Injection of BAE was accompanied both by a decrease in the free fatty acid content and by restoration of the original glycerophosphate dehydrogenase activity. In the writers' view, this can be interpreted as a special manifestation of the normalizing effect on liver function, together with regulation of the LP level and the fatty acid composition of individual PL (Tables 1-4). A fact of special interest is the maximal normalization of the fatty acid composition of the phosphatidylcholines which, as we know, play the role of principal carriers of polyunsaturated fatty acids and play an active role in the structural organization of biological membranes and in regulation of the activity of the most important enzyme systems of the cell organelles, especially the liver microsomes [11].

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ACTION OF CYCLIC AMP ON GLYCOLYSIS AND GLYCOGENOLYSIS IN THE ALBINO RAT LIVER AND ADRENALS

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KEY WORDS: glycolysis; glycogenolysis; cyclic AMP; starvation.

The rate of glycolysis in different tissues is modified in different ways under the influence of subextremal and extremal factors: It is slowed in the liver, muscles, and heart, but is unchanged, for example, in the brain [3]. Glycogenolysis undergoes phasic changes: Activation in the initial period of action of an extremal stimulus is followed by inhibition, but no such changes are found in the brain [3]. Inhibition of glycolysis (and glycogenolysis) during stress is associated with an increase in production of catecholamines and glucocorticoids, which act indirectly through cyclic AMP-dependent mechanisms [3-5]. The cyclic AMP content in the adrenals is increased under the influence of ACTH [6]. A similar situation arises during stress, when a decrease in the rate of glycolysis and glycogenolysis would inhibit steroid production as a result of insufficient formation of glucose-6-phosphate (G6P) and, consequently, of NADPH. Activity of phosphorylase and glucose-6-phosphate dehydrogenase in the adrenals rises during stress [1, 7]. Conditions enabling different physiological effects to be produced by the action of the same hormones are thus formed in different tissues in the course of differentiation. Changes in the direction of action of a hormone in the same hormone depending on the conditions are also possible [3, 4]. However, these conditions are as yet unknown.

The object of this investigation was to study changes in glycolysis and glycogenolysis in two organs with high functional specialization – the liver and adrenals – during stress and to examine the role of cyclic AMP in these changes.

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TABLE 1. Changes in Glycolysis and Glycogenolysis (in nmoles lactate/min/mg protein) in Liver and Adrenals of Rats during Starvation (M ± m)

Organ	Experimental conditions	Substrate			
		glycogen	glucose	G6P	FDP
Liver	Control	8,30±0,48 (8)	6,8±0,3 (9)	24,2±0,8 (8)	31,0±1,3 (9)
	Starvation for 3 days	1,90±0,23* (8)	1,80±0,13* (10)	16,9±0,8* (10)	28,5±2,0 (10)
Adrenals	Control	15,4±1,1 (11)	11,3±0,7 (15)	30,5±2,1 (13)	44,5±3,9 (11)
	Starvation for 3 days	19,8±1,6* (13)	15,6±0,8* (17)	38,8±2,3* (15)	41,0±2,7 (11)

Legend. Number of animals in parentheses.
*Differences significant compared with control.

EXPERIMENTAL METHOD

Female Wistar albino rats weighing 150-200 g were used. The rate of glycolysis (glycogenolysis) in the liver and adrenals was determined in a reconstituted system by the method described previously [4, 8]. Starvation of the animals for 3 days, with water *ad lib,* was used as the model of the state of stress. The rate of glycolysis (glycogenolysis) in the test tissues was determined with the use of both initial (glucose, glycogen) and intermediate (G6P, fructose diphosphate) substrates. Hexokinase, phosphofructokinase (PFK), and pyruvate kinase are among the key enzymes of glycolysis [2]. Phosphorylase plays the role of key enzymes in glycogenolysis [3]. The use of G6P and fructose-1,6-diphosphate (FDP) together with glucose and glycogen as substrates, by-passing the initial key enzymes, enables the role of the latter to be distinguished and their effect on the process as a whole to be assessed. To study the action of cyclic AMP on glycolysis and glycogenolysis, cytosol obtained at 105,000g was used. The cytosol was preincubated with cyclic AMP (10^{-5} M), ATP (10^{-3} M), and $MgCl_2$ (50 mM) for 10 min. Cyclic AMP was used in a higher than physiological concentration, on the grounds that under stress conditions its concentration in the tissues rises [5]. To assess the specificity of action of cyclic AMP on glycolysis and glycogenolysis, 2′, 3′-AMP ($5 \cdot 10^{-5}$ M) also was used, and in some cases 2′, 3′-AMP or protein kinase protein inhibitor was added to the cytosol together with cyclic AMP.* The rate of glycolysis (glycogenolysis) with different substrates was estimated and expressed in nanomoles lactate/min/mg protein. Lactate was determined by an enzymic method.

