ACTIVITY OF AN INSULIN-DEPENDENT CYTOPLASMIC REGULATOR IN THE RAT LIVER AND HEART DURING STARVATION AND MUSCULAR WORK

M. Kh. Gainutdinov, É. B. Abibova, Z. Z. Gizatulina, and Ya. Kh. Turakulov

UDC 612.013.1-08:612.126.41.014.462.4

KEY WORDS: starvation; muscular activity; insulin-dependent regulator.

The writers demonstrated previously that the rat liver contains a thermostable glycolipopeptide with a molecular weight of about 6000 [6, 14], to which the name insulin-dependent cytoplasmic regulator (IDR) was given because its activity increased after injection of insulin into the rats. IDR increases the accumulation of Ca^{++} ions in liver mitochondira *in vitro* [6], inhibits pyruvate transport into the liver mitochondira [14], inhibits gluconeogenesis in fragments of liver [1] and of the renal cortex [5], stimulates glycogen synthesis in the isolated diaphragm [2], and has a hypoglycemic action after injection *in vivo* [1].

It is well know that starvation and muscular work stimulate the outflow of glucose from the liver through activation of glycogenolysis and gluconeogensis [4, 10]. Gluconeogenesis and glycogenolysis in the liver are controlled by cAMP and Ca^{++} ions, which activate them [10, 13], and by IDR, which is an endogenous inhibitor of glycogenolysis and gluconeogenesis in the liver [1, 5]. It can accordingly be postualted that during muscular work and starvation the cause of stimulation of gluconeogenesis and glycogenolysis is not only an increase in the concentrations of the intracellular activators (cAMP and Ca^{++} ions), but also a decrease in activity of the inhibitor (IDR).

The object of this investigation was to study the possiblity that IDR participates in the regulation of glycogenolysis, gluconeogenesis, and glycogen synthesis during starvation and muscular work and in the recovery period after muscular work.

EXPERIMENTAL METHOD

Male albino rats weighing 150-160 g were used. The rats swam in a bath for 2 h at 31-32°C.

Activity of IDR in the thermostable fraction of cytoplasm from the rat's lifer and heart was measured by the method described previously [1]. Cytosol was obtained by centrifuging the liver or heart homogenate (30,000g, 20 min) with 0.25 M sucrose and 5 mM Tris-HCl, pH 7.4 in the ratio of 1 ml incubation medium to 1 g. The cytosol fraction was heated to 97°C for 7 min and the proteins then removed by centrifugation. The rat liver mitochondria were isolated in 0.3 M sucrose, 5 mM Tris-HCl, pH 7.4, at 5000g.

Transport of Ca^{++} ions into the mitochondria was measured by a pH-metric method based on the kinetics of exchange of Ca^{++} ions for H^{+} ions in cubation medium containing 0.1 M KCl, 5 mM succinate, 0.7 mM phosphate, 0.7 μg/ml rotenone, 2 mM Tris-HCl, pH 7.0. Glycogen was measured by the anthrone method [8].

EXPERIMENTAL RESULTS

As shown in Fig. 1a, starvation for 48 h caused weakening of the stimulating action of the thermostable fraction of liver cytosol on the calcium capacity of rat liver mitochondria. The increase in this parameter on the addition of small quantities of cytosol was due to the presence of IDR in the thermostable fraction of cytoplasm [6]. Consequently, activity of IDR in the liver falls during starvation. A similar effect of a fall in IDR activity also was found in the experiments with liver cytosol from rats which had been swimming for 2 h (Fig.

Institute of Biochemistry, Academy of Sciences of the Uzbek SSR. Institute of Regional Medicine, Ministry of Health of the Uzbek SSR. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 37-40, July, 1982. Original article submitted January 19, 1982.

 0007-4888/82/9407-0896$07.50

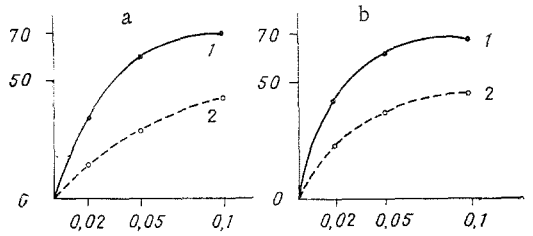

Fig. 1. IDR activity in rat liver under normal conditions, after swimming and after starvation. a) Swimming for 2 h, b) starvation for 48 h. 1) Control cytoplasm, 2) experimental cytoplasm. Abscissa, quantity of cytosol (in ml), ordinate, calcium capacity of liver mitochondria (in present).

TABLE 1. Changes in Glycogen Content in Liver and Heart of Rats (in mg/g tissue) during Swimming and in Recovery Period after Swimming (M ± m)

Experimental conditions	Liver	Heart
Control (n= 12)	12,0±1,7	6,0±0,5
Swimming for 2 h (n= 9)	2,0±0,5	1,4±0,2
Swimming for 2 h + recovery period (n= 8)	4,2±0,8	9,8±0,8

Note. Control and experimental rats starved for 12 h.

