

## ADRENALINE AND GLYCOGEN SYNTHASE ACTIVITY IN FAST AND SLOW MUSCLES OF RAT

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### 1. Introduction

Glycogen synthase and glycogen phosphorylase are subjected to complex regulations by allosteric effectors and by a phosphorylating and dephosphorylating enzymic apparatus [1,2]. In muscle, these reversible covalent changes of the enzyme molecule can be triggered by different physiological stimuli, including hormones. In particular, epinephrine stimulates glycogenolysis and activates glycogen phosphorylase and inactivates glycogen synthase by its action on protein kinase [2], in a few seconds [3].

Most authors agree that interconversion of the latter enzyme should not lead to any changes of the total activity as measured in the usual glucose-6-phosphate plus/minus assay (see table 1 in [4]). Therefore, it was an interesting observation that epinephrine decreases the I+D glycogen synthase levels in a fast (the extensor digitorum longus, EDL) but not in a slow (the soleus, S) muscle of rat [5]. Here we describe the temporal pattern of the decline and of the subsequent restoration of the enzyme activity and give an explanation of this phenomenon together with a hypothesis concerning its physiological role.

### 2. Methods

Albino rats (Sprague Dawley) (180–200 g body wt) under nembutal anaesthesia (50 mg/kg body wt) were used in all experiments. To obtain maximal and long lasting stimulation, epinephrine (freshly dissolved in buffered saline containing 0.8% ascorbic acid) was injected both intravenously (2 µg/rat) and intra-

peritoneally (80 µg/rat). The muscles were immediately frozen with Wollenberger clamps precooled in liquid nitrogen. They were then stored at  $-70^{\circ}\text{C}$  until processed. Except when otherwise stated, homogenization was performed in 20 vol. (w/v) KF (50 mM) and EDTA (1.2 mM, pH 6.1) at  $0^{\circ}\text{C}$  in a Potter Elvehjem apparatus with Teflon pestle. In a few experiments, isotonic KCl or Tris-HCl 50 mM were present. The latter was pH 7.7. The glycogen synthase I and total (i.e., I+D) activities were assayed on  $1000 \times g$ , 10 min supernatants or on whole homogenates by the filter paper method [6], but glycogen was purified from any unreacted UDPG by ascending chromatography in ethanol/water (2/1) [7]. Briefly the incubation mixture contained (final conc.): Tris-HCl 33 mM (pH 7.8); EDTA 14 mM; KF 33 mM;  $\text{Na}_2\text{SO}_4$  10 mM; UDPG 4.4 mM; glycogen 6.7 mg/ml. When present, glucose-6-phosphate was 7.0 mM. In some experiments glycogen synthase was also assayed on myofibrillar fractions, partially purified by the procedure in [8]. Glucose-6-phosphate (G-6-P) and glucose-1-phosphate (G-1-P) were assayed substantially according to [9] after acidic extraction ( $\text{HClO}_4$  0.7 N) of the frozen tissues and neutralization by  $\text{KHCO}_3$  (2 M). In some experiments, purified phosphatase or the catalytic subunit of protein kinase were added to the homogenate. These enzymes had been prepared according to [10] and [11], respectively.

Oyster glycogen, UDPG, G-6-P, cyclic AMP and NADP were products of Sigma Chem. Co. (St Louis, MO). Crystalline G-6-P-dehydrogenase and phosphoglucomutase were purchased from Böhringer GmbH (Mannheim).  $[\text{U-}^{14}\text{C}]$ Glucose was obtained from the Radiochemical Centre (Amersham).  $\text{UDP}[^{14}\text{C}]$ Glucose was prepared by Dr Anna De Paoli Roach according to [6]. All products used were of analytical grade.

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### 3. Results and discussion

Fig.1 shows that the administration of adrenaline, in addition to the well known changes of glycogen synthase I (which can be observed both in the fast EDL and in the slow S) depresses the I+D glycogen synthase activity (in the EDL but not in the S) in a few seconds. As it should be expected when dealing with physiological effects of this hormone, both enzyme changes are rapidly reversible (they disappear in 20 min, fig.2).

