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EFFECT OF CHOLERA ENTEROTOXIN ON CARBOHYDRATE METABOLISM IN THE LIVER AND SMALL INTESTINAL MUCOSA OF RABBITS

P. R. Vengrov, T. D. Cherkasova, V. A. Yurkiv, and V. I. Pokrovskii

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Carbohydrate metabolism in the liver is connected with changes in the cAMP level in the cell. An increase in the cAMP concentration is accompanied by release of glucose from glycogen, stimulation of gluconeogenesis, and inhibition of glycolysis. Cholera enterotoxin is a factor known to increase adenylate cyclase activity, and thereby to increase the cAMP concentration in various tissues, including the liver [9]. There is evidence in the literature that cholera toxin, in primary hepatocyte culture, activates glycogenolysis and inhibits lactic acid formation, probable evidence of the inhibition of glycolysis [9]. This effect of the toxin on carbohydrate metabolism is in agreement on the whole with the action of other factors such as glucagon and adrenalin, which raise the cAMP level in the liver.

Cholera enterotoxin irreversibly activates adenylate cyclase in the mucosa of the small intestine [6]. It has been shown in biopsy specimens from the mucosa of the jejunum in vitro that cholera toxin inhibits pyruvate kinase, which catalyzes an irreversible glycolysis reaction, and stimulates fructose-1,6-diphosphatase [12, 13].

One of the least studied enzymes of carbohydrate metabolism both in the liver and in the small intestinal mucosa, is glucose-6-phosphatase, which unites two metabolic pathways in the liver: gluconeogenesis and glycogenolysis. Glucose-6-phosphatase catalyzes the irreversible reaction of glucose-6-phosphate hydrolysis to glucose and inorganic phosphate and it is possibly one of the regulatory enzymes of gluconeogenesis. The view is held that this enzyme also discharges dephosphorylated glucose into the channels of the endoplasmic reticulum, and consequently into the intercellular space.

In the mucosa of the small intestine glucose-6-phosphatase evidently plays an additional role in glucose transport, for glucose is found inside the cell in a phosphorylated form [3].

Laboratory of Molecular Bases of Pathogenesis of Infectious Diseases, Central Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 4, pp. 410-413, April, 1987. Original article submitted March 13, 1986.

TABLE 1. Changes in Intensity of Gluconeogenesis in the Liver under the Influence of Cholera Enterotoxin (M ± m)

Experimental conditions	Intensity of gluconeogenesis in liver	
	μmoles glucose / g tissue	nmoles glucose/ mg protein
Control	4.93±0.68	12.0±1.7
Injection of cholera toxin	8.35±0.09	18,9±0.2

TABLE 2. Changes in Glucose-6-Phospatase Activity in the Liver under the Influence of Cholera Enterotoxin (M ± m)

Experimental conditions	Glucose-6-phosphatase activity in liver	
	μmoles P_i /g tissue	nmoles P_i/mg protein
Control	770±7	1791±16
Injection of cholera toxin	630±21	1465±50
Feeding	485	1010

Legend. During feeding the animals had free access to food.

The effect of cholera enterotoxin on the activity of this enzyme in the mucosa of the small intestine and in the liver has not been studied, and accordingly, in the investigation described below, the effect of cholera enterotoxin, injected in vivo, on glucose formation from alanine, and also on glucose-6-phosphatase activity in the liver and mucosa of the small intestine, was studied in rabbits.

EXPERIMENTAL METHOD

Cholera enterotoxin (Schwarz/Mann) was injected in a dose of 125 μg into the lumen of an isolated loop of jejunum, 15 cm long, in a rabbit weighing 1.5 kg (the animals were deprived of food for 48 h before the experiment). Physiological saline was injected into a neighboring loop of the jejunum, 15 cm long. The animals were killed 3 h after injection of the reagents.

