·62 ± 0·20	108·37 ± 15·58	0·69 ± 0·04	49·12 ± 2·22
Propranolol + epinephrine ($N=8$)	3·46 ± 0·37	3·47 ± 0·26	3·64 ± 0·57	64·10 ± 17·16	0·89 ± 0·19	11·72 ± 1·30

	pHe		pHi	
	Initial	Final	Initial	Final
B				
Saline control	7·84 ± 0·03	7·90 ± 0·05	7·41 ± 0·02	7·42 ± 0·03
Epinephrine	7·84 ± 0·03	7·89 ± 0·05	7·39 ± 0·03	7·33 ± 0·05
Propranolol	7·80 ± 0·03	7·79 ± 0·02	7·47 ± 0·05	7·41 ± 0·04
Propranolol + epinephrine	7·81 ± 0·03	7·80 ± 0·04	7·47 ± 0·03	7·37 ± 0·03

Initial samples were taken 30 min after the pre-injection (saline/propranolol) and final samples were taken 15 min after the experimental injection (saline/epinephrine).

Units for glucose are μmol g^{-1} and for glycogen μmol glucosyl units g^{-1} liver or muscle tissue.

Means ± 1 S.E. (N = number of animals).

* Significantly different from pre-injection value, paired t-test, $P<0·05$.

Table 3. *(A) Liver pyruvate kinase activity for series I at 0·2 and 5·0 mmol l^{-1} phosphoenolpyruvate (PEP) concentrations and the ratio of activities at 0·2/5·0 PEP and (B) glycogen phosphorylase activity presented as total GPase* (a + b), *GPase* a, *and the relative percentage of the active form to the total activity [percent GPase* a = a/(a + b)*]*

	PEP (mmol l^{-1})		
	0·2	5·0	0·2/5·0
A			
Saline control ($N = 6$)	0·68 ± 0·12	9·23 ± 1·06	0·07 ± 0·01
Epinephrine ($N = 6$)	0·28 ± 0·04*	10·63 ± 1·09	0·03 ± 0·00*
Propranolol control ($N = 8$)	1·30 ± 0·29†	12·21 ± 1·23	0·12 ± 0·03†
Propranolol + epinephrine ($N = 8$)	1·06 ± 0·21†	15·19 ± 2·13	0·08 ± 0·02†

	Total GPase	GPase *a*	Percent GPase *a*
B			
Saline control	1·27 ± 0·33	0·50 ± 0·19	34 ± 7
Epinephrine	1·36 ± 0·10	0·69 ± 0·14	49 ± 7
Propranolol control	0·40 ± 0·08*·†	0·10 ± 0·03*·†	23 ± 5†
Propranolol + epinephrine	0·47 ± 0·10*·†	0·13 ± 0·05†	24 ± 5†

Activities in μmol min^{-1} g^{-1} at 15°C.
Means ± 1 S.E.
* Significantly different from control, unpaired *t*-test, $P < 0·05$.
† Significantly different from epinephrine, unpaired *t*-test, $P < 0·05$.

muscle glycogen levels were well above values in the other treatments. pHe and pHi were not altered by any of the experimental treatments (Table 2B).

Epinephrine significantly modified PK activity (Table 3A). At low PEP concentrations (0·2 mmol l^{-1}), activity was reduced two- to fivefold compared with the control and the other treatments. The ratio of activity rates measured at low (0·2 mmol l^{-1}) and saturating (5 mmol l^{-1}) concentrations of PEP provides an indication of the phosphorylation state of PK: that is, a decrease in the ratio corresponds to an increase in PK phosphorylation. The ratio of activities (Table 3A), was significantly lower in fish treated with epinephrine compared with values for saline and both propranolol treatments; as maximal activities (5 mmol l^{-1} PEP) remained unchanged, this decrease indicates an increase in the $S_{0·5}$ (PEP) value. Propranolol alone increased the ratio and eliminated the inhibitory effect of epinephrine on PK.

In preliminary experiments, PK activities were estimated in the presence of [redacted] mmol l^{-1} L-alanine and 0·1 mmol l^{-1} F-1,6-BP under all experimental treatments. F-1,6-BP had a strong stimulatory effect on PK activity, increasing the

0·2/5·0 PEP ratio; however, this effect was independent of experimental treatment. Alanine did not significantly modify PK activity in any of the experimental manipulations. These data are not shown since they do not add any new information to that presented in Fig. 1.

GPase values in epinephrine-treated fish were not significantly different from control values. The active form of GPase (percent GPase *a*), total GPase (*a*+*b*) and GPase *a* activities, however, were significantly higher in epinephrine-treated fish than in the propranolol control and propranolol + epinephrine experiments (Table 3B). Propranolol alone significantly reduced GPase activity (total and *a*) compared with the saline control value.

