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J. RUTKIEWICZ, D. CZARNOWSKI, J. GÓRSKI

EFFECT OF DENERVATION AND TENOTOMY ON 5'-NUCLEOTIDASE ACTIVITY IN RAT SKELETAL MUSCLES

Department of Physiology, Medical School, Białystok, Poland

Activity of 5'-nucleotidase was shown to be higher in the slow-twitch muscle than in the fast-twitch oxydative-glycolytic muscle and in the latter it was higher than that in the fast-twitch glycolytic muscle of the rat. Both denervation and tenotomy resulted in marked elevation of the enzyme activity in each muscle type.

Key words: *5'nucleotidase, skeletal muscle, denervation, tenotomy, rat.*

INTRODUCTION

5'-nucleotidase (EC 3.1.3.5.) catalyzes dephosphorylation of AMP (or IMP) to adenosine (or inosine) (1). There have been numerous studies on 5'-nucleotidase activity in various tissues, both under physiological and pathological conditions (2). Surprisingly, there are only scarce data on the enzyme activity in skeletal muscles. It has been reported that in rats (3) and cats (4) the enzyme activity in muscles composed of the slow-twitch fibers is much higher than in those composed of the fast-twitch ones. Studies in the rat muscles showed that 5'-nucleotidase activity remained unchanged after starvation, adrenalectomy and physical training and was elevated after exposure to cold (3). Insulin (5) and ageing (3) reduced activity of the enzyme. The aim of this study was to examine the effect of denervation and tenotomy on 5'-nucleotidase activity in different types of rat skeletal muscles.

MATERIAL AND METHODS

The experiments were carried out on male Wistar rats, 220—250 g of body weight, fed *ad libitum* a commercial pellet diet for rodents. They were divided into three groups: 1 — control, 2 — denervated and 3 — tenotomized. Rats assigned to the second group were anaesthetized with

ether and approximatelly 5 mm of the proximal section of the left sciatic nerve was removed. The muscle samples (the name of the muscles are given in *table 1*) were taken from the denervated limb at 24 h, 1, 2 and 4 weeks after the surgery. The rats in the third group were also anaesthetized with ether and the left Achilles tendon was cut through. The muscle samples were taken in the same time intervals as after denervation. The rats were anaesthetized with pentobarbital sodium administered intraperitoneally before muscle sampling. The muscle samples were homogenized in 0.5 M Tris-HCl buffer at pH 7.4. The homogenate was centrifuged at 11000 rpm for 15 min, at +4°C. Activity of 5'-nucleotidase was measured by the method of Campbell (6). Concentration of protein in homogenates was measured by the method of Lowry et al. (7). Student's t-test for unpaired data was used to evaluate the results.

RESULTS

The activity of 5'-nucleotidase in the soleus was significantly higher than that in the red portion of gastrocnemius and in the latter it was higher than that in the white portion of the same muscle. 24 hrs after denervation the enzyme activity in the white portion doubled. In one week after denervation or tenotomy the activity of the enzyme was significantly elevated in each muscle and stabilized thereafter (*Table 1*).

Table 1. The effect of denervation (D) and tenotomy (T) on 5'-nucleotidase activity (nmol $P_i \cdot min^{-1} \cdot mg$ of $protein^{-1}$) in rat muscles. The numbers are means ± SD · N = 10 for each mean. G – gastrocnemius. Numbers in parentheses are control values.

Muscle	Group	Time after surgery			
		24 h	1 week	2 weeks	4 weeks
Soleus (3.35 ± 0.63)	D	3.91 ± 0.3	7.18 ± 1.8 ***	6.39 ± 0.83 ***	8.76 ± 1.17 ***
	T	4.13 ± 0.65	5.82 ± 1.14 **	6.92 ± 1.21 ***	5.45 ± 0.71 **
Red G. (2.70 ± 0.62) [a]	D	3.37 ± 0.65	5.90 ± 1.3 ***	7.15 ± 1.1 ***	6.59 ± 0.88 ***
	T	2.97 ± 0.7	3.63 ± 0.95 **	4.79 ± 1.17 **	3.80 ± 0.87 *
White G. (1.56 ± 0.72) [b]	D	2.97 ± 0.93 **	4.47 ± 1.25 ***	4.36 ± 0.9 ***	8.97 ± 1.23 ***
	T	2.01 ± 1.01	2.78 ± 0.8 **	3.34 ± 0.79 ***	3.53 ± 0.79 ***

* – $p < 0.05$
** – $p < 0.01$ vs the respective control value
*** – $p < 0.001$
[a] – $p < 0.05$ vs the control value in the soleus
[b] – $p < 0.01$ vs the control value in red G.

