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AN *IN VITRO* MODEL OF POST-EXERCISE HEPATIC GLUCONEOGENESIS IN THE GULF TOADFISH *OPSANUS BETA*

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Summary

During strenuous exercise, fish develop substantial proton and lactate loads. Although acidosis is usually rapidly corrected during recovery (1–2 h), lactate levels often remain elevated for up to 8–12 h. The quantitative role of the liver in clearance of the lactate load during recovery from exercise in fish has received little direct examination. The purposes of this study were (1) to attempt to quantify hepatic contribution to lactate clearance, and (2) to identify factors that regulate hepatic gluconeogenesis during recovery from exercise in fish. Both *in vivo* and *in vitro* (isolated hepatocytes) approaches were used. Important blood parameters (pHe, C_{CO_2}, [lactate], [glucose], [epinephrine] and [norepinephrine]) were measured in the gulf toadfish (*Opsanus beta* Goode and Bean) during recovery from strenuous exercise, and they conformed to the general patterns for sluggish benthic species noted in earlier studies. When toadfish hepatocytes were exposed to simulated post-exercise conditions *in vitro*, gluconeogenesis from lactate was stimulated by over 2.5-fold in '0–1 h-' and '1–2 h-post-exercise periods'. Variation of the extracellular parameters in controlled combinations indicated that exercise-induced changes in [glucose], [epinephrine], [norepinephrine], P_{CO_2} and [HCO_3^-] had no significant effects on rates of gluconeogenesis. The observed stimulation of gluconeogenesis could be induced independently by either decreased pH (which lowered K_m for lactate) or increased [lactate] (by simple hyperbolic kinetic effects), but the effects were not additive. Despite this potentially adaptive stimulation of gluconeogenesis, I estimate, based on observed *in vitro* rates and *in vivo* estimates of lactate load, that hepatic gluconeogenesis accounts for less than 2% of the lactate load clearance in toadfish.

Introduction

Exhaustive exercise in fish leads to a pronounced acidosis with a respiratory

Key words: *Opsanus beta*, gluconeogenesis, hepatocytes, exercise.

component (i.e. an increase in carbon dioxide tension, P_{CO_2}) as well as a metabolic component (i.e. an increase in [lactate$^-$] and metabolically generated protons) (Wood and Perry, 1985; Heisler, 1986). Since the study of Wardle (1978) demonstrating 'non-release' of lactate from post-exercise muscle in plaice, it has been suspected that the Cori cycle plays a small role in the final disposal of the lactate generated by exercising muscle in fish. Recent studies have suggested that a large portion of this lactate is indeed retained intramuscularly and reprocessed to glycogen *in situ* in teleosts (Weber *et al.* 1986; Milligan and Wood, 1986, 1987*a*; Milligan and McDonald, 1988). Although it appears that the role of liver in processing of lactate produced during exercise in fish may be small, its contribution has not been quantified.

The lack of knowledge concerning hepatic processing of lactate to glucose in fish is related to the difficulty of specifically measuring this process *in vivo*; even studies of lactate turnover rates cannot place a precise estimate on the contribution by the liver, in that these measurements include turnover by all tissues. In only two studies, employing double-label techniques to measure glucose and lactate fluxes simultaneously, has hepatic glucose production from lactate been estimated. Cornish and Moon (1985) found substantial channelling of lactate to glucose (35 %) in resting American eels with very low plasma [lactate] ($<0.1\,mmol\,l^{-1}$). However, their results cannot be extrapolated to exercise conditions. Weber *et al.* (1986) measured minimal Cori cycle activity in exercising skipjack tuna, but the capabilities of this exceptional fish may not be applicable to teleosts in general.

Recently, we have begun to take an *in vitro* modelling approach to the problem of estimating the importance of hepatic gluconeogenesis in fish by measuring gluconeogenesis in isolated fish hepatocytes exposed to conditions which mimic *in vivo* physiological states. First, we focused on the respiratory acidosis associated with the initial post-exercise recovery period in fish. Uncompensated hypercapnia (decreasing pHe by 0.4 units, increasing CO_2 content from 0.25 to 1 %, and increasing [HCO_3^-] by $3\,mmol\,l^{-1}$) led to marked depressions of gluconeogenesis in rainbow trout hepatocytes, and this depression was the sum of independent depressions due to decreased pHe and increased P_{CO_2}, and stimulation by increased [HCO_3^-] (Walsh *et al.* 1988). These depressions in gluconeogenic rate appear to be compensated *in vivo* in part by endogenous catecholamines in the rainbow trout (Mommsen *et al.* 1988; Perry *et al.* 1988).

