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EFFECT OF "MURINE" TOXIN OF *Yersinia pestis* ON CARBOHYDRATE METABOLISM IN RATS

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KEY WORDS: "murine" toxin of *Yersinia pestis*; carbohydrate metabolism; rat blood and liver.

Many bacterial toxins, when administered intravenously and parenterally, give rise to toxic-infectious shock and to death of the experimental animals, which develops against the background of a profound disturbance of carbohydrate metabolism. Endotoxin shock is known to be accompanied by phasic changes in the blood glucose level, by hyperlactatemia [7, 10], and also by an increase in the blood concentrations of insulin and glucagon [9]. Accumulation of glucose in the blood plasma in the early stages of the action of lipopolysaccharides takes place on account of intensive catecholamine release and an increase in the intensity of glycogenolysis in the liver [5]. Besides an endotoxin of lipopolysaccharide nature, *Yersinia pestis* also contains a so-called "murine" toxin, which is firmly bound with the bacterial cell wall. The "murine" toxin is a thermolabile protein [8], and its parenteral or intravenous administration causes the development of shock and death of mice and rats [11]. The pathogenetic importance and biochemical mechanisms lying at the basis of the action of the "murine" toxin of *Y. pestis* has not been adequately studied. It is suggested that its toxic action is connected with its ability to induce "functional adrenalectomy" in animals [8, 12]. The sympathicoadrenal system plays an important role in the regulation of metabolic processes and, in particular, of carbohydrate metabolism.

The aim of the present investigation was to accordingly study carbohydrate metabolism in rats in the course of development of toxic-infectious shock due to "murine" plague toxin.

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TABLE 1. Blood Glucose and Lactate Concentrations during Development of Toxic-Infectious Plague Shock (M ± m; n = 8)

Time, h	Glucose, mg%	Lactate, μM
0	127+4	3,2±0,2
1/2	106±0,3	4,4±0,6
1	111±3	3,5±0,3
2	88±5*	2,8±0,2
5	59±9*	3,8±0,4

Legend. Here and in Table 2, *p < 0.05 compared with control.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 150-200 g. Toxic effects were induced by injection of 0.5 ml of physiological saline, containing 0.5 mg (LD_{50}) of a preparation of toxin (Becker's fraction II), into the caudal vein. The control animals were given an injection of an equal volume of physiological saline into the caudal vein. All procedures were carried out under superficial ether anesthesia. The animals were autopsied 30 min and 2 and 5 h after injection of the toxin, and the liver was removed and frozen in liquid nitrogen. Blood was collected from the heart in a cooled test tube containing 5000 KIU of trasylol and 14 mg of EDTA to 10 ml of blood. After sedimentation of the cells by centrifugation, the lactate concentration in the blood plasma was determined by an enzymic method [6] and concentrations of glucagon and insulin were determined by radioimmunoassay, using kits from DRG (USA) and of Soviet origin respectively. The glucose level was determined in whole blood by the orthotoluidine method. Frozen liver tissue was weighed and homogenized in a homogenizer of "Politron" type. After extraction (of cAMP) with ethanol it was determined quantitatively by radioimmunoassay using kits from "Amersham International" (England). All the results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that injection of "murine" plague toxin causes the blood glucose level of the experimental animals to fall compared with that found in intact rats at all times of observation. After 5 h of toxic action the blood glucose level was reduced to half of that in the control. Unlike in shock caused by endotoxins of Gram-negative bacteria, the hyperglycemic phase in the initial stages of action of the "murine" plague toxin was not observed.

The hypoglycemia caused by the toxin may be explained in several ways. We know that the main cause of the fall in the glucose level in the late stages of action of lipopolysaccharides is its increased demand by the peripheral tissues and blood cells [14]. In some cases this is connected with the insulin-like action of the toxins [13]. The more rapid utilization of glucose by the peripheral tissues is accompanied by lactate accumulation in the blood and by hyperinsulinemia [9]. Under the influence of "murine" plague toxin we found no statistically significant increase in the blood lactate concentration (Table 1) and the plasma insulin level fell sharply compared with that in the control at all times of observation (Table 2). After 5 h of action of the toxin the plasma insulin concentration was reduced eightfold compared with the control. Such a sharp fall of the insulin level was evidently due to lowering of the plasma glucose concentration [3].

Thus the hypoglycemia arising in the course of development of toxic-infectious plague shock is unconnected with any reduction of the demands for glucose by peripheral tissues.

Inhibition of enzymes of glycogenolysis and gluconeogenesis of the hormonal regulation of these metabolic processes may be another cause of the hypoglycemia developing under the influence of toxins. An important role in the regulation of glycogenolysis and gluconeogenesis in the liver is played by adrenalin and glucagon. When the blood glucose concentration falls, adrenalin is released from the adrenal medulla and glucagon from the pancreas. In the present experiments the hypoglycemia developing under the influence of the plague toxin causes a sharp rise in the blood glucagon level (Table 2). The 50% reduction in the glucose concentration

TABLE 2. Plasma Glucagon and Insulin Concentrations and cAMP Concentration in Liver during Development of Toxic-Infectious Plague Shock (M ± m; n = 7-9)

Time, h	Glucagon, pg/ml	Insulin, pmoles/liter	cAMP, pmoles/g tissue
0	580±71	565±83	101,9±12,3
½	708±30	263±38*	161,5±18,0*
1	701±24	366±83	—
2	805±69*	237±45*	106,9±8,4
5	5193±713*	71±32*	179,5±18,6*

observed 5 h after injection of the toxin was accompanied by a seven-eightfold increase in glucagon accumulation in the blood plasma.

