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CHANGES IN ACTIVITY OF KEY ENZYMES OF GLYCONEOGENESIS IN THE LIVER AND KIDNEY OF RATS EXPOSED TO SUBEXTREMAL AND EXTREMAL FACTORS

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UDC 612.351.11+612.46.015.1].014.4

Activity of the key enzymes of glyconeogenesis – phosphoenolypyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase (FDPase), and glucose-6-phosphatase (G6Pase) – in the liver and kidneys of rats was investigated during simultaneous exposure of the animals to subextremal and extremal factors. The low initial PEPCK activity in the liver and its high variability under the influence of extremal stimuli are evidence that this enzyme plays the role of limiting stage of glyconeogenesis. PEPCK activity in the kidneys was comparable with FDPase activity and significantly higher than G6Pase activity, PEPCK activity in the kidneys was 5-6 times higher than in the liver. Under the influence of extremal factors, PEPCK and G6Pase activity in the kidneys rose whereas FDPase activity was practically unchanged. The absence of any distinctly synchronized changes in the activity of the key enzymes of glyconeogenesis in the liver and kidneys indicates that there is no single operon in the cells of these organs for PEPCK, FDPase, and G6Pase with a common mechanism of regulation.

KEY WORDS: glyconeogenesis; phosphoenolpyruvate carboxykinase; fructose-1,6-diphosphatase; glucose-6-phosphatase; subextremal and extremal factors.

Glyconeogenesis plays and extremely important role in the maintenance of carbohydrate homeostasis in the body when in a state of prolonged functional stress. For example, in states such as starvation this process is the only source of glucose. The effectiveness of glyconeogenesis in vivo depends primarily on the combined operation of three key enzymes, which can reverse the processes of glycolysis in the tissues: phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase (FDPase), and glucose-6-phosphatase (G6Pase). Some workers regard these enzymes as a single genetically determined enzyme ensemble with common mechanisms of regulation [1, 11]. Glyconeogenesis is known to take place in only two organs, the liver and kidneys. This is because the activity of enzymes such as PEPCK is absent in the other organs [6, 10]. Investigations to study the coordination of functions of the key enzymes on which the effectiveness of operation of the corresponding "metabolic conveyors" depends have virtually not been undertaken.

In this study an attempt was made to examine coordination of the function of PEPCK, FDPase, and G6Pase simultaneously in the liver and kidneys during exposure of animals to subextremal and extremal factors. Within the framework of this problem the results of a comparative evaluation of activity of the

Laboratory of Biochemistry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. P. Kazanacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 6, pp. 544-547, June, 1979. Original article submitted June 2, 1978.

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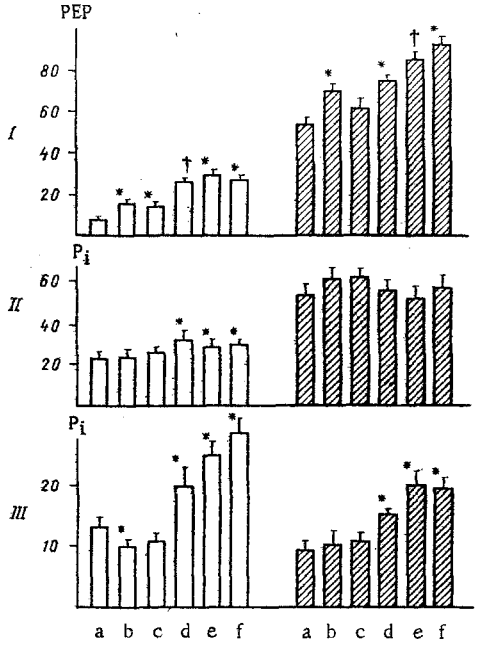

Fig. 1. Changes in activity of key enzymes of glyconeogenesis in rat liver and kidneys during exposure to subextremal and extremal factors. Activity of: I) PEPCK, II) FDPase, III) G6Pase. Unshaded columns represent liver enzymes, shaded columns kidney enzymes. a) Control; b) swimming; c) immobilization; d) starvation; e) starvation + swimming; f) starvation + immobilization. The activity of all enzymes is expressed in nanomoles of product formed per milligram protein per minute at 37°C. Number of animals in each experiment between 9 and 15. *) $P < 0.05$ compared with the control, †) $P < 0.05$ compared with previous state.