With this background in mind, the purpose of the present study was to model post-exercise hepatic gluconeogenesis in a teleost fish, including effects of changes in [lactate] in an attempt to evaluate the contribution of the liver to lactate clearance *in vivo*. We chose the gulf toadfish, *Opsanus beta*, as our experimental organism because of the availability of background information concerning lactate uptake and gluconeogenic flux (Walsh, 1987; Mommsen and Walsh, 1989). Also, *Opsanus beta* is a relatively inactive benthic species with a restricted home range (Gray and Winn, 1961; Avise *et al.* 1987) and, as such, would extend our observations for rainbow trout to a species with different metabolic scope for exercise potential.

Materials and methods

Animals and in vivo *exercise experiments*

Specimens of mature gulf toadfish, *Opsanus beta* (body mass 80–496 g) were collected by roller trawl in Biscayne Bay, Florida, by a local fisherman during June to October 1987 and 1988. Upon return to the laboratory, toadfish were held in running saltwater aquaria (salinity 33 ‰, temperature 26–28 °C) and fed a diet of chopped squid twice a week. Fish were fasted for 3 days before use.

Toadfish used for exercise experiments were fitted with catheters in the caudal vein in a manner similar to that previously applied to American eels (Walsh and Moon, 1982). Toadfish (>100 g body mass) were anaesthetized with 0.5 g l^{-1} MS-222 neutralized with $NaHCO_3$ and a 2.5 cm incision was made just below the lateral line approximately 2.5 cm behind the anus. A length of PE 10 tubing was threaded through a minute puncture in the caudal vein and sewn to the skin by means of a collar. The wound was dusted with oxytetracycline–HCl and closed with 000 gauge silk. The catheter was flushed with toadfish saline (Walsh, 1987) containing 1500 units ml^{-1} of sodium heparin. Fish were allowed to recover in 9-l plastic trays supplied with running sea water for 48 h before use, and blood samples with stable acid–base parameters could be obtained from patent catheters for at least 1 week.

Fish were stimulated to swim in their 9-l trays with a glass probe for 5 min at 26 °C. Typically, during the first 2 min several bouts of vigorous caudal swimming were noted, but in the final 3 min fish swam more slowly, often using their large pectoral fins. By 5 min, fish were so fatigued that they could have been picked up, were it not for a strong biting response. Blood (approx. 1 ml) was sampled anaerobically with heparinized syringes *via* the catheter from toadfish at rest (*R*) or after exercise (1.5 min=0 h, and 1, 2, 4 and 12 h). Only one blood sample was taken per fish. Blood was immediately analysed for pH (100 μl), with a Radiometer PHM 84 pH meter and glass capillary electrode thermostatted to 26 °C, and total CO_2 (C_{CO_2}) (100 μl), with a Corning 965 total carbon dioxide analyser. The remaining blood was centrifuged at 13 000 *g* for 1 min, the plasma drawn off and frozen in liquid nitrogen and stored at −80 °C for subsequent enzymatic analysis of lactate and glucose (Bergmeyer, 1974). In a separate group of fish, skeletal muscle [lactate] was determined on freeze-clamped samples from fish at rest and 0 h post-exercise as previously described for longjaw mudsuckers (Walsh and Somero, 1982).

In a separate group of fish, blood was sampled (also *via* a catheter) at rest and immediately post-exercise for analysis of epinephrine and norepinephrine according to the methods of Woodward (1982). Briefly, blood was immediately centrifuged at 13 000 *g* for 1 min and 500 μl of plasma was drawn off and added to a tube with 50 μl of 5 mmol l^{-1} sodium bisulphite and frozen in liquid nitrogen until subsequent analysis 48 h later. Samples were protected from light at all times. Catecholamines were extracted from plasma by shaking plasma samples for 1 h with 10 mg of acid-washed alumina (Bioanalytic Systems, W. Lafayette, IN), 500 μl of 3.0 mol l^{-1} Tris–HCl, pH 8.8 with 1.5 mol l^{-1} EDTA. Alumina was

pelleted by centrifugation and the remaining plasma discarded. The alumina was washed twice with 1 ml of distilled water adjusted to pH 12.0 with NaOH. Catecholamines were eluted from the alumina using 150 μl of 0.1 mol l^{-1} perchloric acid with 1 mmol l^{-1} sodium bisulphite. Samples were spiked with known amounts of 3,4-hydroxybenzylamine (DHBA), and standards of DHBA, epinephrine and norepinephrine prepared in distilled water were treated identically to plasma. Samples were analysed for catecholamines by HPLC using a LDC/Milton Roy IIIG pump and C1-10 printing integrator, a Techsphere 5 ODS column (25 cm×0.5 cm) and a Bioanalytic Systems LC-4B amperometric detector (range 0–2 nA, approx. voltage 0.78 V). Elution was isocratic with a mobile phase of 0.1 mol l^{-1} monochloroacetic acid, 2 mmol l^{-1} EDTA, pH 3.35, at a flow rate of 3.0 ml min^{-1}. Catecholamines were quantified by comparison of peak areas with standards.

