EFFECT OF TETANUS TOXIN ON THE MYOCARDIUM

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UDC 616.127-02:579.852.11

KEY WORDS: tetranochemolysin; creatine phosphokinase; tetanus toxin; myocardium.

Many workers consider that lesions in the heart, frequently found in tetanus, are due to hypercatecholemia [11, 14, 17], and they suggest the use of β-adrenoblockers for their prevention [8, 9, 15].

Meanwhile experiments on animals infected with *Clostridium tetani* or receiving injections of tetanus hemolysin have nearly always revealed lesions of parenchymatous organs, including the myocardium, even in the prodromal period, before manifestation of any symptoms of the disease [1].

The present writers observed disturbances of the circulation by the use of light microscopy 6 h after injection of native tetanus toxin into animals, in the form of vascular congestion, diapedetic hemorrhages, and lesions of parenchymatous organs, namely cloudy swelling degeneration, necrobiosis, and fibrinoid necrosis. In experiments on dogs, rabbits, and albino rats receiving injections of isopropylnoradrenalin, the present writers also found that the dose of this substance, in order to cause myocardial damage, must be an order of magnitude greater than that which proves lethal in experimental tetanus.

The object of this investigation was to study the role of tetanohemolysin in myocardial damage.

EXPERIMENTAL METHOD

Experimental tetanus was simulated in conventional albino rats weighing 180 ± 10 g, which were given an intravenous injection of 1 MLD (10 μg/kg) tetanus toxin not containing active hemolysin, or 200 hemolytic units (HU)/kg of lyophilized tetanohemolysin, purified by gel-filtration on Sephadex G-100 [5], containing very small traces of spasmin as impurity. This dose of hemolysin was the minimal dose which, if injected simultaneously with 1 MLD tetanospasmin, accelerated and potentiated its action on albino rats.

Under anesthesia thoracotomy was performed on the animals 5, 10, 15, and 30 min, 1, 3, and 24 h after the injection, and thereafter daily, and the largest possible volume of blood was withdrawn by cardiac puncture. Plasma was separated by centrifugation and analyzed quantitatively for free hemoglobin, using the absorption spectrum at 415 nm, and creatine phosphokinase (CPK), by the method of Oliver and Rosalki [10, 13], in the writers' modification. The catecholamine level also was determined spectrofluorometrically in the blood samples.

The hearts were removed in the cold and small pieces of tissue exised from the left ventricle and immersed in a 2% solution of OsO_4 in 0.1 M cacodylate buffer, pH 7.36. Later, pieces measuring 1 mm^3 were fixed in the same solution for 2 h in the cold. Tissue blocks were washed in the same buffer and dehydrated in alcohols and acetone of increasing concentration. The dehydrated pieces of tissue were embedded in a mixture of Epon and Araldite. Sections were cut from the blocks on a UMPT-3 ultramicrotome, stained in a 2% solution of uranyl acetate [16] and lead citrate [12], and examined in the UÉMV-100 K electron microscope with an accelerating voltage of 75 kV.

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 0007-4888/83/9501-0130$07.50

