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Electrical stimulation partly reverses the muscle insulin resistance caused by tenotomy

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It was shown that 15-min electrical stimulation of the rat sciatic nerve greatly increases the in vitro measured sensitivity of lactate formation, glucose transport, and glycogen synthesis to insulin, impaired by previous tenotomy. The insulin sensitivity of all these processes was, however, still below that found in the stimulated intact soleus muscle. Extending the stimulation up to 30 min did not cause any further changes in insulin sensitivity either in tenotomized or in intact muscles.

Muscle inactivity; Electrical stimulation; Insulin sensitivity; Glucose transport; Lactate production; Glycogen synthesis

1. INTRODUCTION

Insulin resistance is a state in which higher than normal concentrations of the hormone are required to produce a given biological effect. This occurs e.g. in obesity, thyroid hormone deficiency, aging and in most cases of non-insulin-dependent diabetes mellitus. Insulin resistance may be caused by the presence of antibodies to the hormone, production of abnormal, less effective insulin as well as by decreased responsiveness or/and sensitivity of the target tissues to insulin.

Skeletal muscles are probably in quantity the most important among other tissues for glucose utilization [5] especially during enhanced physical activity. On the other hand, restriction of physical activity is known to impair considerably glucose tolerance in spite of an excessive response of insulin secretion to glucose load [9]. These observations are consistent with the rapid reduction of the whole body insulin sensitivity in trained individuals following their subsequent inactivity lasting for 10 days [7]. Similar results have been reported in experimental animals after immobilization [12,16]. This effect can be attributed to decreasing skeletal muscle mass [17], although immobilization causes also a marked reduction in the activity of mitochondrial oxidative enzymes, decreases muscle capillary density which may lead to a reduced rate of glucose utilization [15].

In studies of Myllynen et al. [11] it was found that the insulin resistance persists for a few months after starting remobilization, and during this time improvement of insulin sensitivity is only slightly less than in control subjects.

Our previous studies [1] have shown that muscle inactivity, caused by tenotomy, results in a prompt decrease of the rat soleus muscle sensitivity to insulin estimated on the basis of lactate production in vitro. Since there is evidence that both acute exercise and physical training improve insulin sensitivity and glucose metabolism in skeletal muscles, most probably due to locally released contraction-induced factors [8,13], the question arises whether muscle stimulation can increase insulin sensitivity of glucose utilization of tenotomized muscles.

2. MATERIALS AND METHODS

Male Wistar rats (body weight 160–180 g) were kept in a temperature controlled room at 22°C with a light period from 06.00 to 18.00 h and received standard laboratory chow and water, ad libitum, except for the 12-hours before sacrifice when food was withdrawn. Tenotomy (Achilles tendon cutting) was performed in one hindlimb under light ether anesthesia, 12 h before isolation of muscles. Both the intact and tenotomized muscles were stimulated via the sciatic nerve for 15 or 30 min before sacrifice.

2.1. *Electrical stimulation in vivo*

Rats were anesthetized by an intraperitoneal injection of sodium pentobarbitone (50 mg/kg body weight). The skin was removed from a hindlimb, the sciatic nerve was prepared for stimulation and then the gastrocnemius-soleus-plantaris muscle group was prepared for isometric contractions as described by Górski et al. [6]. The nerve was stimulated with supramaximal tetanic pulses (7 V, 0.05 ms duration, delivered in 100 ms trains at 100 Hz) with one pulse per second.

2.2. *Soleus muscle isolation and incubation procedure*

Immediately after sacrifice, soleus muscles were isolated from both hind limbs and each of them was dissected longitudinally to produce two halves of a similar weight as described by Crettaz et al. [4]. The

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muscle strips were tied under tension to stainless steel clips and immediately put into the preincubation medium. The whole procedure did not exceed 1 min. The muscle strips were preincubated for 15 min at 37°C in 5 ml of modified Krebs–Ringer bicarbonate buffer (pH 7.4) containing 5 mM glucose and 1.5% (w/v) defatted serum albumin. Next, the muscles were transferred to Erlenmeyer flasks containing fresh incubation medium plus 0.5 μCi of [U-^{14}C]glucose · ml^{-1} and insulin at various concentrations (1–10,000 μU · ml^{-1}). The flasks were gassed continuously with O_2/CO_2 (19:1) during the preincubation and for the first 45 min of the 60 min incubation period. At the end of incubation, the muscles were removed from the flasks and freeze-clamped. The incorporation of [U-^{14}C]glucose into glycogen was assayed as described by Challiss et al. [3]. The concentrations of lactate (LA) in the incubation medium were measured enzymatically using the commercial kits (Boehringer Diagnostica, Mannheim, Germany)