Isolation of hepatocytes and in vitro *modelling protocol*

In a different group of toadfish (i.e. not exercised or catheterized), hepatocytes were isolated by the collagenase perfusion method as previously applied (Walsh, 1987). In the first series of experiments, CO_2 and glucose production from lactate were measured in hepatocytes subjected to a combination of factors (pHe, C_{CO_2}, P_{CO_2}, [lactate] and [glucose]) designed to reflect the measured *in vivo* parameters at rest and following 0, 1, 2, 4 and 12 h post-exercise recovery. When significant effects on metabolism were observed in the exercise simulation, individual parameters in subsequent experiments were varied in a controlled manner to elucidate their effects on metabolism.

All metabolic incubations were performed at 24°C using the open system extensively described in Walsh *et al.* (1988). Hepatocyte suspensions (approx. 25 mg wet cell mass ml^{-1} suspension medium) were continuously gassed with the appropriate CO_2 content (0.0, 0.25, 0.5 or 1.0%) in the presence of L-[U-^{14}C]lactate (Amersham, Arlington Hts, IL; final specific activity 0.15 μCi μmol^{-1}) for 1.5 h. $^{14}CO_2$ was trapped in exiting gas by bubbling through a trapping solution (1 ml Carbotrap II, Baker Chemicals, +1 ml ethanol), and also at the end of the experiment, when the cells were acidified with perchloric acid, by a hyamine-hydroxide-soaked filter paper in a centre well suspended in the flask. [^{14}C]Glucose production was measured in the acidified suspension, subsequently neutralized, by an ion-exchange method (Walsh *et al.* 1988).

In selected experiments, intracellular pH (pHi) was measured in hepatocytes by the [^{14}C]DMO (5,5-dimethyl-2,4-oxazolidine dione) method, as previously described (Walsh, 1986). ^{14}C radioactivity was counted in a Tracor Analytic Beta Trac 6895 liquid scintillation counter.

Chemicals, calculations and statistical analyses

All biochemicals were purchased from Sigma Chemical (St Louis, MO) unless otherwise noted and all chemicals were reagent grade. Carbon dioxide partial pressures were calculated using measured C_{CO_2} and pHe, and the equations and

constants of Boutilier *et al.* (1984). Absolute metabolic rates (μmol glucose or CO_2 production g^{-1} cell mass h^{-1}) were calculated as previously described using measured specific activities, packed hepatocyte wet masses, and incubation time (Walsh *et al.* 1988). All values are reported as means±1 S.E. Each measurement for each preparation was performed in duplicate and these values were averaged to give a single sample value. Significant differences between means were tested using a paired Student's *t*-test (when comparing two treatments) or a model III, two-way ANOVA for more than two treatments (Zar, 1974). Linear regressions and comparison of slopes and intercepts by *t*-test were made by standard techniques (Zar, 1974).

Results

In vivo *exercise experiments*

Immediately following strenuous exercise in the toadfish, there was a marked depression of pHe (approx. 0.55 units) and an increase in P_{CO_2} (approx. 0.6 kPa=4.5 mmHg) (Fig. 1A,C). The increase in P_{CO_2} was largely corrected by 1 h after exercise, but the decrease in pHe took longer to return to pre-exercise levels (2 h). Increases in C_{CO_2} at 2 and 4 h post-exercise (Fig. 1B) were not statistically significant. Plasma [lactate] was elevated at 0 h and 1 h post-exercise, after which it rapidly returned to near resting levels (Fig. 1D). Our sampling protocol may have missed the peak of plasma [lactate], but we estimate this (graphically, Fig. 1D) to be at most about 5 mmol l^{-1}. Skeletal muscle lactate values increased significantly from 1.86±0.66 mmol kg^{-1} (N=3) at rest to 7.28±2.62 mmol kg^{-1} (N=3) at 0 h. Plasma [glucose] rose steadily until 4 h into the post-exercise period, and was still significantly elevated at 12 h post-exercise (Fig. 1E).

Resting plasma catecholamine levels were below the limits of detection by our methods (<1 nmol l^{-1}), despite high recovery of standards and added DHBA (>90 %). However, immediately following exercise, plasma catecholamines rose to detectable levels ([epinephrine]=7.95 nmol l^{-1}±1.06, N=4; [norepinephrine]=1.69 nmol l^{-1}±0.65, N=3).

