VOLTAGE-DEPENDENT Ca^{2+}-CHANNELS AND CARDIAC CONTRACTILE FUNCTION DURING TOXICOINFECTIOUS SHOCK ASSOCIATED WITH PLAGUE

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

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KEY WORDS: murine plague toxin; toxicoinfectious shock; rat heart; voltage-dependent Ca^{2+}-channels; adrenergic and cholinergic receptors

Disturbance of cardiac function under the influence of the bacterium *Yersinia pestis,* and also of the various toxic substances produced by this microorganism, plays an important role in the pathogenesis of toxicoinfectious shock, which may lead to death of the experimental animals. However, the biochemical mechanisms lying at the basis of damage to the contractile function of the myocardium in plague toxemia have not been adequately studied. An important role in the regulation of the contractile function and metabolism of the heart is played by ionized calcium, whose level in the myocyte increases mainly through activation of voltage-receptor dependent calcium channels and the transport systems of the endoplasmic reticulum [5, 7]. Mediators of the parasympathetic and sympathoadrenal systems, also acting through specific receptors of cardiomyocyte plasma membranes, modify the intracellular Ca^{2+} level through a complex cascade of biochemical reactions, and in that way exert a chronotropic and inotropic effect on the heart [5, 15].

The aim of the present investigation was to study the effect of murine plague toxin on cardiac contractile function and on the state of alpha- and beta-adrenergic and muscarinic (M)-cholinergic receptors and on voltage-dependent Ca^{2+}-channels in the myocardium during the course of toxicoinfectious shock.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 150-220 g. Toxemia was produced by intraperitoneal injection of 0.5 ml of physiological saline, containing 1 mg (LD_{100}) of an original preparation of the toxin (Becker's fraction 2). The animals were killed 4.5-5 h after exposure to the toxin. Control animals were given an intraperitoneal injection of an equal volume of physiological saline. The plasma membranes of the rat heart were isolated by the method in [2]. Binding of [—]-[^{3}H]-dihydroalprenolol with beta-adrenoreceptors of the heart membranes was carried out by the method in [11]. Measurement of binding of [^{3}H]-prazocin with alpha-adrenoreceptors was carried out by the method in [12], and binding of [^{3}H]-quinuclidinyl benzylate with muscarinic cholinergic receptors by the method in [14]. To study binding of the ligand with dihydropyridine receptors of voltage-dependent Ca^{2+}-channels, plasma membranes were isolated from the rat myocardium by the method in [7]. Binding of [^{3}H]-P200-110 with dihydropyridine receptors of the voltage-dependent Ca^{2+}-channel was carried out by the method in [7] with some modification. The reaction of ligand-receptor binding was stopped by rapid addition of 15 ml of cold buffer, followed by passage through GF filters (Whatman, England). The filters were placed in flasks containing dioxan scintillator and their radioactivity was measured on a RackBeta scintillation counter (LKB, Sweden). The number of receptors (B_{max}) and the dissociation constant of the ligand (K_d), the reciprocal of affinity of the receptors, were calculated on a personal computer, using the EBDA (Ligand) program for the IBM

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TABLE 1. Contractile Function of the Heart in Toxicoinfectious Shock Caused by Plague Toxin

Parameter	Group of animals	Before compression of aorta	After compression of aorta	
			30 sec	120 sec
Heart rate, beats/min	Control	438±15	342±18	322±10
	1 h	398±9	312±15	302±27
	2 h	355±10*	285±11	294±19
	5 h	208±39*	167±40*	130±37*
Diastolic pressure	Control	3.6±0.4	28,8±2.9	22,8±3.6
	1 h	5.0±0.7	26±6	20,2±4,2
	2 h	4.9±0.4	27±6	21,7±5
	5 h	3.6±0.6	9,4±3,1*	12,4±4,5*
Systolic pressure	Control	138±11	220±7	183±11
	1 h	75±6*	225±9	240±6*
	2 h	68±4*	232±6	222±12*
	5 h	55±12*	118±51*	126±60*
Pulse pressure	Control	134±11	191±9	160±10
	1 h	70±6*	176±23	218±7*
	2 h	63±4*	205±11	201±12*
	5 h	51±12*	109±49*	99±5*
IFS	Control	97±9	107±7	85±5
	1 h	51±6*	99±14	127±6*
	2 h	42±3*	109±6	110±10
	5 h	21±6*	42±21*	32±25*
Rate of contraction	Control	11 532±1648	9246±1145	6963±1044
	1 h	5142±561*	7592±1309	7177±630*
	2 h	4201±541*	11 885±865	8729±578
	5 h	1719±547*	4251±2307*	3767±2071
Rate of relaxation	Control	4266±427	3201±486	2649±169
	1 h	1719±176*	2676±420	4082±386*
	2 h	1736±189*	3276±327	3506±496*
	5 h	1050±370*	1399±642*	1051±504*