Series II: norepinephrine

The results of the norepinephrine experiments (Tables 4, 5) were similar to those of series I for epinephrine. The major difference was that at the same circulating concentration as epinephrine (60 nmol l^{-1}, Fig. 2B), the effects of norepinephrine on the measured variables were smaller. Following norepinephrine injection, plasma glucose level increased significantly (16 %), but this increase

Table 4. *(A) Plasma glucose, liver and muscle glucose and glycogen and (B) blood pHe and pHi data for series II norepinephrine experiments*

	Plasma glucose		Liver		Muscle	
	Initial	Final	Glucose	Glycogen	Glucose	Glycogen
A						
Saline control ($N=6$)	3·93 ± 0·46	3·76 ± 0·32	2·65 ± 0·24	115·59 ± 31·62	0·72 ± 0·12	8·22 ± 1·53
Norepinephrine ($N=7$)	3·64 ± 0·22	4·25 ± 0·40*	3·28 ± 0·47	62·89 ± 13·56	0·91 ± 0·13	8·84 ± 1·29
Propranolol control ($N=5$)	4·02 ± 0·39	3·89 ± 0·42	2·73 ± 0·07	132·23 ± 40·87	0·95 ± 0·22	11·46 ± 2·72
Propranolol + norepinephrine ($N=6$)	2·83 ± 0·54	2·88 ± 0·43	2·58 ± 0·50	80·63 ± 33·57	0·87 ± 0·25	11·17 ± 2·68

	pHe		pHi	
	Initial	Final	Initial	Final
B				
Saline control	7·86 ± 0·02	7·88 ± 0·02	7·22 ± 0·05	7·26 ± 0·01
Norepinephrine	7·91 ± 0·02	7·89 ± 0·04	7·19 ± 0·03	7·22 ± 0·04
Propranolol	7·77 ± 0·03	7·75 ± 0·02	7·31 ± 0·06	7·19 ± 0·10
Propranolol + norepinephrine	7·81 ± 0·03	7·81 ± 0·05	7·36 ± 0·07	7·39 ± 0·08

Initial samples were taken 30 min after the pre-injection (saline/propranolol) and final samples were taken 15 min after the experimental injection (saline/norepinephrine).

Units for glucose are μmol g^{-1} and for glycogen μmol glucosyl units g^{-1} liver or muscle tissue.

Means ± 1 S.E. (N = number of animals).

* Significantly different from pre-injection value, paired t-test, $P<0·05$.

Table 5. *(A) Liver pyruvate kinase activity for series II at 0·2 and 5·0 mmol l^{-1} phosphoenolpyruvate (PEP) concentrations and the ratio of activities at 0·2/5·0 PEP and (B) glycogen phosphorylase activity presented as total GPase* (a + b), *GPase* a, *and the relative percentage of the active form to the total activity [percent GPase* a = a/(a + b)*]*

	PEP (mmol l^{-1})		
	0·2	5·0	0·2/5·0
A			
Saline control ($N = 6$)	0·64 ± 0·26	10·26 ± 1·07	0·10 ± 0·03
Norepinephrine ($N = 7$)	0·39 ± 0·07	11·49 ± 1·64	0·03 ± 0·01*
Propranolol control ($N = 5$)	1·08 ± 0·58	8·62 ± 1·22	0·13 ± 0·05
Propranolol + norepinephrine ($N = 8$)	0·76 ± 0·25	10·20 ± 1·18	0·07 ± 0·02

	Total GPase	GPase *a*	Percent GPase *a*
B			
Saline control	0·75 ± 0·20	0·17 ± 0·06	19 ± 4
Norepinephrine	1·24 ± 0·38	0·53 ± 0·23	30 ± 7
Propranolol control	0·48 ± 0·13	0·13 ± 0·06	24 ± 4
Propranolol + norepinephrine	0·70 ± 0·27	0·28 ± 0·15	29 ± 7

Activities in μmol min^{-1} g^{-1} at 18°C.
Means ± 1 S.E.
* Significantly different from control, unpaired *t*-test, $P < 0·05$.

was only half of that observed with epinephrine (Table 2). Pre-injection with propranolol eliminated the increase in glucose associated with norepinephrine treatment, again suggesting mediation by β-receptors. pHe and pHi were not altered by any of the experimental treatments (Table 4B).

PK activity at 0·2 mmol l^{-1} PEP was reduced by norepinephrine compared to the other treatments, but this effect was not significant. Norepinephrine, however, did significantly depress the 0·2/5·0 PEP ratio compared with the saline control value.

Norepinephrine increased total GPase, GPase *a* and percent GPase *a* activities (Table 5A), but these trends were not significant.

Series III: hypoxia

Plasma epinephrine and norepinephrine were both elevated after 30 min of hypoxia, although epinephrine levels were three times those of norepinephrine (Fig. 2). Plasma glucose levels were constant during hypoxia. The combination of propranolol and hypoxia, however, caused a significant reduction of plasma

glucose (20%) compared to pre-treatment levels. There were no significant changes in liver or muscle glucose and glycogen levels. Red cell pHi was significantly elevated during hypoxia, which indicates β-adrenoreceptor stimulation (Tetens & Christensen, 1987). Blood Pa_{O_2} and Ca_{O_2} were severely depressed during hypoxia. Pre-treatment with propranolol significantly reduced initial blood oxygen levels, and hypoxia compounded this effect by lowering Pa_{O_2} and Ca_{O_2} values below those seen with hypoxia alone (Table 6C). Hypoxia resulted in a three- to fourfold increase in plasma [lactate], indicating an activation of anaerobic metabolic pathways.