In vitro *modelling of metabolism in hepatocytes*

As previously described for toadfish (Walsh, 1987), the collagenase perfusion technique yielded hepatocyte preparations with consistently high viability as judged by Trypan Blue exclusion and ATP contents. However, since natural populations were sampled, considerable variation from fish to fish existed in gluconeogenic rates (see Tables 2–4; Fig. 2). Therefore, a paired experimental design was used to test for significant effects of experimental variables. Given the apparently important role of catecholamines in adaptation of metabolism to acid–base stress in rainbow trout hepatocytes (Mommsen *et al.* 1988), preliminary experiments were conducted on the effects of epinephrine and norepinephrine on gluconeogenesis in toadfish hepatocytes. Even [catecholamine] approaching peak

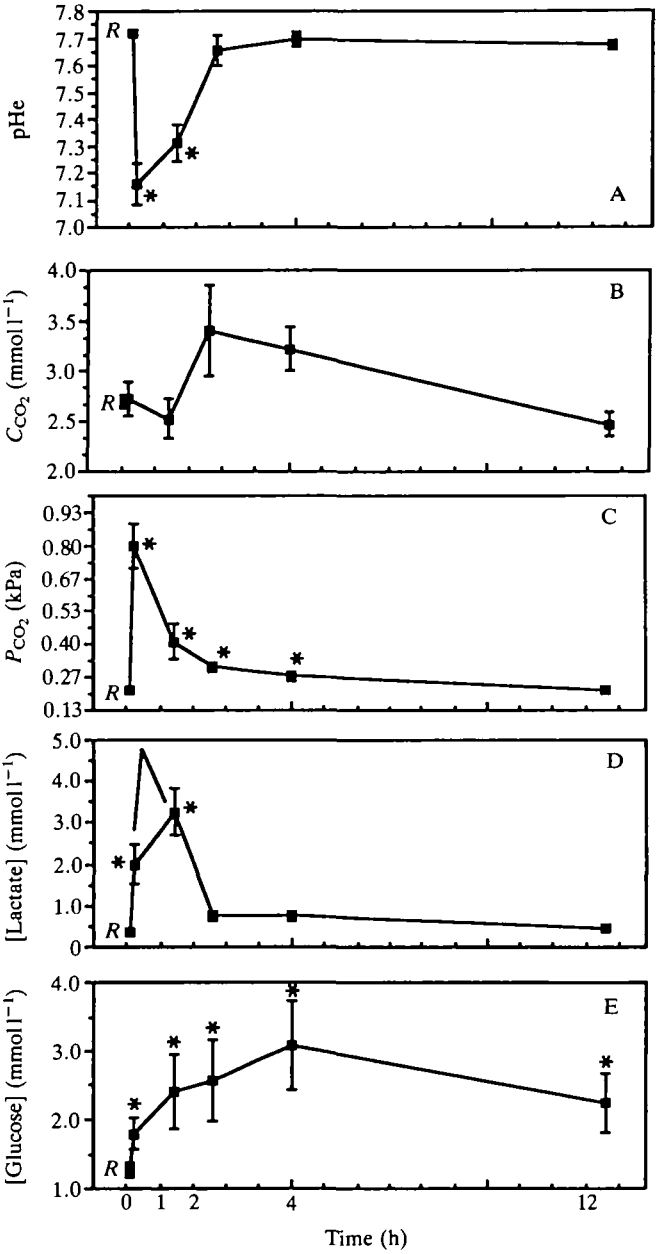

Fig. 1. Blood pH (A), C_{CO_2} (B), P_{CO_2} (C), [lactate] (D) and [glucose] (E) in the toadfish at rest (*R*) and at 0, 1, 2, 4 and 12 h post-exercise. Values are means±S.E.M. (N=4) for each group (i.e. 20 fish in total). Error bars not shown are smaller than symbol size. * denotes significantly different from resting values at $P<0.05$ level, unpaired Student's *t*-test.