When exploring the causes of this phenomenon, we noticed that it was not due to the lower ionic strength and pH. We did not observe any parallel change of the total activity of other glycogen metabolizing enzymes (e.g., of glycogen phosphorylase, [5]). Extensive dialysis did not lead to recovery of the lost activity. Treating the extracts with purified phosphatase restored and increased the I-levels depressed by adrenaline expressed in percentage of total activity but did not reverse the effect of the hormone on the (I+D) activity. However, no changes of the (I+D) activity could be observed when the activity was assayed in the whole homogenate and not in the  $1000 \times g$  supernatant (control  $3.91 \pm 0.11$ ; adrenaline  $3.98 \pm 0.13$  IU/g wet wt; 9 expt). We may

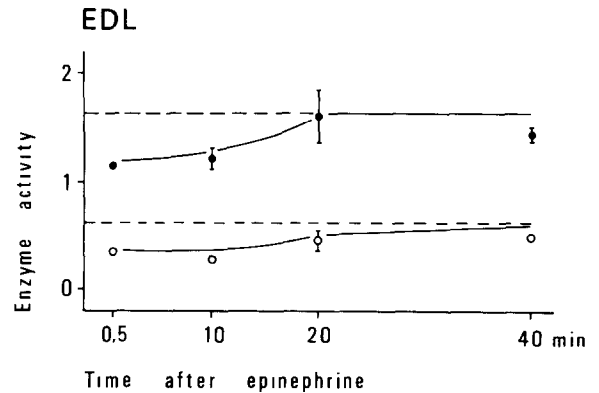


Fig.2. Recovery after epinephrine injection (see the text). GSI (○) and (I+D) (●) levels have been followed for 40 min after the hormone injection. The horizontal lines give the mean values of GSI and (I+D) obtained in 12 controls. Means of 4 expt  $\pm$  SEM are given.

mention here that assays performed on the suspended sediment confirmed the presence of a larger amount of the enzyme activity after epinephrine (see table 2). Finally, the enzyme levels in the supernatant could be restored by incubating the whole homogenate with purified phosphatase or with activators of the endogenous enzyme before centrifugation (table 1). KF

