The increase in diuresis in rats receiving parathyroid extract is evidence of normalization of renal function under the influence of this preparation. In that case, the mechanism of action of parathyroid hormone may be based on blockade of the peripheral effects of vasopressin [4] and also on an increase in velocity of the renal blood flow [8].

A fall of the plasma calcium concentration is an unfavorable prognostic sign in myocardial infarction [3] and is connected with parathyroid insufficiency [1]. Consequently, normalization of the parameters of calcium exchange under the influence of parathyroid extract in the present experiments can also be interpreted as a favorable effect of the treatment given.

It can thus be concluded from the results that administration of parathyroid extract in acute experimental myocardial ischemia has a beneficial action. Solid evidence in support of this conclusion is given by the reduction in mortality of the experimental animals from acute myocardial ischemia by 1.8 times after administration of parathyroidin.

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EFFECT OF STAPHYLOCOCCAL TOXIN ALONE AND TOGETHER WITH ANTISTAPHYLOCOCCAL GAMMA-GLOBULIN ON ELECTRICAL AND CONTRACTILE ACTIVITY OF THE GUINEA PIG MYOCARDIUM

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UDC 616.127-008.3-02:[616.919:579.861.2+615.373:547.962.4]-092.9

KEY WORDS: staphylococcal toxin; myocardium; action potential; amplitude of contractions.

One of the key factors in septicemic shock is the direct action of microbial toxins and, in particular, of staphylococcal toxin (ST) on the cardiovascular system. Observations on patients in a state of septicemic shock have shown that the stroke ejection of the heart and cardiac output may be either reduced [1, 2, 14] or increased [7, 10, 12, 15]. Electrophysiological experiments have shown that ST reduces the amplitude of intracellular potentials and the amplitude of contractions of preparations of the guinea pig and rabbit myocardium [9]. Differences in values of the cardiac output obtained in patients with septicemic shock suggest that ST may have not only an inhibitory, but also a stimulating action on the myocardium.

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 16-19, October, 1983. Original article submitted March 25, 1983.

0007-4888/83/9610-1357$07.50

Since no direct data on the stimulating action of ST on the myocardium could be found in the literature, and since it evidently may depend on the dose of the toxin and on the presence of antitoxin in the patients' blood, the investigation described below was undertaken. Using different doses of ST and also a combination of ST and antistaphylococcal gamma-globulin (ASGG), isometric contractions of preparations of the auricles of the guinea pig heart and transmembrane potentials of myocardial fibers were measured.

The aim of the investigation was to determine the character of the direct action of ST on the myocardium: to compare its action with that of toxoid, and to study the ability of ASGG to prevent the cardiodepressive action of ST.

EXPERIMENTAL METHOD

Experiments were carried out on the auricles of 21 guinea pigs. After decapitation of the animals and thoracotomy, the heart was excised and placed in Tyrode solution of the following composition (in mM): NaCl 136.9, KCl 2.68, $NaHCO_3$ 11.95, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $CaCl_2 \cdot 2H_2O$ 18, glucose 5.6, oxygenated with carbogen (95% O_2 + 5% CO_2). The temperature of the perfusion fluid in the working chamber (volume 2 ml) was kept at 30-32°C and the pH at 7.2. The preparation was placed in the chamber and stretched by means of metal hooks, one of which was secured to the bottom of the chamber, whereas the other was fixed to the rod of a 6MKh.1S mechanotron, by means of which isometric contractions were recorded. The initial load on the preparation was 1-1.5 g. Maximal rates of rise ($\dot{V}_r$) and fall ($\dot{V}_f$) of the contractions were calculated as the tangent of the angle of slope of the leading and trailing edges.

Some preparations possessed spontaneous activity. If the spontaneous activity was regular, the whole experiment was carried out without the use of external electrical stimulation, for neither ST nor ASGG had any significant effect on the spontaneous frequency. In cases when spontaneous activity was irregular, it was suppressed by raising the KCl concentration in the Tyrode solution to 13.5 mM. These preparations were stimulated by above-threshold square pulses of voltage, 1-3 msec in duration and with a frequency of 1 Hz. External silver electrodes made in the form of disks and placed in the chamber along the preparation, were used for stimulation. The running in time of the preparations was 1-1.5 h. Intracellular potentials were recorded by means of glass microelectrodes filled with 2.5 M KCl solution.

