EFFECT OF DALARGIN, A SYNTHETIC ENDOGENOUS OPIOID ANALOG, ON FUNCTION OF THE ISOLATED RAT HEART IN EXPERIMENTAL TOXEMIA IN VITRO

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The severity of the course and the outcome of generalized pyogenic infection, in most cases staphylococcal in its etiology, are largely determined by the consequences of pyrogenic intoxication of the form of a disturbance of the homeostasis system [2, 8, 10]. This combination of disturbances affecting many organs is due primarily to the appearance of exogenous protein products of Staphylococcus aureus in the circulation and, in particular, of one of the principal factors determining the pathogenicity of this organism, namely its α-toxin, which has a marked damaging action on vitally important organs, including the cardiovascular system [7, 11, 12]. However, the direct action of α-toxin on the basic functional parameters of activity of the isolated heart has not yet been studied. Optimal methods of limiting the cardiodepressive action of the toxin virtually do not exist, because in many cases specific treatment with immune serum preparations is unable to inactivate the toxin, after its fixation in the tissues [16], for example, and the selective action of α-toxin on smooth muscle, leading in particular to loss of vascular tone [15], greatly hinder the effective use of vasoactive cardiotonic agents. Consequently, the urgency of the search and prospects of the development of a basically new method of pharmacologic correction of toxic damage to the myocardium will be evident. The most interesting aspect of this problem is accordingly the study of the phenomenologic effects of enzyme-resistant synthetic analogs of endogenous regulatory opioid peptides, which are involved in the maintenance of homeostasis in different types of pathology [3, 5, 6, 9], against the background of experimental toxemia both in vitro and in vivo.

The aim of this investigation was to study the effect of dalargin, a synthetic endogenous opioid and an analog of Leu-enkephalin, on the basic parameters of cardiac activity of rats during experimental staphylococcal toxemia in vitro, and the direct effect of different concentrations of α-toxin on function of the isolated rat heart, with a working left ventricle, also was determined.

EXPERIMENTAL METHOD

The investigation was conducted on isolated hearts of male Wistar rats (n = 21) weighing 230-250 g. The hearts were isolated under pentobarbital anesthesia (1 ml of 1% solution + 300 U heparin) and perfused with Krebs–Henseleit solution of the following composition (in mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 3.0; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose 12 (pH 7.4), oxygenated with carbogen (95% O_2 + 5% CO_2). The heart was perfused at 37°C through cannulas introduced into the left atrium and aorta, with initial values of filling pressure and resistance of 15 and 100 cm water, respectively. During the first 5 min (to allow the isolated heart to run in) retrograde perfusion of the coronary vessels was carried out through the aorta by Langendorff's method [13]. This was followed by perfusion according to [14] through the left atrium with the left ventricle working. Under these conditions the main funtional parameters characterizing work of the isolated heart were determined: aortic and coronary flow (AF and CF), and cardiac output (CO), as the sum of AF + CF. The systolic and mean arterial pressure (BP_{sys} and $BP_{\bar{m}}$) and the heart rate (HR) was recorded on the pressure curve in the aorta by means of EMT-35 transducers on a "Mingograph 34" instrument ("Siemens-Elema," Sweden), and the stroke volume (SV) and total, stroke, and external work of

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TABLE 1. Dose-Dependent Action of S. aureus α-Toxin on Parameters of Function of Isolated Perfused Heart of Intact Rats (M ± m)

Parameter	Initial value	After perfusion with α-toxin in dilution of 1:100	Initial value	After perfusion with α-toxin in dilution of 1:500	After perfusion with α-toxin in dilution of 1:50
HR, beats/min	283,0±27,7	143,3±51,6*	256,6±74,4	303,3±23,5	0
CF, ml/min	15,0±2,5	5,1±1,4*	11,3±1,1	14,2±1,4	0
AF, ml/min	30,1±2,9	3,1±1,6*	30,6±2,4	38,8±0,4*	0
CO, ml/min	45,1±2,7	8,3±2,4*	41,9±3,2	53±1,1*	0
BP_{sys}, mm Hg	116,4±15,3	63,3±22,7	128,3±8,3	133,3±10,9	0
BP_m, mm Hg	88,2±11,6	49,1±17,4	91,0±7,3	98,3±8,3	0
A_{tot}, conventional units	4209±796	604,8±258,8*	3639,4±215,4	5215,5±457,4*	0
A_{str}, conventional units	14,8±1,8	2,9±1,28*	16,1±3,3	17,1±0,23	0
A_{ext}, conventional units	32 916±6 419	14 425±6 242	32 050±8 087	40191,6±3013	0
SV, ml	0,165±0,015	0,036±0,015*	0,184±0,045	0,176±0,012	0

Legend Here and in Table 2: all results obtained during 15-min working perfusion of isolated heart as in [14]; *$p < 0.05$ compared with corresponding values in initial state.

the isolated heart (A_{tot}, A_{str}, A_{ext}, in conventional units) were calculated from these parameters.