PK activity remained constant during hypoxia (Table 7). Pre-treatment with propranolol plus hypoxia, however, caused a significant increase in PK activity at 0·2 mmol l^{-1} PEP, and an increase in the 0·2/5·0 PEP ratio (Table 7). Propranolol alone decreased maximal PK activity (at 5·0 mmol l^{-1} PEP), although a similar change at 0·2 mmol l^{-1} PEP meant that there was no change in the 0·2/5·0 PEP ratio.

Hypoxia increased total GPase activity by two- to threefold over saline and propranolol control treatments. GPase *a* activity was 5–7 times greater during hypoxia compared to values for propranolol treatment with or without hypoxia. Percent GPase *a* activity was significantly higher during hypoxia compared with values during treatments with propranolol + hypoxia (Table 7).

Cyclic AMP

Liver cyclic AMP levels increased slightly with epinephrine, norepinephrine and hypoxia, but none of these treatments resulted in significant changes compared with control levels (Table 8).

Discussion

Liver pyruvate kinase and glycogen phosphorylase activities in vivo

Pyruvate kinase in teleost liver tissue was initially reported to be nonregulatory (Somero & Hochachka, 1968; Guderley *et al.* 1978; Moon & Hulbert, 1980; Guderley & Cardenas, 1980). The results of this study, however, and those of four recent investigations (Petersen *et al.* 1988; Rahman & Storey, 1988; Sand, 1988; Moon *et al.* 1989) indicate the contrary. An explanation for this discrepancy is that differences in the physiological state of the fish and/or the preparation of the liver homogenate result in variable states of phosphorylation of the enzyme. The sensitivity of PK to allosteric modulators is dependent on whether the enzyme is phosphorylated (inactive state) or dephosphorylated (active state) (Pilkis *et al.* 1988). Furthermore, the procedure for isolating hepatocytes in rats, which is similar to that in trout, results in the progressive dephosphorylation of PK (Riou *et al.* 1985). The substrate concentration required for half-maximal saturation ($S_{0.5}$) is dependent on the phosphorylation state, and this may explain why our *in vivo* $S_{0.5}$ value (0·6 mmol l^{-1} PEP) is an order of magnitude greater than th measured by Petersen *et al.* (1988) in trout hepatocytes. Hence, kinetic parameters

Table 6. *(A) Plasma glucose, liver and muscle glucose and glycogen, (B) pHe and pHi and (C)* Pa_{O_2}, Ca_{O_2} *and plasma lactate data for series III hypoxia experiments*

	Plasma glucose		Liver		Muscle	
	Initial	Final	Glucose	Glycogen	Glucose	Glycogen
A						
Saline control ($N=8$)	3·12 ± 0·95	3·37 ± 1·03	2·28 ± 0·66	64·98 ± 25·07	0·60 ± 0·17	8·67 ± 1·97
Hypoxia ($N=11$)	5·72 ± 1·69	6·12 ± 1·75	4·36 ± 1·08	105·13 ± 32·16	0·76 ± 0·18	8·58 ± 2·00
Propranolol control ($N=5$)‡	4·02 ± 0·39	3·89 ± 0·42	2·73 ± 0·07	132·23 ± 40·87	0·95 ± 0·22	11·46 ± 2·72
Propranolol + hypoxia ($N=6$)	4·45 ± 0·87	3·54 ± 0·65*	2·50 ± 0·40	139·00 ± 33·72	0·69 ± 0·18	10·48 ± 1·60

	pHe		pHi	
	Initial	Final	Initial	Final
B				
Saline control	7·91 ± 0·04	7·94 ± 0·02	7·38 ± 0·04	7·35 ± 0·04†
Hypoxia	7·83 ± 0·03	7·76 ± 0·05	7·27 ± 0·02	7·46 ± 0·03*
Propranolol control	7·77 ± 0·03	7·75 ± 0·02	7·31 ± 0·06	7·19 ± 0·10†
Propranolol + hypoxia	7·78 ± 0·02	7·68 ± 0·10	7·22 ± 0·03	7·23 ± 0·08†

	Pa_{O_2} (kPa)		Ca_{O_2} (vols%)		[Lactate] (μmol l^{-1})	
	Initial	Final	Initial	Final	Initial	Final
C						
Saline control	16·0 ± 1·3	18·3 ± 0·9	7·37 ± 0·72	6·67 ± 0·47	1·13 ± 0·35	1·28 ± 0·31
Hypoxia	15·5 ± 1·2	5·9 ± 1·5*	8·38 ± 0·62	3·84 ± 0·29*	1·16 ± 0·20	4·47 ± 0·67*,**
Propranolol + hypoxia	9·3 ± 0·003**,†	3·3 ± 0·01*	6·50 ± 0·03†	2·04 ± 0·08*	0·96 ± 0·30	2·66 ± 0·76*

Initial samples were taken 30 min after the pre-injection (saline/propranolol) and final samples 30 min after the initiation of external hypoxia.

Units for glucose are μmol g^{-1} and for glycogen μmol glucosyl units g^{-1} liver or muscle tissue.

Means ± 1 S.E. (N = number of animals).