There were four series of experiments: in series I the dose–effect curve of ST was studied; in series II the effect of toxoid in the same dilution was studied — this series acted as control for I; in series II and IV the effect of ASGG was studied against the background of the action of ST and toxoid respectively.

ST was used (in an initial concentration of $18 \cdot 10^{-2}$ L_h), and the toxoid, which was inactivated toxin (with an initial concentration of 10 BU/ml) (BU = binding unit), was obtained from the N. F. Gamalaya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR; ASGG was given in a dose of 20 IU/ml (IU = International Unit). The original substances were dissolved in Tyrode solution (normal or containing 13.5 mM KCl) in dilutions of 1000, 100, 10, and sometimes 5 times. The solution with the test substances passed from the vessel into the working chamber containing the myocardial preparation, and from the chamber it was returned by means of a peristaltic pump into the original vessel, so that it circulated.

After the amplitude of contractions of the preparation had reached a steady level and had remained constant for 15-20 min, intracellular potentials and contractions were measured in Tyrode solution. After increasing the concentration of ST or toxoid successively in the solution, the dose–effect curve was determined. The duration of action of ST and toxoid for each dilution was standard (20 min). In experiments with a combination of ST + ASGG, the ST concentration which usually reduced the amplitude of contractions by 10-20% was chosen: In our experiments this occurred with a 100-fold dilution of ST. ASGG was used in three ratios with ST: deficit — dilution of ASGG 1:1000, equivalent concentration — dilution 1:300, excess of ASGG — dilution 1:100. The action of each combination was recorded several times in the course of 20 min. Toxoid together with ASGG was used in a dilution of 1:100, and the dilutions of ASGG were the same as in the combination with ST.

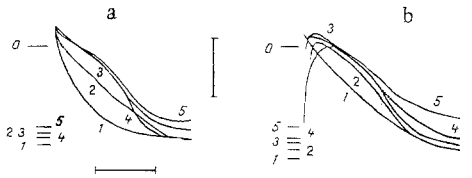

Fig. 1. Effect of ST on spontaneous (a) and evoked (b) AP of guinea pig auricle. a: 0) Isoelectric line; 1) Tyrode solution of normal composition; 2-5) dilution of original ST by 1000, 100, 10, and 5 times, corresponding to concentrations of $18 \cdot 10^{-5}$, $18 \cdot 10^{-4}$, $18 \cdot 10^{-3}$, and $9 \cdot 10^{-3}$ L_h. Spontaneous frequency 3 Hz. b: 0) isoelectric line; 1) Tyrode solution; 2) Tyrode solution with 13.5 mM KCl; 3-5) ST in concentrations of $18 \cdot 10^{-5}$, $18 \cdot 10^{-4}$, and $18 \cdot 10^{-3}$ L_h, corresponding to dilution of the original ST by 1000, 100, and 10 times with Tyrode solution containing 13.5 mM KCl. Evoked frequency 1 Hz. Calibration: horizontally 50 msec, vertically 50 mV.

EXPERIMENTAL RESULTS

Data on changes in intracellular resting potentials (RP) and action potentials (AP) of the myocardial fibers of the guinea pig auricle under the influence of ST are given in Fig. 1. During spontaneous activity of the preparations, ST diluted 1000, 100, 10, and 5 times with Tyrode solutions, considerably lengthened the AP plateau phase measured at the 50% level. Lengthening of the AP plateau phase by ST in a dilution of 1:1000 was less than in dilutions of 1:100, 1:10, and 1:5. The overshoot of AP was unchanged with all dilutions of ST, although there were considerable fluctuations in the values of RP. During electrical stimulation of the preparations an increase in the KCl concentration to 13.5 mM caused a decrease in RP, a decrease in steepness of the leading edge of the AP, and the appearance of so-called "calcium" AP [1]. Under the influence of ST in a dilution of 1:1000 the overshoot of AP was increased (Fig. 1b, 3) compared with AP in the control Tyrode solution containing 13.5 mM KCl (Fig. 1b, 2). With a further increase in the ST concentration both the overshoot and the total amplitude of AP were reduced (Fig. 1b, 4, 5). Just as during spontaneous activity of the preparations, ST caused an increase in duration of AP in all dilutions.

