mechanisms of this phenomenon. The effect of Ca^{++} is evidently not mediated by calmodulin, for it is unchanged by trifluoperazine. The impression is gained that Ca^{++} acts directly on ICDH. However, more complex mechanisms cannot yet be ruled out: phosphorylation of some unknown protein intermediary or the participation of another Ca^{++}-receptive protein. For instance, it has recently been shown that phosphorylation of a mitochondrial protein with molecular weight of 3500 daltons is increased by glucagon [12]. Ca^{++} metabolism in the mitochondria is modified by stimulators not only of α-adrenoreceptors, but also of β-adrenoreceptors [5], and also by cAMP [8]. Perhaps Ca^{++} ions activate ICDH through the intermediary of catecholamines and cAMP.

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EFFECT OF CHOLERA ENTEROTOXIN ON THE ANTITOXIC SYSTEM OF THE RAT SMALL INTESTINE AND LIVER

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KEY WORDS: cholera enterotoxin, small intestine, liver.

An important aspect of the study of the effect of cholera toxin (CT) *in vivo* is the state of the detoxication enzyme system (DES). This system has evolved in order to activate potentially harmful cytotoxic substances formed during metabolism and to render harmless toxins entering from outside. The first function has been studied reasonably well. Activity of enzymes of the mono-oxygenase system (stage I of detoxication) and activity of epoxide hydratase (EH) and glutathione-SH-transferase (GT) (stage II) has been investigated in the liver, kidneys, lungs, and intestine [4, 5, 10, 13], and in the blood cells [12]. The second function of DES is only beginning to be investigated [6, 8]. In our view, to understand the role of the antitoxic system in mobilization of the defensive forces of the body in infectious pathology it is essential to examine the state of those of its enzymes, such as EH, cytochrome-450, uridine disphophoglucuronyl-transferase, GT, superoxide dismutase (SOD), and glutathione peroxidase (GP), in the small intestine, which is the target for bacterial toxins, and also in the liver, the principal detoxicating organ.

The aim of this investigation was to study the principal enzyme of nonspecific defense of the body against the toxic action of oxygen (SOD) and of GP in cytosols of the mucous membrane of the small intestine and in the liver of rats exposed to the action of CT.

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EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 140-160 g. The animals were deprived of food for 24 h before the experiment. Intraperitoneal injection of a 2% solution of hexobarbital was used for anesthesia. CT was injected (70 μg/100 g body weight in 0.5 ml of physiological saline) into the jejunum of the anesthetized animals, into a segment isolated by two ligatures at a distance of 18-20 cm apart. Control rats received an injection of physiological saline only. The animals were decapitated in groups of 6 at a time after 30 min and 1, 2, and 4 h. The liver was perfused, and the ligated portion and two neighboring portions of the small intestine were washed with cold 0.15 M Tris-HCl buffer, pH 8.0, containing 0.3 mM phenylmethylsulfonyl fluoride. Cytosol fractions were isolated from the mucosa of the small intestine and from the liver in the above buffer [5]. Cytosol was kept if necessary at −20°C for not more than 3-7 days. GT activity against 1-Cl-2,4-dinitrobenzene was measured at 37°C [9] and GP activity at 30°C with tert-butyl hydroperoxide [14] and SOD activity by the method in [3] at 30°C. Protein was determined by Lowry's method [11]. Significance of differences between experimental and control values was estimated by Student's test [2].

EXPERIMENTAL RESULTS

GP and SOD activity in cytosol of the small intestinal mucosa of intact rats, having been determined for the first time, was compared with activity of these enzymes in the liver. GP activity in the liver (90 ± 15.27 nmoles/mg protein/min) was almost ten times higher than in the mucosa, where the level of GP activity was the same in all parts of the small intestine (in nmoles/mg protein/min): 7.18 ± 1.28 in the proximal portion, 10.56 ± 4.28 in the middle part, and 8.95 ± 1.21 in the distal portion. SOD activity was higher in the liver than in the intestinal mucosa (in units/mg protein): 30.6 ± 9.3 in the liver, 18.7 ± 3.5 in the proximal part of the intestine, 17.5 ± 4.7 in the middle part, and 25.6 ± 5.1 in the distal part of the intestine. Activity of GT, like that of GP, was much higher in the liver than in the mucosa. In the liver it was 892.8 ± 180.1 nmoles/mg protein/min, whereas in the small intestine it was 176.1 ± 15.9, 147.1 ± 32.7, and 57.9 ± 17.1 nmoles/mg protein/min respectively. The main reserves of enzymes of the body's antitoxin system are thus concentrated in the liver.

