Contraction-mediated inactivation of glycogen synthase is accompanied by inactivation of glycogen synthase phosphatase in human skeletal muscle

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Activities of glycogen synthase (GS) and GS phosphatase were determined on human muscle biopsies before and after isometric contraction at $\frac{2}{3}$ maximal voluntary force. Total GS activity did not change during contraction (4.92 ± 0.70 at rest versus 5.00 ± 0.42 mmol/min per kg dry wt.; mean $\pm$ S.E.M.), whereas both the active form of GS and the ratio of active form to total GS decreased by $\sim 35\%$ ($P < 0.01$). GS phosphatase was inactivated in all subjects by an average of 39%, from 5.95 ± 1.30 to 3.63 ± 0.97 mmol/min per kg dry wt. ($P < 0.01$). It is suggested that at least part of the contraction-induced inactivation of GS is due to an inactivation of GS phosphatase.

INTRODUCTION

During intense muscle contraction, glycogen metabolism is dominated by the process of glycogenolysis. To increase the efficiency of this process, it would be desirable to decrease the rate of glycogenesis. Indeed, almost 20 years ago it was found that the activity of the form of glycogen synthase (GS) which is independent of the presence of glucose 6-phosphate, a rate-limiting enzyme for glycogenesis, was decreased after repeated tetanic stimulation of rat skeletal muscle (Staneloni & Piras, 1969). More recently, it has been demonstrated that short-term strenuous muscle contraction in humans also results in an inactivation of GS, and, in contrast with the findings of Staneloni & Piras (1969), prior contraction was not required to observe this phenomenon (Chasiotis *et al.*, 1982, 1983). However, the mechanism for the contraction-mediated inactivation is not known.

GS is generally considered to be active in its dephosphorylated state, and the enzyme responsible for dephosphorylation is GS phosphatase (Nuttall *et al.*, 1976). We therefore determined whether the contraction-induced inactivation of GS is associated with an inactivation of GS phosphatase.

MATERIALS AND METHODS

Subjects

Five healthy men, whose mean (range) age, weight and height were 27 years (21–35), 78 kg (61–93) and 179 cm (168–188) respectively, participated in the study. The subjects were informed about the possible risks of the study before giving their voluntary consent. The experimental protocol was approved by the Arizona State University Human Subjects Research Review Committee.

Experimental design

Isometric maximal voluntary contraction (MVC) force of the left knee extensor muscles was assessed before the experiment (on a separate day) by using a Cybex dynamometer (Lumex, NY, U.S.A.), as previously described (Sahlin & Henriksson, 1984). On the day of the experiment, the subjects reported to the laboratory approx. 2 h after a meal. Staneloni & Piras (1969) suggested that there may be no advantage in diminishing GS activity during contraction if the activity at rest is already low. Consequently, we tried to elevate GS activity by a meal-induced release of insulin. The subjects assumed the supine position for 10–15 min, after which incisions at biopsy sites (35% of the distance from the superior margin of the patella to the anterior superior iliac spine) over the lateral aspects of both quadriceps femoris muscles were made. A pre-exercise biopsy (Bergström, 1962) was then obtained from the right thigh.

The subjects were then seated in the Cybex unit and had a tourniquet placed loosely around the left thigh, which was to be the contracting one. The tourniquet was used to ensure that the blood flow to the muscle remained occluded between the end of each contraction and the post-exercise biopsy, to prevent any aerobic resynthesis of high-energy phosphates or recovery of any other oxygen-dependent processes (Harris *et al.*, 1981). Subjects then performed an isometric contraction with the knee extensor muscles at $\frac{2}{3}$ MVC force. At this intensity, blood flow to the thigh is completely arrested (Edwards *et al.*, 1972) and the muscle becomes anoxic within the first few seconds of contraction (Henriksson *et al.*, 1986). The tourniquet was inflated to 250 mmHg after 15 s of contraction, and after 20 s the second biopsy was taken. The subjects then resumed the contraction at the same force as in the first contraction to fatigue, with the tourniquet being deflated immediately after resuming the

Abbreviation used: GS, glycogen synthase.