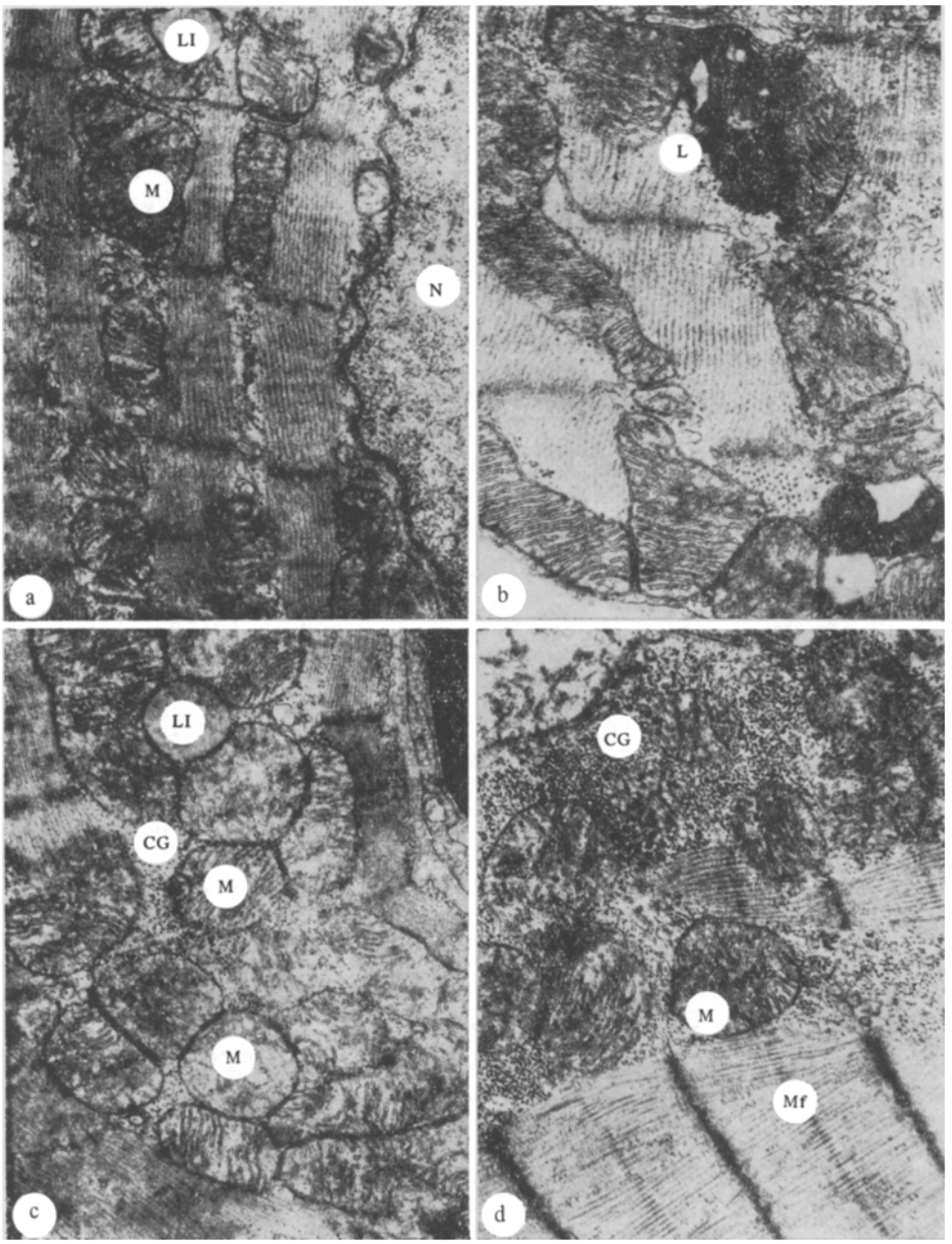

Fig. 1. Ultrastructure of cardiomyocyte of experimental animal after injection of tetanohemolysin. a) Translucency of nucleoplasm (N), tortuosity of outlines of nuclear membranes, increased osmiophilia of mitochondrial matrix (M), and lipid inclusions (LI), 39,000 ×; b) formation of primary lysosomes (L) in cardiomyocytes, 52,000 ×; c) lipid inclusions (LI) present between mitochondria (M) with intact matrix, appearance of cytogranules (CG), 37,000 ×; d) destruction of mitochondrial matrix (M), accumulation of cytogranules (CG), myofilaments (MF), 40,000 ×. a-c) 5 min, d) 10 min, after injection of tetanohemolysin.

The rest of the heart tissue was homogenized in the cold in 0.15 M NaCl solution and CPK activity in it was determined.

The acid resistance of the animals' erythrocytes was determined at the same time [4].

EXPERIMENTAL RESULTS

Translucency of the nucleoplasm, tortuosity of the outlines of the nuclear membranes, and the appearance of lipid inclusions were observed on the electron micrographs 5 min after

injection of tetranohemolysin. Dilatation of the T and L systems of the endoplasmic reticulum took place at these same times and varied osmiophilia of the mitochondrial matrix was observed (Fig. 1).

Electron-microscopic investigation showed the formation of many primary lysosomes in the myocardiocytes with osmiophilic contents and translucent vacuoles (Fig. 1).

Many cytogranules, occupying a large part of the sarcoplasm, could be seen in the ultrastructure of the myocardium 10 min after injection of tetanohemolysin. Marked destruction of individual mitochondria occurred, as shown by destruction of the membrane or of the cristae themselves, and some mitochondria were swollen. There were no visible changes in the myofilaments (Fig. 1).