For the assessment of glucose transport (glucose phosphorylation) in separate experiments the labelled 2-deoxy[2,6-^{3}H]glucose (0.4 μCi · ml^{-1}) was added to the incubation medium, instead of radiolabelled glucose. After 60 min of incubation, muscle strips were removed from the flasks, frozen and homogenized in 4% (w/v) $HClO_4$. After neutralization deoxy[^{3}H]glucose-6-phosphate was separated from deoxy[^{3}H]glucose by Dowex-2 (formate form) ion-exchange chromatography [3] and radioactivity was measured.

The sensitivity of glycolysis, glycogen synthesis and 2-deoxyglucose (2-DG) transport to insulin was expressed as the concentration of insulin (EC_{50}) required to produce a half-maximal effect. The final EC_{50} values, obtained by computer transformation of the relationship between insulin concentrations in the medium and the rates of the above processes into a log-logit plot were presented either as logarithms of insulin concentrations (means ± S.D.) or as their antilogarithms (geometrical means).

Total glycogen content in the soleus muscle was determined according to Carroll et al. [2].

2.3. *Statistical analysis*

The rates of LA production, glycogen synthesis and 2-DG transport at different concentrations of insulin, insulin sensitivity of these processes as well as glycogen content in the muscles were compared between groups by means of a two-way analysis of variance for non-repeated measures followed by the Duncan test. The null hypothesis was rejected when $P < 0.05$. The insulin sensitivity values, expressed as logarithms of insulin concentration, were compared using the Duncan test.

3. RESULTS AND DISCUSSION

Tenotomy significantly decreased the rates of 2-DG transport and glycogen synthesis at all insulin concentrations, whilst the LA production rates were markedly increased. In tenotomized muscles after a 15 min electrical stimulation the rates of 2-DG transport as well as glycogen synthesis were enhanced, and the rate of LA formation was not affected. In the contralateral intact hindlimb muscles, identical stimulation caused a marked increase in the rates of these three processes.

Table I

The effect of tenotomy and electrical stimulation of sciatic nerve on the rates of LA production, 2-deoxyglucose transport and glycogen synthesis measured in the soleus at different insulin concentration

