The results can be summed up as follows. MBLA$^+$-lymphocytes from the Ig$^+$fraction of bone marrow and lymph nodes have a helper action on bone marrow stem cells, expressed as ability of cells of the SC-1$^-$ fraction of bone marrow to induce colony formation in the spleen. Splenic Ig$^+$ MBLA$^+$-cells do not possess this action and suppress the helper effect of SC-1$^+$-PTL and of unknown cells contained in the Ig$^+$-fraction. The Ig$^+$-fraction of bone marrow and lymph nodes contains MBLA$^-$-cells, abolishing the helper action of SC-1$^+$. Consequently, in these experiments effects of Ig$^+$ MBLA$^+$-helpers of colony formation (in the bone marrow and lymph nodes), of suppressors (in the spleen), and also of helpers (in the spleen) and suppressors (in the bone marrow and lymph nodes) of unknown nature, containing cells of these organs in the Ig$^+$-fractions, were recorded. Their nature, their physiological role, the mechanisms of realization of these effects, and also the biological significance of the opposite effects of spleen cells await elucidation by further analysis of Ig$^+$ MBLA$^{+/-}$-cells.

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REGULATORY PROPERTIES OF RAT HEART ADENYLATE CYCLASE DURING THE COURSE OF TOXICOINFECTIOUS SHOCK INDUCED BY *Yersinia pestis* TOXIN

T. D. Cherkasova, V. A. Yurkiv, V. P. Avrorov, and A. V. Griboedov

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Previously the writers found weakening of the contractile function of the heart and a decrease in the number of voltage-dependent Ca^{2+}-channels and beta-adrenergic receptors of the cardiomyocytes in the course of poisoning with murine plague toxin [3]. At the present time the effects of catecholamines responsible for activation of beta-receptors are linked with elevation of the intracellular cAMP level, which causes an increase in adenylate cyclase activity. Hormone-dependent adenylate cyclase (AC) is a key enzyme responsible for the regulatory effect of many hormones and biologically active substances, such as glucagon, histamine, prostaglandins of the E group, and so on, whose levels change sharply in toxicoinfectious shock due to plague, on the heart [3, 4, 7].

In the investigation described below the effect of murine plague toxin on AC activity of the rat heart and also on its regulation by isoproterenol and glucagon was studied.

Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow.

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TABLE 1. Regulatory Properties of Adenylate Cyclase of the Heart during Poisoning by Murine Plague Toxin

Group of animals	AC activity, pmoles cAMP/min/mg protein			
	nothing added	NaF, 10 mM	GIDP, 0.1 mM	histamine, 10^{-6} M
Control (n = 10)	22.5±2.3	141.2±16.0	67.0±5.4	37.1±4.0
Experimental, 1 h exposure to toxin (n = 10)	21.5±2.4	146.5±13.5	68.4±7.9	36.3±3.3
2 h exposure to toxin (n = 10)	22.0±3.0	138.0±15.5	69.0±7.2	30.0±3.2
5 h exposure to toxin (n = 8)	25.0±3.0	153.0±14.9	74.2±7.8	22.5±2.4*

Legend. *$p \leq 0.05$ Indicates significant difference between parameter for experimental and control groups.

Murine plague toxin (Becker's fraction 2) was injected intraperitoneally in 1 ml of physiological saline in a dose of 1 mg (LD_{100}) per rat. Control animals were given an equal volume of physiological saline. The rats were decapitated under ether anesthesia 1, 2, and 5 h after injection of the toxin, and their heart was removed and washed with cold physiological saline. Isolation of the membranes and determination of AC activity were carried out by the method described previously [2]. To determine changes in AC activity in response to stimulation, L-isoproterenol (10^{-8}-10^{-4} M), glucagon (10^{-9}-10^{-6} M), sodium fluoride (10^{-2} M), 5'-guanylylimidodiphosphate (GIDP) (10^{-4} M), and histamine (10^{-6} M) were added to the incubation mixture. The [^{32}P]-cAMP formed was separated on columns with aluminum oxide. Radioactivity was determined by using the Cherenkov effect. All measurements were made in three parallel tests. Protein was determined by the method in [12].

