On the role of insulin in regulation of adenosine deaminase activity in rat tissues

Joanna Rutkiewicz and Jan Górski

Department of Physiology, Medical School, Białystok, Poland

Received 30 July 1990

After administration of insulin adenosine deaminase activity was reduced in different skeletal muscle types, the heart and the liver. On the other hand profound reduction in the plasma insulin concentration (streptozotocin diabetes) resulted in elevation of the enzyme activity in the tissues. It is concluded that the local concentration of adenosine may be effected by the concentration of insulin in the plasma.

Adenosine deaminase; Skeletal muscle; Heart; Liver; Insulin; Rat

1. INTRODUCTION

Adnosine has been proved to play an important role in modulation of insulin action on glucose metabolism in different tissues (see, for example, [3,10,13,16]). There are also some data indicating that insulin may affect metabolism of adenosine by changing activities of principal enzymes involved in the nucleoside production and degradation (5′-nucleotidase and adenosine deaminase, respectively) [12]. 5′-Nucleotidase activity in membranes of rat hindquarter muscles was shown to be reduced by insulin [8]. On the other hand, streptozotocin diabetes resulted in elevation of the heart 5′-nucleotidase and adenosine deaminase activity [7]. The results presented in this paper clearly indicate that insulin is involved in the regulation of activity of adenosine deaminase in different rat tissues.

2. METHODS

Male Wistar rats 220–250 g were used in the experiment. They were housed at a temperature of 20 ± 1°C with 12 h light–dark cycle and had free access to food (a commercial pellet diet for rodents) and water. They were divided into three groups: one, control; two, treated with insulin. Insulin (Insulinum maxirapid, Polfa) in a dose of 0.5 IU (international unit) $\cdot$ kg^{-1} was administered intraperitoneally (half of the dose) and subcutaneously (the other half). Then, the rats were divided into two subgroups: one had free access to the food and water and the other one had the food withdrawn. They were sacrificed 3 h after administration of the hormone 3-streptozotocin-diabetic. Streptozotocin (Calbiochem) was freshly dissolved in citric buffer, pH 4.5 and administered intravenously in a dose of 80 mg $\cdot$ kg^{-1}. Rats were fasted 24 h before the drug administration and then allowed free access to the food. They were sacrificed 48 h after the drug administration. Rats were anaesthetized with pentobarbital sodium and tissue samples were taken. Names of the tissues are given in Table I. The tissue samples were homogenized in 0.089 M of the potassium phosphate buffer, pH 6.5, containing 0.18 M KCl and 2 mM 2-mercaptoethanol. 5% (w/v) of the homogenate was centrifuged at 10000 rpm, 10 min, at + 4°C. Adenosine deaminase activity was measured from the amount of ammonia produced during incubation of a mixture composed of 100 mM succinate/K^+ buffer, 100 mM KCl, 10 mM adenosine and the supernatant in a total volume of 5 ml. The mixture was incubated at 30°C for 10 min. The concentration of ammonia in the incubation medium was determined as in [4], the concentration of protein in the homogenate as in [9], and the concentration of glucose in the blood as in [6]. The Student *t*-test was used to evaluate the results statistically.

Correspondence address: J. Rutkiewicz, Department of Physiology, Medical School, ul. Mickiewicza 2c, 15-230 Białystok, Poland

3. RESULTS AND DISCUSSION

Adenosine deaminase activity in the myocardium was found to be two times higher than in the soleus and in the latter it was 3.5-times higher than in the quadriceps in the rat [11]. We have confirmed (Table I) the existing inter-muscle differences. However, the differences found in our work are not as great as those in the paper quoted above and the reason for this discrepancy is unclear. The cause for the inter-muscle differences in adenosine deaminase activity hasn't been elucidated. The muscles are composed of different fiber types: the soleus mostly of the slow-twitch oxidative fibers, the red gastrocnemius mostly of the fast-twitch oxidative fibers, the white gastrocnemius and plantaris mostly of the fast-twitch glycolytic fibers [1]. The capillary surface area in the muscles with high oxidative potential is much bigger than that with high glycolytic potential [5,14]. The 5′-nucleotidase activity in rat skeletal muscles was found to be localized principally in endothelium and in localized zones within myocytes in close proximity to blood vessels [15]. A localization of adenosine deaminase within the muscles has not been established, as yet. If it was similar to the localization of 5′-nucleotidase, the richer capillarization of the

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

Table I

Adenosine deaminase activity (μmol $NH_3 \cdot min^{-1} \cdot$ mg of protein^{-1}) in tissues of insulin-treated and streptozotocin-diabetic rats

G, gastrocnemius. The numbers are means ± SD. $N = 10$ for each mean.