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second contraction. When the subjects demonstrated difficulty in maintaining the predetermined force, the tourniquet was rapidly inflated to 250 mmHg and the subjects were encouraged to continue exercise until fatigue. The third biopsy was taken at fatigue and the tourniquet was deflated. The time between the end of each contraction and freezing of the biopsy sample averaged 13 s (range 9–18). The biopsies were rapidly plunged (< 2 s after incision) into liquid Freon, maintained at its freezing point (-150°C) with liquid N_2 .

The time between the end of the first contraction and the beginning of the second contraction averaged 22 s (range 20–26). It should be noted that during this period under the present conditions the metabolites (high-energy phosphates and glycogenolytic intermediates) remain at their steady-state concentrations (Harris *et al.*, 1981).

Analytical methods and calculations

The biopsies were freeze-dried, dissected free from blood, fat and all visible connective tissue, and powdered. The powder was thoroughly mixed and stored at -70°C until analysis.

For measurement of GS activity, the method of Guinovart *et al.* (1979), using filter paper (Thomas *et al.*, 1968), was used. Briefly, powder was homogenized with a buffer (200 μl /mg dry wt.) containing 30% (v/v) glycerol, 10 mM-EDTA and 50 mM-KF, pH 7, with a ground-glass homogenizer at 4°C . The homogenate was centrifuged at 10000 *g* for 20 min at 4°C . The supernatant was diluted with a buffer containing 50 mM-Tris, 20 mM-EDTA and 130 mM-KF, pH 7.8, at 4°C , and then used for assay of GS. The active form of GS was assayed at low glucose 6-phosphate concentration (0.17 mM) (GS_{low}), whereas total GS (GS_{tot}) was assayed at high glucose 6-phosphate concentration (7.2 mM). UDP-glucose concentration was 0.14 mM. Activities are expressed as mmol of glucosyl units from UDP- ^{14}C glucose incorporated into glycogen/min per kg dry wt. at 30°C . The fractional activity was calculated as $\text{GS}_{\text{low}}/\text{GS}_{\text{tot}}$.

GS phosphatase was assayed by the method of Miller (1979), as modified by Okubo *et al.* (1988). Briefly, commercial rabbit GS D (Sigma, G2259) was purified as described by Okubo *et al.* (1988), and was $\sim 85\%$ glucose 6-phosphate-dependent (i.e. D-form) when assayed in the absence and presence of 7.2 mM-glucose 6-phosphate and in the presence of 7 mM-UDP-glucose. Muscle powder was homogenized with a buffer (133 μl /mg dry wt.) containing 50 mM-Tris, 10 mM-EDTA and 50 mM-2-mercaptoethanol, pH 7.8, at 4°C . The homogenate was centrifuged at 10000 *g* for 20 min at 4°C . Supernatant (100 μl) was incubated in homogenization buffer, containing 25 munits of purified rabbit GS D (30 μl) which was preincubated with 0.3% glycogen, 10 mM-Tris, 1 mM-EDTA and 5 mM-dithiothreitol for 20 min at 30°C , in a final volume of 175 μl for 20 min at 30°C . The reaction was stopped by diluting 25 μl of the incubation mixture with 80 vol. of 130 mM-KF/50 mM-Tris/2 mM-EDTA, pH 7.8 at 4°C . GS phosphatase activity was determined from the change in GS_{low} per unit time, i.e. mmol of glucosyl units from UDP- ^{14}C glucose incorporated into glycogen/min per kg dry wt. The assays were linear with time. Protein in the supernatant fractions of the homogenates was measured by the Bradford (1976) method.

Because GS phosphatase activity has, to our knowledge, never been reported in freeze-dried muscle, pre-

liminary tests were undertaken to determine the effect of freeze-drying on the phosphatase activity. Biopsies from six humans and muscle from one rat were each divided into two pieces and frozen in liquid N_2 . One piece was freeze-dried and powdered. GS phosphatase activity was measured on both pieces, and averaged 5.67 ± 0.84 mmol/min per kg dry wt. for freeze-dried and 1.41 ± 0.22 mmol/min per kg wet wt. for frozen wet muscle. When corrected for water content (77% of wet muscle is water), the mean value for wet muscle becomes 6.11 mmol/min per kg dry wt., i.e. about 8% higher than in the freeze-dried muscle, but the difference was not statistically significant ($P > 0.05$). We conclude that freeze-drying has no major effect on GS phosphatase activity. The inter-assay (day-to-day) variation with rat dry muscle was 7% ($n = 7$).

