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A regulatory role for cortisol in muscle glycogen metabolism in rainbow trout *Oncorhynchus mykiss* Walbaum

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Summary

To test the hypothesis that cortisol has a regulatory role in fish muscle glycogenesis post-exercise, rainbow trout were treated 1 h prior to exercise with either saline (control) or metyrapone (2-methyl-1, 2-di-3-pyridyl-1-propanone) to block cortisol synthesis. Following exercise (time 0), half of the metyrapone-treated fish received a single injection of cortisol, to mimic the post-exercise rise usually observed. Muscle glycogen and the relative activities of glycogen phosphorylase *a* (Phos *a*) and glycogen synthase *I* (GSase *I*), regulatory enzymes for glycogen resynthesis, were monitored 4 h post-exercise. Metyrapone treatment succeeded in blocking the post-exercise rise in plasma cortisol (17±2 *vs* 118±13 ng ml^{-1} in controls at time 0), and cortisol injection resulted in a larger and more prolonged cortisol increase than in controls (159±22 *vs* 121±14 ng ml^{-1} in controls at 1 h). Muscle glycogen was completely restored in the metyrapone-treated fish within 2 h after exercise (8.3±0.6 *vs* 8±0.7 μmol g^{-1} pre-exercise), only partially restored in control fish at 4 h (5.4±01.4 *vs* 8.8±1.3 μmol g^{-1} pre-exercise), and not at all in cortisol-treated fish (1.0±0.5 μmol g^{-1} at 4 h). The rapid glycogen resynthesis in the metyrapone-treated fish was associated with a more rapid inactivation of Phos *a* and stimulation of GSase *I* compared to controls. In cortisol-treated fish, Phos *a* activity persisted throughout 4 h post-exercise; there was also a significant stimulation of GSase *I* activity. As a consequence of dual activation of Phos *a* and GSase *I*, glycogen cycling probably occurred, thus preventing net synthesis. This explains why the post-exercise elevation of cortisol inhibits net glycogen synthesis in trout muscle.

Key words: rainbow trout, *Oncorhynchus mykiss*, cortisol, muscle, glycogen, exercise, glycogen phosphorylase, glycogen synthase, metapyrone.

Introduction

In fish, high intensity exercise results in a near total depletion of white muscle glycogen stores. Glycogen replenishment and hence restoration of sprint performance, can be a slow process, often requiring more than 6 h (for reviews, see Milligan, 1996; Kieffer, 2000). Exercise to exhaustion also results in an elevation of plasma cortisol levels, which can reach 5 times those at rest and remain elevated for 6 h after exercise (see Milligan, 1996). The elevated plasma cortisol level appears to be inhibitory to glycogenesis, as there is no evidence of net muscle glycogen synthesis until cortisol levels begin to decline (e.g. Pagnotta et al., 1994). Preventing the rise in plasma cortisol, either by blocking its release or its synthesis (Pagnotta et al., 1994; Eros and Milligan, 1996) hastens the restoration of muscle glycogen stores. Allowing the fish to swim slowly after exercise prevents the post-exercise cortisol elevation and also hastens muscle glycogen resynthesis, compared to fish held in still water (Milligan et al., 2000). These observations strongly suggest that the persistent elevation of cortisol after exhaustive exercise is inhibitory to muscle glycogen resynthesis. Whether this is an effect of cortisol directly on muscle glycogen metabolism or a non-specific effect is not understood.

The rate at which muscle glycogen is synthesized is a function of the relative activities of glycogen synthase (GSase) and glycogen phosphorylase (Phos). The activity of these enzymes is regulated by a complex phosphorylation/dephosphorylation cycle. Both Phos and GSase exist in two interconvertible forms, active and inactive (nominally). Phosphorylation of glycogen phosphorylase (converting Phos *b* to Phos *a*), leads to an increase in activity, whereas phosphorylation of glycogen synthase (converting GSase *I* to GSase *D*) decreases its activity (e.g. Connett and Sahlin, 1996). These transformations are regulated hormonally (by, for example, adrenaline, glucagon) *via* cAMP-mediated activation of kinases and contraction-induced changes in intracellular Ca^{2+} (for a review, see Connett and Sahlin, 1996). Glucocorticoids have also been implicated in regulating these enzymes, at least in liver (see Mommsen et al., 1999). However, little evidence exists to suggest a similar regulatory role for glucocorticoids in muscle. In addition, both Phos *a* and

GSase *I* activities are influenced by substrate availability (P_i and glucose-6-phosphate, respectively) and changes in allosteric modifiers (e.g. AMP and ADP; Shulman et al., 1995; Shulman and Rothman, 1996; Howlett et al., 1998). Relative levels of Phos *a* are likely to be the dominant factor controlling glycogenolysis because this enzyme not only catalyzes the initial rate-limiting step in glycogenolysis, but also inhibits glycogenesis by blocking conversion of GSase *D* to GSase *I* (Connett and Sahlin, 1996).