Insulin conc. (μV · ml^{-1})	Controls	Controls after 15 min stimulation	Controls after 30 min stimulation	Tenotomized	Tenotomized after 15 min stimulation	Tenotomized after 30 min stimulation
	Lactate production (μmol · g^{-1} · h^{-1})					
1	6.05 ± 0.65 (10)	9.31 ± 0.56 (10)*	9.71 ± 0.38 (8)*	11.12 ± 0.80 (10)*$^+$	11.20 ± 1.08 (10)*$^+$	10.99 ± 0.55 (8)*
10	6.01 ± 0.66 (10)	10.33 ± 0.71 (10)*	10.63 ± 0.29 (8)*	10.97 ± 0.68 (10)*	11.58 ± 0.69 (10)*	11.88 ± 0.42 (8)*
100	9.54 ± 0.61 (10)	13.27 ± 0.92 (10)*	13.82 ± 0.41 (8)*	12.33 ± 0.97 (10)*	13.53 ± 0.83 (10)*$^\#$	13.80 ± 0.58 (8)*$^\#$
1,000	13.04 ± 0.83 (10)	16.28 ± 0.63 (10)*	16.58 ± 0.49 (8)*	15.02 ± 0.78 (10)*	15.17 ± 0.67 (10)*	15.45 ± 0.61 (8)*
10,000	13.07 ± 0.76 (10)	16.94 ± 0.76 (10)*	17.02 ± 0.33 (8)*	14.93 ± 0.77 (10)*	15.51 ± 0.84 (10)*	15.62 ± 0.49 (8)*
log EC_{50}	2.01 ± 0.07 (10)	1.62 ± 0.10 (10)*	1.65 ± 0.09 (8)*	2.51 ± 0.13 (10)*$^+$	1.93 ± 0.08 (10)$^{\#+}$	1.89 ± 0.05 (8)
	2-Deoxyglucose transport (μmol · g^{-1} · h^{-1})					
1	1.42 ± 0.10 (5)	2.61 ± 0.18 (5)*	2.59 ± 0.23 (5)*	0.52 ± 0.11 (5)*	1.44 ± 0.24 (5)$^{+\#}$	1.62 ± 0.19 (5)$^{+\#}$
10	1.70 ± 0.05 (5)	3.22 ± 0.16 (5)*	3.41 ± 0.25 (5)*	0.57 ± 0.10 (5)*	1.74 ± 0.25 (5)$^{+\#}$	1.73 ± 0.25 (5)$^{+\#}$
100	2.52 ± 0.16 (5)	3.54 ± 0.13 (5)*	3.62 ± 0.18 (5)*	1.11 ± 0.16 (5)*	2.43 ± 0.13 (5)$^{+\#}$	2.68 ± 0.39 (5)$^{+\#}$
1,000	3.24 ± 0.14 (5)	4.02 ± 0.14 (5)*	4.22 ± 0.29 (5)*	2.05 ± 0.13 (5)*	3.21 ± 0.19 (5)$^{+\#}$	3.41 ± 0.18 (5)$^{+\#}$
10,000	3.44 ± 0.12 (5)	3.91 ± 0.16 (5)*	4.32 ± 0.15 (5)*	2.46 ± 0.11 (5)*	3.25 ± 0.19 (5)$^{+\#}$	3.53 ± 0.27 (5)$^{+\#}$
log EC_{50}	1.99 ± 0.08 (5)	1.46 ± 0.10 (5)*	1.39 ± 0.08 (5)*	2.77 ± 0.12 (5)*	2.04 ± 0.21 (5)$^{+\#}$	2.01 ± 0.18 (5)$^{+\#}$
	Glycogen synthesis (μmol · g^{-1} · h^{-1})					
1	1.10 ± 0.28 (10)	2.30 ± 0.40 (10)*	2.29 ± 0.39 (8)*	0.58 ± 0.29 (10)*$^+$	1.72 ± 0.61 (10)$^\#$	1.91 ± 0.42 (8)$^\#$
10	1.27 ± 0.28 (10)	2.56 ± 0.38 (10)*	2.59 ± 0.28 (8)*	0.62 ± 0.35 (10)*$^+$	1.84 ± 0.50 (10)$^\#$	1.99 ± 0.39 (8)$^\#$
100	2.20 ± 0.30 (10)	3.26 ± 0.31 (10)*	3.34 ± 0.31 (8)*	1.14 ± 0.41 (10)*$^+$	2.45 ± 0.46 (10)$^\#$	2.55 ± 0.42 (8)$^\#$
1,000	3.23 ± 0.31 (10)	3.37 ± 0.37 (10)	3.39 ± 0.25 (8)	2.59 ± 0.43 (10)*$^+$	3.05 ± 0.42 (10)	3.18 ± 0.35 (8)
10,000	3.42 ± 0.37 (10)	3.39 ± 0.33 (10)	3.52 ± 0.38 (8)	2.70 ± 0.47 (10)*$^+$	3.12 ± 0.46 (10)	3.19 ± 0.43 (8)
log EC_{50}	2.06 ± 0.07 (10)	1.41 ± 0.07 (10)*	1.59 ± 0.11 (8)	2.30 ± 0.05 (10)*$^+$	1.95 ± 0.07 (10)$^{+\#}$	2.01 ± 0.09 (8)$^{+\#}$

Results are presented as means ± S.D. The values significantly different ($P < 0.05$) from controls (intact, unstimulated muscle) are indicated by *, from controls 15 min stimulated by $^+$, and from tenotomized unstimulated by $^\#$.

Table II

Effects of tenotomy and electrical stimulation on the level of glycogen (μmol of glucose · g^{-1}) in soleus muscle of rats

Controls	Controls after 15 min stimulation	Controls after 30 min stimulation	Tenotomized	Tenotomized after 15 min stimulation	Tenotomized after 30 min stimulation
29.72 ± 1.51 (10)	22.50 ± 2.52 (5)*	22.17 ± 2.97 (5)*	36.42 ± 3.19 (5)*+	24.98 ± 1.57 (5)*#	24.12 ± 2.27 (5)*#

Results are presented as means ± S.D. The values significantly different ($P < 0.05$) from controls (intact, unstimulated muscles) are indicated *, from controls 15 min stimulated by +, and from tenotomized unstimulated by #.

