Hormonal regulation of glycogen metabolism in white muscle slices from rainbow trout (Oncorhynchus mykiss Walbaum)

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Frolow, Jason, and C. Louise Milligan. Hormonal regulation of glycogen metabolism in white muscle slices from rainbow trout (Oncorhynchus mykiss Walbaum). Am J Physiol Regul Integr Comp Physiol 287: R1344–R1353, 2004. First published August 19, 2004; doi:10.1152/ajpregu.00532.2003. —To test the hypothesis that cortisol and epinephrine have direct regulatory roles in muscle glycogen metabolism and to determine what those roles might be, we developed an in vitro white muscle slice preparation from rainbow trout (Oncorhynchus mykiss Walbaum). In the absence of hormones, glycogen-depleted muscle slices obtained from exercised trout were capable of significant glycogen synthesis, and the amount of glycogen synthesized was inversely correlated with the initial postexercise glycogen content. When postexercise glycogen levels were <5 μmol/g, about 4.3 μmol/g of glycogen were synthesized, but when postexercise glycogen levels were >5 μmol/g, only about 1.7 μmol/g of glycogen was synthesized. This difference in the amount of glycogen synthesized was reflected in the degree of activation of glycogen synthase. Postexercise glycogen content also influenced the response of the muscle to 10−8 M epinephrine and 10−8 M dexamethasone (a glucocorticoid analog). At high glycogen levels (>5 μmol/g), epinephrine and dexamethasone stimulated glycogen phosphorylase activity and net glycogenolysis, whereas at low (<5 μmol/g) glycogen levels, glycogenesis and activation of glycogen synthase activity prevailed. These data clearly indicate not only is trout muscle capable of in situ glycogen synthesis, but the amount of glycogen synthesized is a function of initial glycogen content. Furthermore, whereas dexamethasone and epinephrine directly stimulate muscle glycogen metabolism, the net effect is dependent on initial glycogen content.

cortisol; glycogen phosphorylase; glycogen synthase

Most of the muscle mass in teleost fish is white or “anaerobic” muscle, consisting of fast-twitch, glycolytic muscle fibers. These fibers are responsible for powering more intensive bursts of exercise and use the breakdown of glycogen (glycogenolysis) as an energy source (24, 25, 30). During high-speed swimming in trout, glycogenolysis is rapidly activated in white muscle (42) and, when exhausted, reserves of glycogen are reduced and lactate accumulates.

Whereas there is increasing evidence that muscle glycogen is resynthesized in situ with lactate as the main substrate (32, 39, 51), little is known how this process is regulated. Glycogen phosphorylase (GPase, E.C. 2.4.1.1) and glycogen synthase (GSase, E.C. 2.4.1.11) are likely to be key control points. The rate of net muscle glycogen synthesis is a function of the rate of glycogenolysis, which is regulated by a complex phosphorylation-dephosphorylation cycle. Both GPase and GSase exist in two interconvertible forms, active (a) and inactive (b) (nominally). Phosphorylation of GPase by phosphorylase kinase, which converts GPase b to GPase a, leads to an increase in activity, whereas phosphorylation of GSase, converting GSase I (active form) to GSase D (inactive form), decreases its activity (29, 32). These enzymes are regulated by hormones, including cortisol; glycogen phosphorylase; glycogen synthase

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hormones return toward resting levels that muscle glycogen resynthesis begins (38). Therefore, the hypothesis has been put forward suggesting that cortisol, alone or in conjunction with catecholamines, exerts an inhibitory effect on glycogenesis via inhibition of G6ase activity.

To test the hypothesis that cortisol and epinephrine are acting directly at the muscle to regulate glycogen metabolism, an in vitro white muscle slice preparation has been developed. In vivo studies (13, 31) have demonstrated a regulatory role of cortisol in muscle glycogen metabolism, but it is not clear whether the effect is directly at the muscle or an indirect effect. Furthermore, it is not clear whether cortisol is acting alone or in conjunction with catecholamines, in a permissive-type role (e.g., 19). With the use of an isolated white muscle tissue slice preparation, the direct effects of cortisol, epinephrine, and the two in combination at the level of the muscle can be readily observed. Because a white muscle slice preparation from rainbow trout has not been used in this context before, it was necessary to ensure that the preparation was viable and responsive to hormones. To ascertain the former, O2 consumption and metabolite levels were monitored throughout the incubation period, and hormonal responsiveness was determined by incubating the slices with glucagon, which is known to act on fish muscle (34).