The rate-limiting step to glycogen synthesis, at least in mammalian muscle, appears to be shared between glucose transport and glycogen synthase activity, depending upon the nutritional state of the organism (Shulman and Rothman, 1996; Howlett et al., 1998; Fisher et al., 2002). The story is likely to be somewhat different in fish muscle because of the relatively low rates of glucose uptake in the latter (West et al., 1993) and the minor contribution made by blood glucose to muscle glycogen synthesis (Pagnotta and Milligan, 1991). Although glucose transporters have been identified in fish muscle (Legate et al., 2001; Teerijoki et al., 2001), their physiological role is unclear. As has been described for mammalian muscle (Montell et al., 1999), glycogen levels themselves may dictate the activity of GSase and subsequently, the rate of net glycogen synthesis (e.g. Parkhouse et al., 1988).

The purpose of this study was therefore to investigate the role of cortisol in regulating muscle glycogen synthesis. Changes in white muscle glycogen synthase and phosphorylase activities in rainbow trout during recovery from exhaustive exercise were followed in fish that had been treated prior to exercise with metyrapone to block cortisol synthesis (see Eros and Milligan, 1996). To confirm that the effects seen were cortisol-specific, metyrapone-treated fish were infused with cortisol after exercise, in an attempt to mimic the post-exercise rise in cortisol seen in the control fish.

Materials and methods

Experimental animals

Rainbow trout *Oncorhynchus mykiss* Walbaum of both sexes, 150–230 g, were obtained from Rainbow Springs Trout Hatchery at various times of the year and held indoors in 900 liter plastic tanks continuously aerated and supplied with dechlorinated London (Ontario) tapwater at 15±1°C. Fish were fed a daily maintenance ration of commercial trout pellets, and fish were held for at least 2 weeks prior to experimentation.

Fish were surgically fitted with a dorsal aorta cannula (Soivio et al., 1972) while under MS-222 anesthesia (1:10,000 dilution, adjusted to pH 7.0 with $NaHCO_3$). Fish were allowed to recover for at least 48 h in black acrylic fish boxes continuously supplied with aerated, dechlorinated tapwater at the experimental temperature. During this period fish were not fed. The catheters were checked daily and filled with heparinized (50 i.u. ml^{-1} sodium-heparin, Sigma) saline.

Experimental protocol

Fish were randomly assigned to one of three experimental groups: saline injected (control, *N*=8), metyrapone + saline injected (metyrapone group, *N*=8) or metyrapone + cortisol injected (cortisol group, *N*=8). 1 h prior to exercise, fish were injected with either 3 mg 100 g^{-1} metyrapone (2-methyl-1, 2-di-3-pyridyl-1-propanone; Sigma Chemical, St Louis, MO, USA) or an equivalent volume, 20 μl 100 g^{-1}, of 0.9% NaCl. Fish were then transferred to a large circular tank (300 liter) and manually chased around the tank for 5 min, at which point they were unresponsive to further stimuli and considered exhausted. This method of exhausting fish has been used extensively and results in consistent metabolic disturbances (for a review, see Milligan, 1996). Fish were returned to their individual boxes, where they were able to freely move about, until sampled.

Half of the metyrapone-treated fish were injected with either 30 μg 100 g^{-1} cortisol (hemisuccinate cortisol, Sigma; cortisol group) and the other half received an equivalent volume (20 μl 100 g^{-1}) of 0.9% NaCl (metyrapone group). The control fish were injected with 20 μl 100 g^{-1} 0.9% NaCl.

Individual fish were sampled only once, either 1 h after metyrapone or saline injection (termed 'rest'), immediately after exercise to exhaustion (time 0) or at 1, 2 or 4 h post-exercise. Fish sampled at time 0 had only been injected with either saline or metyrapone. Cortisol injection was administered after exercise, so the cortisol group was not sampled until 1 h post-exercise. At the appointed time after exercise, 500 μl of blood was withdrawn from the catheter into a gas-tight Hamilton syringe and placed on ice until analyzed. The fish was then killed by anaesthetic overdose (2.0 g l^{-1} buffered MS-222) and a sample of white muscle was excised from the dorsal-epaxial muscle mass. The muscle sample was freeze-clamped between aluminum plates pre-cooled with liquid N_2 and stored at –80°C prior to analysis. A 50 μl sample of whole blood was added to 200 μl of ice-cold 8% $HClO_3$, mixed, then centrifuged at 10 000 ***g*** for 5 min. The supernatant was withdrawn and stored at 4°C until analysis for lactate. The remaining blood was centrifuged at 10 000 ***g*** for 5 min, and the plasma stored at –80°C for analysis of cortisol and catecholamines.

