

J. RUTKIEWICZ, J. GÓRSKI

## EFFECT OF EXERCISE ON ADENOSINE DEAMINASE ACTIVITY IN RAT SKELETAL MUSCLES

Department of Physiology, Medical School, Białystok, Poland

Adenosine deaminase activity was shown to decrease in each skeletal muscle type (the slow-twitch oxydative, fast-twitch oxydative — glycolytic and fast-twitch glycolytic) at the beginning of exercise of moderate intensity and to return to the control when exercise was continued till exhaustion. 5 min occlusion of the femoral artery had no effect on the enzyme activity in either muscle. The reduction of the enzyme activity at the onset of exercise could result in reduction of adenosine breakdown and thus contribute to vasodilation at this stage of increased contractile activity of the muscles.

**Key words:** *Adenosine deaminase, skeletal muscles, exercise, artery occlusion, rat.*

### INTRODUCTION

Adenosine is one of the most important local factors responsible for vasodilation in contracting skeletal muscles (1). The concentration of the nucleoside in the muscles and its release to the venous blood has been repeatedly shown to increase during exercise (2, 3). That increase was attributed to increased production (1). Adenosine is rapidly degraded to inosine and ammonia by the enzyme adenosine deaminase. This enzyme is present also in skeletal muscles (4,5). It is, therefore, reasonable to assume that changes in the enzyme activity may also contribute to local concentration of the nucleoside in the muscles. However, there is no information on the effect of contractions on adenosine deaminase activity in the muscles, and it was the aim of the present study to examine this question.

## 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. Two experiments were carried out: 1-exercise on a treadmill, 2-ligation of the femoral artery.

Ad. 1. Rats were made to run 5 min or to exhaustion on a motor-driven treadmill set at  $+10^{\circ}$  incline and moving at a speed of 20 n/min. The animals were familiarized with the treadmill by forcing them to run as above 10 min daily, for five days preceding the test-exercise. Exhaustion was defined as the point when the animals refused further running. The rats were anaesthetized with pentobarbital sodium administered intraperitoneally and muscle samples were taken at the above time intervals. The following muscles were sampled: the soleus, the red and white portion of the gastrocnemius. These muscles are composed mostly of the slow-twitch oxidative, fast-twitch oxidative-glycolytic and fast-twitch glycolytic fibres, respectively (6).

Ad. 2. Rats were anaesthetized as above, and one femoral artery was ligated right below the ligamentum inguinale. Muscle samples were taken (as above) 5 min after the ligation. The activity of adenosine deaminase and the concentration of protein in the muscle samples were determined as previously (5). The Student t-test for unpaired data was used to evaluate the results statistically. Each mean was calculated from data obtained in ten rats.

## RESULTS

Adenosine deaminase activity was reduced in each muscle after 5 min exercise. It returned to the resting level after exercise till exhaustion (time of running till exhaustion was  $220 \pm 29$  min). 5 min occlusion of the femoral artery had no effect on the enzyme activity in either muscle (Table 1).

Table 1. Effect of running and ligation of the femoral artery on adenosine deaminase activity ( $\mu\text{mol NH}_3 \cdot \text{min}^{-1} \text{mg of protein}^{-1}$ ) in different rat muscles. The numbers are means  $\pm$  standard deviation. N=10. G — Gastrocnemius

Muscle	Control	Exercise		Femoral artery ligation (5 min)
		5 min	exhaustion	
Soleus	$27.82 \pm 2.39$	$20.78 \pm 3.56^{**}$	$31.56 \pm 6.90$	$30.23 \pm 3.71$
Red G	$29.06 \pm 4.63$	$19.34 \pm 2.53^{**}$	$26.48 \pm 7.80$	$28.67 \pm 3.00$
White G	$21.36 \pm 3.33$	$16.08 \pm 2.82^*$	$23.44 \pm 3.32$	$21.35 \pm 3.30$