EXPERIMENTAL RESULTS

The rate of glycolysis and glycogenolysis in the adrenals of intact animals was significantly higher than in the liver (Table 1). This is shown by the results obtained by the use of all four substrates, but more convincingly when glucose and glycogen were used. The rate of lactate formation from G6P was higher than from glucose and glycogen, and from FDP it was higher from G6P. This indicates that the first limiting enzyme of glycolysis is hexokinase and of glycogenolysis it is phosphorylase, and that the second limiting enzyme is PFK. The combined rate of the two processes (glycolysis and glycogenolysis) also was lower than the rate of lactate formation from G6P. This indicates that PFK under normal conditions does not limit anaerobic degradation of carbohydrates in the liver and adrenals. Under starvation conditions glycolysis and glycogenolysis in the liver are inhibited. The rate of lactate formation from G6P is reduced. The rate of lactate formation from FDP was not significantly changed (Table 1). The opposite changes were found in the adrenals, where glycolysis and glycogenolysis increased, as did the rate of lactate formation from G6P. Under stress (starvation) conditions, the changes discovered in glycolysis and glycogenolysis thus affected only three substrates: glycogen, glucose, and G6P. This is evidence that under stress conditions activity mainly of only three enzymes is changed in the tissues: phosphorylase, hexokinase, and PFK. These changes, moreover, were opposite in character: A decrease in their activity was found in the liver and an increase in the adrenals. Under the influence of starvation no changes were found in the glycolytic chain in either organ, starting with FDP. The role of hexokinase as limiting enzyme of glycolysis and of phosphorylase as limiting enzyme of glycogenolysis in the liver becomes more prominent still in starvation. In the adrenals changes were in the opposite direction, so that the power of the glycolytic system was significantly increased.

An attempt was made to elucidate some of the mechanisms responsible for the different patterns of regulation of glycolysis and glycogenolysis in the liver and adrenals. Preincubation of liver cytosol with cyclic AMP, ATP, and $MgCl_2$ led to marked inhibition of glycolysis (Fig. 1). No significant changes were found when glycogen, G6P, and FDP were used. These results indicate that under these experimental conditions only hexokinase activity was inhibited. The inhibitory effect

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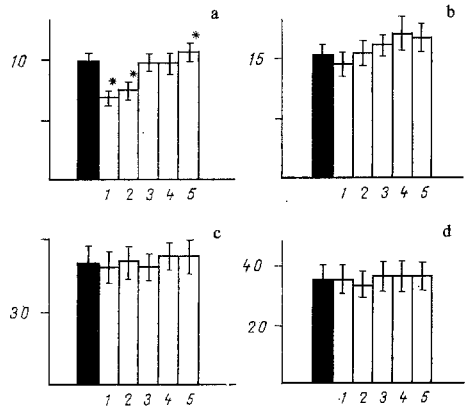

Fig. 1

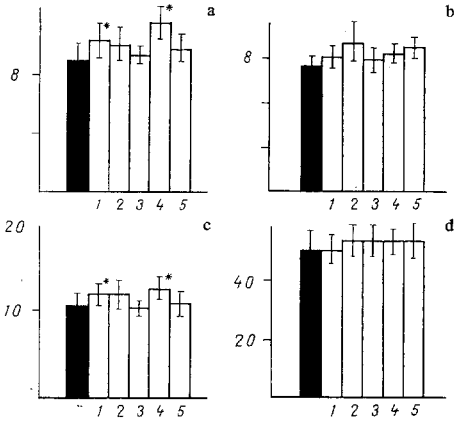

Fig. 2

Fig. 1. Liver. Changes in rate of glycolysis and glycogenolysis in reconstituted system under the influence of cyclic AMP. Black columns – control (without additives); 1) cyclic AMP; 2) cyclic AMP + 2′, 3′-AMP; 3) 2′, 3′-AMP: 4) cyclic AMP + protein inhibitor. *) Significance of differences compared with control. a) Glucose, b) glycogen, c) G6P; d) FDP. Ordinate, rate of glycolysis (glycogenolysis) in nmoles lactate/min/mg protein.

Fig. 2. Adrenals. Changes in rate of glycolysis and glycogenolysis in reconstitured system under the influence of cyclic AMP. Legend as to Fig. 1.

of cyclic AMP was highly specific. No similar action was found with 2′, 3′-AMP. This likewise did not abolish the inhibitory action of cyclic AMP on hexokinase. The decrease in activity of the enzyme under the influence of cyclic AMP was probably connected with its phosphorylation by the corresponding protein kinase. Addition of protein kinase protein inhibitor and cyclic AMP to the incubation medium in fact completely abolished the inhibitory effect of the latter on hexokinase and ensured an adequately high rate of glycolysis (at the control level) in the liver. Under these conditions inhibition of glycogenolysis and the decrease in the rate of lactate formation from G6P in the liver, observed *in vivo* during starvation, could not be reproduced *in vitro*. This indicates that the regulation of phosphorylation and PFK is a more complex process than is generally considered [9]. It has recently been shown that the phosphatase of PFK and, probably, the phosphatase of liver phosphorylase are under cyclic AMP-dependent control. This effect is realized through inactivation of the low-molecular-weight protein inhibitor of PFK phosphatase. The molecular mechanisms of the action of cyclic AMP on the protein inhibitor are not yet known. It is stated in the same citation that phosphorylation of PFK in the liver is carried out by a cyclic AMP-independent protein kinase.