Analytical techniques and calculations

Whole blood lactate was measured enzymatically on 100 μl of the deproteinized $HClO_3$ extract using Sigma lactate reagents and procedures described by Bergmeyer (1965). Cortisol was measured on 25 μl duplicate samples of plasma using a commercially available radio-immunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA). The lower limit of detection of this assay was 1.5 ng ml^{-1} and the intra- and inter-assay coefficients of variation were 7.3 and 8.5%, respectively. Plasma adrenaline and noradrenaline levels were measured on alumina-extracted samples using high pressure liquid chromatography (Beckman System Gold, Fullerton, CA, USA) with electrochemical detection (ESA Coulochem II, Chelmsford, MA, USA), according to the method of Woodward (1982). Dihydroxybenzylamine hydrobromide (DHBA) was used as an internal standard in all samples.

For measurement of muscle lactate, glucose and glucose-6-phosphate, muscle was ground to a fine powder in a liquid N_2-cooled mortar. Approximately 100 mg of powdered tissue were added to 1.0 ml of ice-cold 8% $HClO_3$ and vigorously mixed with a vortex mixer for 1 min. Homogenates were centrifuged for 5 min at 10 000 ***g*** and the supernatant withdrawn and stored at 4°C for up to 1 week until analyzed for lactate concentration, as described above for blood. Muscle glycogen was measured on approximately 100 mg of frozen muscle, which was placed directly into 1.0 ml of 30% KOH and digested in a boiling water bath. Glycogen was isolated as described by Hassid and Abraham (1957) and measured as free glucose following digestion with amyloglucosidase (Bergmeyer, 1965).

Glycogen phosphorylase (Phos, EC 2.4.1.1) was measured on muscle ground to a fine powder under liquid nitrogen in a liquid nitrogen-cooled mortar as described by Storey (1991). In brief, ~150 mg of powdered, frozen tissue was transferred to a test tube containing 1 ml ice-cold homogenization buffer (50 mmol l^{-1} imidazole, 100 mmol l^{-1} NaF, 5 mmol l^{-1} EGTA, 2 mmol l^{-1} EDTA, 30 mmol l^{-1} 2-mercaptoethanol, 0.1 mmol l^{-1} phenylmethylsulfonylflouride) and homogenized on ice for 3× 10 s bursts with a Tissue Tearor (Biospec Products, Bartlesville, OK, USA) set to speed 6. The samples were allowed to settle on ice for 15 min and the supernatant was assayed for enzyme activity. Phos *a* activity was measured in 50 mmol l^{-1} potassium-phosphate buffer (pH 7.0) containing 2 mg ml^{-1} glycogen (oyster muscle, dialyzed), 0.4 mmol l^{-1} NAD, 10 μmol l^{-1} glucose-1,6-bisphosphate, 0.25 mmol l^{-1} EDTA, 150 mmol l^{-1} $MgCl_2$ and 1.4 U ml^{-1} phosphoglucomutase and 0.5 U ml^{-1} glucose-6-phosphate dehydrogenase. Total Phos (*a*+*b*) activity was measured in the presence of 1.6 mmol l^{-1} 5′AMP. Absorbance changes were measured at 340 nm on a UV-160 recording spectrophotometer (Shimadzu, Columbia, MD, USA). All enzyme activities were measured at 15°C. Enzyme activities are expressed per unit wet mass.

Glycogen synthase (GSase; EC 2.4.1.11) was measured on ~100 mg of powdered muscle mixed vigorously for 2 min on a vortex mixer at maximum speed, with 1 ml homogenization buffer (50 mmol l^{-1} Tris, 0.5 mmol l^{-1} dithiothrietol, 1.0 mmol l^{-1} EDTA, 2.0 mmol l^{-1} $MgCl_2$, pH 7.8 (Schallin-Jantti et al., 1992). GSase activity measured in samples processed this way were less variable and more consistent with previously published values, compared to values obtained from tissues processed using a mechanical tissue homogenizer. The sample was briefly centrifuged (1 min at 10 000 ***g***), and the supernatant assayed for glycogen synthase activity. Glycogen synthase activity in the supernatant was measured in the presence (*I* + *D* forms) or absence (*I* active form only) of 5 mmol l^{-1} glucose-6-phosphate. Glycogen synthase activity is expressed as the ratio of *I*:*I*+*D*. The assay mixture contained 70 mmol l^{-1} KCl, 50 mmol l^{-1} Tris, 7 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} phosphoenolpyruvate, 0.15 mmol l^{-1} NADH, 2.5 U 10 ml^{-1} lactate dehydrogenase, 5 U 10 ml^{-1} pyruvate kinase and 20 mmol l^{-1} UDP-glucose. A blank from each homogenate, which did not contain UDP-glucose, was assayed simultaneously and subtracted from all GSase measurements. The change in absorbance over time at 340 nm was monitored at 15°C, as described above.

All biochemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA) or Boehringer-Mannheim (Lachine, Quebec, Canada); all other reagents were acquired from local suppliers and were of the highest available purity.

Statistical analysis

All values are presented as means ± 1 S.E.M. Significant differences within a group were assessed with a one-way analysis of variance (ANOVA) and, where appropriate, followed by a Dunnett's analysis to compare within-group times to the respective pre-exercise value. Significant differences between groups at a given time were assessed using Student's *t*-test, unpaired design. Differences were considered significant if $P<0.05$.

Results

In the control group, plasma cortisol levels were significantly increased immediately after cessation of exercise through to at least 2 h; by 4 h post-exercise, cortisol levels had returned to pre-exercise levels (Fig. 1). Metyrapone treatment effectively blocked the post-exercise rise in plasma cortisol levels (Fig. 1), as plasma cortisol levels in this group of fish did not differ significantly from the pre-exercise value at any time post-exercise. Plasma cortisol levels in the cortisol-injected group remained elevated throughout the 4 h post-exercise period, and were significantly greater than the control group at all times (Fig. 1).

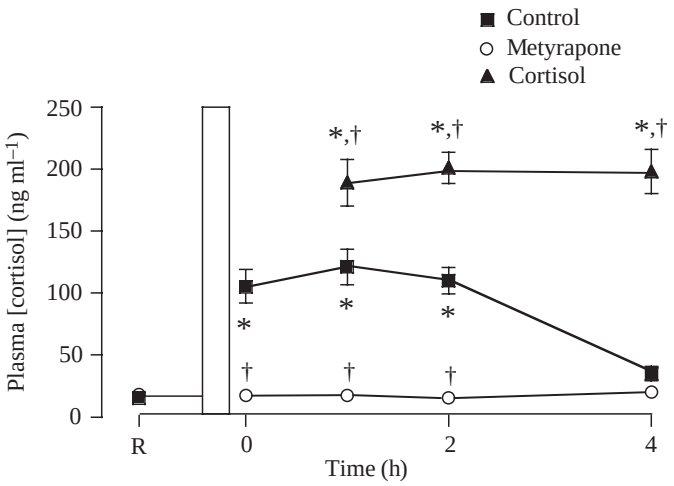

Fig. 1. Plasma cortisol levels in fish injected with either saline (control, *N*=8; filled squares), metyrapone (*N*=8; open circles) prior to exercise or cortisol (*N*=8; filled triangles) after a 5 min period of exercise (indicated by rectangular bar). R, the pre-exercise, post-injection value. Values are means ± 1 S.E.M. *Significant difference ($P<0.05$) from the pre-exercise value; †significant difference ($P<0.05$) from the corresponding control value.

Concentrations of circulating catecholamines were significantly elevated post-exercise in all groups (Fig. 2A,B) and had declined to levels not different from pre-exercise rest values by 1 h post-exercise.

In all groups, the exercise regime resulted in a decrease in muscle glycogen and increase in muscle lactate levels (Fig. 3A,B). The decrease in glycogen and increase in lactate levels were equivalent in all groups. In control fish, muscle lactate levels did not show any signs of decline until 4 h post-exercise (Fig. 3B), at which time there was evidence of net glycogen synthesis (Fig. 3A). However, glycogen restoration and lactate clearance were still incomplete in control fish at 4 h. In contrast, in metyrapone-treated fish, muscle glycogen levels were completely restored within 2 h post-exercise (Fig. 3A), and this was associated with clearance of the lactate load to pre-exercise levels (Fig. 3B). Restoration of muscle glycogen and clearance of muscle lactate levels were delayed in the cortisol-treated group; there was no glycogen synthesis or clearance of lactate by 4 h after exercise in these fish (Fig. 3A,B).

