EFFECT OF LYSOSOMAL ENZYMES ON MYOCARDIAL ELECTRICAL ACTIVITY AND CONTRACTILITY IN BURN SHOCK

E. G. Vornovitskii, N. A. Len'kova,
N. I. Kochetygov, and M. I. Remizova

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A leading role in the genesis of the early hemodynamic disturbances of burn shock is played by a decrease in cardiac output [3, 5], one cause of which after severe thermal trauma is a decrease of myocardial contractility [6, 13]. It has been suggested that the heart muscle may be damaged as a result of the action of a toxic factor, formed in burns [8]. Experiments with perfusion of an isolated papillary muscle of the rabbit heart with blood plasma from burned animals have in fact shown that "burn" plasma obtained as early as 20-60 min after thermal trauma inhibits myocardial contractility [1]. A definite role in the formation of this toxic factor in severe burns, just as in hemorrhagic shock, is perhaps played by lysosomal enzymes circulating in the blood [4, 11]. It was shown previously that lysosomal enzymes can aggravate hemodynamic disorders existing during shock, by lowering, in particular, the cardiac ejection [4]. However, it is difficult in experiments on the intact animal to reveal the direct action of lysosomal enzymes on cardiac function.

The object of this investigation was to compare changes in myocardial contractility and electrical activity under the influence of "burn" plasma and lysosomal enzymes and thus to determine the contribution of lysosomal enzymes appearing in the blood in burn shock to the disturbance of myocardial contractility.

EXPERIMENTAL METHOD

To obtain plasma from burned animals, burn trauma affecting 30% of the body surface was inflicted with boiling water on 16 rabbits anesthetized with urethane (1 g/kg body weight). Plasma was taken 20 and 60 min after burning. Activity of lysosomal enzymes — acid phosphatase (AP) [9] and cathepsin D [7], and also the protein concentration [12], were determined in the plasma of six animals before and 20 and 60 min after thermal trauma.

Electrical activity and contractility of isolated papillary muscles of the normal rabbit heart were studied in two series of experiments. In series I the preparations were perfused with blood plasma from burned animals (16 experiments). In series II they were perfused with plasma from healthy normal animals (nine experiments) to which lysosomal enzymes had been added, in the form of an enriched fraction isolated from normal rabbit liver [10]. The plasma used for perfusion was diluted in both series of experiments with Tyrode solution in the ratio of 1:1. The original volume of perfusate, circulating in a closed system, was 60 ml. In the experiments of series II the fraction of lysosomal enzymes was added in a volume of 10 ml three times in the course of each experiment. The perfusate was continuously saturated with carbogen (95% O_2 + 5% CO_2). The temperature of the perfusate was 31-33°C and its pH 7.25-7.3. Papillary muscles were isolated from the right ventricle. The technique of isolation, the apparatus used, and the composition of the Tyrode solution were described previously [2]. Intracellular action potentials (AP) of myocardial fibers were recorded by means of intracellular glass microelectrodes filled with 2.5M KCl; the frequency of stimulation was 1 Hz. Isometric contractions of the papillary muscles were recorded with a 6MKhIS mechanotron with successive change of frequencies of stimulation: 0.1, 0.2, 0.5, 1, and 2 Hz.

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TABLE 1. Activity of Lysosomal Enzymes in "Burn" and Normal Plasma after Addition of Lysosomal Enzymes

Perfusate	AP, μg P_i/mg protein/h	Cathepsin D, μg tyrosine/mg protein/h
Burn plasma (n=6):		
20 min after burning	1,57±0,25	1,1±0,4
60 min after burning	2,8±0,7	3,8±1,4
Tyrode solution + normal plasma + L_1 (n=9)	0,56±0,06	4,83±0,37
Same + L_2 (n=8)	0,98±0,11	8,5±0,65
Same + L_3 (n=5)	1,30±0,15	11,3±0,86

Legend. L_1, L_2, and L_3) Consecutive additions of lysosomal enzymes to normal plasma.

TABLE 2. Action of "Burn" and Normal Plasma with Lysosomal Enzymes on Intracellular AP of Rabbit Heart Papillary Muscles

Perfusate	AP, mV	Duration, of AP, for undermentioned level of repolarization		
		20 %	50 %	80 %
Burn plasma				
Normal plasma (n=7)	102±6,3	65,4±10	122±12,9	163±8,7
Burn plasma (n=7)	95±4,7	97±12,6	154±15,5	186±12,7
P		<0,01	<0,01	<0,05
Normal plasma with lysosomal enzymes				
Normal plasma (n=4)	133±12	94±14,3	154±16,8	206±25,2
Same + L_1 (n=4)	114±9	100±16	161±20	216±28,5
Same + L_2 (n=4)	112±6,75	106±14,6	164±19,3	210±26,4

Legend. L_1 and L_2) First and second additions of lysosomal enzymes respectively to normal plasma. Significance of differences determined by method of comparison of pairs.