TABLE 1. Changes in Some Biochemical Indices in the Blood and Tissues of Rats Exposed to Subextremal and Extremal Factors (M ± m)

Experimental state	Lactate, mg % (n = 10)	Glucose, mg % (n = 10)	11-HCS, μg % (n = 10)	Cyclic AMP	Nanomoles/g
				liver (n = 8)	renal cortex (n = 8)
Control	20,6±1,0	86,6±5,7	20,9±2,5	2,05±0,52	1,51±0,06
Swimming	31,2±2,9*	99,3±5,6	60,3±2,1*	2,68±0,38	2,67±0,42*
Immobilization	21,2±2,3	88,7±4,2	24,7±3,1	2,47±0,32	1,54±0,08
Starvation	18,4±1,4	88,7±5,2	31,8±2,3*	2,12±0,28	1,49±0,08
Swimming after starvation	20,8±1,7	83,7±3,1	65,5±3,9*	2,70±0,36	1,83±0,21

Legend. Asterisk denotes significance ($P < 0.05$) of difference from control; n) number of experiments.

key enzymes of glyconeogenesis in these organs and of the differential role of the liver and kidneys in the maintenance of carbohydrate homeostasis of the body during its adaptation to the action of extremal stimuli also must be interesting.

EXPERIMENTAL METHOD

Female Wistar albino rats weighing 120-150 g were used. Control animals formed group 1; animals compelled to undertake intensive physical exertion (swimming in an aquarium with a load of 4% of the body weight for 4 h in water at a temperature of 30-32°C) formed group 2, animals in a state of immobilization (produced by

keeping them in a close-fitting Plexiglas box of variable volume for 4 h) formed group 3, animals starved for 72 h without restriction of water formed group 4, animals exposed to the combined effect of starvation for 72 h followed by swimming for 4 h formed group 5, and animals also exposed to a combination of factors (starvation for 72 h followed by immobilization) formed group 6. Activity of PEPCK, FDPase, and G6Pase was determined by methods described previously [3]. The activity of all these enzymes was expressed in nanomoles of product (phosphoenolpyruvate and P_i) formed per minute per milligram protein. The serum 11-hydroxycorticosteroid (11-HCS) level was determined fluorometrically [4], the blood lactate concentration was determined by an enzymic method [7], and the glucose concentration by the o-toluidine method [5]. The cyclic AMP concentration in the tissues was estimated by an immunoradioactive method using standard test kits from the Radiochemical Centre, Amersham.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the initial PEPCK activity was 4-5 times higher, and FDPase activity 2-3 times higher in the renal cortex than in the liver. The specific G6Pase activity was virtually identical in the two organs. Under the influence of subextremal and extremal factors the most marked changes were discovered in the "flank" enzymes of glyconeogenesis, namely PEPCK and G6Pase. FDPase activity was virtually unchanged; a very small but significant increase was observed only in the liver in such subextremal states as starvation, and also immobilization and a combination of starvation and subsequent physical exertion. The high initial activity and the low variability of activity of this enzyme suggest that it does not limit the velocity of the process as a whole and, consequently, it is not a limiting factor. Of the two flank enzymes, liver PEPCK was most sensitive to the action of extremal external environmental factors. A significant increase in the activity of this enzyme was observed under the influence of procedures such as immobilization and intensive physical exertion. In the latter case the activity of the enzyme was significantly increased in the renal cortex also. G6Pase activity was actually reduced in these states. The results are evidence that of the three key enzymes of glyconeogenesis, it is PEPCK which plays the role of limiting factor. The short period of exposure (4 h) indicates that the increase in the activity of this enzyme in these states was due to certain allosteric mechanisms rather than to induction of de novo synthesis of the enzyme protein. The more marked stepwise increase in PEPCK activity during starvation for 3 days and during a combination of starvation with immobilization and intensive physical exertion suggests that in these states the increase in enzyme activity is due to the action of both mechanisms.