At rest the maximum total activity of muscle glycogen phosphorylase (Phos) was 0.63±0.08 U g wet tissue^{-1} (N=8) that of glycogen synthase (GSase) was 1.06±0.11 g wet tissue^{-1} (N=8). The total activities of these enzymes did not change with exercise, metyrapone or cortisol treatment. Instead, the relative activities of the active form of the enzymes (Phos *a* and GSase *I*) changed with exercise; the rate and direction of change were dependent upon the cortisol level (Fig. 4A,B). Phos *a* activity increased from 0.12 g wet tissue^{-1} (N=8) at rest to 0.54 g wet tissue^{-1} (N=8), attaining approximately 85% of maximal activity immediately after exercise (Fig. 4A). In control fish, Phos *a* activity declined slowly, attaining pre-exercise levels by 4 h post-exercise. However, in metyrapone-treated fish, Phos *a* activity declined rapidly, returning to pre-exercise levels within 1 h of exercise, whereas Phos *a* activity remained elevated throughout the 4 h period in the cortisol-treated fish (Fig. 4A). GSase *I* activity was slow to increase in the control group; it was not until 2 h post-exercise that evidence of activation was seen, with activity further increased at 4 h, coincident with the time-frame of glycogen synthesis (Fig. 3A). Significant increases in GSase *I* activity were seen as early as 1 h post-

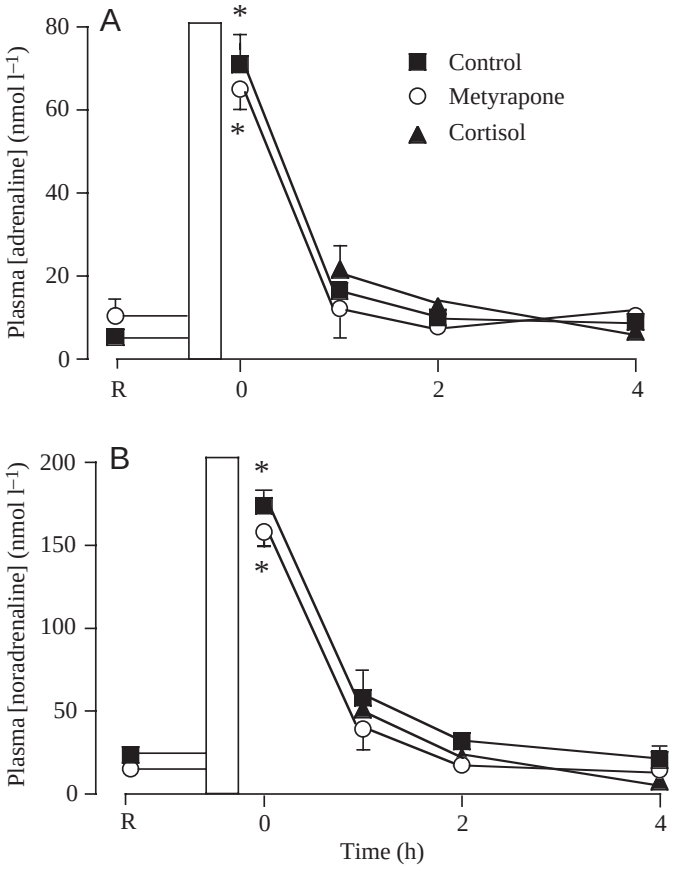

Fig. 2. Plasma adrenaline (A) and noradrenaline (B) levels prior to and following 5 min of exercise in fish treated with metyrapone or saline prior to exercise, or cortisol after exercise. R, pre-exercise, post-injection value. Values are means ± 1 S.E.M. (N=8 for each group). *Significant difference (P<0.05) from pre-exercise value.

