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Morphology of cardiac muscle in septic shock. Observations with a porcine septic shock model

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Abstract The morphology of cardiac muscle was investigated in a porcine model of septic shock, created by intermitted application of *Escherichia coli*-endotoxin. The earliest lesions, found after 18 h of septic shock, were endothelial cell swelling, marked leucostasis and slight ischaemic alterations of the muscle fibres. At the end point of the experiments, after 48 h, some fibrin thrombi were found associated with more pronounced ischaemic alterations of cardiac muscle cells and some necrotic fibres. Comparing these findings with the severe endothelial and muscle fibre lesions found in skeletal muscle, the endothelial cells of the heart microvasculature, are clearly more resistant to the attack of the endotoxins and mediators liberated in septic shock.

Key words Cardiac muscle · Microvasculature · Septic shock · Porcine shock model

Introduction

In the septic shock syndrome all organs are injured, primarily due to endothelial cell damage and its consequences [2]. The severity of damage, however, differs markedly from one organ to another and it is reasonable to assume that the responsiveness and sensitivity of the endothelial cells in septic shock is most probably organ specific. In a recent study using a porcine model of endotoxaemia to mimic human septic shock we showed that severe morphological lesions were present even in sekeletal muscle [7].

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Because myocardial insufficiency is an important factor in septic shock, we compared the morphology of the cardiac muscle with that of the skeletal muscle in the same experimental animals. To evaluate the functional consequences of heart muscle damage the cardiac output was monitored continuously.

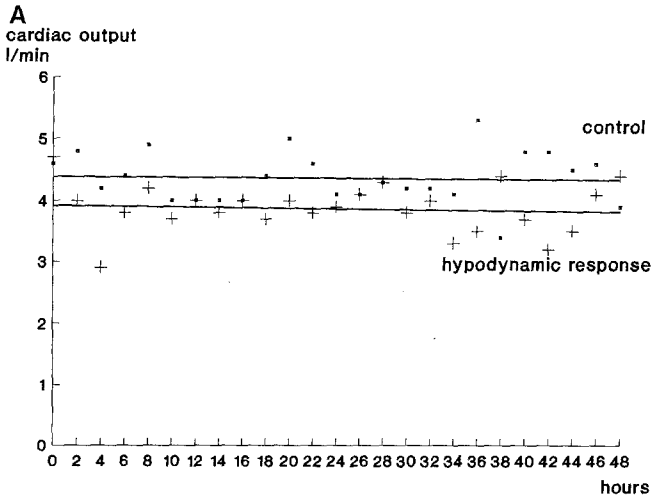
Materials and methods

The animal model has been described previously [11, 12]. Briefly, domestig pigs (28–32 kg) were in quarantine and were treated with antibiotics to prevent pulmonary infections for at least 1 week. The animals were divided into three groups. The 48 h test group (six animals) received endotoxin intravenously (0.5 µg/kg *Escherichia coli*-endotoxin WO111:B4 over 60 min; Difco Laboratories; Detroit, Mich., USA) at two time points, after 1 h and 22 h. The 18 h test group (two animals) received 0.5 µg/kg of endotoxin over 30 min at the beginning of the experiment and at 5 and 10 h.The control group (two animals for each experimental period) received physiological saline solution. The animals were sacrificed by an intravenous injection of 10% potassium chloride. Tissue samples from the left and right ventricle of all animals of all groups were removed immediately. The present study was performed in accordance to the National Institute of Health guidelines for the use of experimental animals and the German Law for the Protection of Animals (Tierschutzgesetz). Permission was also granted by the local authorities.

Tissue sampes of the animals were fixed in 10% neutral buffered formaldehyde and embedded in paraffin. Sections 5 µm thick were cut and staind with haematoxylin and eosin.