To correct for variability in non-muscle constituents between biopsies, the enzyme activities were divided by the protein contents and then multiplied by the mean protein content for the whole material to express activities per unit weight of muscle. The soluble protein contents averaged 388 ± 31 g/kg dry wt. for the GS supernatants and 204 ± 11 for the GS phosphatase supernatants. Contraction did not affect the protein contents (390 ± 51 g/kg dry wt. at rest versus 386 ± 43 after contraction for GS supernatants, $P > 0.05$; 190 ± 22 at rest versus 215 ± 11 for the GS phosphatase supernatants, $P > 0.05$).

Statistics

Significant ($P < 0.05$) changes were determined with Student's paired *t* test. Data are expressed as means $\pm$ S.E.M. unless otherwise indicated.

RESULTS

Total GS activity averaged 4.92 ± 0.70 and 5.00 ± 0.42 mmol/min per kg dry wt. at rest and after contraction respectively ($P > 0.05$; Table 1). GS_{low} decreased in all subjects from 3.28 ± 0.53 at rest to 2.19 ± 0.28 mmol/min per kg dry wt. after contraction ($P < 0.01$). Similarly, fractional activity decreased in all subjects from 0.69 ± 0.08 at rest to 0.45 ± 0.06 after contraction ($P < 0.01$). The fractional activities are higher than those generally observed in humans fasted overnight. This is probably because a meal was ingested before the experiment, and possibly because of the nature of the GS_{low} assay (low instead of no glucose 6-phosphate).

GS phosphatase activity decreased in all subjects, from 5.95 ± 1.30 at rest to 3.63 ± 0.97 mmol/min per kg dry wt. after contraction ($P < 0.01$; Table 2). Moreover, the decrease in GS phosphatase was positively related to the decrease in GS_{low} ($r = 0.62$; $n = 7$). However, this relationship did not reach statistical significance ($P > 0.05$), possibly owing to the limited number of observations.

DISCUSSION

The present findings demonstrate that short-term intense contraction results in a marked decrease in GS which is active at low glucose 6-phosphate concentration, without change in total GS activity, which is in accordance with previous findings (Chasiotis *et al.*, 1982, 1983; Staneloni & Piras, 1969). Moreover, this decrease does not need to be associated with an interconversion of phosphorylase *b* into *a* (see Staneloni & Piras, 1969), since

Table 1. Effect of isometric contraction on muscle glycogen synthase activity at 30 °C

Values are given in mmol/min per kg dry wt. Contraction duration to fatigue averaged 64.0 ± 4.7 s. A 50 μ l portion of diluted supernatant was incubated in a final volume of 75 μ l in a buffer containing 50 mM-Tris, 20 mM-EDTA, 130 mM-KF and 0.14 mM-UDP-glucose, pH 7.8, with either 0.17 mM-glucose 6-phosphate (GS_{low}) or 7.2 mM-glucose 6-phosphate (GS_{tot}) for 20 min.

Subject	GS _{low}			GS _{tot}			GS _{low} /GS _{tot}		
	Rest	20 s	Fatigue	Rest	20 s	Fatigue	Rest	20 s	Fatigue
1	2.60	2.03	1.65	3.00	3.78	3.60	0.87	0.54	0.46
2	2.73	—	2.45	3.90	—	5.08	0.70	—	0.48
3	2.90	1.68	1.68	6.53	6.58	6.23	0.44	0.25	0.27
4	5.40	3.75	—	6.45	5.05	—	0.84	0.74	—
5	2.78	—	2.10	4.70	—	4.68	0.59	—	0.45

Table 2. Effect of isometric contraction on muscle GS phosphatase activity at 30 °C

Values are given in mmol/min per kg dry wt. A 100 μ l portion of supernatant was incubated in a final volume of 175 μ l in a buffer containing 50 mM-Tris, 10 mM-EDTA, 50 mM-2-mercaptoethanol and 25 munits of preincubated GS D, pH 7.8, for 20 min. Phosphatase activity was determined from the change in GS activity.