The action of ST on contractility of the myocardial preparations for a frequency of stimulation of 1 Hz is shown in Fig. 2. Changes in the amplitude of the contractions and where maximal rates of rise ($\dot{V}_r$) and fall ($\dot{V}_f$) under the influence of ST in dilutions of 1000, 100, and 10 times, are shown compared with changes produced by toxoid in the same dilutions. It will be clear from Fig. 2 that, unlike toxoid, which caused no significant change in the parameters of contractions measured, ST in a concentration of $18 \cdot 10^{-5}$ L_h increased both the amplitude of the contractions and the maximal rate of their rise and fall. The amplitude of contractions increased up to 124.5 ± 6.7%, $\dot{V}_r$ rose to 139.3 ± 10.7%, and $\dot{V}_f$ rose to 132.1 ± 9.5% (n = 4; values for all parameters obtained in Tyrode solution are taken as 100). At a concentration of $18 \cdot 10^{-4}$ L_h, ST reduced the amplitude of contractions to 82.75 ± 7.2%, $\dot{V}_r$ to 83.4 ± 8%, and $\dot{V}_f$ to 85.1 ± 6.7% (n = 6). A further tenfold increase in the ST concentration led to even stronger inhibition of contractile activity.

ASGG, added to the perfusate when contractile activity was depressed by ST diluted 100 times, not only did not abolish the inhibitory action of ST but, on the contrary, potentiated it. This effect is shown in Fig. 3. Regardless of whether ASGG was present in a deficient, equivalent, or excess amount relative to ST, the cardiodepressant action of the combination was well marked. It must be pointed out that not only the ST + ASGG combination, but also the toxoid + ASGG combination had a cardiodepressant action, although it was weaker. As Fig. 3 shows, under the influence of the ST + ASGG combination the steepness of rise and fall of the contractions was reduced although their amplitude showed no significant change.

During the development of the AP plateau, Ca^{++} ions are known to enter heart cells [5, 13] and to participate in the development of contraction. The amplitude of contractions can be used as an indirect indicator of the intracellular free calcium concentration [3, 6]. The increase in the amplitude and duration of the "calcium" AP with a simultaneous increase in the amplitude of contractions under the influence of ST in a dilution of 1:1000 is evidence

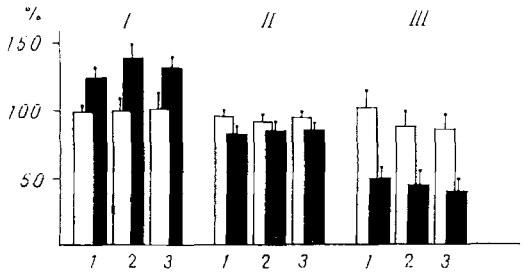

Fig. 2

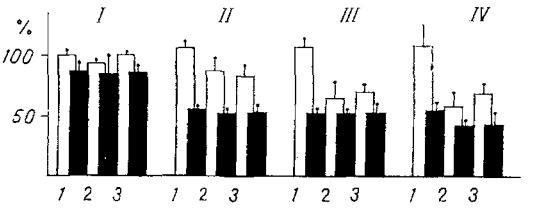

Fig. 3

Fig. 2. Dose-dependent action of ST (black columns) and toxoid (white columns) on amplitude of contractions of guinea pig auricle (1) and their maximal rate of rise (2) and fall (3). I) Dilution of original ST and toxoid by 1000 times (corresponding to concentrations of ST of $18 \cdot 10^{-5}$ L_h and of toxoid of 10^{-4} BU/ml); II and III) dilutions of ST and toxoid 100 and 10 times respectively. All parameters given as percentages of values observed in Tyrode solution.

Fig. 3. Action of combination of ST and ASGG on amplitude of contractions of guinea pig atrium (1) and of their rates of rise (2) and fall (3). Black columns — ASGG added to ST dilution 100 times; white columns — ASGG added to toxoid dilution 100 times. All parameters given as percentages of those observed in Tyrode solution. I) ST and toxoid diluted 100 times; II, III, IV) three different ratios of ASGG with ST and toxoid: II) deficit of ASGG (ST/100 + ASGG/1000 and toxoid/100 + ASGG/1000), III) equivalent ratio (ST/100 + ASGG/300 and toxoid/100 + ASGG/300), IV) excess of ASGG (SG/100 + ASGG/100 and toxoid/100 + ASGG/100).

of an increase in the entry of Ca^{++} ions into the heart cells. The subsequent decrease in amplitude of the "calcium" AP and the amplitude of contractions under the influence of ST in dilutions of 100, 10, and 5 times was probably connected with a decrease in the calcium inflow. The combination of an increase in the duration of AP and depression of contractile activity, observed under the influence of ST in spontaneously contracting preparations with an increase in the dose of the toxin, may be due to a decrease in Ca^{++}, which delays the outflow of K^+ ions from the cell and lengthens AP [4, 8, 11].