Legend. *$p < 0.05$) Significance of difference between parameters for animals of control and experimental groups. Ten animals in each group.

PC (McPherson, 1984). Cardiac contractile function was studied at the same stages of the experiment as the biochemical parameters. The pressure within the left ventricle was recorded under urethane anesthesia under open chest conditions and artificial respiration, by means of a cannula introduced into the apex of the left ventricle and connected to the transducer of a Mingograf-34 electromanometer (Elema, Sweden). The heart rate (HR), systolic (Ps), diastolic (Pd), and pulse (Pp) pressures, and the rate of rise (Vs) and rate of fall (Vd) of pressure were measured on the pressure curve. Intensity of functioning of structures (IFS) was calculated by the formula

$$Pp \times HR/\text{mass of left ventricle.}$$

The same parameters were determined when the ascending aorta was compressed for 30 and 120 sec. Protein was determined by the method in [13]. All the results were analyzed by Student's test.

EXPERIMENTAL RESULTS

The physiological experiments showed that after 1 h of toxemia produced by plague toxin the systolic and pulse pressure, the rate of relaxation and contraction of the ventricle, and the index of functioning of structures were 50-60% lower than their levels in the control animals. Longer exposure to the toxin caused an even greater fall in the values of these parameters. The hart rate was significantly lower only after 2 and 5 h of exposure to the toxin. Under maximal resistance loading due to compression of the aorta while the nervous regulation of the heart remained intact, the dynamics of the parameters was similar in both control and experimental rats, but the degree of change of these parameters differed. Both in control rats and in rats exposed for 1 and 2 h to the toxin, the parameters of cardiac contractility studied did not differ significantly after compression of the aorta for 30 sec. Continued compression of the aorta led to a significant increase in Ps, Pp, IFS, Vs, and Vd on average by 30-50% in animals of the experimental groups compared with the control rats (Table 1). Compression of the aorta in rats in a terminal state led to some increase in the values of the various parameters, but they were significantly lower than in the control. It must be emphasized that Pd at all points of observation

TABLE 2. Characteristics of Receptors of Myocardial Plasma Membranes during Toxicoinfectious Shock Due to Plague Toxin

Experimental conditions	Dihydropyridine receptors of voltage-dependent Ca^{2+}-channels		Adrenergic receptors				M-cholinergic receptors	
			β_1-receptors		α_1-receptors			
	B_{max}, fmoles/mg	K_d, nM	B_{max}, fmoles/mg	K_d, nM	B_{max}, fmoles/mg	K_d, nM	B_{max}, fmoles/g	K_d, nM
Control, n = 10	270±15	0,25±0,03	52±5,1	1,55±0,05	148±16	0,55±0,03	380±27	0,15±0,01
1 h of toxemia, n = 10	169±18*	0,51±0,06*	50±5,6	1,6±0,07	140±13	0,45±0,05	420±40	0,16±0,01
2 h of toxemia, n = 10	140±13*	0,55±0,05*	44±4,8	2,17±0,15*	150±15	0,67±0,04	390±16	0,12±0,03
5 h of toxemia, n = 8	135±14*	0,45±0,05*	37±4,0*	2,05±0,13*	158±12	0,52±0,03	400±36	0,14±0,02

Legend: n) number of animals. *$p < 0.05$) Significance of differences between values for control and experimental groups.

was identical with that in intact rats. Only compression of the aorta in the group of animals in the terminal state led to reduction of this parameter by 50-70% compared with its value in the control rats.