The increase in PEPCK activity, the absence of change in FDPase activity, and the decrease in G6Pase activity in the liver during immobilization and intensive physical exertion point to considerable variability of regulation of all three key enzymes of glyconeogenesis. The uniform character of the changes (an increase in activity) was observed only during starvation and immobilization and intensive physical exertion preceded by starvation, but the percentage of increase of activity differed for all three enzymes: The increase in PEPCK activity was greatest. In the kidney cortex the changes in activity were uniform in character only for PEPCK and G6Pase. As already noted, FDPase activity was unchanged. These results indicate the absence of synchronized changes and they question the existence of a common operon with a single mechanism for regulation of the synthesis of PEPCK, FDPase, and G6Pase. There is no doubt about the fact that the key enzymes of glyconeogenesis carry different functional loads: Whereas PEPCK is the initial enzyme of glyconeogenesis and determines the velocity of glucose formation in the body, G6Pase is not only a key enzyme of glyconeogenesis, but it also plays a role in the formation of glucose from glycogen in the liver. The two enzymes thus play different functional roles and must have their own mechanisms of regulation. As we have already seen, FDPase behaves in the same way.

Regulation of the activity of the key enzymes of glyconeogenesis in vivo is largely determined by changes in the concentration of glucocorticoids in the blood and cyclic AMP in the corresponding tissues. The molecular mechanisms of hormonal regulation of the key enzymes of glyconeogenesis are not yet clear. A noteworthy feature in the present investigations was the high variability of the 11-HCS concentration in the blood and the much lower variability of the cyclic AMP concentration in the liver and kidneys during exposure to different subextremal and extremal states (Table 1). For example, after starvation for 3 days the 11-HCS concentration in the blood serum was significantly higher, whereas the cyclic AMP concentration did not differ from the control either in the liver or in the kidneys. In this case it is preferable to speak of steroid induction, at least of PEPCK in the liver, independent of cyclic AMP. After intensive physical exertion for 4 h the serum glucocorticoid concentration was twice as high as in the previous case; the cyclic AMP concentration in the tissues (kidney) also was considerably higher. In this case it is probably preferable to speak of allosteric activation of PEPCK, mediated through cyclic AMP. In the case of a combination of these states, i.e., physical

exertion after previous starvation, the serum 11-HCS concentration was just as high as after physical exertion, but the cyclic AMP concentration in the tissues showed only a tendency to rise. This was probably a case when both mechanisms of regulation were in operation. In fact, PEPCK activity in the liver was very low, but in the kidney cortex it was significantly higher during exposure to the combined factors than during ordinary starvation. It is interesting to note that during immobilization no significant increase was found in either the glucocorticoid concentration in the serum or the cyclic AMP concentration in the tissues, although a tendency to rise was observed in the liver. This shift in the cyclic AMP concentration was probably enough to ensure a significant increase in PEPCK activity.

Research into the role of acidosis in the regulation of activity of the key enzymes of glyconeogenesis in the kidneys has recently been published [8, 9]. An increase in the concentration of ketone bodies in the blood was observed by the present writers during starvation [2] and an increase in the blood lactate concentration was found during intensive physical exertion (Table 1). The combined activity of two organs thus reliably stabilizes the blood sugar level (Table 1); under these circumstances the liver plays the role of the principal adaptive organ, whereas the kidneys are probably brought in as a compensatory organ only in critical situations: during prolonged starvation, starvation combined with physical exertion, and immobilization.

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