* Significantly different from initial value, paired t-test, $P<0.05$.

** Significantly different from control value, unpaired t-test, $P<0.05$.

† Significantly different from hypoxia value, unpaired t-test, $P<0.05$.

‡ Data from series II.

for PK measured in this study, using freeze-clamped liver tissue and a phosphorylation–dephosphorylation stopping buffer, may be closer to *in situ* values than those measured on isolated hepatocytes or in many of the previous studies made on this enzyme in fish.

Alanine had virtually no effect on PK activity in the present study, but is a strong inhibitor of mammalian liver and invertebrate hepatopancreas PK (see Munday

Table 7. *(A) Pyruvate kinase activity for series III at 0·2 and 5·0 mmol l^{-1} phosphoenolpyruvate (PEP) concentrations and the ratio of activities at 0·2/5·0 PEP and (B) glycogen phosphorylase activity presented as total GPase* (a + b), *GPase* a, *and the relative percentage of the active form to the total activity [percent GPase* a = a/(a + b)*]*

	PEP (mmol l^{-1})		
	0·2	5·0	0·2/5·0
A			
Saline control ($N = 8$)	1·30 ± 0·31	13·18 ± 0·82	0·11 ± 0·03
Hypoxia ($N = 11$)	1·13 ± 0·23	13·23 ± 1·45	0·09 ± 0·02
Propranolol control ($N = 5$)‡	1·08 ± 0·58	8·62 ± 1·22*	0·13 ± 0·05
Propranolol + hypoxia ($N = 6$)	2·36 ± 0·57†	10·02 ± 1·42	0·24 + 0·04*,†

	Total GPase	GPase *a*	Percent GPase *a*
B			
Saline control	0·77 ± 0·16	0·22 ± 0·08	23 ± 4
Hypoxia	1·39 ± 0·18*	0·52 ± 0·10	34 ± 4
Propranolol control‡	0·48 ± 0·13†	0·13 ± 0·06†	24 ± 4
Propranolol + hypoxia	0·36 ± 0·11†	0·07 ± 0·03†	13 ± 6†

Activities in μmol min^{-1} g^{-1} at 16°C.
Means ± 1 S.E.
* Significantly different from control, unpaired *t*-test, $P < 0·05$.
† Significantly different from hypoxia, unpaired *t*-test, $P < 0·05$.
‡ Data from series II.

et al. 1980). Moon *et al.* (1989) have also shown an effect of alanine on rainbow trout liver PK; the reason for this discrepancy is not understood. In contrast, relatively low concentrations of F-1,6-BP activated PK, lowered the $S_{0·5}$ value for PEP and modified the shape of the PEP saturation curve to a hyperbola. The sensitivity of trout PK to F-1,6-BP activation has been reported previously in rainbow trout (Moon *et al.* 1989), goldfish (Rahman & Storey, 1988) and flounder (Sand, 1988).

The net rate of glycogen breakdown depends largely on the relative activity of the active form of GPase or percent GPase *a*. Glycogenolysis is stimulated during stress in many vertebrates (Hems & Whitton, 1980) and, therefore, one would expect activity of the *a* form to be low in resting animals and increase with stress. Published liver percent GPase *a* values for resting fish are high, ranging from 60 to 90 % (Ottolenghi *et al.* 1985, 1986; Perry *et al.* 1988; Mommsen *et al.* 1988; Moon *et al.* 1989), suggesting near maximal rates of glycogen breakdown under resti[illegible] conditions. These high percent GPase *a* values may possibly be an artifact of the

Table 8. *Liver cyclic AMP levels*

	[cyclic AMP] (pmol g^{-1} liver tissue)
Control ($N = 19$)	538 ± 29
Propranolol ($N = 13$)	562 ± 48
Epinephrine ($N = 5$)	644 ± 81
Epinephrine + propranolol ($N = 8$)	484 ± 56
Norepinephrine ($N = 7$)	601 ± 34
Norepinephrine + propranolol ($N = 6$)	510 ± 44
Hypoxia ($N = 10$)	580 ± 63
Hypoxia + propranolol ($N = 5$)	468 ± 72

Data from control and propranolol experiments were not significantly different and therefore were pooled.
Means ± 1 S.E.

assay technique. Stalmans & Hers (1975) reported that AMP activated both the *a* and *b* forms of GPase and only when caffeine was added to liver homogenates was the *b* form completely inhibited. Their technique provided a more accurate estimate of percent GPase *a*. This study is one of the first to use the criteria of Stalman & Hers (1975), and reports percent GPase *a* values of between 10 and 50 %, depending upon conditions. These values, to our knowledge, are the lowest reported for any teleost fish, including a recent study on rainbow trout (Moon *et al.* 1989), or for mammalian liver (e.g. 70–85 %; Stalmans & Hers, 1975; Hems & Whitton, 1980).

It should be noted that total GPase ($a+b$) was not constant between different experimental treatments (Tables 3B, 5B, 7B). Although this observation has been reported by others (Ottolenghi *et al.* 1986, 1988; Mommsen *et al.* 1988; Perry *et al.* 1988), we do not have an explanation for these results.