MATERIALS AND METHODS

Experimental Animals

Male and female rainbow trout (Oncorhynchus mykiss Walbaum; 150–250 g) were purchased from Rainbow Springs Trout Farm (Thamesford, Ontario, Canada) throughout the year. The trout were held in a large circular tank (1,000 liters) and provided with a continuous flow of dechlorinated London, Ontario city water at 14 ± 1°C on a 12:12-h light-dark light cycle. The fish were fed daily to satiation with commercial trout pellets. Twenty-four hours before experimentation, fish were isolated in black, acrylic, 4-liter boxes continuously aerated and supplied with dechlorinated London, Ontario city water at 14 ± 1°C. Isolation of fish in individual boxes before experimentation facilitated sampling of fish at rest and transfer of individuals to the swimming tank. During this holding period fish were not fed. A total of 50 fish were used in these experiments. The University of Western Ontario Animal Use Subcommittee in accordance with the Canada Council on Animal Care guidelines approved these experiments.

Experimental Protocol

On the day of the experiment, fish were individually transferred from their holding boxes to a 300-liter circular tank and manually chased 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 (25, 30). Immediately before the tissue sampling, fish were killed with an overdose of tricaine methane sulfonate (0.1 g/l, MS-222, Syndel Labs, Vancouver, Canada) buffered to pH 7.8 with NaHCO3. Resting fish were treated in a similar manner, except they were not exercised. A block of muscle tissue (~1.5 cm3) was rapidly excised from the dorsal epaxial muscle mass and placed in ice-cold modified Cortland’s saline. The saline contained (in mM) 124 NaCl, 5.1 KCl, 1.9 MgSO4, 2.9 Na2HPO4, 11.9 NaHCO3, 1.4 MgCl2, 5 glucose, 5 l-lactate, and 10 sodium pyruvate; pH was adjusted to 7.8 with 1 mM NaOH and equilibrated with 99.5% O2-0.5% CO2 (22). The muscle block was cut along a longitudinal axis using a Stadie-Riggs microtome (Thomas Scientific; Swedesboro, NJ) into slices with an average thickness of 1.32 ± 0.02 mm (n = 21) and mass of 0.235 ± 0.011 g (n = 27).

Immediately after slices were obtained, one slice from each fish was blotted dry and frozen between aluminum blocks precooled with liquid nitrogen. This slice was used to determine the metabolic status of the muscle at the time of sampling and referred to as the “time 0 slice.” The remaining slices were incubated individually in 3.5 ml of modified Cortland’s saline (as above) in a 25-ml Erlenmeyer flask and continuously aerated with humidified 99.5% O2-0.5% CO2 for 1 h at 15°C in a shaking water bath. At the end of 1 h, tissue slices were removed, blotted dry, and freeze-clamped as described. Frozen tissue was then ground into a fine powder with a pestle and mortar cooled with liquid nitrogen, and the powder was stored at −80°C until analyzed for metabolites and enzyme activities.

Experimental Series

For each experimental trial, up to 10 muscle slices from a single fish were incubated as described, with each treatment carried out in duplicate. For each experimental series, tissue slices were obtained from 8 to 10 different fish. Therefore, in the figures, n refers to the total number of fish from which tissue slices were obtained.

Preparation viability. To determine the viability of the tissue slices over time, oxygen consumption was measured at 10-min intervals for 60 min with a Gilson respirometer (Gilson Medical Electronics; Middleton, WI) at 15°C. In addition, at time 0 and after 60 min of incubation, slices were blotted dry and freeze-clamped for measurement of lactate, ATP, phosphocreatine (PCr), and glycogen content.

