CHANGES IN GLYCOGEN SYNTHETASE AND PHOSPHORYLASE DURING MUSCULAR CONTRACTION

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Summary: Tetanic contraction of rat skeletal muscle, previously stimulated to increase its glucose-6-P independent proportion of glycogen synthetase (I-GS), results in the transformation of GS from the independent to the dependent (D-GS) form. This I to D conversion is very rapid, since it takes place during the first 5 sec of stimulation, and occurs simultaneously with an increase of phosphorylase a. Possible mechanisms of this conversion have been investigated. During the ensueing recovery period glycogen phosphorylase is reverted to the less active b form, and GS to the more active I form. The simultaneous and opposite changes in the active forms of both enzymes are mantained, at least, through four cycles of contraction and recovery, suggesting the presence of a system which seems to interlock both enzymes to allow maximal efficiency during glycogenolysis or glycogenesis.

The effect of several hormones on glycogen metabolism is due to their antagonizing action on the conversions of the active forms of both glycogen phosphorylase(∝1,4 glucan: orthophosphate glucosyltransferase, E.C. 2.4.1.1) and glycogen synthetase (UDP-glucose: 1,4 glucan∝-4 glucosyltransferase, E.C. 2.4.1.11) (e.g. Litwack and Kritchevsky, 1964; Leloir, 1967). This and the similarities in the two enzymatic systems have led to the generally accepted view that GS becomes inactivated when phosphorylase is activated, and viceversa. (Larner, 1966; Leloir, 1967; Stadman, 1966). The effect of electrical stimulation of skeletal muscle, on the other hand, is a rapid and extensive transformation to phosphorylase a (Danforth et al., 1962), followed only by a much slower conversion of GS from the D to the I form (Danforth, 1965; Piras and Staneloni, 1969). Therefore, there was a lack of evidence that muscular contraction can a) produce an I-GS to D-GS conversion, and b) that synthetase too can undergo a very rapid transformation.

Experimental Procedure: The posterior muscles from the thigh of male rats were exposed bilaterally and stimulated electrically in situ through the sciatic nerve to obtain a tetanic contraction. Most of the experimental details regarding the

stimulation of the muscles, as well as their handling and the enzymatic determinations have been described elsewhere (Piras and Staneloni, 1969).

Results: Electrical stimulation in situ of resting rat skeletal muscles rapidly activates phosphorylase, but does not decrease the level of I-GS (Table I), in accordance with previous results (Danforth, 1965; Piras and Staneloni, 1969). After a 4-min recovery the level of phosphorylase a is very low and the proportion of GS in the I form is maximal. It was thought that a new stimulation in this situation could give rise to a transformation of the two enzymatic systems in opposite directions. The results of Table I indicate that, indeed, the second contraction decreases the percentage of I-GS from 52 to 22 %. Essentially similar results were obtained with 5-sec or 20-sec stimulations (not shown). Therefore, muscular contraction can not only elicit an I to D transformation, but this can take place at a rather rapid rate. Simultaneously with the I-GS decrease, phosphorylase a rises from 5 to 35 % (Table I). Thus, a synchronous and opposite change in the forms of the two enzymes does occur under these conditions. Total phosphorylase and GS activities remain constant throughout the experiment.

Exploratory experiments were carried out in order to ascertain if the in vivo I to D transformation took place by one of the known mechanism for GS conversions

Table I.- Effect of tetanic stimulations and recovery on phosphorylase a and I-GS of rat skeletal muscle[a].

Condition	Phosphorylase a (%)		I-GS (%)		Glycogen (mg/g)	
Rest	22 ± 4	(6)	32 ± 4	(6)	8.7 ± 0.3	(8)
S	53 ± 6	(7)	30 ± 4	(6)	5.8 ± 0.4	(6)
S+R	5 ± 1	(6)	52 ± 5	(8)	7.2 ± 0.3	(6)
S+R+S	35 ± 6	(5)	22 ± 4	(8)	5.4 ± 0.4	(6)

[a] S stands for a 10-sec tetanic stimulation, and R for a 4-min recovery period. Percentages of phosphorylase a and I-GS and glycogen concentration were measured as described (Piras and Staneloni, 1969). Muscles from each rat were paired to obtain results corresponding to the condition of Rest vs. S, S vs. S+R, and S+R vs. S+R+S. The results are presented as average of all values obtained for each condition ± standard error of the mean.