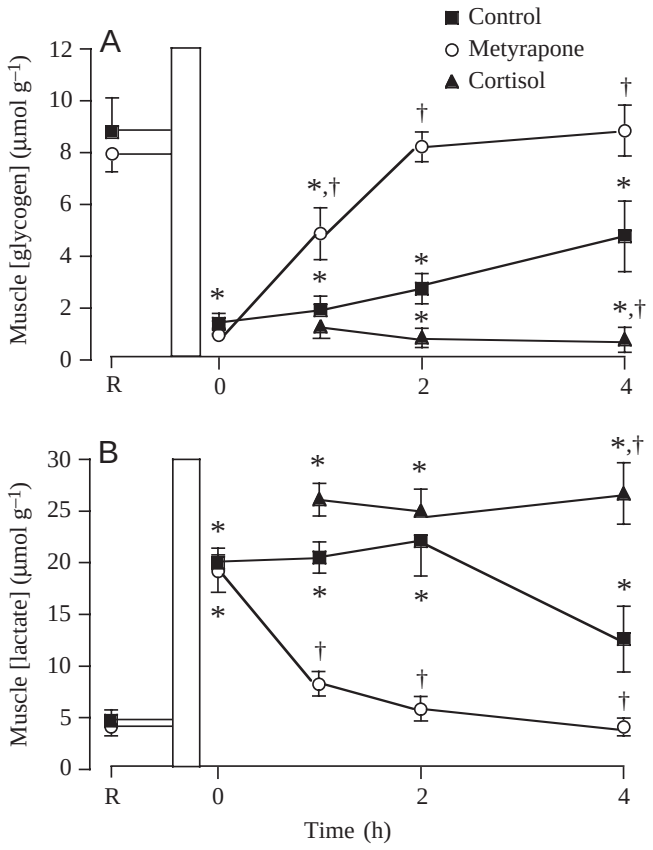

Fig. 3. Muscle glycogen (A) and lactate (B) concentrations in fish treated with metyrapone or saline prior to exercise or cortisol after 5 min of exercise. Muscle glycogen concentration is expressed as glycosyl units. R indicates pre-exercise, post-injection value. Values are means ± 1 S.E.M. (N=8 for each group). *Significant difference (P<0.05) from pre-exercise value; †significant difference (P<0.05) from corresponding control value.

exercise in metyrapone-treated fish, with peak activation [0.59± 0.11 g wet tissue^{-1} (*N*=8), ~55% of total activity] seen at 2 h post-exercise. Again, these changes in GSase *I* activity were coincident with glycogen resynthesis (Fig. 3A). The activation of GSase *I* in cortisol-treated fish was similar to that seen in the controls (Fig. 4B), yet there was no net glycogen synthesis in this group (Fig. 3A).

Muscle free glucose concentration increased late (2–4 h) into the post-exercise period in the control and cortisol-treated fish (Fig. 5A), but there was no change in glucose concentration in the metyrapone-treated fish. Muscle glucose-6-phosphate concentration increased immediately after exercise in the control and cortisol-treated groups and remained elevated throughout the 4 h post-exercise period. No change in glucose-6-phosphate concentration was seen in the metyrapone-treated fish (Fig. 5B).

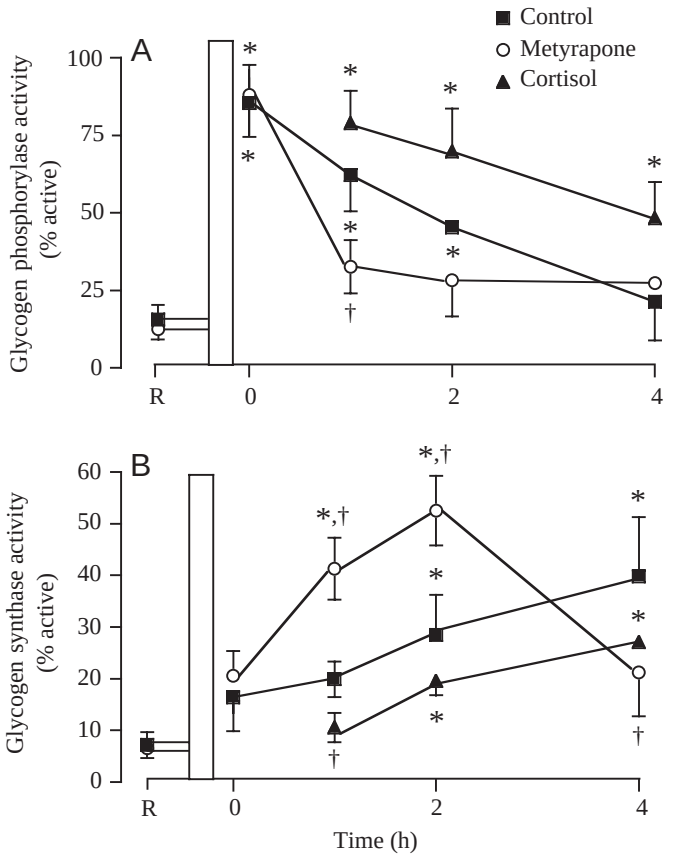

Fig. 4. Muscle glycogen phosphorylase *a* (A) and glycogen synthase *I* (B) activities prior to and following 5 min of exercise. Glycogen phosphorylase activity is expressed as the percentage of the total activity (measured in the presence of 5′AMP) in the active form (measured in the absence of 5′AMP). Glycogen synthase activity is expressed as the percentage of the total activity (measured in the presence of glucose-6-phosphate) in the active form (measured in the absence of glucose-6-phosphate). R, pre-exercise, post-injection value. Values are means ± 1 S.E.M. (*N*=8 for each group). *Significant difference (P<0.05) from the pre-exercise value; †significant difference (P<0.05) from the corresponding control value.