Effects of glucagon, epinephrine, cortisol, and dexamethasone on muscle glycogen metabolism. Tissue slices obtained from fish either at rest or immediately following exercise were incubated in modified Cortland’s saline containing a final concentration of either 10−8 M epinephrine bitartrate, 10−8 M dexamethasone-21-phosphate, 10−8 M bovine glucagon (Sigma Chemical; Mississauga, ON, Canada), or 10−8 M cortisol (hydrocortisone, gift of Dr. J. P. Wiebe, Dept. of Biology, University of Western Ontario) dissolved in 2% ethanol. Control slices were incubated in saline or saline containing 2% ethanol. Slices incubated in 2% ethanol were not different from those incubated in saline alone, thus all control values were pooled.

At the end of 60 min, the slices were removed, blotted to remove excess saline, and frozen between aluminum blocks precooled with liquid nitrogen and stored at −80°C until later analyzed for glycogen, G6ase, and GPase activities.

Analytic Techniques

Muscle glycogen content was assayed by isolating the glycogen (20) and measuring the free glucose after digestion of the glycogen with amyloglucosidase (Worthington Biochemical; Lakewood, NJ; 4). In our laboratory, we typically recover 95–100% of the glycogen with this method. Muscle concentrations of lactate, ATP, and PCr were measured in ~100 mg of muscle ground to a fine powder in a liquid N2-cooled mortar and vigorously resuspended in 1 ml 8% HClO4. Lactate, ATP, and PCr were then measured in the neutralized supernatant (4).

The relative activities of GPase were measured spectrophotometrically by using a modification of the method outlined by Storey (47). GPase exists in two forms: the more active a and the less active b (47). To determine the amount of GPase a, 40–50 mg of powdered tissue were homogenized on ice for 3 × 10–s bursts with a Tissue Tearer (Bisspec Products; Bartlesville, OK) set to speed 6. The homogenization buffer contained (in mM) 50 imidazole HCl (pH 7.0), 100 NaF, 5 EGTA, 2 EDTA, 30 2-mercaptoethanol, and 0.1 phenylmethylsulfonylfluoride. Aliquots of 5 μl were then added to wells in a microplate reader containing 245 μl of assay buffer consisting of 50 mM potassium phosphate (pH 7.0), 2 mg/ml glycogen (oyster muscle; dialedylated), 0.4 mM NAD, 10 μM glucose 1,6 bisphosphate, 0.25 mM EDTA, 15 mM MgCl2, 0.5 U glucose-6-phosphate dehydrogenase (Boehringer-Mannheim Chemical, Laval, PQ, Canada), and 1.4 units phosphoglucomutase (Worthington Biochemical). The wells were
mixed and allowed to stand for 5 min at 20°C, and then absorbance at 340 nm was measured for 5 min with a Spectromax 340 PC microplate reader. Total activity $a + b$ was measured by adding 4 μl of 5'-AMP to bring the well to a final concentration of 1.6 mM 5'-AMP, and the absorbance was measured for 5 min at 20°C. One unit of enzyme activity is defined as the amount of enzyme that produces 1 μmol of product per minute at 20°C. GPase activity is expressed as the ratio of $(a + b) \times 100$.

GSase also exists in two forms, the more active $I$ and the less active $D$, and their activities were measured by the method of Schallin-Jantti et al. (44). The assay couples the conversion of UDP-glucose (UDP-G) to UDP and glycogen with the oxidation of NADH catalyzed by two enzymatic steps that involve pyruvate kinase and lactate dehydrogenase. To determine GSase activity, 40–50 mg of frozen, powdered tissue were suspended in buffer containing (in mM) 50 KF, 50 Tris (pH 7.8), 0.5 dithiothretiol, 1.0 EDTA, and 2.0 MgCl₂. The homogenate was mixed vigorously (2 min on a vortex mixer set to maximum speed) and briefly centrifuged (1 min at 10,000 g), and the supernatant was assayed for GSase activity. GSase activity measured in samples processed this way was less variable and more consistent with previously published values compared with tissues processed with a mechanical tissue homogenizer. A 100-μl aliquot of the homogenate was added to 820 μl of assay mixture containing 70 mM KCl, 50 mM Tris (pH 7.8), 7 mM MgCl₂, 10 mM glycogen, 5 mM phosphoenolpyruvate 0.42 mM NADH, 50 units pyruvate kinase, and 25 units lactate dehydrogenase (both enzymes from Worthington Biochemical). Activity was measured in either the presence or absence of glucose-6-phosphate (GSase $I$ activity), 0.1 mM (GSase fractional activity), or 10 mM (GSase $I + D$ activity) glucose-6-phosphate. To start the reaction, UDP-G was added to a final concentration of 20 mM. Absorbance at 340 nm was measured at 20°C for 20 min with a Shimadzu UV-160 recording spectrophotometer. GSase activity is expressed as the ratio of $[I]/[I + D] \times 100$.