Table II.- I-GS kinase and D-GS phosphatase activities of muscles homogenates[a]

HOMOGENATE	I-GS KINASE		D-GS PHOSPHATASE
	no cyclic AMP	75 μM cyclic AMP	
Rest	70	100	100
S + R	78	108	104
S + R + S	82	98	110

[a] Activities were measured with reaction mixtures and muscle extracts prepared as described by Larner et al. (1968), using purified D- or I-GS as substrates. No F^- was present in the kinase assay. Values are expressed as percentage of the total activity obtained with resting muscles, and are average of two experiments. Abbreviations are those of Table I.

(Friedman and Larner, 1963; Appleman et al., 1964). The results of Table II show that no significant difference could be detected in the total or cyclic-AMP independent activity of GS kinase (Larner et al.,1968) from SR and SRS muscles. GS phosphatase also did not show any change. Since no direct evidence could be

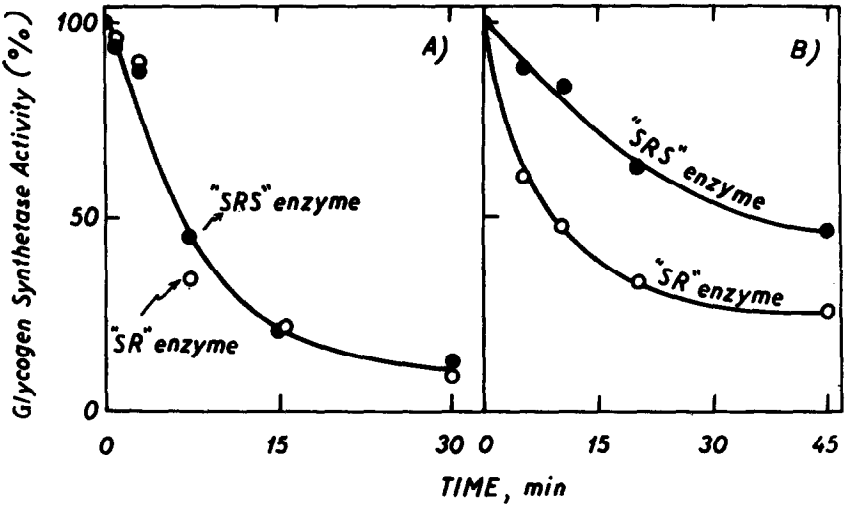

FIGURE 1: Trypsin and heat sensitivities of GS. Muscles stimulated for 10-sec and allowed to recover for 4-min ("SR" enzyme, -O-) or muscles which received and additional 10-sec stimulation ("SRS" enzyme, -●-) were homogenized with 0.05 M tris-HCl buffer, pH 7.5, 0.1 M NaF, 5 mM EDTA and centrifuged at 6.000 x g for 10 min. The I-form content for each enzyme corresponds to that of Table I. The extracts were treated with trypsin (A) at a concentration of 1 mg/ml and at 30°. The reaction was stopped with soybean trypsin inhibitor. Heat treatment (B) was carried out at 42°. Assays were done at 30° for 5 min in the presence of 10 mM glucose-6-phosphate.

obtained for the participation of the kinase-phosphatase mechanism, the possibility was explored that the I to D conversion brought about *in vivo* during the second stimulation took place as described *in vitro* with Ca^{2+} by Appleman *et al.* (1964). The *in vitro* Ca^{2+} - transformed GS is more sensitive to both a trypsin and temperature treatment than an I-GS, while the ATP-transformed GS has a trypsin sensitivity similar to that of I-GS, but it is more stable to the temperature treatment (Belocopitow *et al.*, 1967). Therefore, these two properties were studied with the SR and SRS enzymes. It was found that both have a similar trypsin sensitivity (Fig. 1A), but that the SRS enzyme is more stable at 42° than the SR enzyme (Fig. 1B). These results suggest that the *in vivo* transformation is not due to a mechanism similar to that described for Ca^{2+} *in vitro*. Further support to this interpretation was obtained with the experiment shown in Table III, where muscles received sequential stimulations and periods of recovery. If the decrease in I-GS were mediated through the Ca^{2+} conversion as described *in vitro* (Belocopitow *et al.*, 1967), then on each stimulation approximately 30 % of the GS would be irreversibly converted to the D-form, and at the end of the fourth cycle no I-GS should be present[1]. The results of Table III show that 4 min after the fourth

Table III.- Effect of sequential stimulations and recoveries on phosphorylase *a* and I-GS[a].