Discussion

The results from this study clearly demonstrate that cortisol plays a pivotal, and probably direct, role in the regulation of muscle glycogen resynthesis after a bout of exhaustive exercise in rainbow trout. In metyrapone-treated fish, there was no post-exercise increase in plasma cortisol levels (Fig. 1). This was associated with a rapid decrease in glycogen phosphorylase *a* (Phos *a*) activity and rapid increase in glycogen synthase (GSase *I*) activity, compared to controls (Fig. 4A,B). These changes in enzyme activities were correlated with a rapid restoration of muscle glycogen levels, which was complete within 2 h after exercise. In contrast, glycogen restoration was only partially complete at 4 h after exercise in control fish. (Fig. 3A,B) and totally inhibited in cortisol-treated fish, even though GSase *I* activity increased in the latter. The observations suggest a direct link between continuous elevation of plasma cortisol levels and inhibition of muscle glycogenesis.

Regulation of muscle glycogen metabolism in fish is not well understood but, at least for glycogenolysis, seems to be similar to that described for other vertebrates. The two most

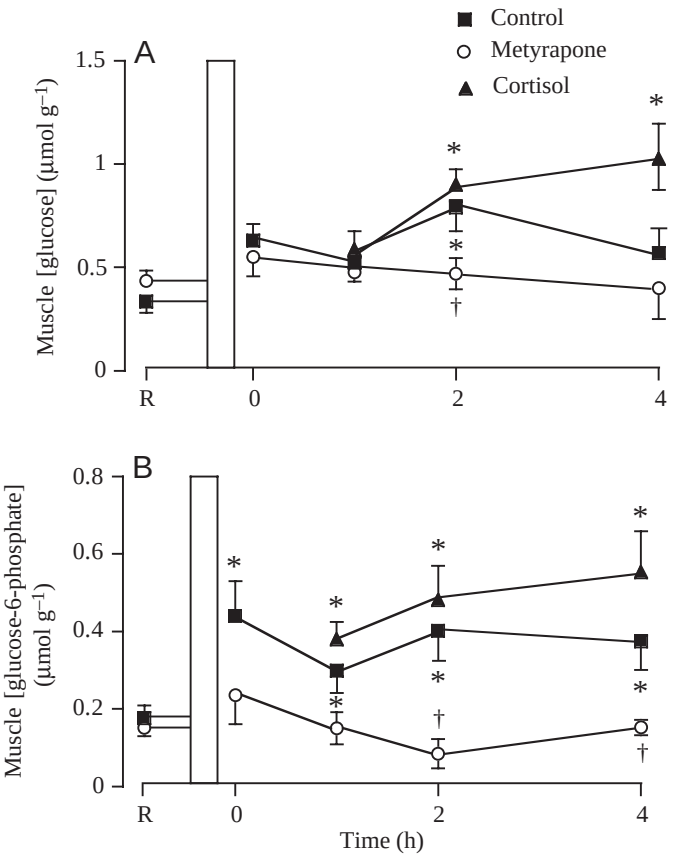

Fig. 5. Muscle glucose (A) and glucose-6-phosphate (B) concentrations in fish treated with metyrapone or saline prior to exercise or cortisol after 5 min of exercise. R, pre-exercise, post-injection value. Values are means ± 1 S.E.M. (*N*=8 for each group). *Significant difference (P<0.05) from the pre-exercise value; †significant difference (P<0.05) from the corresponding control value.

important stimuli of muscle glycogen breakdown are contractile activity and increased circulating adrenaline levels (Jensen et al., 1999). Contractile-induced increases in intracellular [Ca^{2+}] activate phosphorylase kinase, which in turn, stimulates glycogen phosphorylase activity and glycogenolysis (Yamamoto, 1968). Adrenaline stimulates glycogen phosphorylase activity and glycogenolysis *via* β-adrenergic receptors, linked to the cAMP signaling cascade (Jensen et al., 1999). A similar scenario probably occurs in trout, as adrenaline infusion into resting catfish and trout stimulates glycogen phosphorylase activity and glycogenolysis in white muscle (Ottolenghi et al., 1984; Frolow, 1999). This effect is probably mediated by cAMP-linked β-adrenergic receptors, which have recently been identified in trout white muscle (Lortie and Moon, 2003).