Subject	GS phosphatase		
	Rest	20 s	Fatigue
1	3.85	2.39	0.81
2	3.87	—	2.47
3	9.69	7.52	5.49
4	8.51	5.63	—
5	3.85	—	1.11

under the present conditions phosphorylase *a* formed during the first contraction (i.e. at 20 s) has reverted to the *b* form at fatigue (Chasiotis *et al.*, 1982, 1983).

The phosphorylated and dephosphorylated forms of GS are interconvertible (Danforth, 1965). Thus at least two mechanisms can be considered to account for the decreased activity of GS_{low}. One is the enhancement of phosphorylation of the enzyme by the protein kinase systems, and the second is an inhibition of the dephosphorylation by the protein phosphatase systems.

It is possible that activation of cyclic AMP-dependent protein kinase could account for the inactivation of GS (Walkenbach *et al.*, 1980). However, under the present conditions the cyclic AMP content in muscle does not change (Chasiotis *et al.*, 1983). Moreover, there is no indication of an increase in cyclic AMP-dependent protein kinase activity in the electrically stimulated perfused psoas muscle of rabbit (Bauer *et al.*, 1986) or stimulated rat muscle (Staneloni & Piras, 1969). However, the sensitivity of protein kinase to cyclic AMP was determined at 75 μ M- (Staneloni & Piras, 1969) and 100 μ M-cyclic AMP (Bauer *et al.*, 1986), which apparently are saturating concentrations, whereas the sensitivities may be altered at much lower cyclic AMP contents (Walkenbach *et al.*, 1978). Nevertheless, if one accepts the hypothesis that cyclic AMP-dependent protein kinase is activated during muscle contraction, this should result in an activation of inhibitor 1 and thereby induce an

inhibition of GS phosphatase (Cohen, 1983), and consequently an inactivation of GS_{low}.

Several other kinase systems, such as Ca²⁺-dependent phosphorylase kinase (Cohen, 1982), GS kinase 3 (Hemmings *et al.*, 1981) and casein kinase (Itarte *et al.*, 1983), are known to exist. The activation of any of these systems during contraction could at least partly explain the decrease in the fractional activity of GS in the present study. Since muscle contraction results in the release of Ca²⁺ from the sarcoplasmic reticulum into the cytosol, the activation of phosphorylase kinase (Heilmeyer *et al.*, 1970) could explain the inactivation of GS (Cohen, 1983). It has, however, been shown that intense exercise to fatigue in humans does not result in changes in the activity of phosphorylase *b* kinase (i.e. conversion of the *b* into the *a* form) in muscle biopsies (Toews *et al.*, 1979), which is consistent with the phosphorylase results (see above). On the other hand, troponin C and Ca²⁺ in the physiological range can activate the dephosphorylated form of phosphorylase *b* kinase, and this is not associated with a conversion of phosphorylase *b* kinase *b* into phosphorylase *b* kinase *a* (Cohen, 1980). Thus the importance of activation of phosphorylase kinase for contraction-mediated inactivation of GS is debatable. Studies on muscle deficient in phosphorylase *b* kinase may help to resolve this issue.

It is possible that the inactivation of GS is due at least partly to the inactivation of GS phosphatase. The present findings would support this explanation. The inactivation of GS phosphatase in the present study contrasts with the earlier findings by Staneloni & Piras (1969), who showed no change in the GS phosphatase activity of two muscle homogenates. The reason for this apparent discrepancy is not clear, but it may be related to the requirement of prior contraction to observe the contraction-mediated inactivation of GS and/or differences in the assay of phosphatase (Staneloni & Piras, 1969).

The inactivation of GS phosphatase is probably due to covalent modification of the enzyme and/or binding of inhibitors that were not released in the processing of the muscle. However, the mechanism for the inactivation of GS phosphatase activity is not clear. Covalent modification, resulting in decreased GS phosphatase activity, could be accounted for by an increase in inhibitor 1 (Cohen, 1983). On the other hand, the finding that protein phosphatase 2B is Ca²⁺-dependent ($K_a = 0.5$ – 1 μ M) and accounts for ~ 70 % of the total potential activity on inhibitor 1 dephosphorylation in skeletal-

muscle extracts in the presence of micromolar concentrations of Ca^{2+} (Ingebritsen *et al.*, 1983) would predict an increased GS phosphatase activity, and consequently an activation of GS. The significance of this explanation (i.e. Ca^{2+} -dependent inhibition of inhibitor 1) for conditions *in vivo* is questionable, in view of the present findings (i.e. inactivation of both GS and GS phosphatase).