For transmission electron microscopy (TEM), tissue specimens were fixed in 3% cacodylate-buffered glutaraldehyde for 30 min. After postfixation in 0.1 M cacodylate-buffered osmium, the specimen were dehydrated in ethanol and embedded in epon. Semi-thin (1 µm) sections were stained with methylene blue-azure II. Ultra-thin sections (70 nm) were mounted on copper grids, stained with uranyl acetate and lead citrate and examined with a TEM (Philips EM400T). From five blocks of each observation period, serial sections were made to search for endothelial fenestrations.

For monitoring of cardiac output (CO) a 5 Foley paediatric Swan-Ganz catheter was placed through the right jugular vein into an interlobular pulmonary artery. CO was measured in triplicate by a thermodilution method using a CO computer and injections of 5 ml of 0.9% ice-cold saline. Strain gauges were zeroed to the level of the mid-axilla. Body temperature was recorded from the thermistor in the pulmonary artery.



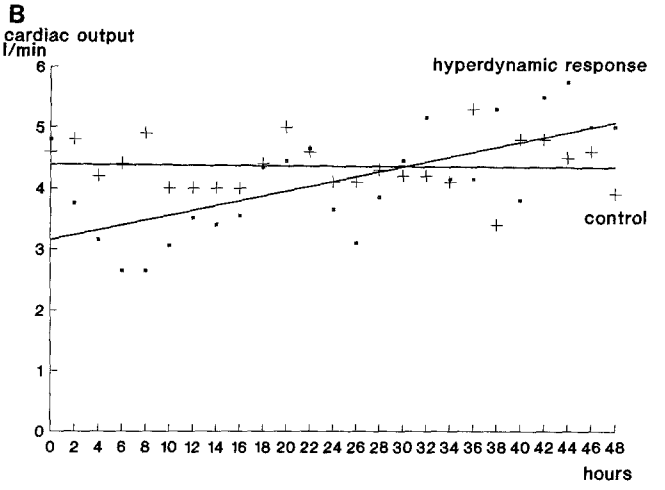
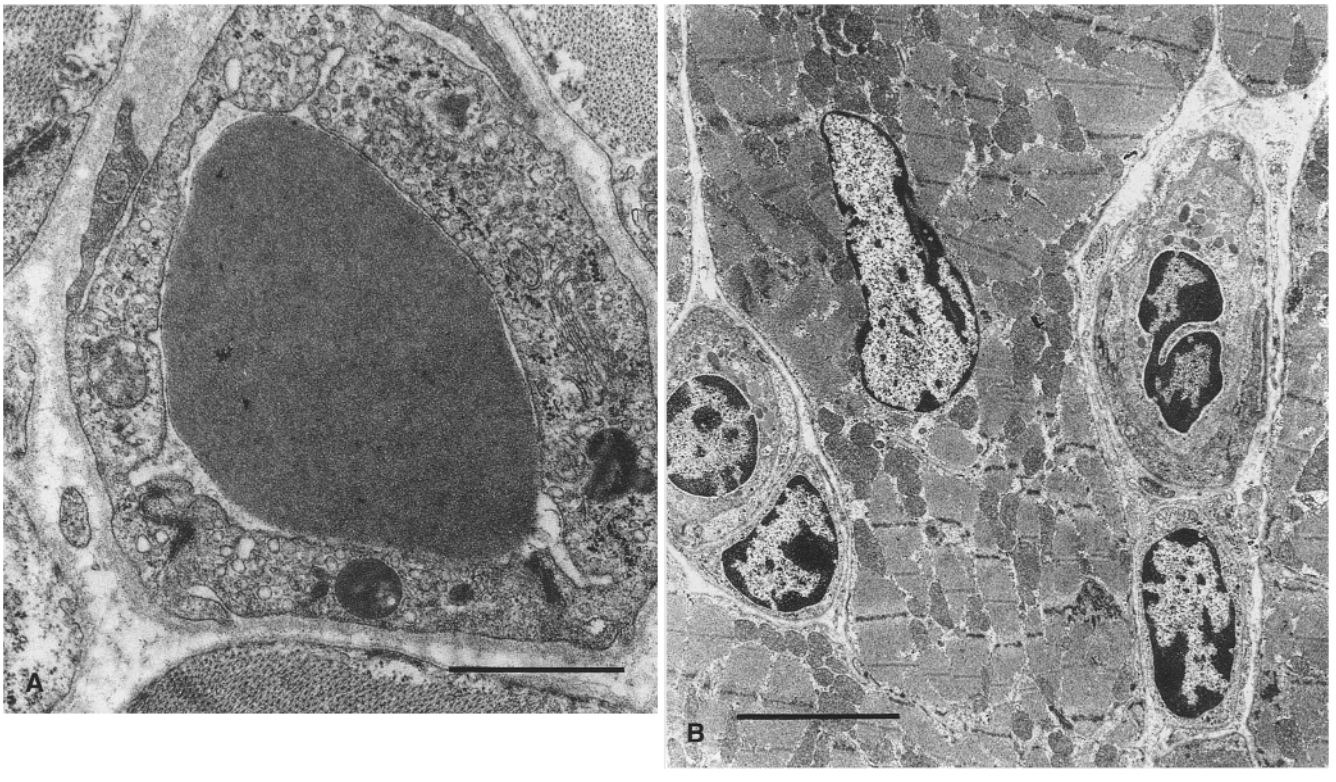