DISCUSSION

There are three different fibre types in rat skeletal muscles: slow-twitch oxidative (SO), fast-twitch oxidative-glycolytic (FOG) and fast-twitch glycolytic (FG). The soleus is composed almost exclusively of SO fibers, the

red portion of gastrocnemius mostly of FOG fibers and the white portion of this muscle mostly of FG fibers (8). In the available papers (3, 4) activity of 5'-nucleotidase in fast-twitch muscles was determined in muscle samples being a mixture of FOG and FG fibers and our study is the first to report the enzyme activity in the two fast-twitch muscle types separately. And we showed that activity in the muscle composed of FG fibers was nearly half that of the muscle composed of FOG fibers. According to Newsholme et al. (3) activity of 5'-nucleotidase in the rat soleus is severalfold higher than that in the quadriceps (which is a mixture of FOG and FG fibers (8)). The difference in the present study is much smaller than that described by Newsholme et al. (8) and it is difficult to explain the reason for this discrepancy. In skeletal muscles 5'-nucleotidase is localized mostly in endothelium and in myocytes with the greatest density of activity close to the blood vessels (9). The capillary network in muscles with high oxidative potential is denser than that in muscles with low oxidative capacity (10, 11). This could account for the differences in the enzyme activity between the muscle types. Both denervation and tenotomy markedly increased the enzyme activity in each muscle type. The reason for this phenomenon is obscure. Available data indicate that the effect of denervation on the activity of different enzymes varies. Simard et al. (12) showed reduced activities of glycolytic enzymes with concomitantly elevated activity of hexokinase in rat fast-twitch muscles three weeks after denervation. In denervated chicken breast muscle a reduction in the activities of glycolytic enzymes was also observed. At the same time activity of creatine kinase and the rate of its synthesis was stable (13). Denervation was also shown to markedly increase glutamine synthetase activity in the rat soleus and plantaris muscles and an inhibition of the enzyme degradation was hypothetized to be the main reason for this phenomenon (14). The present data showed that 5'-nucleotidase activity increased not only after denervation but also after tenotomy. This may be assumed to be a consequence of disuse or/and desintegration of the muscle fibers. As it was mentioned above different activity of 5'-nucleotidase between the muscle types depends on its capillary network. Capillary density (capillaries/mm^2) in the soleus muscle was shown to increase 34% after 5 wk of disuse (15), and this factor could contribute to elevation in the enzyme activity. However, if it were the case one might expect a gradual elevation in the enzyme activity with time, i.e. when disuse or desintegration continued but that did not happen. It would suggest that other factors should be taken into account. As it was already mentioned insulin inhibits 5'-nucleotidase activity in rat muscles (5). On the other hand both denervation and tenotomy reduce skeletal muscle sensitivity to insulin (16, 17). It may be, therefore, hypothetized that the elevation of 5'-nucleotidase activity in denervated and tenotomized muscles was, at least in part, a consequence of reduction of the muscles sensitivity to an inhibitory action of insulin. Our

previous study (18) showing "tonic" inhibitory action of insulin on adenosine deaminase activity in different muscles would favour such a hypothesis. The increased activity of 5'-nucleotidase in denervated and tenotomized muscles seems to increase the muscle's potential for irreversible degradation of the adenine nucleotides pool. In consequence, it may contribute to deterioration of the muscle's function.

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Author's address: J. Górski, Department of Physiology, Medical School, ul. Mickiewicza 2c, 15-230 Białystok, Poland.