Ca^{2+} in the micromolar range has been shown to inhibit the form of GS phosphatase which binds tightly to glycogen particles in rat liver extracts (Mvumbi *et al.*, 1985). Thus a possible explanation for the observed decrease in the activity of GS phosphatase could be Ca^{2+} -mediated inactivation of GS phosphatase.

In conclusion, these results demonstrate that contraction of human skeletal muscle leads to an inactivation of GS, which appears to be, at least in part, caused by an inactivation of GS phosphatase. The mechanism for phosphatase inactivation, however, requires further investigation.

REFERENCES

- Bauer, H. P., Reichmann, H. & Hofer, H. W. (1986) *Int. J. Biochem.* **18**, 67–72
- Bergström, J. (1962) *Scand. J. Clin. Lab. Invest. Suppl.* **68**, 1–110
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Chasiotis, D., Sahlin, K. & Hultman, E. (1982) *J. Appl. Physiol.* **53**, 708–715
- Chasiotis, D., Brandt, R., Harris, R. C. & Hultman, E. (1983) *Am. J. Physiol.* **245**, E166–E170
- Cohen, P. (1980) *Eur. J. Biochem.* **111**, 563–574
- Cohen, P. (1982) *Nature (London)* **296**, 613–620
- Cohen, P. (1983) *Philos. Trans. R. Soc. London B* **302**, 13–25
- Danforth, W. H. (1965) *J. Biol. Chem.* **250**, 588–593
- Edwards, R. H. T., Hill, D. K. & McDonnell, M. (1972) *J. Physiol. (London)* **224**, 58P–59P
- Guinovart, J. J., Salavert, A., Massague, J., Ciudad, C. J., Salsas, E. & Itarte, E. (1979) *FEBS Lett.* **106**, 284–288
- Harris, R. C., Hultman, E. & Sahlin, K. (1981) *Pflügers Arch.* **389**, 277–282
- Heilmeyer, L. M. G., Meyer, F., Haschke, R. H. & Fisher, E. (1970) *J. Biol. Chem.* **245**, 6649–6656
- Hemmings, B. A., Yellowlees, D., Kernohan, J. C. & Cohen, P. (1981) *Eur. J. Biochem.* **119**, 443–451
- Henriksson, J., Katz, A. & Sahlin, K. (1986) *J. Physiol. (London)* **380**, 441–451
- Ingebritsen, T. S., Steward, A. A. & Cohen, P. (1983) *Eur. J. Biochem.* **132**, 297–307
- Itarte, E., Plana, M., Gausch, M. D. & Martos, C. C. (1983) *Biochem. Biophys. Res. Commun.* **117**, 631–636
- Miller, T. B. (1979) *Biochim. Biophys. Acta* **583**, 36–46
- Mvumbi, L., Bollen, M. & Stalmans, W. (1985) *Biochem. J.* **232**, 697–704
- Nuttall, F. Q., Gannon, M. C., Corbett, V. A. & Wheeler, M. P. (1976) *J. Biol. Chem.* **251**, 6724–6729
- Okubo, M., Bogardus, C., Lillioja, S. & Mott, D. M. (1988) *Metab. Clin. Exp.* **37**, 1171–1176
- Sahlin, K. & Henriksson, J. (1984) *Acta Physiol. Scand.* **122**, 331–339
- Staneloni, R. & Piras, R. (1969) *Biochem. Biophys. Res. Commun.* **36**, 1032–1038
- Thomas, J. A., Schlender, K. K. & Larner, J. (1968) *Anal. Biochem.* **25**, 486–499
- Toews, C. J., Ward, G. R., Leveille, R., Sutton, J. R. & Jones, N. L. (1979) *Diabetes* **28**, Suppl. 1, 100–102
- Walkenbach, R. J., Hazen, R. & Larner, J. (1978) *Mol. Cell. Biochem.* **19**, 31–41
- Walkenbach, R. J., Hazen, R. & Larner, J. (1980) *Biochim. Biophys. Acta* **629**, 421–430

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