Dalargin (developed and synthesized by Professor M. I. Titov and V. A. Vinogradov, at the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR), a stable synthetic analog of endogenous opiate receptor ligands, D-Ala^2-Arg^6-enkephalin, in a concentration of $5 \cdot 10^{-8}$ M, and staphylococcus aureus α-toxin (produced by the N. F. Gamaleya Research Institute of Immunlogy, Epidemiology, and Microbiology, batch 250-G), with an initial concentration of $16 \cdot 10^{-2}$ Lh, diluted 500, 100, and 50 times with Krebs–Henseleit solution, to correspond to its concentration in the system for perfusion of the isolated heart, namely $8 \cdot 10^{-5}$ Lh, $16 \cdot 10^{-4}$ Lh, and $8 \cdot 10^{-4}$ Lh, respectively, were used. There were three series of experiments: in I the dose-dependent effect of the α-toxin was studied; in II the action of the α-toxin on the myocardium was investigated after preliminary perfusion of the latter with dalargin (to assess its possible preventive and protective action) in series II dalargin was added to the perfusion fluid after α-toxin against the background of an already developed cardiotoxin action.

Experiments of series I (n = 10): After recording of the initial parameters of the functional state of the isolated heart with a working left ventricle (at the 15th minute of perfusion according to [14]) the myocardium was perfused for a further 15 min through the coronary vessels under the conditions in [13] with Krebs–Henseleit solution containing one of the concentrations of α-toxin to be investigated. For the next 5 min the heart was perfused under the same conditions with pure (without α-toxin) Krebs–Henseleit solution, after which the parameters of cardiac activity were assessed for 15 min during working perfusion of the myocardium as in [14] with standard Krebs–Henseleit solution.

Experiments of seres II (n = 5): After the initial data had been recorded the heart was perfused as in [13] with Krebs–Henseleit solution containing dalargin in a concentration of $5 \cdot 10^{-8}$ M, after which the state of function of the isolated heart was recorded. After replacement of the initial perfusion solution with dalargin by Krebs–Henseleit solution containing α-toxin in a concentration of $16 \cdot 10^{-4}$ Lh (dilution 1:100), retrograde perfusion of the coronary vessels was repeated for 15 min. Changes in functional parameters of the isolated heart were determined at the 15th minute of work of the heart under the conditions in [14]. Experiments of series III (n = 6): The order of perfusion was the same as in the previous series, but unlike in that series dalargin was added to the perfusion fluid after the α-toxin, when its cardiodepressive action was already manifested.

EXPERIMENTAL RESULTS

Staphylococcus aureus α-toxin has a marked and dose-dependent action on the principal functional parameters of the isolated perfusion heart of intact rats (Table 1). Perfusion of the myocardium with low concentrations of α-toxin ($8 \cdot 10^{-5}$ Lh) led to intensification of the pumping function of the myocardium, as was judged from the increase in aortic flow. The increase in CO and A_{tot} of the isolated heart also was due to an increase in the ejection fraction of the left ventricle, for values of the coronary flow and BP_m respectively were not statistically significantly increased. On 100-fold dilution of the α-toxin ($16 \cdot 10^{-4}$ Lh) marked inhibition of function of the isolated heart was observed in the form of

TABLE 2. Effect of Dalargin ($5 \cdot 10^{-8}$ M) on Functional Parameters of Isolated Rat Heart with Working Left Ventricle before and after Perfusion with S. aureus α-Toxin in a Dilution of 1:100 ($16 \cdot 10^{-4}$ Lh; M ± m)

Parameter	Initial value	Preliminary perfusion with dalargin	Subsequent perfusion with α-toxin	Initial value	Preliminary perfusion with α-toxin	Subsequent perfusion with dalargin
HR, beats/min	300±20,4	298±21,4	84±52,3*	236,6±32,6	100±50,8*	236±23,6**
CF, ml/min	12,8±1,5	14,6±1,4	3,16±1,93*	12,4±2,2	2,6±1,2*	9,5±1,12**
AF, ml/min	29,7±1,1	33,6±2,4	0	29±2,39	0,83±0,58*	10,9±1,2**
CO, ml/min	42,5±1,1	48,2±3,5	3,16±1,93*	41,4±0,9	3,43±1,71*	20,6±1,56**
BP_{sys}, mm Hg	97,8±11,7	92,4±11,3	18±11*	123,5±9,9	48,3±21,9*	90±8,6
BP_m, mm Hg	78±9,2	74±8,1	15±9,2*	86,5±6,8	36,6±16,5*	66,6±6,4
A_{tot}, conventional units	3326±404	3526±537	117,6±72,8	3579±280	256±133*	1395±195**
A_{str}, conventional units	11,2±1,5	12,1±1,47	0,56±0,347*	16,9±3,0	1,7±1,02*	6,46±1,25**
A_{ext}, conventional units	29 512±4 568	28 190±4 915	3 780±2 353*	28 954±4 868	9 308±4 481*	21 195±2 850**
SV, ml	0,143±0,010	0,165±0,018	0,015±0,009*	0,195±0,032	0,021±0,012*	0,093±0,015**