Catecholamine regulation of glucose metabolism during hypoxia

The results of this study support the hypothesis that catecholamines, released during hypoxia, have an important role in regulating the availability of glucose. Injections of epinephrine or norepinephrine into resting fish increased plasma glucose levels, and this hyperglycemia was blocked by pre-treatment with the β-adrenoreceptor antagonist propranolol. During hypoxia, plasma catecholamine levels increased but there was no change in plasma glucose levels. We suggest that the reason we did not observe catecholamine-mediated hyperglycemia was

because there was an increase in tissue glucose utilization, as a result of inhibition of aerobic, and activation of anaerobic, metabolism (Dunn & Hochachka, 1987; Boutilier *et al.* 1988). Plasma lactate levels increased fourfold during hypoxia, supporting an enhancement in anaerobic metabolism. Finally, plasma glucose levels were significantly depressed during hypoxia after blockade of β-adrenoreceptors. This indicates an essential role for β-adrenoreceptor-mediated mobilization of glucose during hypoxia to meet metabolic requirements.

Catecholamines regulate glucose metabolism, in part by modifying the activity of two key regulatory enzymes: PK, a regulatory enzyme in glycolysis and gluconeogenesis, and GPase, the enzyme directly responsible for glycogen breakdown. Injections of epinephrine, and to a lesser extent norepinephrine, increased the percent GPase *a* (statistically significant compared with propranolol-treated fish) and decreased PK activity in the liver, indicating an activation of glycogenolysis and an inhibition of glycolysis and/or stimulation of gluconeogenesis, respectively. The catecholamine effects on GPase and PK were blocked completely by propranolol, identifying β-receptors as the mediators of the trout hepatic catecholamine response. Blockade of β-adrenoreceptors caused substantial reductions of percent GPase *a* and total GPase activities, while PK activity was markedly stimulated. Thus, it is apparent that the maintenance of blood glucose levels during hypoxia was due to adrenergic activation of GPase and inhibition of PK. In turn, these enzymatic changes accelerated hepatic glycogenolysis and gluconeogenesis while inhibiting glycolysis. The unusually high activity of PK (assayed at low PEP concentrations) and low activity of GPase during hypoxia after β-adrenoreceptor blockade suggest that internal hypoxia is specifically affecting the phosphorylation state, that is dephosphorylating these two enzymes. Thus, it would appear that the mobilization of catecholamines during hypoxia serves to offset the deleterious effects of hypoxemia on PK and GPase.

The hyperglycemic effect of catecholamines and involvement of β-adrenoreceptors have been demonstrated *in vitro* in trout (Mommsen *et al.* 1988) and in catfish (Brighenti *et al.* 1987*b*; Janssens & Lowrey, 1987). The present study is the first to show β-adrenoreceptor-mediated catecholamine effects on glucose metabolism in the intact trout with administration of physiologically relevant epinephrine or norepinephrine levels. In addition, we have shown that liver percent GPase *a* is twofold greater in epinephrine-treated fish than in propranolol- or propranolol + epinephrine-treated fish, whereas previous hepatocyte studies have failed to demonstrate a marked change (Ottolenghi *et al.* 1985, 1986; Mommsen *et al.* 1988). Epinephrine inhibits PK activity in trout hepatocytes (Mommsen *et al.* 1988), although the magnitude of the effect was considerably less than we report in the present study. Mommsen and co-workers found that the 0·1/5·0 PEP ratio decreased by 20%, compared to our results in which the 0·2/5·0 PEP ratio was reduced by 60%. As discussed above, the hepatocyte isolation procedure may have altered the phosphorylation state of PK, resulting in a reduced sensitivity to catecholamines. Norepinephrine mimicked the effects of epinephrine but was less potent (Tables 4, 5). Similar results were obtained by

Brighenti *et al.* (1987*a*,*b*) in catfish hepatocytes. In trout hepatocytes, however, both hormones induced similar changes in glucose production and lactate gluconeogenesis (Mommsen *et al.* 1988).

In many animals, the immediate response to oxygen deprivation is the breakdown of liver glycogen and subsequent mobilization of glucose *via* the blood to other tissues for anaerobic utilization. In the longer term, as glycogen levels decline, liver gluconeogenesis must be activated to supply glucose required for anaerobic metabolism or, alternatively, overall metabolism may be depressed. Our results did not show a significant drop in liver or muscle glycogen stores following 30 min of hypoxia (Table 6A), but the increases in liver total GPase and percent GPase *a* activity (Table 7B) suggest that glycogen catabolism may have been enhanced. Liver glycogen levels are extremely variable in fish and without initial glycogen contents it is difficult to evaluate the glycogenolytic effect of hypoxia. In fish hepatocytes and perfused livers, glycogen level decreases in the presence of catecholamines (Janssens *et al.* 1983; Ottolenghi *et al.* 1985, 1986; Brighenti *et al.* 1987*a*; Janssens & Lowery, 1987). Dunn & Hochachka (1987) found that trout exposed to water with a P_{O_2} of 4 kPa for 3 h showed no significant fall in liver glycogen. Thus, it may be that 30 min of moderate hypoxia ($Pw_{O_2} = 7{\cdot}1$ kPa) is not of sufficient intensity and duration to cause a discernible depletion of glycogen stores, despite elevated catecholamine levels.