Tissue or muscle	Control	Insulin-treated		Diabetic
		Food withheld	Food present	
Soleus	27.8 ± 2.4^{x}	18.0 ± 2.5*,^{x}	20.6 × 2.8*,^{x}	42.7 ± 6.4*,^{x}
Plantaris	22.9 ± 5.6	15.5 ± 2.4*	14.9 ± 3.3*	33.8 ± 3.9*
Red G.	29.1 ± 4.6^{x}	18.3 ± 2.4*,^{x}	17.6 ± 4.2*,^{xx}	43.8 ± 4.5*,^{xx}
White G.	21.4 ± 3.3	13.7 ± 1.9*	12.9 ± 1.6*	27.0 ± 4.1**
Heart	38.2 ± 2.6^{x}	26.3 ± 2.1*,^{x}	27.4 ± 2.9*,^{x}	46.6 ± 3.3*,^{x}
Liver	29.8 ± 2.9	22.9 ± 3.5*	21.2 ± 4.3*	44.7 ± 7.0*
Blood glucose (mmol $\cdot l^{-1}$)	7.1 ± 0.4	3.9 ± 0.8*	6.4 ± 1.3	28.1 ± 2.8*

* $P<0.001$ vs. the respective control value; ** $P<0.01$ vs. the respective control value; ^{x} $P<0.001$ vs. the white gastrocnemius; ^{xx} $P<0.01$ vs. the white gastrocnemius

muscles with high oxidative potential would, at least partially, account for higher activity of the enzyme in the muscles than in those with low oxidative potential. Insulin reduced the activity of the enzyme in each tissue studied of both hypoglycemic and normoglycemic rats (Table I). This indicates that the inhibitory effect of insulin on adenosine deaminase activity is not a consequence of hypoglycemia but results from a direct action of the hormone. On the other hand, rats with acute streptozotocin-diabetes showed significant elevation in the enzyme activity also in each tissue studied. The latter results would suggest that insulin exerts a 'tonic' inhibitory effect on adenosine deaminase activity in rat tissues. It is an open question as to what extent changes in adenosine deaminase activity accompanying changes in the plasma insulin concentration affect concentration of adenosine in the tissues. Since adenosine deaminase reduces the concentration of adenosine in tissues it is often used as a tool in the examination of the biological role of the nucleoside. Addition of the enzyme, e.g., reverses the reduction in the sensitivity to insulin of the soleus muscle strips isolated from the diet-induced insulin-resistant rats [2]. Therefore, our results allow to hypothesize that insulin may modulate its own action on glucose metabolism in the tissues by changing adenosine deaminase activity and hence local concentration of adenosine.

REFERENCES

[1] Armstrong, R.B. and Phelps, R.O. (1984) Am. J. Anatomy 171, 259–272.
[2] Budohoski, L., Challiss, R.A.J., Cooney, G.J., McManus, B. and Newsholme, E. (1984) Biochem. J. 224, 327–330.
[3] Challiss, R.A.J., Leighton, B., Lozeman, F.J. and Newsholme, E.A. (1987) in: Topics and Perspectives in Adenosine Research (Gerlach, E. and Becker, B.F., eds.) pp. 275–285, Springer-Verlag, Heidelberg.
[4] Chaney, A.L. and Marbach, E.P. (1962) Clin. Chem. 8, 130–132.
[5] Folkow, B. and Halicka, H.D. (1968) Microvasc. Res. 1, 1–14.
[6] Hultman, E. (1959) Nature 183, 108–109.
[7] Jenkins, R.L., McDaniel, H.G., Digerness, S., Parrish, S.W. and Ong, R.L. (1988) Diabetes 37, 629–636.
[8] Klip, A., Ramlad, T., Douen, A.G., Burdett, E., Young, D., Cartee, G.D. and Holloszy, J.O. (1988) FEBS Lett. 238, 419–423.
[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[10] McLane, M.P., Black, P.R., Law, W.R. and Raymond, R.M. (1990) Diabetes 39, 62–69.
[11] Newsholme, E.A., Blomstrand, E., Newell, J. and Pitcher, J. (1985) FEBS Lett. 181, 189–192.
[12] Ohisalo, J.J. (1987) Med. Biol. 65, 181–191.
[13] Richelsen, B., Hjøllund, E., Pedersen, O. and Sørensen, N.S. (1985) Biochim. Biophys. Acta 844, 359–366.
[14] Romanul, F.C.A. (1965) Arch. Neurol. 12, 497–509.
[15] Rubio, R., Berne, R.M. and Dohson, J.G.Jr. (1973) Am. J. Physiol. 225, 938–953.
[16] Sharahan, M.F., Edwards, B.M. and Ruoho, A.E. (1986) Biochim. Biophys. Acta 887, 121–129.