Unless otherwise stated, all biochemicals were purchased from Sigma Chemical. All other reagents were acquired from local suppliers and were of the highest available purity.

Statistical Analyses

Values presented are means ± SE ($n$). A repeated measures ANOVA was performed on the oxygen consumption data to assess any differences through time. A series of one-way ANOVAs was used for comparison between treatments, followed by a Tukey-Kramer honest significant difference (HSD) analysis. All statistical analyses were done using the software JMP (SAS Institute, Cary, NC), and significant differences were accepted when $P < 0.05$.

RESULTS

Tissue Viability

Tissue slices obtained from both resting and exercised fish remained viable for the entire 60-min incubation period, as indicated by the maintenance of oxygen consumption rates (Fig. 1) and volatile metabolites such as ATP and PCr (Fig. 2). Those slices taken from exercised fish were capable of enhanced oxygen consumption compared with slices from resting fish (Fig. 1) for the first 40 min of incubation. From 40 min onward, the oxygen consumption of muscle slices from exercised fish was not different from the slices obtained from fish at rest. A second indicator of the viability of the muscle slices is seen in Fig. 2, which demonstrates that not only are ATP, PCr, glycogen, and lactate levels unchanged over the 60-min period in slices from resting fish (Fig. 2A), but more importantly, these metabolites, which were altered as a result of exercise, show recovery (Fig. 2B). Glycogen, ATP, and PCr are all lower at time 0 than at 60 min in exercised fish, whereas lactate levels are reduced at 60 min compared with time 0 levels. This indicates that the muscle slice is a metabolically viable preparation and that trout white muscle is capable of significant glycogenolysis.

Hormonal Influences on Muscle Glycogen Metabolism

In the absence of any hormonal stimulation, muscle glycogen levels in muscle slices obtained from resting fish did not change over the 60-min incubation period. Incubation of slices in 10⁻⁸ M bovine glucagon stimulated glycogenolysis, reducing glycogen by ~50% (Fig. 3A). Tissue slices obtained from fish postexercise synthesized glycogen in the absence of any hormone (control), with a net synthesis of about 3.4 μmol/g (Fig. 3B). Incubation of muscle slices from exercised fish with 10⁻⁸ M glucagon inhibited glycogen synthesis compared with control (i.e., no treatment).

In slices obtained from resting fish in which glycogen levels at time 0 were between 7 and 10 μmol/g, 10⁻⁸ M dexamethasone-21-phosphate, 10⁻⁸ M cortisol, and 10⁻⁸ M epinephrine all stimulated net glycogenolysis, with an average decline in glycogen of about 55–60% (Fig. 4A). There was no further stimulation of glycogenolysis in muscle slices incubated with 10⁻⁸ M dexamethasone and 10⁻⁸ M epinephrine in combination. Coincident with the stimulation of glycogenolysis was a significant threefold increase in the proportion of GPase in the active form (Fig. 4B) and a reduction in the active proportion of GSase (Fig. 4C). The maximal total activities of GPase (0.71 ± 0.05 U/g wet tissue, $n = 8$) and GSase (1.19 ± 0.07, U/g wet tissue, $n = 8$) were unaffected by any treatment.