Table 1. *Simulated conditions for the exercise-modelling experiment (see Fig. 1 for experimental values)*

		Simulation group				
	Rest	0 h	1 h	2 h	4 h	12 h
pHe	7.772±0.019	7.147±0.023	7.347±0.049	7.769±0.021	7.790±0.021	7.784±0.022
C_{CO_2} (mmol l^{-1})	2.5±0.2	2.3±0.1	2.0±0.3	2.5±0.1	2.4±0.1	2.5±0.1
CO_2 gas (%)	0.25	1.0	0.5	0.25	0.25	0.25
[Lactate] (mmol l^{-1})	0.4	2.0	3.3	0.8	0.8	0.4
[Glucose] (mmol l^{-1})	1.3	1.8	2.4	2.6	3.1	2.3

Measured values are means±S.E.M. (N=5).

in vivo levels did not significantly alter rates of gluconeogenic flux or rates of CO_2 production *in vitro*, although a slight (<10 %) upward trend could be discerned; furthermore this pattern did not change at low pHe (7.10) or elevated [lactate] (3.3 mmol l^{-1}) (results not shown). Significant effects of catecholamines on gluconeogenesis in toadfish hepatocytes have only been observed at supraphysiological levels (i.e. 50–100 nmol l^{-1}) (E. Danulat, T. P. Mommsen and P. J. Walsh, unpublished data). Therefore catecholamines were deleted from further *in vitro* modelling experiments.

Simulated post-exercise conditions (Table 1) markedly stimulated rates of gluconeogenesis from lactate in toadfish hepatocytes at 0 and 1 h post-exercise: after this time, rates slowly returned to resting levels by 12 h post-exercise (Fig. 2). This effect appears to be specific in that the simulation led to relatively small and non-significant changes in rates of oxidation of lactate to CO_2 (Fig. 2).

Further experiments were designed to determine the basis for the increase in gluconeogenic rates at 0 and 1 h post-exercise. Variation of extracellular [glucose] from 1.0 to 3.5 mmol l^{-1} under resting acid–base conditions (similar to those in Table 1) and 1 mmol l^{-1} lactate had no significant effect on rates of gluconeogenesis (results not shown). The changes in acid–base variables (pHe, P_{CO_2}, C_{CO_2} or $[HCO_3^-]$) in simulated post-exercise conditions were relatively complex and, since only one of these variables can be held constant at a time, a series of multifactorial experiments at constant [lactate] (1 mmol l^{-1}), similar to experiments previously conducted on rainbow trout hepatocytes (Walsh *et al.* 1988), was performed to determine if any of these variables exerted an effect on gluconeogenesis (Tables 2–4). Uncompensated hypercapnia (i.e. the respiratory acidosis component of the 0 h post-exercise conditions) led to a significant increase in gluconeogenesis from lactate (Table 2), which disappeared when the acidosis was compensated with increased $[HCO_3^-]$ (Table 2). When pHe and $[HCO_3^-]$ were co-varied while holding P_{CO_2} constant, acidosis again led to a similar stimulation of gluconeogenesis (Table 3), indicating the lack of a specific P_{CO_2} effect. Finally,

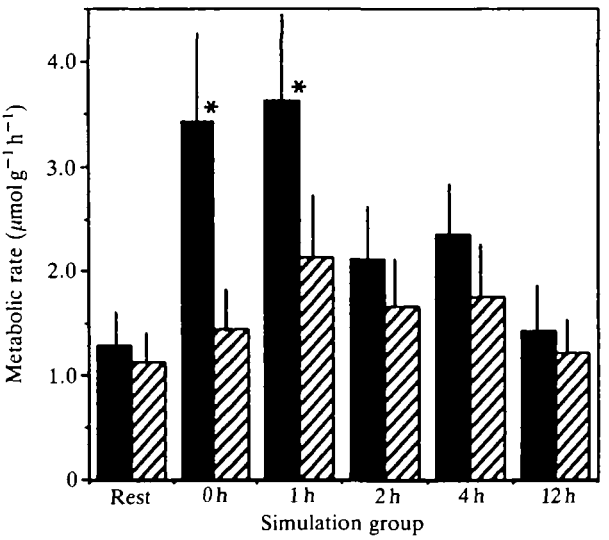

Fig. 2. Rates of glucose (solid bars) and CO_2 (cross-hatched bars) production by toadfish hepatocytes under simulated conditions of rest and 0, 1, 2, 4 and 12 h post-exercise. Conditions of pHe, C_{CO_2}, [lactate] and [glucose] are given in Table 1. Values are means±S.E.M. (N=5). Incubation time was 1.5 h. * denotes significantly different from resting values at P<0.05 level, model III, two-way ANOVA.

when experiments were repeated in the nominal absence of HCO_3^-, acidosis again led to stimulation of gluconeogenesis (Table 4). These experiments lead to the conclusion that a portion of the stimulation of gluconeogenesis in simulated 0 h post-exercise conditions may be related directly to a decrease in pHe. Summarizing the data in Tables 2–4 for percentage stimulation of gluconeogenesis *vs* extent of decrease in pHe, the following equation is obtained: fold-stimulation=$-1.48+4.69(-\Delta pHe)$, r=0.97. The data are summarized in this manner because of the individual variation noted for absolute rates of gluconeogenesis (see the differences between 'Resting', Table 2 and 'Normal', Table 3).