Neither in tenotomized nor in intact muscle did extension of stimulation by up to 30 min cause further change in the rates of LA production, 2-DG transport or glycogen synthesis in vitro (Table I).

In the intact, unstimulated muscles, the values of EC_{50} for LA production, 2-DG transport and glycogen synthesis, retransformed from logarithms were similar, approximating to 100 μU of insulin · ml^{-1}. Stimulation of the muscles for 15 min decreased EC_{50} for LA formation to 41.7 μU · ml^{-1}, for 2-DG transport to 28.8 μU · ml^{-1}, and for glycogen synthesis to 25.7 μU · ml^{-1}, thus indicating a marked increase in the sensitivity of these processes to insulin. Similar results were obtained after a 30-min stimulation. In tenotomized muscles, insulin sensitivity was dramatically reduced. The values of EC_{50} for LA production, 2-DG transport and glycogen synthesis were 323.6 μU · ml^{-1}, 588.8 μU · ml^{-1} and 199.5 μU of insulin · ml^{-1}, respectively. Electrical stimulation of tenotomized muscles for 15 min considerably improved their insulin sensitivity. After this procedure the values of EC_{50} became similar to the control, unstimulated muscles (86.1 μU · ml^{-1} for LA production, 109.6 μU · ml^{-1} for 2-DG transport, and 89.1 μU of insulin · ml^{-1} for glycogen synthesis). No further improvement in insulin sensitivity of these processes was noted when the tenotomized muscles were stimulated for 30 min (Table I).

As can be seen from Table II, after tenotomy the soleus muscle glycogen content was significantly elevated in comparision with the content of this substrate in the contralateral intact muscle. Electrical stimulation caused similar depletion of muscle glycogen in intact and tenotomized muscles.

The present study confirmed the data obtained previously in this laboratory [1], indicating that for several hours following tenotomy the rates of lactate formation in the soleus muscle are increased. It may be connected with some mitochondrial disfunction shown in immobilized muscles [10]. It seems that the accelerated LA production results from the enhanced glycogenolysis, since the rates of glucose transport were diminished under this condition. Similarly, as in the previous study, tenotomy was shown to decrease considerably the muscle insulin sensitivity.

The most important finding obtained in these investigations is that a short (15 min) contractile activity of tenotomized muscle is able to greatly improve the sensitivity to insulin of LA production, 2-DG transport and glycogen synthesis. Although the insulin concentrations necessary to produce half of the maximal effects after stimulation were still higher in the tenotomized muscles than in the stimulated, intact ones, the net effect of stimulation, i.e. the absolute difference in EC_{50} values, was greater in the former.

It is of interest that when the nerve stimulation had been maintained for a longer period (30 min) there was no further improvement of the insulin sensitivity either in the tenotomized or intact muscles, thus suggesting that the maximal effect of muscle contractions on insulin sensitivity might be achieved within 15 min of stimulation. On the other hand, it was reported [8,13] that a much longer time was necessary to increase the soleus muscle insulin sensitivity when rats were running on a treadmill at moderate intensity, and no beneficial effect was achieved by repeated bouts of short-term exercise performed by rats at the maximal speed [8]. The differences between the effects of electrical stimulation and treadmill exercise might depend on the relatively lower involvement of the soleus contractions during running.

The finding that even after 30 min of stimulation insulin sensitivity of the tenotomized muscle and the rates of 2-DG transport were significantly below those of the intact stimulated muscles suggests a decrease in the total pool of glucose transporters [14], or some other not so easily reversible changes occurring during the 12 h period of muscle inactivity.

The present data showing that extending of stimulation from 15 to 30 min did not cause any further changes in the content of muscle glycogen confirmed the previously reported results [6]. The lack of accelerated depletion of glycogen is most probably, due to an equilibrium between glycogenolysis and glycogen synthesis. It seems of importance that the rates of glycogen synthesis and lactate production measured in vitro, increased after stimulation in comparison with unstimulated muscles, but they were not influenced by duration of stimulation.

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