The response of muscle slices isolated from exercised fish differed from that of resting fish and appears to be related to endogenous glycogen levels at time 0. Even though all slices were obtained from fish that had been exercised, the extent of glycogen depletion varied; such that at time 0, muscle slices from some fish had glycogen levels of 5–7 μmol/g, whereas those from other fish were 1–4 μmol/g. The variation in postexercise glycogen levels among the fish may reflect dif-
ferences in how well they exercised. On the basis of observations during the swimming period, some fish simply did not swim as long or with as much vigor as did others. Nonetheless, it became immediately obvious from the data that the responses of muscle glycogen metabolism to the various treatments were dependent on the initial (i.e., time 0) glycogen level (Figs. 5, 6, and 7). In all muscle slices from exercised fish, in the absence of any hormonal stimulation, there was net glycogen synthesis, a reduction in relative GPase activity (Figs. 5B and 6B), and increase in relative GSase activity (Figs. 5C and 6C). However, the total amount of glycogen synthesized and the relative stimulation of GSase activity over the 60-min period was greater in the fish where time 0 glycogen was compared with muscle slices with >5 μmol/g (high glycogen). This was also reflected in the relative stimulation of GSase activity. In the low-glycogen muscle slices, fractional GSase activity increased to ~50% compared with ~35% in the high-glycogen muscle slices (Fig. 5C vs. Fig. 6C; Fig. 7, A vs. C).

Muscle slices with postexercise glycogen levels greater than 5 μmol/g responded to hormonal stimulation in much the same manner as did slices from resting fish (Fig. 5A vs. Fig. 4A). Dexamethasone, cortisol, epinephrine, and epinephrine plus dexamethasone in combination all stimulated net glycogenolysis and the relative activity of GPase (Fig. 5B and Fig. 7D) and inhibited GSase activity, relative to the control (no hormone) slices (Fig. 5C). However, when glycogen levels were <5 μmol/g at time 0, glycogenesis and the relative activity of GSase were stimulated in all slices (Fig. 6 and Fig. 7A). In the low-glycogen slices, hormonally stimulated glycogenesis was about 2.5 times greater than in the absence of hormonal

Fig. 2. Levels of ATP, phosphocreatine (PCr), glycogen (expressed as μmol glucose/g), and lactate in white muscle slices taken from rainbow trout either at rest (A; n = 8 fish) or after exhaustive exercise (B; n = 8 fish). Time 0 (open bars) represents immediately after the slice was obtained and the start of the 60-min incubation period. The solid bars are values at the end of the 60-min incubation period. Values are means ± SE. *Significant difference from corresponding time 0 value.
stimulation, and GSase activity increased by about another 15% (Fig. 7A). The total activities of GSase (1.13 ± 0.08 U/g; n = 23) and GPase (0.74 ± 0.06 U/g; n = 23) were not different in any treatment.

The influence of time 0 (or initial) glycogen content on the response of muscle GSase and GPase to hormonal stimulation is best illustrated in Fig. 7, in which the relative GSase (Fig. 7, A and C) and GPase (Fig. 7, B and D) activities are plotted against the corresponding initial glycogen levels for each individual tissue slice. The relationship between relative GSase (Fig. 7, A and C) or GPase activities (Fig. 7, B and D) and initial glycogen content, as defined solely by time 0 and the control values (no hormone treatment; dashed lines) did not differ between those slices with low or high glycogen content. However, when the treatment groups are included (Fig. 7, solid lines), the relationship between GSase and glycogen is not altered in the low-glycogen (<5 μmol/g) group (Fig. 7A), rather it is simply extended. In contrast, in the tissue slices with time 0 glycogen levels >5 μmol/g, the relationship changes significantly (slopes of the two lines are significantly different, P < 0.01). Similarly, when defined solely by the time 0 and control data, the effect of glycogen levels on the relative activity of GPase was independent of initial glycogen content. The lines defining this relationship are not different between slices with low and high glycogen content (Fig. 7, B and D; dashed lines). Inclusion of the treatment groups (Fig. 7, B and D; solid lines) altered the relationship between GPase activity and glycogen content compared with that defined by the time 0 and controls (Fig. 7, B and D; dashed lines significantly

Fig. 4. Glycogen (A) and the relative percent activities of glycogen phosphorylase (B) and glycogen synthase (C) in muscle slices obtained from resting fish. Time 0 refers to values at the time of sampling. Slices were incubated for 60 min in either modified Cortland’s saline alone (stippled bar), 10⁻⁸ M dexamethasone (bold hatched bar), 10⁻⁸ M epinephrine (solid bar), 10⁻⁸ M cortisol (light hatched bar), or 10⁻⁸ M epinephrine plus 10⁻⁸ M dexamethasone (crosshatched bar). Values are means ± SE; n = 10 fish for all treatments. Columns with different letters are significantly different (P < 0.05).