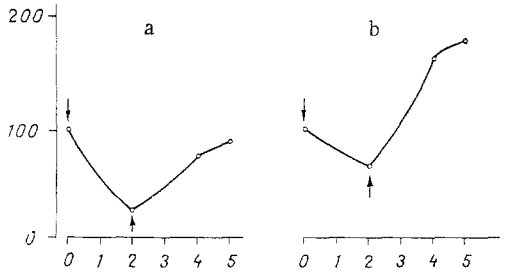

Fig. 2. IDR activity in liver (a) and heart (b) of rats during swimming and in recovery period after swimming. Arrow pointing downward denotes beginning, arrow pointing upward denotes end of swimming. Abscissa, time (in h); ordinate, IDR activity (in percent of normal).

1b). However one of the most likely causes of the fall in liver IDR activity during starvation and muscular work is a fall in the plasma insulin concentration, which has been described for these physiological states [4, 7]. Nevertheless, the possibility cannot be ruled out that the fall in IDR activity is a result of an increase in the concentration of catecholamines, glucagon, and somatotrophic hormone in the plasma [15], for a decrease in IDR activity was observed after injection of adrenalin into rats [6]. In this connection it must be noted that the maximal level of increase of calcium capacity of the mitochondria obtained from rats after starvation and swimming was reduced by the addition of thermostable cytoplasm (Fig. 1), although previously, on injection of insulin into the rats, no change was observed in the maximal effect of cytosol [6].

It is well known that gluconeogenesis in the hepatocytes may be limited by the velocity of pyruvate transport from cytosol into mitochondira, for pyruvate carboxylase is located in the mitochondria and the efficiency of the monocarboxylate carrier in liver mitochondria is very low [6]. The normally high IDR activity is one mechanism that determines the low velocity of pyruvate transport from cytosol into mitochondria and, correspondingly, the low ac-

tivity of gluconeogenesis, for IDR inhibits pyruvate transport [14]. A fall in IDR activity as a result of starvation and muscular work ought to lead to stimulation of pyruvate transport from cytosol into mitochondria and stimulation of gluconeogenesis in the hepatocytes. Stimulation of pyruvate transport from cytosol into mitochondria during starvation and muscular work is evidently not entirely the result of a decrease in IDR activity, for glucagon and catecholamines, whose concentration is increased in the physiological states under examination [4, 7, 15], stimulate pyruvate transport by increasing the H^+ ion gradient on the mitochondrial membrane [11]. It is difficult to conclude which of the mechanisms of stimulation of pyruvate transport examined above is more efficient. Most likely synergism is exhibited in their action. One conclusion of the present investigation is that correlation exists between inhibition of insulin secretion by the pancreatic beta cells [4, 7] and IDR activity in the liver during starvation and muscular work; the fall in IDR activity, moreover, is one cause of stimulation of glyconeogenesis.

Consequently, both during starvation and during muscular work IDR activity falls in the rat liver. Since IDR is a inhibitor of glycogenolysis and gluconeogenesis in the liver [1, 5], it will be evident that a fall in IDR activity is one of the mechanisms of stimulation of glycogenolysis and gluconeogenesis in the liver during starvation and muscular work. Besides a fall in IDR activity during starvation and muscular activity, an increase also is observed in the cAMP concentration in the liver, due to secretion of glucagon and catecholamines [4, 15], and this strongly stimulates gluconeogenesis and glycogenolysis. The writers showed previously that cAMP has no direct action on IDR activity in liver homogenates. Nevertheless the possibility cannot be ruled out that in intact hepatocytes cAMP may reduce IDR activity by increasing the Ca^{++} concentration in the cytoplasm [13], for Ca^{++} ions in physiological concentrations depress IDR activity in liver homogenates [3].

Catecholamines increase the concentration of ionized Ca^{++} in the cytoplasm during interaction with the α-adrenoreceptor; this effect, moreover, is not connected with an increase in cAMP concentration [13]. Consequently, the fall in IDR activity in the liver during starvation and muscular activity is a result both of a fall in the plasma insulin concentration and of a rise in the ionized Ca^{++} concentration in the cytoplasm.

Glycogenolysis in the liver and heart, which is stimulated by swimming [7], is replaced by glycogen resynthesis after the end of swimming (Table 1). There is a parallel increase in IDR activity in the liver and heart (Fig. 2). If the decrease in IDR activity during swimming is due mainly to a fall in the plasma insulin concentration, the increase in IDR activity after the end of swimming must evidently be the result of the rapid rise in the plasma insulin concentration, which takes place on the cessation of muscular work [7]. Glycogen resynthesis in the liver and heart is also connected with an increase in the insulin concentration.

As Fig. 2 shows, the decrease in IDR activity during swimming is manifested more strongly in the liver than in the heart. In the recovery period after swimming the increase in IDR activity takes place much more strongly in the heart than in the liver. IDR activity in the heart 2 h after the end of swimming was significantly higher than before swimming, whereas in the liver IDR activity was slightly weaker than initially (Fig. 2a). Since IDR inhibits glycogenolysis [1] and stimulates glycogen synthesis [2], it can be postulated that the increase in IDR activity in the recovery period after muscular work is one cause of glycogen resynthesis in the liver and heart. As Table 1 shows, glycogen resynthesis takes place faster in the heart than in the liver. It was found previously that glycogen synthetase activity in the heart is much higher after muscular work than initially, although this effect was not found in the liver [12]. Consequently, correlation exists between the rate of glycogen resynthesis, the change in glycogen synthetase activity, and IDR activity in the heart and liver in the recovery period after muscular work.