Catecholamine release during hypoxia is dependent on the intensity and duration of the hypoxic stress (Tetens & Christensen, 1987; Boutilier *et al.* 1988). Plasma epinephrine levels in trout exposed to hypoxia (water $Pw_{O_2} = 7{\cdot}1$ kPa for 30 min, Fig. 2A) were two- to 10-fold greater than that reported by Fievet *et al.* (1987) and Tetens & Christensen (1987) for trout in water of $Pw_{O_2} = 5{\cdot}3$ kPa for 20–30 min. Norepinephrine levels (Fig. 2B) were lower than in the other two studies (Fievet *et al.* 1987; Tetens & Christensen, 1987) and, therefore, it may be more instructive to look at the total catecholamine levels ([epinephrine + norepinephrine]; Fig. 2C). Total plasma catecholamine concentration was 29 nmol l^{-1} during hypoxia (Fig. 2C), a value very similar to the total level in the Fievet *et al.* (1987) study and 2·5-fold higher than levels reported by Tetens & Christensen (1987). Control or initial catecholamine levels were approximately 1 nmol l^{-1}, which is representative of true resting values (Mazeaud & Mazeaud, 1985). Stress levels of epinephrine and norepinephrine may be as high as 200–400 nmol l^{-1} and, therefore, the measured levels of either catecholamine (60 nmol l^{-1}) after injection in the present study were well within the physiological range, although higher than those achieved during hypoxia. An interesting observation is that propranolol alone stimulated catecholamine release and, during hypoxia, resulted in a higher plasma catecholamine concentration (Fig. 2). Tetens & Christensen (1987) also noted a catecholamine stimulation with propranolol, although they did not comment on its significance. The fact that pre-treatment with propranolol reduced blood Pa_{O_2} and Ca_{O_2} (Table 6C) suggests that fish were hypoxic before the experimental treatment (epinephrine, norepinephrine or hypoxia). This might explain the initial small rise in both epinephrine and norepinephrine in fish treated

with propranolol. The marked increase in catecholamines with both propranolol and hypoxia, compared to hypoxia alone, is presumably related to the much greater hypoxic stress ($Pa_{O_2} = 3{\cdot}3$ kPa, $Ca_{O_2} = 2$ vols%, Table 6C) in these fish.

Resting or baseline catecholamine levels (1 nmol l^{-1}) may be important in the regulation of glucose metabolism. When propranolol was administered to resting fish in series I, total GPase and GPase *a* activities were reduced by three- to fivefold compared to control values (Table 3B). (The same trends were observed in series II, but the differences were not statistically significant.) There are two possibilities; catecholamines tonically regulate glycogenolysis in the liver, or *pre-mortem* handling induced a catecholamine surge that resulted in an immediate β-adrenoreceptor response (Moon *et al.* 1988). In this study, the procedure of killing the fish, excising the liver and freezing the tissue in liquid N_2 took less than 10 s. It is possible, however, that some catecholamines were released within this short time. To differentiate between tonic adrenergic control of GPase or *pre-mortem* stress, further studies on intact fish are necessary where the potential problem of stress-related handling is reduced or eliminated.

Binding of catecholamines to the β-adrenoreceptor activates adenylate cyclase and increases cellular cyclic AMP levels (see Levitzki, 1988). In the present study, cyclic AMP levels did increase slightly with catecholamines and hypoxia, although the changes were not significant. In contrast, cyclic AMP levels increased rapidly with both epinephrine or norepinephrine treatment in fish hepatocyte studies (Janssens & Lowrey, 1987; Brighenti *et al.* 1987*a*). There are several possible explanations for the discrepancy between *in vivo* and *in vitro* results. First, stimulation of cellular cyclic AMP levels is dose-dependent. A plasma catecholamine level *in vivo*, however, is probably not physiologically equivalent to the same *in vitro* dose, because of the more complex structure and circulation of the whole organ *versus* isolated hepatocytes. Second, the sampling time of our *in vivo* study may not have corresponded with the maximum surge of intracellular cyclic AMP levels. Based on the ubiquitous nature of cyclic AMP as the β-adrenoceptor intracellular messenger in higher vertebrates (Levitzki, 1988), teleosts (Janssens & Lowrey, 1987; Brighenti *et al.* 1987*a*) and amphibians (Janssens *et al.* 1986), it is probable that the low (but physiological) catecholamine dose used in our study, the inherent variability of *in vivo* data and transient surges have masked the role of cyclic AMP as the intracellular messenger in trout liver tissue.

We thank Dr Tom Mommsen for his helpful discussions during the initial stages of this study. This work was supported by NSERC of Canada operating and equipment grants to TWM and SFP. PAW was the recipient of a Killam Postdoctoral Fellowship.

References

BERGMEYER, H. U. (1974). *Methods of Enzymatic Analysis*. New York: Academic Press.

BIRNBAUM, M. J., SCHULTZ, J. & FAIN, J. N. (1976). Hormone-stimulated glycogenolysis in isolated goldfish hepatocytes. *Am. J. Physiol.* **231**, 191–197.