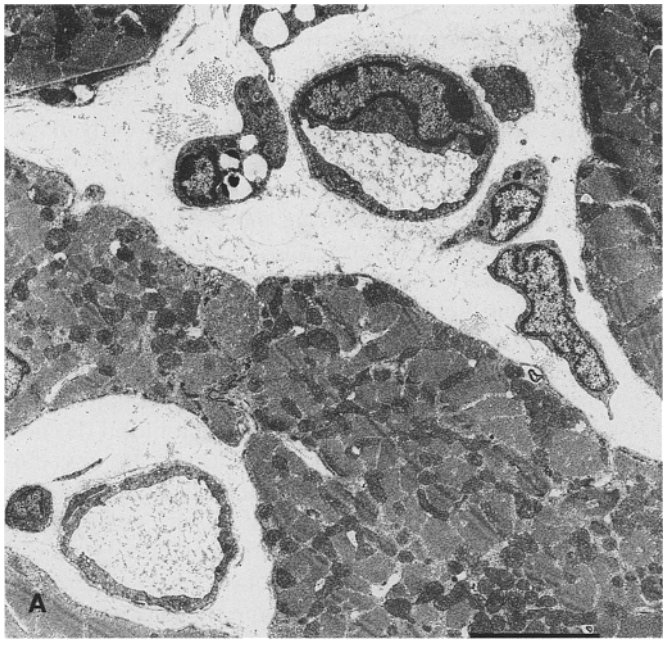
Fig. 1 Cardiac output measurement demonstrating markedly impaired cardial function after treatment with endotoxin for 48 h in some animals (**A**) and hyperdynamic septic shock in others (**B**)

Results

The CO measurements revealed one group of animals with a hyperdynamic response and another with hypodynamic behaviour (Fig. 1A, B). Whereas the former group is characterized by an initially decreased CO with a tendency to increasing, the latter remain at the hypodynamic level throughout the observation period. The CO of the 18 h animals was hypodynamic, and it becomes clear from Fig. 1A, B that the CO from both the hypodynamic and the hyperdynamic group is below the control level within the first 18 h. On comparing the morphological findings of both the hypodynamic and the hyperdynamic group, no differences were detected.