Fig. 5. Glycogen (A) and the relative percent activities of glycogen phosphorylase (B) and glycogen synthase (C) in muscle slices obtained from exercised fish in which time 0 muscle glycogen was >5 μmol/g. Time 0 refers to values at the time of sampling, which was immediately after exercise. Slices were incubated for 60 min in either modified Cortland’s saline alone (stippled bar), 10⁻⁸ M dexamethasone (bold hatched bar), 10⁻⁸ M epinephrine (solid bar), 10⁻⁸ M cortisol (light hatched bar), or 10⁻⁸ M epinephrine plus 10⁻⁸ M dexamethasone (crosshatched bar). Values are means ± SE; n = 5 fish for all treatments. Columns with different letters are significantly different (P < 0.05).
more, and most importantly from the point of view from this study, muscle slices were responsive to hormonal stimulation, as indicated by the increase in glycogenesis in response to glucagon (Fig. 3A).

Resynthesis of Muscle Glycogen After Exercise

Glycogen-depleting contractile activity, in the absence of any hormones, stimulated glycogen synthase activity and net glycogenesis, with the amount of glycogen synthesized and the relative increase in glycogen synthase activity dependent on the glycogen levels at time 0 (Fig. 7, A and C). The variation in postexercise muscle glycogen levels most likely reflects a difference in the amount depleted, because it was observed during the exercise protocol that some fish did not exercise to the same extent as others. Such individual variation in swimming performance is not unusual in domestic rainbow trout (e.g., 25). Muscle slices with an average postexercise glycogen level of 2.8 μmol/g synthesized, on average, 3.4 μmol/g of glycogen (Figs. 3 and 6A) compared with only 1.3 μmol/g synthesized when postexercise glycogen was higher, 6.5 μmol/g (Fig. 5A). The relative activity of GSase 1 was significantly correlated with initial glycogen content; the lower the postexercise glycogen, the greater the relative activity of GSase, the greater the amount of glycogen synthesized (Fig. 7, A and C). The relative activity of GPase in the absence of hormonal stimulation was significantly correlated with glycogen content, but the relationship between GPase activity and glycogen levels was independent of initial glycogen content. These data suggest that in trout muscle, endogenous glycogen regulates its own synthesis via alterations in the relative activities of GSase, such that at lower glycogen contents, there is greater synthesis.

Glycogen autoregulation, the ability of glycogen to regulate its own synthesis, has been previously observed in human and rat muscle (5, 21, 33, 36) and is thought to be due to a combination of glycogen feedback inhibition on glucose transport, hexokinase activity, and GSase activity (e.g., 15, 27, 41). Glucose transport and hexokinase activities are probably not important regulatory sites for glycogen synthesis in trout white muscle because, even though muscle does have glucose transporters, (9, 28, 48), glucose is only poorly utilized by muscle (e.g., 39, 40, 51) and hexokinase activity is extremely low (26, 35, 47). Rather, the major regulatory site for trout white muscle glycogenesis is most likely glycogen synthase.

There are several factors that can regulate GSase activity, including covalent modification (via phosphorylation-dephosphorylation) and allosteric regulation (e.g., changes in glucose-6-phosphate, P_i, ATP, and AMP; for reviews, see Refs. 1, 11, 23). Allosteric effects on GSase activity could not be detected by the assay methods used in the present study, so only covalent modifications (i.e., phosphorylation-dephosphorylation) could cause the observed changes in GSase activity. How the actual phosphorylation state of GSase is regulated in fish muscle remains to be determined.

In mammalian skeletal muscle, the relative activity of GSase is a function of its phosphorylation state; with dephosphorylation activating GSase, thus promoting glycogen synthesis. Mammalian muscle GSase has multiple phosphorylation sites, which are dephosphorylated in a hierarchical manner (1, 43), setting the stage for differential regulation of GSase activity. In

**DISCUSSION**

**Preparation Viability**

The muscle slice preparation is a viable and useful system in which to study the regulation of glycogen metabolism in trout muscle. Evidence of viability includes the observations that slices from resting fish maintained oxygen consumption at a steady rate and slices from exercised fish showed an enhanced oxygen consumption, which probably reflects the considerable synthetic activity occurring in these slices. Exercise reduced ATP, PCR, and glycogen levels and elevated lactate levels in muscle, and within 60 min of incubation, all had returned to levels typical of a resting fish (e.g., Fig. 2, B vs. A). Furthermore, columns with different letters are significantly different (P < 0.05). Different from solid lines) but was independent of initial glycogen content (Fig. 7, B and D; solid lines are not different).