CONDITION[b]	PHOSPHORYLASE *a* (%)	I-GS(%)
(S+R)	5 ± 1 (6)	52 ± 5 (8)
(S+R)+S	35 ± 6 (5)	22 ± 4 (8)
$(S+R)_2$	4 ± 1 (5)	60 ± 5 (5)
$(S+R)_2$+S	42 ± 3 (5)	31 ± 4 (5)
$(S+R)_3$	2 ± 1 (5)	55 ± 2 (4)
$(S+R)_3$+S	29 ± 2 (6)	34 ± 2 (6)
$(S+R)_4$	5 ± 1 (4)	50 ± 4 (4)

[a] The abbreviations and experimental conditions are similar to those of Table I.

[b] The subindex indicates the number of stimulation-recovery cycles.

1. This assumption implies that no appreciable *de novo* synthesis of I-GS takes place during the experiment.

stimulation there was still 50 % I-GS.

DISCUSSION: In vivo conversions of muscle GS from the D to the I form occur after electrical stimulation (Danforth, 1965; Piras and Staneloni, 1969) or insulin treatment (Larner et al.,1968). The inverse conversion (I to D) occurs only as a reversion to rest of these mechanisms, or after epinephrine treatment (Danforth, 1965; Williams and Mayer, 1966). It would be expected that during muscular con - traction the I-GS content should decrease to facilitate glycogenolysis. So far, however, electrical stimulation was known to increase only phosphorylase a, without a concomitant I- to D-GS transformation (Danforth, 1965; Piras and Staneloni, 1969). It has been now possible to show that, indeed, electrical stimulation in situ of rat skeletal muscle can elicit an I to D conversion. This could be demonstrated by producing the tetanic contraction at a time when GS was pre - sent mainly in the I form[2]. It should be pointed out that the D- to I-GS conversion produced after electrical stimulation requires 3-4 min (Danforth, 1965; Piras and Staneloni, 1969), but the I-to D-GS conversion here described ocurrs within 5 sec. This suggests, even in the absence of data for shorter stimulations, that the rate of this transformation approaches the order of magnitude described for the in vivo activation of phosphorylase (Danforth et al., 1962; Danforth and Lyon, 1964). The evidence obtained for the possible mechanism of the in vivo I- to D-GS conversion only indicates that this is reversible (Table III), and that it is not mediated by a transformation similar to that obtained in vitro with Ca^{2+} (Appleman et al.,1964). In this regard, it has been recently shown that the in vitro Ca^{2+}- mediated activation of phosphorylase b kinase, a system similar to GS, is irreversible (Houston and Krebs, 1968; Drummond and Duncan, 1968). Other studies, however, indicate a rol for Ca^{2+} in the reversible activation of phospho rylase b kinase, both in vitro (Ozawa and Ebashi,1967) and in vivo (Namm et al., 1968). Therefore, the possibility that a similar mechanism might be involved in

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2.It is difficult to understand, however, why stimulation of resting muscles does not decrease I-GS. Possible explanations could be a) that since GS activity at rest is low (Piras and Staneloni,1969) there might be no special advantage in further diminishing the I content of GS b) If D-GS has some activity even in the absence of Glucose-6-P, then the plus-minus assay of GS would not reflect the true proportion of I-GS.If the activity found at rest reflected this point, then one cannot expect this activity to decrease upon stimulation,since I-GS would be already zero.

the GS conversions should be kept in mind. If phosphorylation is involved (Friedman and Larner, 1963) this could be due either to increased GS kinase activity or to GS phosphatase inhibition. No differences could be detected in the activity of these enzymes upon stimulation (Table III), suggesting that if the conversion took place through these enzymes it occured by metabolite modulation of their activities rather than through the increase of an active form. In this regard, the negative feedback regulation by glycogen of GS phosphatase (Danforth, 1965; Larner, 1966; Saheki and Tsuiki, 1968), seems to have been overrun in this instance by other factors, since the glycogen level actually falls down when I-GS is converted to the D form (Table I).

The physiological implications of the coordinate and reverse relationship between the changes in the active forms of GS and phosphorylase are obvious. In fact, under each circunstance, glycogenesis or glycogenolysis would be switched on or off to prevent unnecessary recycling of glucosyl residues. These closely geared changes (Table III) suggest that they might be due to either the fluctuations in the concentration of a metabolite which triggers both systems, or to the activation of a single, common enzyme to both pathways. Finally, the tetanic contractions not only give rise to the transformations described, but also produce alterations in the sedimentability of GS and of phosphorylase. In fact, these enzymes, which are normally associated with glycogen, are partially released from it during stimulation. A full account of these experiments will be reported (Staneloni and Piras, in preparation).

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