If there were no effect of [lactate] on gluconeogenesis, the change in pHe

Table 2. *Effects of hypercapnia and compensated hypercapnia on gluconeogenesis from lactate and pHi in toadfish hepatocytes*

	Resting	Hypercapnic	Compensated
Glucose production (μmol g^{-1} h^{-1})	3.24±1.10	5.49±1.73*	3.61±1.35
pHi	7.503±0.049	7.106±0.030*	7.403±0.038
pHe	7.771±0.056	7.249±0.051	7.685±0.057
C_{CO_2} (mmol l^{-1})	3.1±0.4	4.0±0.4	10.7±1.0
P_{CO_2} (kPa)	0.22±0.06	0.96±0.14	0.94±0.21

Values are means±S.E.M. (N=6).
* Significantly different, Model III, two-way ANOVA, P<0.05.

Table 3. *Effects of variable pHe and $[HCO_3^-]$ at constant P_{CO_2} on gluconeogenesis from lactate and pHi in toadfish hepatocytes*

	Low (pHe=7.337±0.033)	Normal (7.854±0.043)	High (8.294±0.015)
C_{CO_2} (mmol l^{-1})	1.1±0.1	3.2±0.3	9.0±0.3
Glucose production (μmol g^{-1} h^{-1})	6.94±0.60*	5.45±0.67*	3.24±0.56*
pHi	7.157±0.043*	7.559±0.052*	7.904±0.020*
P_{CO_2} (kPa)	0.20±0.03	0.19±0.04	0.17±0.01

Values are means±1 S.E.M. ($N=6$).
* Significantly different, Model III, two-way ANOVA, $P<0.05$.

between rest and 0 h in the modelling experiment (−0.625; Table 1) would have stimulated gluconeogenesis by only 1.45-fold. In fact a 2.64-fold increase was observed. Furthermore, despite the fact that pHe had 'recovered' by 1 h in the simulation, gluconeogenic rates remained elevated, suggesting that elevated [lactate] also stimulates gluconeogenesis. Further experiments revealed the nature of this stimulation. Gluconeogenesis responds to increasing [lactate] with simple Michaelis–Menten type kinetics (Fig. 3). Under resting acid–base conditions, the pathway approaches saturation between 3 and 5 mmol l^{-1} (Fig. 3) and the K_m is 1.53 mmol l^{-1} (determined from a Lineweaver–Burk plot). Exposure of hepatocytes to 0 h post-exercise acid–base conditions failed to alter V_m (Fig. 3), but it did decrease K_m to 0.954 mmol l^{-1} ($P<0.05$). Thus, at low [lactate], gluconeogenesis can be stimulated by a decrease in pHe or an increase in [lactate], but these effects are not strictly additive; pHe only affects gluconeogenic rates at low [lactate] through effects on the 'pathway K_m' for lactate.

Discussion

The results of this study demonstrate: (1) that the gulf toadfish is a sluggish fish

Table 4. *Effects of variable pHe at nominal $[HCO_3^-]$ on gluconeogenesis from lactate and pHi in toadfish hepatocytes*

	Low (pHe=7.330±0.029)	Normal (7.690±0.029)	High (8.050±0.037)
Glucose production (μmol g^{-1} h^{-1})	4.59±1.33*	3.64±1.19*	2.75±0.98*
pHi	7.174±0.025*	7.427±0.030*	7.719±0.048*
C_{CO_2} (mmol l^{-1})	0.1±0.1	0.2±0.1	0.7±0.1
P_{CO_2} (kPa)	0.02±0.01	0.02±0.01	0.03±0.01

Values are means±S.E.M. ($N=6$).
* Significantly different, model III, two-way ANOVA, $P<0.05$.