In the adrenals, preincubation of the cytosol with cyclic AMP, ATP, and $MgCl_2$ did not lower the rate of anaerobic breakdown of carbohydrates on any substrate. Moreover, there was even a small increase in the rate of glycolysis and of lactate formation from G6P (Fig. 2). Activation was not abolished in the presence of protein kinase protein inhibitor. In the writers' view, this effect was not connected with regulation of activity of the enzymes of glycolysis by a phosphorylation – dephosphorylation mechanism, but was due to the effector influence of cyclic AMP on hexokinase and PFK. The absence of an inhibitory action of cyclic AMP on hexokinase in the adrenals is evidence that in these glands there is no cyclic AMP-dependent protein kinase which could phosphorylate the enzyme and reduce its activity. No changes were found in the adrenals when glycogen and FDP were used.

The results thus indicate that changes in glycolysis and glycogenolysis in the liver under stress conditions (starvation for 3 days) are connected with inhibition of probably only three enzymes: phosphorylase, hexokinase, and PFK. Changes in the adrenals under similar conditions were reversible in character. Analysis of the molecular mechanisms of the changes thus revealed points to the conclusion that inhibition of hexokinase in the liver in stress is due to phosphorylation of the enzyme under the influence of a cyclic AMP-dependent kinase. Evidently because of the absence of such an enzyme in the adrenals the rate of glycolysis in these glands is not inhibited during stress. The slight activation of glycolysis observed in the adrenals under the influence of cyclic AMP *in vivo* was probably due to the effector influence of this substance. The marked rise in hexokinase activity and in the rate of glycolysis in the adrenals *in vivo* under stress conditions requires further analysis.

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POLYPEPTIDES ISOLATED FROM THE GASTRIC MUCOSA AND THEIR ACTION ON PEPSIN BIOSYNTHESIS BY GASTRIC GLANDS

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KEY WORDS: stomach; pepsin biosynthesis; polypeptides.

Administration of natural and artificial gastric juice is based on the principle of replacement therapy, in which a patient is prescribed a remedy containing pepsin [5, 6, 11]. However, it has recently been noted that preparations of the pepsidil type and new artificial gastric juice (NAGJ) contain a hydrochloric acid solution of products of enzymic hydrolysis of gastric mucosal tissue [4, 11], which has the power to excite gastric secretion [1, 12]. It has also been shown that a mixture of polypeptides, formed during the production of pepsin preparations, the technology of which is based on the principle of autolysis [13], possesses a similar property. These polypeptides have been isolated by the writers from the autolyzate by chromatography with Sephadex G-100, after which they were separated by repeated chromatography on Sephadex G-25 columns into three fractions, conventionally termed A (mol. wt. ∿2800), B (mol. wt. ∿2500), and C (mol. wt. ∿2200).

The object of the present investigation was to study which of these polypeptides has the greatest ability to stimulate pepsin biosynthesis in the gastric mucosa and whether this property of the peptides is modified through the action of proteinases (pepsin and trypsin) on them in the digestive tract.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male albino rats weighing 120-150 g. A food stimulus (1 ml milk) was introduced simultaneously with one of the test substances into the stomach of all the rats through a tube. After 1 h the stomach was removed quickly from the peritoneal cavity and opened, after which it was washed twice with cold distilled water. A homogenate was prepared from the gastric mucosa and 0.01 N HCl in the ratio of 1:10. To convert the whole of the pepsinogen into pepsin, the homogenate was incubated for 1 h at 37°C. Pepsin activity in the homogenate was determined as its proteolytic action at pH 3.0 [15], for it is in such a medium that pepsin mainly exhibits its action.

The milk-curdling ability of pepsin is due to rupture of peptide bonds in the caseinogen molecule, which is converted into the unstable form – casein [3]. This test is widely used to determine the activity of the enzyme at pH 5.0, on the basis of its milk-curdling action [8].

A mixture of the food stimulus and 1 ml of 0.01 N HCl, into which 30 mg of polypeptide B, denatured at 100°C, had first been dissolved, was introduced into the stomach of the rats of group 1 (control). Besides the food stimulus, the animals of groups 2, 3, and 4 each received 1 ml of 0.01 N HCl in which 30 mg of polypeptides A, B, and C, respectively, had been dissolved. The rats of group 5 received the food stimulus alone: 0.75 ml 0.01 N HCl and 0.25 ml NAGJ (containing 2 mg/ml pepsin and 106 mg/ml of a mixture of polypeptides). The results of these series were subjected to statistical analysis [2].

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