Fig. 2 **A** Marked distension of endothelial cells after 18 h of septic shock with increased pinocytotic vesicles, prominent rough endoplasmatic reticulum, lipid droplets and fine granular matrix in the cytoplasm. Note the intact cell junctions and the absence of degenerative changes. (*Bar*=1 μm). **B** Leucostasis in capillaries after 48 h of septic shock. Note the intact intercalation discs. *Bar*=5 μm)





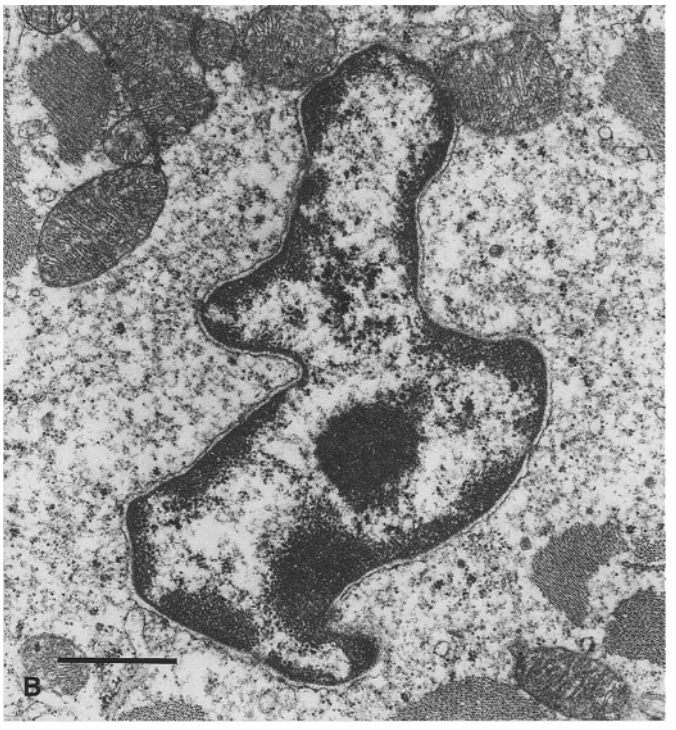


Fig. 3 **A** Interstitial oedema in septic shock of 48 h duration. (*Bar*=5 μm). **B** Perinuclear halo consisting of fluid with fine granular material and a slightly enlarged perinuclear cistern in this cardiomyocyte after 48 h. *Bar*=1 μm)

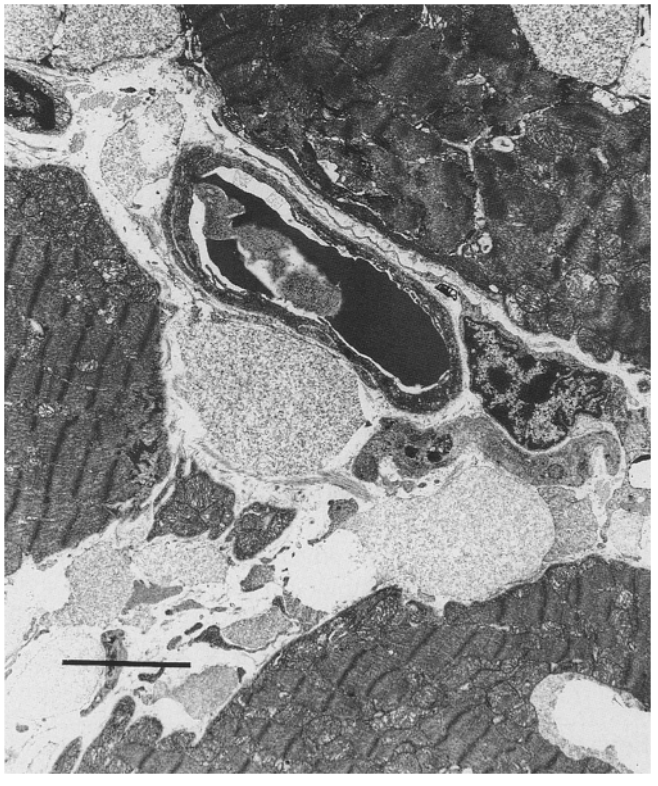


Fig. 4 Fibrin thrombus and contracture bands in a cardiomyocyte. There is focal disintegration of myofilament architecture. Note the marked interstitial oedema. *Bar*=5 μm)