Fig. 6. Glycogen (A) and the relative percent activities of glycogen phosphorylase (B) and glycogen synthase (C) in muscle slices obtained from exercised fish in which time 0 muscle glycogen was <5 μmol/g. Time 0 refers to values at the time of sampling, which was immediately after exercise. Slices were incubated for 60 min in either modified Cortland’s saline alone (stippled bar), 10^{-8} M dexamethasone (bold hatched bar), 10^{-8} M epinephrine (solid bar), 10^{-8} M cortisol (light hatched bar), or 10^{-8} M epinephrine plus 10^{-8} M dexamethasone (crosshatched bar). Values are means ± SE; n = 5 fish for all treatments. Columns with different letters are significantly different (P < 0.05).
mammalian skeletal muscle, glucose-6-phosphate can increase the relative activity of GSase by binding to it and exposing phosphorylated sites to protein phosphatase action (49). However, the influence of glucose-6-phosphate on GSase activity varies depending on the extent of glycogen depletion. For example, in glycogen-depleted human muscle cells in culture, the initial insulin-independent phase of glycogenesis is a function of the amount of glycogen depletion rather than the level of glucose-6-phosphate (33). In muscle cells overexpressing GPase activity, glycogen depletion was greater than in controls when incubated in glucose-free media and showed greater stimulation of GSase activity despite similar glucose-6-phosphate levels (33). Thus the argument has been made that the rapid, insulin-independent phase of glycogenesis commonly seen in mammalian skeletal muscle is due to the stimulating effect of glycogen-depletion on GSase activity (6, 33). A similar mechanism may be operating in glycogen-depleted trout muscle, because in vivo there is a significant increase in GSase activity in the absence of changes in glucose-6-phosphate levels (31). It has been suggested that because GSase and GSphatase are bound together in a glycogen-glycogenin protein complex, depletion of glycogen may increase exposure of the phosphorylated site of GSase to phosphatase activity, an effect that may be enhanced by glucose-6-phosphate (e.g., 23). Certainly the data from the present study (Fig. 7) are consistent with the notion that glycogen depletion per se is a driving force for glycogenesis, because the greater the exercise-induced glycogen depletion, the greater the relative activity of GSase and amount of glycogen synthesized.

Hormonal Regulation of Muscle Glycogen Resynthesis

Both glycogenesis and glycogenolysis were responsive to hormonal stimulation. In slices from resting fish, glucagon stimulated net glycogenolysis (Fig. 3A) but did not further stimulate glycogenolysis in slices from exercised fish with already depressed glycogen levels (Fig. 3B). Rather, in slices from exercised fish, glucagon inhibited net glycogenesis (compare glucagon treated with control, Fig. 3B). This response of muscle to glucagon is consistent with what is known about the physiological role of glucagon in fish, which is primarily that of a hyperglycemic hormone (34). This inhibition of net glycogenesis by glucagon in muscle from exercised fish may reflect glycogen cycling. Whereas GSase activity may increase because of glycogen depletion (see Resynthesis of Muscle Glycogen After Exercise), the presence of glucagon would continue the activation of GPase, thus a situation may arise in which any glycogen synthesized is rapidly broken down. Evidence for such glycogen cycling in fish muscle comes from the study of Pagnotta and Milligan (39), in which muscle incorporated radiolabeled carbon from the blood into glycogen in the absence of any net synthesis.