The time course of GT activity was similar in the cytosol of the mucosa of the proximal, middle, and distal parts of the small intestine, even though only the middle part of the small intestine was exposed to the direct action of CT: a sharp decline in enzyme activity 30 min after injection of the toxin, an increase up to the control level after 2 h, and a further decline after 4 h. Activity of this enzyme in the liver cytosol remained at the control level, without any statistically significant changes.

GP activity in the mucosa of all parts of the small intestine showed similar changes to GT. Activity of this enzyme in the liver rose until 1 h, returned to the control level toward 2 h, and fell sharply by 4 h after injection of the toxin.

Changes in the SOD level in the cytosol of the small intestinal mucosa were as follows: in the proximal part it remained at the control level with no statistically significant deviations. In the middle and distal parts SOD activity fell until 1 h, rose to the control level toward 2 h, and fell toward 4 h after injection of the toxin in the distal part, and remained at a low level until 4 h after injection in the middle part. The time course of SOD activity in the liver was similar to that in the mucosa of the distal portion.

Changes in activity of the DES enzymes under the influence of CT were thus general in character and affected all parts of the mucosa of the small intestine and the liver. The fall in SOD activity indicates an increase in the content of superoxide anions — radicals lead to activation of phospholipases. One result of this could be increased liberation of arachidonic acid and synthesis of prostaglandins (PG), with the consequent development of "inflammatory" lesions — disturbances of cellular permeability and possible loss of enzymes leading to an intracellular deficiency. This mechanism gives rise to a vicious circle: enzyme deficiency leads to accumulation of toxic products of oxygen and to fresh damage to the cells. This hypothesis of a disturbance of permeability is supported by data obtained in the writers' laboratory showing an increase in the content of lysoforms of phospholipids in membranes of the mucosa of the rabbit small intestine [1].

The decline in GP activity also leads to accumulation of peroxidation products, including hydroperoxides of fatty acids, which are activators of cyclo-oxygenase, the key enzyme in PG

biosynthesis and, consequently, to fresh release of PG. It can be expected that minimal GP activity will correspond to the maximal PG level.

GT is the key enzyme of biodegradation of epoxides in the small intestine and one of the principal enzymes of DES in the liver. The sharp decline in its activity is evidence of severe damage to the antitoxic system of the small intestinal mucosa.

We know that CT does not penetrate into the blood stream [7, 15] and, as the writers' experiments *in vitro* have shown, it does not affect enzymes of DES (data not published in this paper). It can be concluded that the general character of changes in enzyme levels in the liver and mucosa is determined by the indirect action of the toxin on the enzymes concerned. Most probably the cause of the changes observed in DES is excessive formation of toxic substances, namely inhibitors of the enzymes indicated above or the systems for their biosynthesis, in this experimental model of a pathological process. Causes of the increase in activity in the second stage are even less clear and may be connected with a compensatory reaction of the body leading to activation of apoenzymes and stimulation of their synthesis. The possibility of an increase in contamination with erythrocytic enzymes, on account of microcirculatory disturbances in the intestinal mucosa, likewise cannot be ruled out.

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EFFECT OF ACTH AND HYDROCORTISONE ON cAMP CONTENT AND PHOSPHODIESTERASE ACTIVITY IN RAT SKELETAL AND SMOOTH MUSCLES

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Adrenocortical hormones are known to have a significant effect on various types of metabolism in muscle tissue [1, 4]. However, the mechanisms of this influence have not yet been explained. It can be postulated that their action on muscles is realized through the adenylate cyclase system. For instance, investigations have shown that ACTH and corticosteroids modify the cyclic AMP (cAMP) content in some organs and tissues [3, 7, 14, 15], and cAMP helps to control activity of the enzymes of glycolysis and the respiratory chain, and of transport ATPases [2, 5, 9, 11-13, 15].

The aim of this investigation was to study the effect of ACTH and hydrocortisone on the cAMP content and cyclic nucleotide phosphodiesterase (PDE) activity in rat skeletal and smooth muscles.

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