Table 2 shows that in the early stages of exposure to the toxin the number of voltage-dependent Ca^{2+}-channels determined from the number of dihydropyridine receptors and their affinity for the ligand decreased by 1.6 and 2 times respectively. After 2 and 5 h of exposure to the toxin these parameters remained lower than in the control rats. A decrease in the number of voltage-dependent Ca^{2+}-channels may evidently limit the inflow of calcium ions into the cardiomyocytes and lead to reduction of the contractile function of the heart in the course of poisoning by plague toxin.

The greatest number of beta-receptors in the membranes of the heart was unchanged both in the early stages of exposure to the toxin and when the animals exhibited a picture of severe shock (2 h; Table 2). The results of the physiological experiments show that it is during this time period, during compression of the ascending aorta in the animals, that the lowered values of cardiac contractility with an intact nervous regulation can be restored to the level observed in intact rats, evidently on account of an increase in the catecholamine concentration acting on the heart [1]. Desensitization of beta-adrenergic receptors, expressed as a decrease in their number and affinity by 30%, was observed only in animals in an agonal state (Table 2). The effect of habituation of many tissues to the action of catecholamines is known to develop as a result of the prolonged (4-12 h) effect of these substances on the cell [6, 10]. A decrease in the number of receptors in the late stages of exposure to the toxin is evidently mediated by catecholamines, the level of which is sharply rai types of shock [3], and is unconnected with the direct blocking action of the murine toxin on receptors of this type.

As will be clear from Table 2, desensitization of alpha-adrenergic and M-cholinergic receptors does not develop in the course of toxicoinfectious shock.

Thus depression of contractile function of the heart in toxicoinfectious shock due to plague toxin may be caused by at least two factors, limiting the increase in the ionized Ca^{2+} level in the myocardial cell: a decrease in the number of voltage-dependent Ca^{2+}-channels and desensitization of beta-adrenergic receptors in the late stages of shock.

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PRODUCTION OF ANTIVIRAL FACTOR BY INFECTED CHICK EMBRYONIC FIBROBLASTS

N. V. Gribkova and V. I. Votyakov

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The writers showed previously that infection of chick embryonic fibroblasts by virus VÉL-230 is accompanied by the production of an antiviral factor of protein nature in the culture fluid [2, 9]. The originality of the antiviral factor was confirmed by properties such as the absence of species specificity, preservation of its activity after heating to 100°C for 30 min, and a molecular weight of not more than 10 kilodaltons. The antiviral factor suppressed reproduction of the viruses of influenza, Venezuelan equine encephalomyelitis, vesicular stomatitis, herpes simplex, and the interferon-resistant virus of vaccinia in vitro, without causing any significant stimulation of synthesis of cellular RNA and proteins. Recent investigations have shown that this antiviral effect is unconnected with interferon induction [4].

The aim of this investigation was to continue the study of the production of this cellular antiviral factor.

EXPERIMENTAL METHOD

A primary trypsinized culture of chick embryonic fibroblasts (CEF) and transplantable green monkey kidney cells (Vero), hamster kidney (BHK-21), and human embryonic fibroblasts (HEF) were used in the experiments. The cell cultures were infected with Venezuelan equine encephalomyelitis virus (VÉL-230), maintained by passage in a culture of CEF. The infectious titer of the virus was determined by the plaque-formation method [8]. Antiviral factor (AF) was obtained by successive hydrolysis of the culture fluid at pH 2.0 and 6.0 [1]. The denatured substances were removed by centrifugation and the supernatant was used as the total AF preparation. Antiviral activity of AF was estimated in conventional antiviral units (AU/ml). Activity of AF was taken to be the reciprocal of the maximal dilution of AF causing suppression of reproduction of the test virus by 2 log PFU/ml.

EXPERIMENTAL RESULTS

To study the distribution of the phenomenon of AF production, the ability of other cell cultures to produce similar activity in response to infection was tested. Intact cultures of HEF, Vero, and BHK-21 cells, and also of CEF, produced virtually no AF, since activity of AF was less than 1 AU/ml and did not cause statistically significant suppression of reproduction of the VÉL-230 virus. Activity of the AF preparation obtained from the culture fluid of infected HEF and Vero was comparable and did not exceed 2 AU/ml. Infection of BHK-21 cells did not lead to production of any AF

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