\* —  $p < 0.01$

\*\* —  $p < 0.001$  vs the respective control value

## DISCUSSION

Elevation of adenosine deaminase (AD) level by means of infusion of the enzyme was reported to reduce vasodilation during contractile activity (7, 8). Therefore, the reduction in the enzyme activity seen in each muscle type after 5 min exercise may be expected to contribute to an increase of adenosine concentration in the muscles at the beginning of exercise. In consequence, it may contribute to vasodilation. Factors responsible for the inhibition of the enzyme activity at the onset of exercise remain to be established. Watanabe et al (9) has reported, that *in vitro*, the enzyme activity is reduced by a reduction of  $P_{O_2}$  and concentration of potassium as well as elevation of  $P_{CO_2}$ , and concentration of ammonia, lactate and inorganic phosphate. All these factors may appear in contracting muscles, depending on intensity and duration of contractions. The exercise employed in the present work was of moderate intensity. Ammonia is not produced during this type of exercise. Moreover, it is not formed in the rat soleus (the slow-twitch muscle) (10). Also, only a small increase in the concentration of lactate, potassium and  $P_{CO_2}$  in the muscles should be expected and these factors are not likely to contribute to inhibition of the enzyme activity in the present experiment. An inadequate oxygen supply might occur at the beginning of exercise due to a delay in the circulatory adaptation. To check if oxygen deficiency may affect AD activity in muscles *in vivo*, the femoral artery was ligated. However, 5 min occlusion of the artery had no effect on the enzyme activity in the muscles. This would therefore suggest that oxygen deficiency was not a factor reducing activity of the enzyme. This is in line with a conclusion of Ballard et al. (2) that a large proportion of adenosine is released during contractions by a mechanism other than lack of oxygen. The concentration of inorganic phosphate ( $P_i$ ) in the muscles should be expected to increase at the beginning of exercise as a result of phosphocreatine breakdown. It may be, therefore, a likely factor reducing the AD activity. When exercise was continued  $P_i$  was re-utilized and its inhibitory action on the enzyme activity would cease.  $P_i$  itself is involved in the production of exercise hyperemia but the mechanism of its vasodilatory action hasn't been fully elucidated (11). Elevation of the phosphate concentration was also shown to reduce the affinity of AD for adenosine (12). It is tempting to speculate that the latter effect of  $P_i$  combined with its possible inhibitory action on AD activity may result in a reduction in adenosine breakdown. In consequence, this mechanism may be, at least partly, responsible for the vasodilatory action of  $P_i$ .

## REFERENCES

1. Karim F, Ballard HJ, Cotterrell D. In: Vasodilatation. Vascular Smooth Muscle, Peptides, Autonomic Nerves, and Endothelium. Ed. Paul M. Vanhoutte, Raven Press. Ltd., New York, 1988; 383—388.

2. Ballard HJ, Cotterrell D, Karim F. The influence of blood flow rate on adenosine release from contracting dog skeletal muscle. *Quart J Exp Physiol* 1989; 74:97—107.
3. Collins MG. The vasodilator role of adenosine. *Pharmac Ther* 1989; 41:143—162.
4. Newsholme EA, Blomstrand E, Newell J, Pitcher J. Maximal activities of enzymes involved in adenosine metabolism in muscle and adipose tissue of rats under conditions of variations in insulin sensitivity. *FEBS Lett* 1985; 181:189—192.
5. Rutkiewicz J, Górski J. On the role of insulin in regulation of adenosine deaminase activity in rat tissues. *FEBS Lett* 1990; 271:79—80.
6. Ariano MA, Armstrong RB, Edgerton VR. Hindlimb muscle fiber populations of five mammals. *J Histochem Cytochem* 1973; 21:51—55.
7. Proctor KG. Reduction of contraction — induced arteriolar vasodilation by adenosine deaminase or theophylline. *Am J Physiol (Heart Circ. Physiol. 16)* 1984; 247:H 195—H 205.
8. Schwartz LM, McKenzie JE. Adenosine and active hyperemia in soleus and gracilis muscle of cats. *Am J Physiol (Heart Circ. Physiol. 28)* 1990; 259:H 1295—H 1304.
9. Watanabe K, Tominaga S, Nakamura T. Effects of lactate, ammonia, inorganic phosphate, potassium, pH  $P_{CO_2}$ , and  $P_{O_2}$  on the activities of adenosine and AMP aminohydrolases from skeletal and cardiac muscles. *Tohoku J Exp Med* 1973; 111:353—360.
10. Banister EW, Rajendra W, Mutch BJC. Ammonia as an indicator to exercise stress implications of recent findings to sports medicine. *Sports Med* 1985; 2:34—46.
11. Hudlicka O, El-Khelly F. In: Vasodilation. Vascular smooth muscle, peptides, autonomic nerves, and endothelium, P. M. Vanhoutte Ed. New York Raven Press, Ltd., 1988; 377—381.
12. Aikawa Y, Aikawa T. Kinetic constant changes by hydrogen and phosphate ions in the clam adenosine deaminase. *Comp Biochem Physiol* 1981; 70 B: 199—208.

Received: November 14, 1991

Accepted: March 18, 1992

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