The quantity of free hemoglobin in the plasma 30 min after injection of hemolysin was increased by 25 $\pm$ 4%, the CFK activity in the homogenates of the hearts was increased by 16 $\pm$ 3%, and its activity in the blood plasma by 68 $\pm$ 9% compared with the control.

To differentiate between CPK of myocytic and erythrocytic origin, 40 HU of tetanohemolysin (the dose of hemolysin per rat) was added to 5-6 ml of heparinized rat blood collected into heparin. In one case the free hemoglobin level rose by 223 $\pm$ 29% whereas the CPK activity was virtually unchanged.

Preliminary intravenous injection of 50 units heparin into rats 15 min before injection of the hemolysin lowered the free hemolgobin level by 38 $\pm$ 6% and, at the same time, increased the CPK activity by 269 $\pm$ 30%. Hence it can be concluded that heparin activated the release of CPK from the cardiomyocytes or prevented self-healing of the injuries in their membranes.

The blood catecholamine level of the animals 30 min after injection of hemolysin was 63 $\pm$ 11 ng/ml, which in the present experiments was 53% above normal, but only one-tenth of the dose capable of inducing pathological changes in cardiomyocytes [7].

In a certain percentage of cases of tetanus lesions in the myocardium may perhaps be due to hypercatecholemia but, since the disturbances of activity of the cardiovascular system are a rather usual event in tetanus [3, 6], it seems likely that they are due primarily to tetanohemolysin. This conclusion is all the more likely because all strains of *Cl. tetani* without exception, even nontoxigenic strains, produce tetanohemolysin [2].

A beneficial effect of β-adrenoblockers in the treatment of tetanus depends mainly on their membrane-stabilizing action.

Determination of the acid resistance of the erythrocytes showed that it was lowered by 5% 1 h after injection of tetanohemolysin, by 10% after 2 h, by 15% after 3 h, by 42% after 24 h, by 50% after two days, and by 58% after three days. The progressive fall in erythrocyte resistance thus demonstrates progression of the destructive changes in the lipoprotein structures and a catabolic trend of metabolic processes in the animals.

The results of investigations of rats receiving tetanus toxin without hemolysin were virtually indistinguishable from the controls during the first few hours.

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ULTRASTRUCTURAL STEREOLOGIC ANALYSIS OF CARDIOMYOCYTES IN SPONTANEOUSLY HYPERTENSIVE RATS

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UDC 616.12-008.331.1-055.5].7-07:616.127-018.1-076.4

KEY WORDS: spontaneous hypertension; hypertrophy of the myocardium; ultrastructure of the cardiomyocyte; morphometry; stereology.

Spontaneous hypertension in experimental animals is linked with the development of hypertrophy of the heart [12, 14], in the course of which ultrastructural reorganization of the cardiomyocyte takes place [10, 15]. Quantitative changes in the mitochondrial and myofibrillary compartments have been studied in the greatest detail, for relations between these organelles are considered to play a key role in compensatory-adaptive reactions developing at the cell level [2, 5-7, 11]. However, for a fuller understanding of the processes taking place in cardiomyocyte hypertrophy it is essential to know the structural state of the transport systems of the cell and their interaction with other organelles.

The aim of this investigation was to study the intracellular organization of cardiomyocytes in rats of different ages with spontaneous genetic hypertension in the course of its development.

EXPERIMENTAL METHOD

Male albino rats (n = 34) with genetic arterial hypertension (SHR line) were used. The arterial blood pressure (BP) was measured by a transducer in the tail under superficial ether anesthesia. A morphometric study of the heart was conducted in twelve rats aged 1 month (body weight 62.0 ± 0.36 g), 4 months (body weight 273.3 ± 23.3 g), and 11 months (body weight 233.3 + 32.8 g). Immediately after sacrifice of the animals the heart was removed from the chest and placed in a cold chamber until it stopped beating completely. The absolute and relative weight of each heart was then determined. Samples of tissue from the left papillary muscle were fixed in a 4% solution of paraformaldehyde, postfixed in 2% OsO_4 solution, dehydrated with ethanol and propylene oxide, and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on the LKB-III Ultratome. Ultrathin sections were stained and examined in the JEM 100 B electron microscope.

The mean diameter of the cardiomyocytes for each groups of animals was determined in semithin sections stained with azure II by means of the MOV-1-15 ocular micrometer. Stereologic analysis was carried out on electron micrographs under a final magnification of

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