The exercise-induced increases in levels of adrenaline, noradrenaline and intracellular [Ca^{2+}] were all probably important to the initial stimulation of Phos *a* and resultant glycogen breakdown. By 2 h into the recovery period, however, circulating catecholamine levels had decreased in all experimental groups, but only in the control and metyrapone-treated fish did Phos *a* activity decline; it was persistently elevated in the cortisol-treated fish. Cortisol can exert a permissive effect on the actions of catecholamines, such that the adrenergic response is enhanced by glucocorticoids (e.g. Reid et al., 1992). In trout this effect is seen in liver after chronic (8–11 days) elevation of cortisol levels, and was associated with an increase in adrenergic receptor density (Reid et al., 1992). Given the short-term elevation of cortisol levels in this study (1–3 h *vs* 8–11 days), it is unlikely that the persistent elevation of Phos *a* in the cortisol-treated fish was the result of a permissive effect on adrenergic signaling. Furthermore, the fact that GSase *I* activity was increased in the cortisol-treated group also argues against any permissive effect on adrenergically mediated signaling, since catecholamines inhibit GSase activity (e.g. Moon et al., 1999). Instead, the persistent elevation of cortisol levels was likely to have been the cause of the continuous stimulation of Phos *a* activity in the cortisol-treated fish. Trout muscle does contain corticosteroid receptors, albeit at relatively low density compared to liver or gill (Chakraborti et al., 1987; Ducouret, 1996). While there is no evidence for any direct effects of cortisol on fish muscle glycogen metabolism, dexamethasone (a glucocorticoid analog) increases the activities of both Phos *a* and GSase *I* in rat hepatocyte primary culture, and stimulates glycogenolysis. These effects are independent of changes in the levels of Phos or GSase mRNA or protein synthesis, but dependent upon extracellular [Ca^{2+}], suggesting glucocorticoids may activate Phos and GSase by modifying their phosphorylation state (Baqué et al., 1986; Gomez-Muñoz et al., 1989). Current hypotheses are that glucocorticoids initiate rapid signaling *via* nongenomic mechanisms, though whether membrane-initiated steroid signaling is the proximate cause remains to be seen (for reviews, see Falkenstein et al., 2000; Lösel and Wehling, 2003). The mechanism by which glucocorticoids activate Phos in trout muscle is highly speculative, but is likely to involve maintenance of the phosphorylation state of Phos, rather than activation *via* alteration of allosteric modifiers or substrates. The method used in this study to assay Phos *a* activity only detects changes in activity due to covalent modification, not any due to allosteric modifiers or substrate concentration, because the enzyme activity is measured in a highly diluted homogenate. The increase in GSase *I* activity may be a consequence of elevated levels of glucose-6-phosphate, which can stimulate the interconversion of GSase *D* to GSase *I* by increasing the sensitivity of GSase *D* to phosphatases (Villar-Palasi, 1991; Villar-Palasi and Guinovart, 1997). Also, reduced glycogen levels themselves can lead to dephosphorylation of GSase *D*, converting it to GSase *I*. In various mammalian muscles, low glycogen levels are known to activate phosphatases and inhibit phosphorylase kinase, and thus reduce Phos *a* activity while stimulating that of GSase *I* (Alonso et al., 1995; Laurent et al., 2000). Whether a similar mechanism is operating in fish muscle remains to be seen, but that fact that there was no change in total activity of either Phos or Gsase in our studies, suggests there was no *de novo* synthesis of these enzymes in response to cortisol treatment.

As a consequence of the dual activation of Phos *a* and GSase *I*, there is no net glycogen synthesis, suggesting that cortisol may be stimulating glycogen cycling in the muscle. This certainly explains why the persistent elevation of cortisol after exercise inhibits net glycogen synthesis in trout (e.g. Pagnotta et al., 1994; Eros and Milligan, 1996; Milligan et al., 2000). The physiological significance of such a response, however, is less clear. One hypothesis suggests that since muscle glycogenolysis results in lactate production and cortisol stimulates hepatic lactate gluconeogenesis, stimulation of glycogenolysis in muscle may provide lactate for hepatic gluconeogenesis (see Mommsen et al., 1999). While lactate certainly accumulates in fish muscle after exercise (see Fig. 3B), the vast majority of it stays there (see Sharpe and Milligan, 2003), making it unavailable as a gluconeogenic substrate for other tissues. Rather, the effect of cortisol on Phos *a* in muscle may reflect its general role as an energy mobilizing hormone, keeping the muscle ‘primed’ for rapid energy mobilization *via* glycogenolysis. The simultaneous stimulation of GSase could have been an indirect consequence of the cortisol-mediated Phos *a* activation, which caused an increase in glucose-6-phophate and decrease in glycogen levels, thus further stimulating GSase *I* activity.

In summary, this study is the first report of an effect of cortisol on muscle glycogen metabolism in fish. Further, these results provide a working model to explain why cortisol elevation post-exercise is inhibitory to muscle glycogen synthesis. The stimulation of Phos *a* and GSase *I* activities in the absence of any change in total enzyme activity, in conjunction with the relatively short time frame of action (~1 h), suggest that cortisol may be exerting its effects on muscle glycogen metabolism *via* nongenomic action. Delineation of the interactions of cortisol and fish muscle

metabolism should prove a fruitful line of inquiry because since the muscle constitutes such a large portion of the whole body mass, even small metabolic changes may have a very large impact on the whole animal.

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