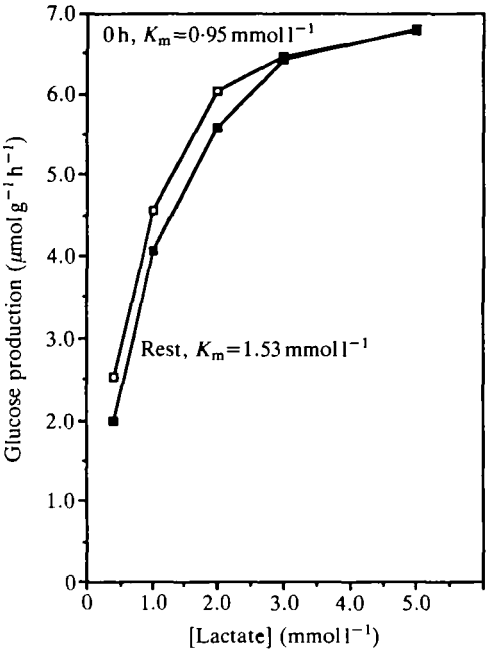

Fig. 3. Rates of glucose production by toadfish hepatocytes as a function of [lactate] at resting (■) and 0 h post-exercise (□) conditions of pHe (7.76 and 7.19) and C_{CO_2} (2.4 and 2.5 mmol l^{-1}). [Glucose]=1.3 mmol l^{-1}. Results are means of duplicate determinations for hepatocytes from a single toadfish in a paired experimental design.

with somewhat different responses to exercise from those of active pelagic species; (2) that post-exercise conditions (specifically decreased pHe and increased [lactate]) stimulate gluconeogenesis; but (3) that hepatic gluconeogenesis can account for only a small portion of post-exercise lactate clearance.

Prior studies of the effects of strenuous exercise in fish have described a spectrum of species-specific responses with respect to lactate dynamics. Although all species generate considerable quantities of lactate intramuscularly, active pelagic species release substantially more of this lactate load to the blood than do sluggish benthic species (Wood and Perry, 1985; Milligan and Wood, 1986, 1987*a*). For example, while the change in plasma [lactate] typically reaches peak values in excess of 16 mmol l^{-1} in rainbow trout and coho salmon (Milligan and Wood, 1986; Milligan and McDonald, 1988), the change in plasma [lactate] in less active species (e.g. flounder, sole, sea raven, etc.) is typically less than 2 mmol l^{-1} (Milligan and Wood, 1987*a*; Wood and Perry, 1985). The gulf toadfish, *Opsanus beta*, falls near the sluggish end of this spectrum: muscle [lactate] increased by 5.4 mmol kg^{-1} (see Results), but plasma [lactate] reached only 3.3 mmol l^{-1} in the present study, a change of less than 2.9 mmol l^{-1} from resting values (Fig. 1D).

Most other aspects of post-exercise acid–base status in the gulf toadfish (i.e. pHe depression, P_{CO_2} elevation, etc.; Fig. 1) are similar in magnitude and timing to that noted for other species of teleostean fishes (Wood and Perry, 1985).

However, a much smaller elevation in plasma catecholamine levels occurs in the toadfish (see Results) than in the rainbow trout (Primmett *et al.* 1986). Unchanged plasma catecholamine levels and absence of effects of *in vivo* adrenoreceptor blockade were observed in the starry flounder, *Platichthys stellatus*, another sluggish fish species (Milligan and Wood, 1987*b*; Wood and Milligan, 1987). These observations, along with the apparent lack of effect of catecholamines *in vitro* on the oxygen transport capability of red blood cells in flounder (Milligan and Wood, 1987*c*) and gluconeogenesis in toadfish hepatocytes (see Results above, and below), suggest that these hormones may play less of an important role in the coordination of responses to acid–base stresses in sluggish fish species than in the rainbow trout.

What is the quantitative role of the liver in processing of lactate to glucose in fish? Recently, Milligan and McDonald (1988) measured lactate turnover rates during a post-exercise period in coho salmon and starry flounder. Using these rates, which represent total processing of lactate, they calculated that non-muscle tissues working at peak rates could clear the lactate load produced during exercise in 21 h in coho salmon and in 28 h in starry flounder. However, the measured time of lactate load clearance in these and other fish species is about 10 h (Wood and Perry, 1985). Furthermore, of the ^{14}C-labelled lactate injected into starry flounder or coho salmon, only 10–20 % appeared in blood-borne components other than lactate (Milligan and McDonald, 1988). Thus, *in vivo* experiments indirectly point to a small quantitative role for the liver in post-exercise processing of lactate.