Tissue from the mucosa and liver was homogenized in Tyrode solution in a homogenizer of Potter type, in the ratio (w/v) of 1:7/1:10, respectively. The homogenates were incubated with L-alanine (Sigma, USA) in a final concentration of 40 mM for 45 min at 37°C. L-[2,3-^{3}H]-alanine was added to the incubation medium in a dose of 4 μCi per sample. To stop the reaction 100 μl of 0.3 N HCl solution was added to a sample of 200 μl. The residue was removed by centrifugation at 1000g for 15 min. The supernatant (pH 2.0) was applied to a column with Dowex 50 × 8 (7 × 15 mm), after which it was washed with 1.5 ml of 0.1 N HCl solution. The eluent from the first column (pH 8.0) was applied to a column with Dowex 1 × 8 (7 × 15 mm). The solution obtained after these two columns was lyophilized, the dry residue was dissolved in 100 μl of distilled water, an aliquot of 10 μl was chosen, and thin-layer chromatography carried out with a reference substance on glass plates: silica-gel G (Serva, West Germany) + 0.1 N boric acid solution. The mobile phase was a system of butanol–acetone–H_2O (4:5:1). Chromatograms were developed with 5% $AgNO_3$ with the addition of an aqueous solution of ammonia. Spots corresponding to glucose (R_f 0.53) were scraped off and extracted with 1 ml H_2O. The quantity of radioactive glucose was determined in a scintillation counter (RackBeta, LKB, Sweden), using Ready Solv MP scintillator (Beckman, USA).

Glucose-6-phosphatase activity was determined by Baginski's method.

EXPERIMENTAL RESULTS

Secretion of fluid in the experimental segment 3 h after injection of cholera enterotoxin into the lumen of the jejunum amounted to 0.44 ml/cm length of intestine. This process was shown to be active in character, i.e., it requires energy [16]. Some workers consider that the main source of energy in the mucosa of the small intestine is glycolysis [7, 11]. Glucose required for intensive working of transport mechanisms (as is observed in secretory diarrhea induced by cholera toxin) cannot be used for the glycogen depots, for during starvation for 48 h the glycogen reserves must be virtually completely exhausted [2]. In the present experiments considerable (by 60%) activation of glucose synthesis was found in the liver under the influence of cholera enterotoxin (Table 1). Possibly it is gluconeogenesis in the liver that is the source of the energy substrate for secretion induced by cholera toxin, although the mechanism of activation of glucose synthesis in this case is not clear. In the present experiments cholera toxin evidently could not have any direct action on the liver cells. Knowing that glyconeogenesis is stimulated by cAMP, it can be tentatively suggested that under the influence of enterotoxin a neurohumoral mechanism is triggered in the mucosa of the small intestine, and its operation leads to elevation of the cAMP level in the hepatocytes.

The stimulating effect of cAMP on gluconeogenesis in the liver cells is connected, in the opinion of most investigators, with activation of fructose-1,6-diphosphatase, the key enzyme of glucose synthesis. Glucose-6-phosphatase occupies a key position in carbohydrate metabolism, for it is responsible for the release of glucose from the cells, but the activity of this enzyme is evidently regulated by the substrate concentration [8]. Whatever the case, the hypothesis regarding phosphorylation and simultaneous activation of glucose-6-phosphatase by cAMP-dependent protein kinase has not been confirmed [5]. The results of determination of glucose-6-phosphatase activity in the liver are given in Table 2.

Activity of this enzyme is strongly dependent on the diet, as data of other authors have confirmed [1].

Glucose-6-phosphatase activity in the liver fell by 22% 3 h after injection of cholera toxin into the intestinal lumen, even though the intensity of glucose synthesis increased. The view is held that glucose-6-phosphatase is connected with membranes of microsomes and consists of three components [4]: 1) a carrier of glucose-6-phosphate, 2) phosphohydrolase, and 3) a carrier of P_i.

It is possible that in the present experiments changes in activity only of the phosphohydrolase component were discovered, and not of the whole enzyme complex. This hypothesis is confirmed by the fact that in von Gierke's disease (type I glycogenosis), the predominant stage in the pathogenesis of which is an inherited disturbance of the glucose-6-phosphate carrier, the phosphohydrolase reaction takes place normally in samples of frozen liver [10].

Glucose-6-phosphatase activity in the mucosa of the small intestine also depends on diet: as will be clear from Tables 2 and 3, starvation for 48 h leads to increased activity of the enzyme in the liver, whereas in the mucosa a tendency is observed for it to fall. This fact can evidently be explained on the grounds that glucose-6-phosphatase activity depends on substrate concentration; during starvation, glucose transport through the epithelium of the small intestine is depressed, and the intracellular glucose-6-phosphate concentration is accordingly limited, and gluconeogenesis in the liver is intensified under these circumstances.