BOUTILIER, R. G., DOBSON, G., HOEGER, U. & RANDALL, D. J. (1988). Acute exposure to graded levels of hypoxia in rainbow trout *Salmo gairdneri*: metabolic and respiratory adaptations. *Respir. Physiol.* **71**, 69–82.

BRIGHENTI, L., PUVIANI, A. C., GAVIOLI, M. E., FABBRI, E. & OTTOLENGHI, C. (1987*a*). Catecholamine effect on cyclic adenosine 3′:5′-monophosphate level in isolated catfish hepatocytes. *Gen. comp. Endocr.* **68**, 216–223.

BRIGHENTI, L., PUVIANI, A. C., GAVIOLI, M. E. & OTTOLENGHI, C. (1987*b*). Mechanisms involved in catecholamine effect on glycogenolysis in catfish hepatocytes. *Gen. comp. Endocr.* **66**, 306–313.

BUTLER, P. J., TAYLOR, E. W. & DAVISON, W. (1979). The effect of long term, moderate hypoxia on acid–base balance, plasma catecholamines and possible anaerobic end products in the unrestrained dogfish *Scyliorhinus canicula*. *J. comp. Physiol.* **132**, 297–303.

CHRISTOPHERSON, R. I. (1983). Desalting protein solutions in a centrifuge column. *Methods Enzymol.* **91**, 278–281.

DEROOS, R. & DEROOS, C. C. (1978). Elevation of plasma glucose levels by catecholamines in elasmobranch fish. *Gen. comp. Endocr.* **34**, 447–452.

DUNN, J. F. & HOCHACHKA, P. W. (1987). Turnover rates of glucose and lactate in rainbow trout during acute hypoxia. *Can. J. Zool.* **65**, 1144–1148.

FIEVET, B., MOTAIS, R. & THOMAS, S. (1987). Role of adrenergic-dependent H^+ release from red cells in acidosis induced by hypoxia in trout. *Am. J. Physiol.* **252**, R269–R275.

GUDERLEY, H. & CARDENAS, J. M. (1980). A study of the catalytic properties of pyruvate kinase isozymes from salmon and an examination of their functional relationships. *J. exp. Zool.* **212**, 269–277.

GUDERLEY, H., FIELDS, J. H. A., CARDENAS, J. & HOCHACHKA, P. W. (1978). Pyruvate kinase from the liver and kidney of *Arapaima gigas*. *Can. J. Zool.* **56**, 852–859.

HEMS, D. A. & WHITTON, P. A. (1980). Control of hepatic glycogenolysis. *Physiol. Rev.* **60**, 1–50.

JANSSENS, P. A., CAINE, A. G. & DIXON, J. E. (1983). Hormonal control of glycogenolysis and the mechanism of action of adrenaline in amphibian liver *in vitro*. *Gen. comp. Endocr.* **49**, 477–484.

JANSSENS, P. A., KLEINEKE, J. & CAINE, A. G. (1986). Calcium-independent stimulation of glycogenolysis by arginine vasotocin and catecholamines in liver of the axolotl (*Ambystoma mexicanum*) *in vitro*. *J. Endocrinol.* **109**, 75–84.

JANSSENS, P. A. & LOWREY, P. (1987). Hormonal regulation of hepatic glycogenolysis in the carp, *Cyprinus carpio*. *Am. J. Physiol.* **252**, R653–R660.

LEVITZKI, A. (1988). From epinephrine to cyclic AMP. *Science* **241**, 800–805.

MAZEAUD, M. M. & MAZEAUD, F. (1985). Adrenergic responses to stress in fish. In *Stress in Fish* (ed. A. D. Pickering), pp. 49–55. New York: Academic Press.

MOMMSEN, T. P., WALSH, P. J., PERRY, S. F. & MOON, T. W. (1988). Interactive effects of catecholamines and hypercapnia on glucose production in isolated trout hepatocytes. *Gen. comp. Endocr.* **70**, 63–73.

MOON, T. W., FOSTER, G. D. & PLISETSHAYA, E. M. (1989). Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for six weeks. *Can. J. Zool.* (in press).

MOON, T. W. & HULBERT, W. C. (1980). Regulatory properties of pyruvate kinases isolated from tissues of the American eel, *Anguilla rostrata* le sueur. *Comp. Biochem. Physiol.* **65B**, 291–296.

MOON, T. W., WALSH, P. J., PERRY, S. F. & MOMMSEN, T. P. (1988). Effects of *in vivo* beta-adrenoceptor blockade on hepatic carbohydrate metabolism in rainbow trout. *Exp. Zool.* **248**, 88–93.

MORATA, P., VARGAS, A. M., PITA, M. L. & SANCHEZ-MEDINA, F. (1982). Hormonal effects on the liver glucose metabolism in rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **72B**, 543–545.

MUNDAY, K. A., GILES, I. G. & POAT, P. C. (1980). Review of the comparative biochemistry of pyruvate kinase. *Comp. Biochem. Physiol.* **67B**, 403–411.

NAKANO, T. & TOMLINSON, T. (1967). Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. *J. Fish Res. Bd Can.* **24**, 1702–1715.