Most prior direct measurements of gluconeogenic rates in fish hepatocytes *in vitro* or in fish *in vivo* have been made under conditions different from *in vivo* post-exercise states, with respect to strict control of acid–base variables or [lactate] (for a review, see Suarez and Mommsen, 1987). Therefore, the data from these prior studies are only of limited value in evaluating the quantitative contribution of post-exercise hepatic gluconeogenesis to lactate clearance. However, the data of the present study support the suggestion that post-exercise processing of lactate by the liver plays a small quantitative role. Using the peak gluconeogenic rate obtained under post-exercise simulation (i.e. 3.62 μmol g^{-1} liver h^{-1} at 1 h post-exercise, Fig. 2) and the hepatosomatic index for the gulf toadfish [HSI=0.956±0.054 g liver 100 g^{-1} body mass, mean±S.E. (N=12); D. Gilchrist and P. J. Walsh, unpublished data], we estimate the maximum hepatic gluconeogenic rate for a 100-g toadfish to be 3.46 μmol h^{-1}. The post-exercise lactate load in the muscle of a hypothetical 100-g toadfish can be estimated to be 189 μmol by using the measured change in muscle [lactate] (5.4 mmol kg^{-1}) and the average caudal skeletal muscle mass of a 100-g toadfish (35 g; C. J. Kennedy, K. A. Gill and P. J. Walsh, unpublished data). We then estimate that the toadfish liver can process only 1.8 % of the total muscular lactate load to glucose in 1 h, and that it would take the liver of this hypothetical 100-g toadfish about 55 h to process this lactate load completely to glucose working at maximal rates! Lactate is cleared from the blood and presumably from the tissues by 12 h in the toadfish (Fig. 1).

A slightly different approach yields a similar conclusion. The maximum

potential for hepatic lactate clearance in the toadfish can also be estimated by assuming that all of the hepatic oxidative metabolism ($10.2\pm0.7\,\mu mol\,O_2$ consumed $g^{-1}\,h^{-1}$, measured with $10\,mmol\,l^{-1}$ lactate as substrate, P. J. Walsh, unpublished data) could be devoted to lactate clearance. Under these conditions, maximum lactate clearance could be 5.1 % of the lactate load per hour, or a time exceeding 19.5 h to clear the entire lactate load.

The data in the present study allow an initial assessment of the mechanisms involved in the increase in gluconeogenic rates observed during simulation of post-exercise conditions. In contrast to studies of gluconeogenesis in rainbow trout hepatocytes (Mommsen *et al.* 1988), the application of catecholamines at concentrations measured *in vivo* had no effect on hepatic gluconeogenesis in the toadfish (see Results). The data from the 0 h post-exercise simulation indicate a role for acid–base variables and/or [lactate] as possible modulators of gluconeogenesis in toadfish hepatocytes. When changes in acid–base variables were studied in a controlled fashion at a single [lactate], gluconeogenesis was affected only by changes in extracellular pH (not changes in P_{CO_2} or $[HCO_3^-]$), and decreased pH stimulated gluconeogenesis (Tables 2–4). Again these results differ from those obtained for rainbow trout hepatocytes, in which decreased pH or increased P_{CO_2} depressed, and increased $[HCO_3^-]$ enhanced, rates of gluconeogenesis (Walsh *et al.* 1988). Increased [lactate] can also enhance rates of gluconeogenesis from lactate in toadfish hepatocytes in a simple saturable manner with saturation occurring at about the highest concentrations of lactate observed in plasma, i.e. $3–5\,mmol\,l^{-1}$ (Fig. 3). However, the stimulatory effects of decreased pHe and increased [lactate] on gluconeogenesis are not additive at higher [lactate], in that decreased pHe appears to act by decreasing the K_m of the pathway for lactate, rather than by adjusting V_m (Fig. 3). Since changes in extracellular pH fail to affect rates of lactate transport across the plasma membrane in toadfish hepatocytes (Walsh, 1987), the observed pHe effects are most probably mediated by changes in pHi, which in fish hepatocytes appears to track pHe with absolute values about 0.15–0.2 units lower than pHe in the physiological range (Walsh and Moon, 1983; Walsh, 1986; Tables 2–4). Direct effects of pH on a rate-limiting enzyme, or direct effects of pH on transfer of metabolites between cellular compartments, are likely candidates for the observed stimulation of gluconeogenesis by acidosis in toadfish hepatocytes.

There are interesting differences in the effects of acid–base stress on hepatic gluconeogenesis in the rainbow trout and the toadfish. Possibly in relation to their differing lifestyles, the rainbow trout and the toadfish display different sensitivity to acid–base variables, and different compensatory mechanisms to cope with these stresses. On the one hand, rainbow trout appear to compensate for the inhibitory effects of acidosis on hepatic gluconeogenesis with catecholamine-mediated stimulation of gluconeogenesis and glycogenolysis (Mommsen *et al.* 1988; Perry *et al.* 1988; Walsh *et al.* 1988). The toadfish liver, on the other hand, appears to respond directly to acidosis with a pH-mediated stimulation of gluconeogenesis. Further comparison of the molecular mechanisms involved in these two very

different responses may shed additional light on the diversity in regulation of hepatic gluconeogenesis noted in piscine systems (Suarez and Mommsen, 1987).

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