We know that, besides elevation of the cAMP level, cholera enterotoxin stimulates fructose-1,6-diphosphatase in the mucosa of the small intestine, but no intensification of glucose synthesis could be discovered in that tissue (Table 4).

Glucose-6-phosphatase activity in the mucosa was unchanged through the action of cholera toxin (Table 3). Glucose synthesis from amino acids in the mucosa of the small intestine evidently has a different mechanisms of regulation from the cAMP-dependent type. This conclusion is supported by data showing that glucagon, which stimulates gluconeogenesis in the liver through elevation of the cAMP level, does not affect adenylate cyclase activity in the mucosa, and moreover, it stimulates lactate formation [14].

The following suggestions can be put forward on the basis of the above remarks. The secretory process induced by cholera enterotoxin in the rabbit small intestine is supplied with energy through intensification of gluconeogenesis in the liver. The toxin may perhaps exert its action through neurohumoral mechanisms, raising the cAMP level in the liver.

Gluconeogenesis in the mucosa of the small intestine, calculated per milligram of protein, is 2.5 times more intensive than glucose synthesis in the liver, and its mechanism of regula-

TABLE 3. Changes in Glucose-6-Phosphatase Activity in Mucosa of Small Intestine under the Influence of Cholera Enterotoxin (M ± m)

Experimental conditions	Glucose-6-phosphatase activity in mucosa of small intestine	
	μmoles P_i/g tissue	nmoles P_i/mg protein
Control	212±19	1080±96
Injection of cholera toxin		
into experimental segments	247±11	1007±44
into control segment	258±8	971±29
Feeding	278	1190

TABLE 4. Changes in Intensity of Gluconeogenesis in Mucosa of Small Intestine under the Influence of Cholera Enterotoxin (M ± m)

Experimental conditions	Intensity of gluconeogenesis in mucosa of small intestine	
	μmoles glucose/g tissue	nmoles glucose/mg protein
Control	5.83±0.41	29.8±2.1
Injection of cholera toxin		
into experimental segments	5.71±0.18	29.1±1.0
into control segments	6.34±0.08	29.2±0.4

tion is evidently different. Elevation of the cAMP level in the mucosa through the action of cholera toxin does not correlate with intensification of glucose synthesis in that organ.

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REPAIR OF HEPATOCYTE MEMBRANES BY PHOSPHATIDYLCHOLINE AFTER HELIOTRINE POISONING

O. V. Dobrynina, S. Z. Shatinina, and A. I. Archakov

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Toxic hepatitis induced by various compounds (CCl_4, heliotrine, etc.) is accompanied by damage to hepatocyte membrane systems. Evidence of this is given by distrubances of the enzyme systems of the endoplasmic reticulum, which metabolize toxic substances entering the body [2, 3], and the marked elevation of the blood enzyme levels, reflecting disturbances of the integrity of the plasma membrane [8]. These lesions are partly abolished by the use of phosphatidylcholine liposomes [4, 7], as has been shown in experimental toxic hepatitis due to CCl_4.

The aim of this investigation was to study the reparative action of phosphatidylcholine liposomes in experimental toxic hepatitis caused by heliotrine.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 100-150 g. A heliotrine model of toxic hepatitis was used. Heliotrine was injected subcutaneously or intraperitoneally (in the form of a neutral aqueous solution) in doses of 10, 20, and 30 mg/100 g body weight. The animals were decapitated 24 h later, blood was collected, and fructose-1-monophosphate aldolase activity in the serum obtained from it was determined by Shapiro's method in Baginski's modification [9]. Egg phosphatidylcholine was isolated by the method in [6]. Dilinoleylphosphatidylcholine (DLPC) and dipalmitoylphosphatidylcholine (DPPC) (both from Sigma, USA), and preparations of Lipostabil and Essentiale (Nattermann, West Germany) were used. Multilayered

Department of Biochemistry, Medico Biological Faculty, N. I. Pirogov Second Moscow Medical Institute.

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