OTTOLENGHI, C., PUVIANI, A. C., BARUFFALDI, A. & BRIGHENTI, L. (1984). Epinephrine effects on carbohydrate metabolism in catfish (*Ictalurus melas*). *Gen. comp. Endocr.* **55**, 378–386.

OTTOLENGHI, C., PUVIANI, A. C., BARUFFALDI, A., GAVIOLI, M. E. & BRIGHENTI, L. (1988). Glucagon control of glycogenolysis in catfish tissue. *Comp. Biochem. Physiol.* **90B**, 285–290.

OTTOLENGHI, C., PUVIANI, A. C., GAVIOLI, M. E. & BRIGHENTI, L. (1985). Epinephrine effect on carbohydrate metabolism in isolated and perfused catfish liver. *Gen. comp. Endocr.* **59**, 219–229.

OTTOLENGHI, C., PUVIANI, A. C., GAVIOLI, M. E. & BRIGHENTI, L. (1986). Epinephrine effect on glycogen phosphorylase activity in catfish liver and muscle. *Gen. comp. Physiol.* **61**, 469–475.

PERRY, S. F. & VERMETTE, M. G. (1987). The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. I. Blood respiratory, ionic and acid–base states. *J. exp. Biol.* **128**, 235–253.

PERRY, S. F., WALSH, P. J., MOMMSEN, T. P. & MOON, T. W. (1988). Metabolic consequences of hypercapnia in the rainbow trout, *Salmo gairdneri*: β-adrenergic effects. *Gen. comp. Endocr.* **69**, 439–447.

PETERSEN, T. D. P., HOCHACHKA, P. W. & SUAREZ, R. K. (1988). Hormonal control of gluconeogenesis in rainbow trout: regulatory role of pyruvate kinase. *J. exp. Zool.* **243**, 173–180.

PILKIS, S. J., RAAFAT EL-MAGHRABI, M. & CLAUS, T. M. (1988). Hormonal regulation of hepatic gluconeogenesis and glycolysis. *A. Rev. Biochem.* **57**, 755–783.

RAHMAN, M. S. & STOREY, K. B. (1988). Role of covalent modification in the control of glycolytic enzymes in response to environmental anoxia in goldfish. *J. comp. Physiol.* **157B**, 813–820.

RIOU, J. P., AUDIGIER, C., LAVILLE, M., BEYLOT, M., PIGEON, P. & MORNEX, R. (1985). Dephosphorylation of L-pyruvate kinase during rat liver hepatocyte isolation. *Archs Biochem. Biophys.* **236**, 321–327.

RISTORI, M. T. & LAURENT, P. (1985). Plasma catecholamines and glucose during moderate exercise in the trout: Comparison with bursts of violent activity. *Exp. Biol.* **44**, 247–253.

RUSH, S. B. & UMMINGER, B. L. (1978). Elimination of stress-induced changes in carbohydrate metabolism of goldfish (*Carassius auratus*) by training. *Comp. Biochem. Physiol.* **60A**, 69–73.

SAND, O. (1988). The effect of fructose 1,6 diphosphate on pyruvate kinase from the liver of the flounder (*Platichthys flesus* L.). *Comp. Biochem. Physiol.* **90B**, 401–407.

SOIVIO, A., WESTMAN, K. & NYHOLM, K. (1972). Improved method of dorsal aorta catheterization: haematological effects followed for three weeks in rainbow trout (*Salmo gairdneri*). *Finn. Fish. Res.* **1**, 11–21.

SOMERO, G. N. & HOCHACHKA, P. W. (1968). The effect of temperature on catalytic and regulatory functions of pyruvate kinases of rainbow trout and the antarctic fish *Trematomus bernacchii*. *Biochem. J.* **110**, 395–400.

STALMANS, W. & HERS, H. G. (1975). The stimulation of liver phosphorylase b by AMP, fluoride and sulfate. *Eur. J. Biochem.* **54**, 341–350.

TETENS, V. & CHRISTENSEN, N. J. (1987). Beta-adrenergic control of blood oxygen affinity in acutely hypoxia exposed rainbow trout. *J. comp. Physiol.* **157B**, 667–675.

THORPE, A. & INCE, B. W. (1974). The effect of pancreatic hormones, catecholamines and glucose loading on blood metabolites in northern pike (*Exos lucius* L.). *Gen. comp. Endocr.* **23**, 29–44.

VERMETTE, M. G. & PERRY, S. F. (1988). Adrenergic involvement in blood oxygen transport and acid–base balance during hypercapnic acidosis in the rainbow trout, *Salmo gairdneri*. *J. comp. Physiol.* B **158**, 107–115.

WOLF, K. (1963). Physiological salines for fresh water teleosts. *Progve Fish Cult.* **25**, 135–140.

WOODWARD, J. J. (1982). Plasma catecholamines in resting rainbow trout, *Salmo gairdneri* Richardson by high pressure liquid chromatography. *J. Fish Biol.* **21**, 429–432.

WOSILAIT, W. D. & SUTHERLAND, E. W. (1956). The relationship of epinephrine and glucagon to liver phosphorylase II enzymatic inactivation of liver phosphorylase. *J. biol. Chem.* **218**, 469–481.