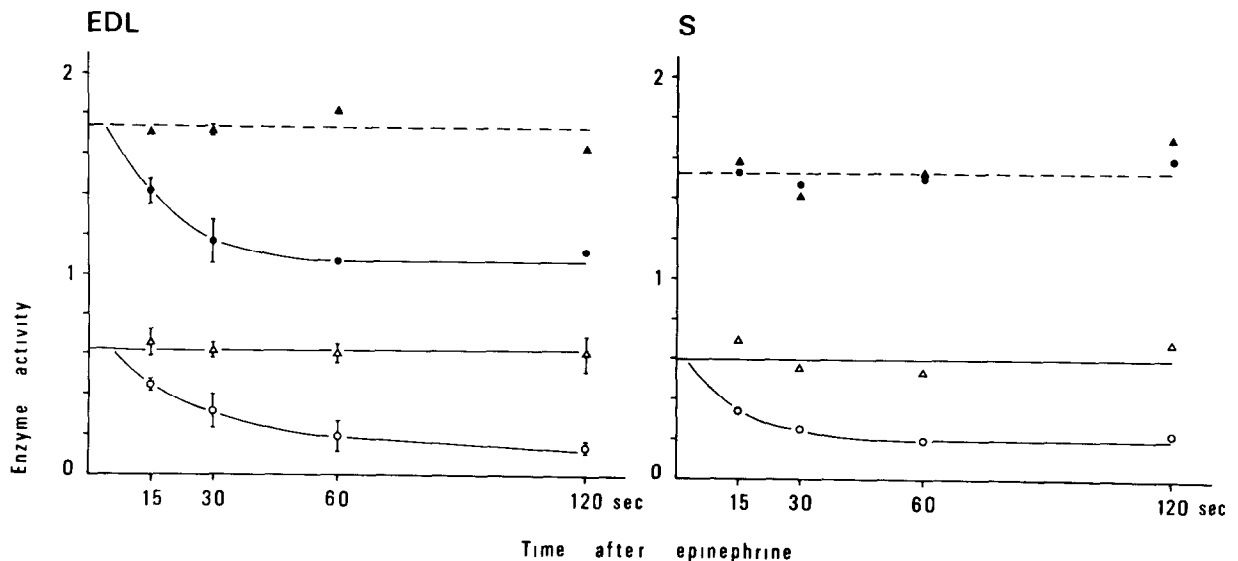


Fig.1. Effects of epinephrine (circles, for dosage see text) on glycogen synthase (GS) I (○,△) and (I+D) (●,▲) levels in the  $1000 \times g$ , 10 min supernatants of fast (extensor digitorum longus, EDL) and slow (soleus, S) muscles of rat. Means of 4 expt are given. Vertical segments represent  $2 \times$  SEM (they have been omitted when SEM is smaller than the symbol used). Abscissa, seconds following the intravenous injection of epinephrine, ordinate, GS activity.

Table 1

Endogenous glycogen synthase (GS) activating enzymes can reverse in vitro the effects of epinephrine administration in vivo on the total GS activity in the supernatant from the EDL muscle in the absence of KF

Treatment	KF absent	KF present
Control	2.42 ± 0.09 (8)	2.07 ± 0.13 (8)
Epinephrine	2.26 ± 0.05 (8)	1.49 ± 0.07 (8)

The homogenates were divided in two parts, and added with KF (100 mM final conc.) or water, and then incubated at 30°C for 4 min and centrifuged at 1000 × *g*, 10 min in the cold. Means ± SEM are given; no. expt in parentheses

100 mM inhibited recovery (table 1). We could not depress the recovery of (I+D) activity by adding purified C-subunits of protein kinase and MgATP to the whole homogenate of control muscles.

In conclusion, our data show that the decrease of the (I+D) activity in the supernatants after adrenaline is due to a larger retention of the enzyme activity in the sediments of the fast muscles. The temporal patterns of the decrease and of the spontaneous recovery of the activity (as compared with the changes of the I activity) and the restoration of the activity by phosphatase(s) in vitro indicate that the degree of phosphorylation of the enzyme molecule may be a relevant factor. We may mention that we found significantly larger amounts of glycogen synthase in the myofibrils when partially purified from the epinephrine-treated tissue (table 2). The contractile proteins may be different in fast and slow muscles [12,13] and this might help to understand the different behaviour of the enzyme in the two types of muscle. Also, the fact that most mammalian muscles contain both fast and

Table 2

Effect of epinephrine administration on the recovery of total glycogen synthase (GS) activity in myofibrillar fractions during myofibrillar purification from the EDL muscle of the rat [8]

Fractions	% Recovery of GS activity	
	Control	Epinephrine
Whole homogenate	100	100
Myofibrillar sediment 1	35 ± 9	47 ± 7
Myofibrillar sediment 2	21 ± 2	34 ± 5
Myofibrillar sediment 3	11 ± 2	20 ± 5

Means of 3 different expt are given ± SEM

Table 3

Effect of epinephrine administration (in vivo) on the levels of G-6-P and G-1-P in the EDL and in the S muscle of the rat (nmol/g wet wt)

Muscle	Epinephrine	G-6-P	G-1-P
EDL	—	329 ± 17 (5)	22 ± 2 (5)
	+	1391 ± 70 (5)	100 ± 5 (5)
S	—	141 ± 2 (5)	8 ± 1 (5)
	+	593 ± 52 (5)	34 ± 2 (5)

Means ± SEM are given; no. expt in parentheses

slow fibres, may explain why this phenomenon has been overlooked so far. These enzyme changes are small and restricted to the fast fibres and may escape detection when slow fibres are present and researchers focus their attention on the effects of adrenaline on the ratio of the I/D activities.

Finally, an answer should be given to the question about the physiological meaning of this finding. The administration of adrenaline has different consequences on the sugar phosphate concentration in fast and slow muscles (table 3). Since the sugar phosphates are confined to the intracellular water (which is <60% muscle wet wt, unpublished) it can be calculated that G-6-P after the administration of adrenaline rises up to 2.5–3 mM in the fast muscle and only to ~1 mM in slow muscle. In fast muscle, the D form of glycogen synthase may undergo partial activation [14]. Therefore to stop glycogen synthesis completely after epinephrine admin., the fast muscle may need some other mechanism in addition to the covalent regulation of glycogen synthase. The hypothesis should be evaluated in that the association of the enzyme to particles other than its substrate may represent this additional regulatory factor.

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