It is difficult to explain differences in the changes in IDR activity in the liver and heart during and after swimming purely by a change in the plasma insulin concentration. Evidently not only insulin, but also other factors are concerned in the regulation of IDR activity. It may be that IDR activity in the heart is also controlled by kinins, for kinins have an insulin-like action on metabolism processes in muscles and their concentration rises during muscular work [9]. If this is so, the increase in the concentration of kinins during muscular work must weaken the depression of IDR activity in the heart caused by hypoinsulinemia during muscular work, and over compensation of IDR activity in the heart in the recovery period is due to summation of the action of kinins and insulin on IDR activity.

Changes in IDR activity in the liver and heart during starvation and muscular work and in the recovery period after muscular work are thus one mechanism of regulation of gluconeogenesis, glycogenolysis, and glycogen resynthesis.

LITERATURE CITED

1. M. Kh. Gainutdinov, N. Kh. Abdullaev, Z. Z. Gizatullina, et al., Dokl. Akad. Nauk SSSR, 243, 1589 (1978).
2. M. Kh. Gainutdinov, Z. Z. Gizatulina, I. I. Lavina, et al., Dokl. Akad. Nauk SSSR, 257, 1265 (1981).
3. M. Kh. Gainutdinov, I. I.Lavina, and Ya. Kh. Turakulov, Vopr. Med. Khim., No. 1, 87 (1981).
4. E. Newsholme and C. Start, Regulation in Metabolism London (1973).
5. Ya. Kh. Turakulov, Z. Z. Gizatullina, M. Kh. Gainutdinov, et al., Byull, Eksp. Biol. Med., No. 3, 321 (1981).
6. Ya. Kh. Turakulov and M. Kh. Gainutdinov, Physiological Regulation of Energy-Producing Reactions of the Mitochondira [in Russian], Tashkent (1980).
7. I. Bottger, E. M. Schlein, G. R. Faloona, et al., J. Clin. Endocrinol., 35, 117 (1972).
8. N. V. Caral, R. W. Longley, and Y. A. Roe, J. Biol. Chem., 220, 583 (1956).
9. G. Dietze and M. Wicklmayr, FEBS Lett., 74, 205 (1977).
10. Y. H. Exton, in: Hormones and Energy Metabolism, New York (1979), pp. 125-167.
11. A. P. Halestrap, Biochem. J., 172, 389 (1978).
12. D. R. Lamb, J. Peter, R. N. Jeffress, et al., Am. J. Physiol., 217, 1628 (1969).
13. H. Rasmussen and D. B. Goodman, Physiol. Rev., 57, 422 (1977).
14. Ya. Kh. Turakulov (I. Ch. Turakulov), in: Frontiers of Bioorganic Chemistry and Molecular Biology, Oxford (1980), pp. 115-121.
15. S. C. Woods and D. Porte, Physiol. Rev., 54, 596 (1974).

COMPONENTS OF THE GABA-ERGIC SYSTEM AND LIPID PEROXIDATION IN ACUTE EXOGENOUS ACRLONITRILE POISONING

V. V. Ivanov and A. M. Al'shanskii

UDC 615.917:547.391.1.239.2].015.4:[616.831-008.934.663]616-008.939.15

KEY WORDS: stress; monoamines; antioxidants; GABA; liver; brain.

The organism responds to stress by a "sterotyped" form of biochemical, functional, and structural changes, the degree of which is assessed on the basis of changes in concentrations of hormones of the pituitary-adrenal system.

The attention of research workers is currently drawn to the study of cellular and molecular mechanisms of stress injuries in effector target organs. The role of the GABA system in limitation of the stress syndrome has been described and data published on the state of receptor membranes [10]. Hormone reception and barrier, detoxication (the cytochrome P-450 system), and other functions of cell membranes are easily disturbed during the action of products of lipid peroxidation (LPO), one of the most common mechanisms of injury to biomembranes that determines the effects of a number of physical and chemical factors [5]. The attention of research workers is thus beginning to be diverted toward other, as yet inadequately studied mechanisms of stress, but which are also, like changes in the endocrine system, general and nonspecific in character.

This paper describes the study of the state of some parameters of the system of the inhibitory neurotransmitter GABA, certain functions of cell membranes, and the possible role of LPO, and it also assesses the effectiveness of antioxidants in the prevention of stress injuries, in a model of poisoning by the widely used industrial monomer acrylonitrile (AN), during the production and use of which cases of acute poisoning have been observed [13].

Central Research Laboratory and Department of Pathophysiology, Krasnoyarsk Medical Institute.

(Presented by Academician of the Academy of Medical Sciences the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 40-43, July, 1982. Original article submitted September 24, 1981.

0007-4888/82/9407-0899$07.50