EXPERIMENTAL RESULTS

In the modern view the AC system consists of three functional units: receptor, catalytic subunit, and GTP-binding proteins (N-proteins), which are responsible for interaction between the activated receptor and the catalytic component of the enzyme [14]. Injury to any component of this system or to their coupling by toxic substances of the plague microorganism may cause changes in the intracellular cAMP level and, consequently, changes in the functional state of the heart also.

As Table 1 shows, the level of basal (not hormone-stimulated) AC activity of the plasma membranes of the heart of animals of the control and experimental groups did not differ significantly in the course of poisoning. Sodium fluoride and GIDP activated the AC of the heart of the control animals by 6.2 and 3 times respectively. The sensitivity of AC to these compounds in rats exposed to the action of murine plague toxin did not differ at all times of poisoning from that in the control animals. The results can be taken as evidence that, unlike many bacterial toxins [15], murine toxin does not affect the GTP-ase activity of the N-proteins of the adenylate cyclase complex or its coupling with the catalytic component in vivo. Meanwhile, it follows from the results described above that the catalytic subunit of AC in the heart may be activated to the greatest degree under the influence of plague toxin. Of course, direct stimulation of the catalytic subunit by forskolin may reflect the state of this component better. However, since we found no difference in the degree of AC activation in the heart membranes of experimental and control rats when using sodium fluoride and GIDP in concentrations giving rise to maximal activation of the enzyme, the experiments with forskolin could hardly have provided any more information.

The activating effect of catecholamines, glucagon, and histamine on the heart is effected through corresponding receptors, linked with the adenylate cyclase complex [1, 8, 10, 11]. As will be clear from Fig. 1, isoproterenol (10^{-6} M-10^{-5} M) activates AC in the cardiac membranes of control and experimental rats after exposure to the toxin for 1 and 2 h by 4 times. In animals in the agonal stage of toxemia, the degree of activation of the cardiomyocyte AC by this agonist was reduced by 30-35% compared with the control groups. Weakening of the stimulating effect of isoproterenol on AC was not accompanied by any change in affinity of the enzyme for catecholamines and their agonists in this group of rats, and it was evidently due to a decrease in the number of beta-adrenergic receptors in the heart. In fact, as we showed in a previous

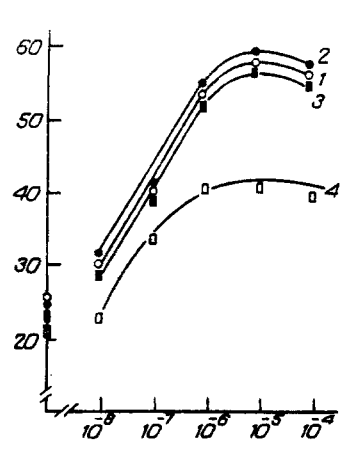

Fig. 1

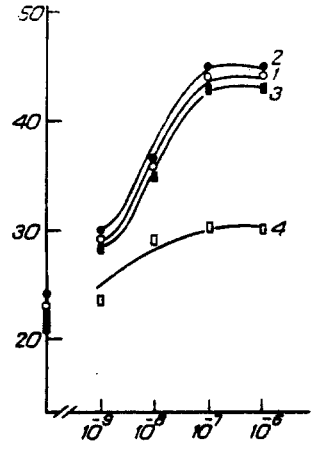

Fig. 2

Fig. 1. Dependence of AC activation in myocardial plasma membranes on isoproterenol concentration. Abscissa, isoproterenol concentration (in M); ordinate, cAMP formation (in pmoles/mg protein/min). 1) Control group (10 animals), 2) after exposure to toxin for 1 h (10 animals), 3) exposure for 2 h (10 animals), 4) exposure for 5 h (eight animals).