*Significance of differences compared with analogous parameters for preliminary perfusion of myocardium with α-toxin ($p < 0.05$).

bradycardia, a threefold reduction of the coronary flow, and a tenfold decrease in the pumping function (as reflected in values of the aortic ejection). With a further increase in concentration of the α-toxin to $8 \cdot 10^{-4}$ Lh, total depression of both pumping and contractile functions of the myocardium was observed. On the basis of the results, to reproduce experimental toxemia in vitro in the subsequent series of experiments, a 100-fold dilution of the initial α-toxin was used, corresponding to its concentration of $16 \cdot 10^{-4}$ Lh in the perfusion solution.

The results of the experiments of series II with preliminary perfusion with dalargin are given in Table 2, from which it follows that the synthetic endogenous opioid analog did not affect the functional parameters of the isolated intact rat heart, and likewise did not prevent the subsequent cardiodepressive action of the α-toxin. However, against the background of toxic myocardial damage (experiments of series III), subsequent perfusion with dalargin abolished the cardiodepressive action of the α-toxin sufficiently effectively, although complete restoration of cardiac activity to the initial values for the state of function of the intact rat myocardium was not observed (Table 2). Nevertheless, the basic parameters of cardiac activity rose by several times after perfusion with dalargin compared with the corresponding values associated with toxic damage to the myocardium before injection of the opioid peptide: CO was increased sixfold, not only due to the increased aortic injection fraction, but also due to the increase in CF. Despite the more than twofold increase in HR, an increase in SV (by 4.4 times) also was observed; values of Aext, Astr, and A_{tot} of the isolated rat heart increased correspondingly 2, 3, and 5 times.

The α-toxin of S. aureus thus has a dose-dependent action on the parameters of function of the isolated rat heart, causing a positive inotropic effect if the concentration of the toxin in the perfusion fluid was low, and inhibition of cardiac activity in the presence of higher concentrations, amounting in some cases to complete depression of contractile and pumping functions. This phenomenon is similar to the effect of α-toxin observed by the writers previously on isolated fragments (strips) of the auricle of the atrial myocardium [4], further confirmation of the ability of the α-toxin to exert a direct action on heart muscle and, in particular, on its contractile activity. The absence of a preventive protective action of dalargin in the present experiments, i.e., its ability to prevent subsequent toxic damage to the myocardium, can evidently be explained on the grounds that the relative pharmacologic activity of dalargin is exhibited when pathological changes have reached a certain level. In fact, the protective action of dalargin was manifested against the background of the cardiodepressive effect of the α-toxin. Incidentally not only dalargin, but many regulatory peptides likewise do not exhibit their biological activity, or do not do so significantly, when acting on the organism in the normal state [1]. The protective effect of dalargin in the present experiments, consisting of abolition of disturbances of function of the working isolated heart, due to the cardiodepressive action of the α-toxin, is most likely to be multifactorial in nature. This may be linked both with the ability of dalargin to abolish microcirculatory disturbances [3, 9] and to block the development of hormonal-metabolic disturbances [5, 9], and with depression of the level of lipid peroxidation products [6], depressed after administration of the synthetic opioid, and, consequently, with the less severe damage to

the lipid bilayer of the cell membranes and the lesser degree of inactivation of membrane-bound proteins.

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EFFECT OF ADAPTATION TO STRESS ON ELECTRICAL ACTIVITY, CONTRACTILITY, AND RESISTANCE OF PAPILLARY MUSCLE TO EXCESS OF INTRACELLULAR CALCIUM

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Recent investigations have shown that adaptation of animals to repeated stress increases the resistance of the heart to ischemic, reperfusion, and adrenergic arrhythmias [1, 3]. This protective effect of adaptation has been shown to be largely realized at the level of the heart itself: it is accompanied by increased efficiency of function of the sarcoplasmic reticulum (SPR) and, correspondingly, by increased resistance of the isolated heart to the direct contractural and arrhythmogenic action of high Ca^{++} concentrations [4]. These data make it very probable that adaptation has a wider effect on mechanisms of membrane transport and, as a result, on bioelectrical activity of the cardiomyocytes. However, the question of the effect of adaptation on electrical activity of the cardiomyocytes and its dynamics during calcium loading has not been settled. The aim of this investigation was to study the effect of adaptation of animals to short periods of stress on the resting potential (RP) of the papillary muscle, on its resistance to sodium-deficient contracture, and also on electrical and contractile activity of the papillary muscle during increases in the frequency of contractions and the calcium concentration.

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