The most interesting and novel finding of this study is the “dual” effect of epinephrine and cortisol on muscle glycogen metabolism. The response of muscle glycogen metabolism to epinephrine, cortisol, and its analog dexamethasone-21-phosphate was dependent on initial glycogen levels (Figs. 4–7). In muscle slices from resting fish in which glycogen levels are “high,” the predominant response to epinephrine, cortisol, and dexamethasone-21-phosphate was stimulation of GPase a activity and net glycogenolysis. This is the expected response of muscle to at least epinephrine, which probably stimulates glycogenolysis via a adrenergic activation of the cAMP-signal- ing pathway (29). The mechanism underlying the stimulatory effect of cortisol on muscle glycogenolysis is less well understood but could be due to a direct effect of cortisol on GPase a activity, because there was no effect on total GPase activity. Cortisol has been shown to activate GPase a activity in rat hepatocytes in the absence of protein synthesis or any change in total GPase activity, arguing for a direct, non-
epinephrine can stimulate muscle glycogenesis. In both red and white muscle isolated from exercised lizards (Dipsosaurus dorsalis; 17), epinephrine stimulated lactate, but not glucose, incorporation into the muscle glycogen pool. The physiological significance of this response may be to accelerate lactate clearance postexercise when both blood lactate and epinephrine levels are elevated, although the underlying mechanism is not at all clear. Epinephrine usually stimulates net glycogenolysis in skeletal muscle and would be expected to inhibit net glycogenesis. This effect is in fact observed in insulin-stimulated glycogenesis in rat skeletal muscle, but surprisingly, epinephrine did not inhibit glycogenesis stimulated by glycogen-depleting contractile activity (16). As might be expected from the effects on net glycogenesis, epinephrine decreased the relative activity of GSase I in insulin-stimulated muscles but increased it in contracted (or exercised) muscles. It was reported (12) some 40 years ago that muscle contraction can reduce the inhibitory effect of epinephrine on glycogen synthesis, although the mechanism is still unresolved.

Given that the relative activity of GSase was greater in those muscle slices with lowest initial glycogen levels (Fig. 7A), there was likely a difference in the degree of phosphorylation at low vs. higher glycogen levels. This differential effect of epinephrine on glycogen metabolism at high and low glycogen content may be influenced by the fact that more (and therefore different) sites of GSase are dephosphorylated, though the nature of the influence is not clear. Nonetheless, these data are consistent with the suggestion that glycogen depletion is a major driving force for glycogenesis.

**Perspectives**

The results of this study clearly demonstrate that muscle is capable of in situ glycogen synthesis, and that the amount of glycogen synthesized is inversely correlated to initial glycogen content and can be modified by epinephrine and cortisol. Glycogenesis in trout muscle in vitro is not a novel observation, because Wang et al. (50) reported significant postexercise glycogen synthesis in a perfused trunk preparation within the first 30 min after exercise. Whereas the absolute amount of glycogen synthesized was greater in the perfused trunk (~30.5 μmol glucose units/g in 30 min) than in the muscle slices (2–4 μmol glucose units/g in 60 min), glycogenesis was greater in both preparations than in trout muscle in vivo (<0.5 μmol glucose units/g in 60 min; 13, 38). Typically, in vivo, there is no net glycogen synthesis within the first hour postexercise (30), whereas in vitro, glycogen levels returned to levels typical of resting fish within the hour if postexercise glycogen levels were “low.” The differences between in vitro and in vivo glycogenic rates cannot be attributed to differences in postexercise glycogen content alone, because at similarly low postexercise glycogen levels (<5 μmol glucose units/g) there is no net glycogenesis in vivo (30). It has been proposed that in vivo, exercise-induced elevations in plasma cortisol may be inhibitory to muscle glycogenesis (13, 38). In the perfused trout trunk, it was suggested that it is because of the absence of cortisol (and perhaps other hormonal stimuli) that glycogenesis proceeds at a greater rate in vitro than in vivo (51). It is here, however, where inconsistencies between the data of the present study and in vivo observations emerge. In vitro, cortisol elevation stimulates glycogenesis when postexercise glycogen levels
were low (<5 μmol glucose units/g), and it stimulates glycogenolysis at higher glycogen levels. In the presence of cortisol in vivo, even when postexercise glycogen levels are <5 μmol glucose units/g, there is no net glycogen synthesis (13, 38). It appears that in vivo, cortisol inhibits glycogenesis, an effect that does not appear to be overridden by the extent of glycogen depletion. This apparent conflict in the role of cortisol in vivo compared with in vitro indicates that regulation of glycogen metabolism is, indeed, complex. Nonetheless, these data indicate that epinephrine and cortisol directly affect muscle glycogen metabolism and that, depending on the extent of glycogen depletion, the nature of the response varies.

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