The experiments thus showed that ST not only has a cardiodepressant action, but is present in low concentration in the external solution, it can evoke a positive inotropic effect, and this is evidently one of the causes of the increase in stroke ejection of the heart which is observed fairly frequently in patients with septicemic shock [7, 10, 12, 15]. The absence of a protective action of ASGG against the background of the developing cardiodepressant effect of ST is of purely practical interest, for it shows that administration of ASGG to patients with septicemic shock in the acute stage of the disease may increase the damage to the heart.

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CONNECTION BETWEEN CARDIOMYOCYTE LYSOSOMES AND SOME CHARACTERISTICS OF FUNCTION, METABOLISM, AND ULTRASTRUCTURE OF THE INTACT RABBIT HEART

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UDC 616.127-018.1-008.9:576.311.342/-092-07

KEY WORDS: heart; lysosomes; factor analysis.

Activation of the lysosomal apparatus of cells may reflect the degree of their injury or, on the other hand, may be associated with intensification of intracellular regeneration processes [4, 8, 12].

To study the role of lysosomes of cardiomyocytes in a multiparametric living object (intact cardiomyocytes, intact heart) we excluded some parameters which have been studied and replaced them by a smaller number of functions of them, in order to establish the most general relations between the various characteristics of function, metabolism, and ultrastructure of the test object. Since the investigation had to be carried out over a long period of time, seasonal influences on the object were excluded and an attempt was made to discover relations between parameters that are independent of season, for the writers previously found seasonal changes in certain physiological, biochemical, and morphological characteristics of the working intact heart [4, 6, 7]. Factor analysis, one of the methods of multidimensional statistical analysis, was used for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 180 intact male chinchilla rabbits weighing 3.5 ± 0.5 kg. In the course of the year, at the middle of the month, during winter, spring, summer, and fall 28 physiological, biochemical, and morphological characteristics of cardiac activity were investigated (from 3 to 10 measurements during each season). The peak systolic pressure under real conditions (BP_p) and during occlusion of the ascending aorta or pulmonary artery for 5 sec, i.e., under conditions when the heart developed its maximal contractile power (BP_m), was measured electromanometrically in the left ventricle (LV) and right ventricle (RV) of the animals. The duration of the diastolic pause (T_d) was calculated from the BP_pLV curve. Activity of lipoprotein lipase (LPL), triacylglycerolipase (TGL), and monoacylglycerolipase (MGL) in pre- and postheparin blood plasma, myocardium, and adipose tissue was determined by the writers' own method [1]: The concentration of free fatty acids (FFA) in the blood was determined [3]. Heart sections were studied histochemically by Burstone's reaction for acid phosphatase, followed by counting the number of formazan granules in the section. For electron-microscopic investigation the heart was perfused through the aorta (after preliminary washing with physiological saline) with a 2.5% solution of glutaraldehyde, followed by postfixation of areas of papillary muscles in a 1% solution of OsO_4 at pH 7.2-7.4 . The material was then embedded in Araldite and sections were cut on a Reichert-Jung Ultracut ultramicrotome and stained with lead hydroxide and uranyl acetate, after which they were examined in the Tesla BS 540 electron microscope under a magnification of 22,000×. For quantitative analysis of the electron micrographs (EM) modified methods suggested by Paukov and Frolov [2, 4] were used. The mean number of mitochondria in one standard EM (M), the mean area of one mitochondrion (AM), the mean total area of the mitochondria in one EM (AM_{em}), the mean number of cristae per mitochondrion (CM), the mean total number of cristae per EM (CM_{em}), the coeffi-

Department of Pathological Physiology, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 19-22, October, 1983. Original article submitted March 25, 1983.

0007-4888/83/9610-1361$07.50