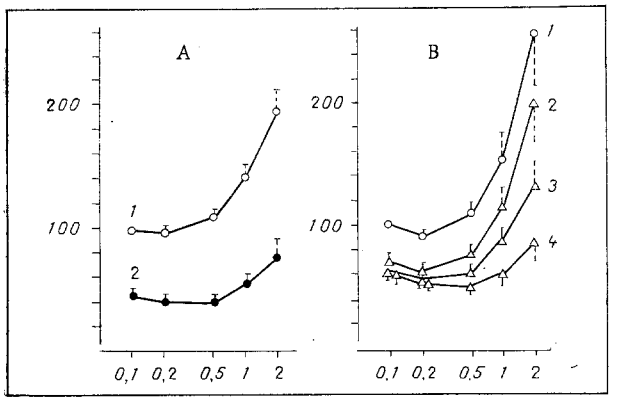

Fig. 1. Changes in frequency-inotropic relations in rat heart papillary muscles under influence of "burn" plasma (A) and lysosomal enzymes (B). Abscissa, frequency of stimulation (in Hz); ordinate, amplitude of contractions (in %; amplitude of contractions in normal plasma, with frequency of 0.1 Hz, taken as 100). A: 1) Normal plasma, 2) "burn" plasma (n = 16). B: 1) Normal plasma, 2, 3, 4) three consecutive additions of lysosomal enzyme fraction to plasma (n = 9).

EXPERIMENTAL RESULTS

Activity of the lysosomal enzymes (AP and cathepsin D) in normal rabbit blood plasma was virtually not detected. Activity of these enzymes 20 min after burning was: for AP 1.57 ± 0.25 μg P_i/mg protein/h, and for cathepsin D 1.1 ± 0.4 μg tyrosine/mg protein/h. Activity of the enzymes 60 min after trauma was increased by 2 and 3 times respectively (Table 1).

AP activity in lysosomal fractions of liver cells used to perfuse the papillary muscle in the experiments of series II was 3.9 ± 0.4 μg P_i/mg protein/h and cathepsin D activity was 34 ± 3.4 μg tyrosine/mg protein/h. Activity of these enzymes in the perfusate on consecutive addition of the lysosomal fractions to it is given in Table 1.

In the experiments of series I replacement of normal plasma in the perfusing solution by "burn" plasma led to a sharp decrease in contractility of the papillary muscles (Fig. 1A). The amplitude of isometric contractions of the papillary muscles was significantly reduced at all frequencies of stimulation.

In the experiments of series II addition of 10 ml of the lysosomal enzyme fraction to normal plasma led to a significant decrease in amplitude of the contractions at all frequencies of stimulation. Addition of the second and third portions of enzymes to the perfusate caused a further decline in amplitude of contractions of the myocardial preparations (Fig. 1B).

Table 2 gives the amplitude and duration of intracellular AP of papillary muscles recorded in normal plasma, "burn" plasma, and normal plasma with the addition of lysosomal enzymes. The duration of AP was measured at three levels of repolarization: 20, 50, and 80%. Replacement of normal plasma in the perfusing solution by "burn" plasma did not change the amplitude of AP but led to a significant increase in the duration of AP at all levels of repolarization. Addition of lysosomal enzymes to normal plasma caused no significant changes in either amplitude or duration of AP.

Consequently, normal plasma, with added lysosomal enzymes, induces marked inhibition of contractility of myocardial preparations similar to the action of "burn" plasma.

However, during the action neither of "burn" plasma nor of normal plasma with added lysosomal enzymes was the decline in contractility of the papillary muscles accompanied by depression of the amplitude of the intracellular AP: "Burn" plasma increased the duration of AP, but lysosomal enzymes, added to normal plasma, did not change AP. Consequently, in both cases a disturbance of electromechanical coupling in the myocardial cells could be deduced.

Plasma of burned rabbits, in which lysosomal enzymes circulate as early as 20-60 min after trauma, thus inhibits contractility of the papillary muscles of the heart. A perfusate with artificially created lysosomal enzyme activity comparable with that found in the plasma of burned animals, also inhibits myocardial contractility. This suggests that lysosomal enzymes in burn shock may be one of the factors inhibiting myocardial contractility, and thereby contributing to hemodynamic disorders.

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