The activating action of adrenalin and glucagon on glycogenolysis and gluconeogenesis in the liver is effected through stimulation of adenylate cyclase through β-adrenergic and glucagon receptors, and a subsequent rise of the intracellular level of cAMP, which plays an important role in the regulation of activity of enzymes of carbohydrate metabolism. Experimental studies have shown that "murine" plague toxin can inhibit the release of free fatty acids from the liver induced by adrenalin, but does not incluence the effects of glucagon or of dibutyryl-cAMP. The lethal effect of intraperitoneal injection of the toxin can be prevented or considerably reduced by injection of cholera toxin and of glucagon, but not of adrenalin. On the basis of these findings it has been suggested that "murine" plague toxin blocks β-adrenoreceptors [4]. Meanwhile the opinion is held that the molecular mechanisms of the action of "murine" plague toxin is determined by inactivation of adenylate cyclase, as a result of which the regulation of intracellular processes mediated not only by adrenalin, but also by glucagon, and also by other biologically active substances which exert their action through receptors coupled with this system [1], is blocked.

It will be clear from Table 2 that under the influence of "murine" plague toxin the cAMP concentration in the liver tissue was increased by 1.6 times after 30 min compared with the intact control. After 2 h of toxic action the normal cAMP concentration was restored, but toward 5 h, it was 1.8 times higher than its level in intact rats. The increase in the cAMP concentration in the liver tissue of experimental animals is evidently due to activation of the adenylate cyclase system under the influence of glucagon, the plasma level of which in the late stages of action of the toxin was sharply increased compared with the intact control. The cAMP level in the liver, incidentally, was increased also in the initial stages of action of the plague toxin (30 min) compared with that in the control. This effect was evidently due to the influence of adrenalin on the liver adenylate cyclase, whose level was sharply increased as a result of the primary stress reaction of the animal to the action of the toxin. It was during this period (30 min-1 h) that the stable levels of glucose, glucagon, and insulin, indistinguishable from the control, were maintained in the blood stream (Tables 1 and 2).

The results indicate that the "murine" toxin does not disturb regulation of the adenylate cyclase system by glucagon, in agreement with data on the protective action of glucagon obtained previously [8, 12].

The biochemical mechanisms lysing at the basis of the disturbance of carbohydrate metabolism in toxic-infectious shock caused by "murine" plague toxin are thus unconnected with any increased demand for glucose by the peripheral tissues or disturbance of cAMP-dependent regulation of glycogenolysis and gluconeogenesis in the liver. "Murine" plague toxin evidently causes a disturbance of carbohydrate homeostasis by direct inhibition of enzymes involved in glucose synthesis in the liver. A similar mechanism of development of hypoglycemia due to blocking of phosphoenolpyruvate carboxykinase, with simultaneous inhibition of glycogen breakdown in the liver, has been demonstrated under the influence of several endotoxins of Gram-negative bacteria [2].

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ESTIMATION OF EFFECTIVENESS OF SPECIFIC PLASMA PERFUSION WHEN USED IN EXPERIMENTAL ACUTE PANCREATITIS

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Acute pancreatitis is a serious disease of the pancreas which is difficult to diagnose and treat. The overall mortality from acute pancreatitis is 8.3%, and in destructive forms it may reach 30-60% [9]. An important role in the pathogenesis of acute pancreatitis is played by proteolytic enzymes, which enter the blood stream as a result of activation of zymogens of proteinases, synthesized in the pancreas (trypsin, chymotrypsin, elastase), as a result of accumulation of granulocytes in the region of the affected organ (elastase of granulocytes etc.), and of tissue destruction (cellular cathepsins).

In the blood stream these proteinases, by activating the most important proteolytic systems, lead to changes in hemostasis and to the development of a disseminated clotting syndrome and a state of collapse and shock [2, 4]. A method of removing proteinases from the blood stream by specific hemoperfusion in experiments on animals is known. A soy bean inhibitor immobilized on silica-gel [5], a proteinase inhibitor from bovine organs of the Kunitz type (BPTI), and duck ovomucoid, covalently cross-linked with a polyacrylamide carrier [3], has been suggested as specific sorbents for proteinases.

The aim of this investigation was to develop a more effective sorbent of proteinases from the blood plasma of animals with acute pancreatitis, consisting of an acid-stable inhibitor (ASI) of proteolytic enzymes from human urine, immobilized on sepharose.

EXPERIMENTAL METHOD

The ASI was isolated by a modified method [6, 11] from the urine of patients with nephritis. The ASI was immobilized (virtually 100%) on sepharose by the cyanogen bromide method, with the addition of 2 mg of ASI to 1 ml of swelling sorbent to the reaction mixture. Granylocytic elastase was isolated from the buffy coat by a modified method [7].

Experiments were carried out on mongrel dogs of both sexes weighing 10-16 kg. In the animals (9) of group 1 (control), blood was taken under adequate anesthesia into heparin from

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