Fig. 2. Dependence of AC activity in myocardial plasma membranes on glucagon concentration. Abscissa, glucagon concentration (in M); ordinate, cAMP formation (in pmoles/mg protein/min). 1) Control group, 2) after exposure to toxin for 1 h, 3) exposure for 2 h, 4) exposure for 5 h.

study [3], it is in this group of animals that a decrease in number of beta-adrenergic receptors and their affinity for the ligand that is observed.

In the cardiac membranes of the control rats glucagon (10^{-7} M-10^{-6} M) produced twofold activation of AC (Fig. 2). In animals in the early stage of toxemia, and with a marked picture of shock, the degree of activation of the enzyme by this hormone in a concentration of 10^{-9} M-10^{-5} M did not differ from that in the control animals. The stimulating effect of glucagon on the enzyme was significantly reduced by 40-50% in the group of animals in the terminal stage of poisoning by murine plague toxin (Fig. 2). Weakening of the stimulating effect of glucagon on the enzyme was not accompanied by any change in the affinity of AC for this hormone.

As Table 1 shows, histamine (10^{-6} M) increased activity of cardiac AC in the control and experimental groups of rats after poisoning for 1 and 2 h by 60%. In animals in the terminal stage of shock, the level of histamine-stimulated enzyme activity was the same as the basal level.

Thus after exposure to plague toxin for 2 h the sensitivity of the cardiac AC to the regulatory influence of catecholamines, glucagon, and histamine was unchanged, suggesting, evidently, that the molecular mechanism of the damaging action of murine plague toxin is not due to inactivation of AC, coupled with these hormonal receptors. In the late stages of toxicosis weakening of the stimulating effect of the hormones tested on cardiac AC may be due to desensitization of the corresponding types of hormonal receptors arising, through the prolonged action of the hormones [8], whose level rose sharply not only in toxicoinfectious shock due to plague toxin [3, 4, 7], but also in other types of shock [5, 6]. Possibly in this period of plague toxicoinfectious shock coupling of the hormonal receptors with AC may be disturbed, due to a change in the lipid composition of the plasma membranes of the heart in the experimental animals. A similar mechanism of a decrease in sensitivity of AC in membranes of the heart and liver to hormones has been demonstrated in endotoxic shock [9, 13].

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ANTIGEN-SPECIFIC DETERMINATION OF SERUM LEVELS OF HBsAg/IgM and HBsAg/IgG CIRCULATING IMMUNE COMPLEXES IN HBV-INFECTED PATIENTS BY ELISA

A. A. Sokolenko, K. L. Shakhanina, and A. N. Kurilov

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During the recognition and elimination of hepatitis B virus its surface antigen (HBsAg) is present in patients' blood sera mainly in the form of infectious and noninfectious circulating immune complexes (HBsAg-containing CIC) [5, 10]. During the formation of these CIC, HBsAg may form complexes not only with specific antibodies to it (pre-S_1, pre-S_2, pre-S), but also with modified host proteins: polymerized human serum albumin (pHSA) [9], immunoglobulin M [11] and G [6], and with the corresponding autoantibodies to them.

Correspondingly, the qualitative and quantitative composition of HBsAg-containing CIC and, in particular, the ratio of virus-specific antibodies and autoantibodies to host proteins, are largely determined by the character and outcome of immunologic resolution of HBV infection (or HBV + HDV infection on account of HBsAg common to them).

It has recently been shown that injection of human HBsAg/anti-HBs CIC obtained in vitro (or monoclonal anti-HB "a" of class G with a preserved Fc-fragment) stimulates the more effective proliferative response of HBsAg-specific T lymphocytes and a more effective anti-HBs-response, requiring a 100-500 times lower concentration of HBsAg for this purpose [7, 8].

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow.

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