In the early phase of septic shock (18 h after beginning the experiment) no morphological changes were detectable by light microscopy. However, by EM, cardiac endothelial cells in the endotoxin group showed marked swelling with an increase in fine granular matrix in the cytoplasm and numerous pinocytotic vesicles (Fig. 2A). Loosening of the endothelial cell junctions, endothelial cell fenestrations, and endothelial cell necrosis was not observed. Leucostasis was prominent (Fig. 2B). Numerous cardiac muscle cell mitochondria had widened cristae and a reduced number of granulations.

After 48 h a slight interstitial and perinuclear oedema was found by EM, which was accompanied by an increase in the fine granular matrix of endothelial cells (Fig. 3A, B). Several blood vessels were obstructed by fibrin thrombi. In cardiac muscle cells, mitochondrial oedema, vacuolar distension of mitochondrial cristae, and cristolyis had progressed. In numerous heart muscle cells intracellular oedema, open intercalated discs, an enlargement of the T-tubules, broad contracture bands and focal necrosis were found (Fig. 4).

Discussion

Whereas petechial haemorrhages and necrotizing myocardial lesions were a common finding in patients that had died from hypovolaemic shock [15], reports about myocardial alterations in patients that had died from septic shock indicate only slight structural alterations of the heart muscle [20].

The findings in our experimental model are very similar to those described in humans [20]. We found morpho-

logical alterations in endothelial cells indicating a metabolically activated state in the early phase of endotoxin shock. Because the endothelial cell damage was only mild, no oedema was visible by light microscopy and thus few signs of hypoxic damage of heart muscle cells were present. A prominent feature was marked leucostasis. Later, fibrin thrombi occurred but again without necrosis of endothelial cells. The endothelial cell changes observed are probably related to the cascade of inflammatory mediators which is activated in septic shock [7, 12]. Platelet activating factor and oxygen radicals reduce the anionic charges of endothelial cells [7, 26] resulting in a loss of normal permeability of the capillary wall, which facilitates the formation of microthrombi and leads to intracellular oedema of endothelial cells.

In the later stage of septic shock the findings of hypoxic alteration of heart muscle cells were increased, including intracellular oedema, open intercalated discs, enlargement of the T-tubules, broad contracture bands, and isolated necrotic myofibrils. These alterations in the heart muscle fibres could be the result of a direct toxic endotoxin effect and/or hypoxic injury. Ischaemia as a result of the impaired microcirculation [8] due to both formation of fibrin thrombi and the considerable leucostasis as well as cardiomyocyte swelling that leads to constriction of the capillaries [4] might be aggravated by coronary hypoperfusion [5] during the stage of low CO.

From the various animal models of septic shock using sheep, rats and dogs, the results obtained with sheep that had undergone caecal ligature and perforation [8] are comparable to our findings. However, in rats and dogs, marked structural alterations and endothelial cells were found including disruption of interendothelial tight junctions and all degrees of mitochondrial damage [6, 7, 11, 17, 22]. Because of these models other dosages and different application modes has been used, the results are not directly comparable with our findings.

The findings in the heart are in contrast to the severe damage observed in skeletal muscle where in the early phase, the endothelial cells showed necrosis causing an oedema rich in proteins [7]. Moreover, in the microvasculature of skeletal muscle fibrin thrombi and marked leucocyte sticking were missed. The endothelial cells of heart muscle seem to be much more resistant to damage than endothelial cells in skeletal muscle. In skeletal muscle necrosis of endothelial cells is quite often seen, however, the endothelial cells in the heart are obviously activated, become adhesive for leucocytes and reduce their fibrinolytic potential. This different behaviour of cardiac endothelial cells protects the heart from serious morphological alterations and is probably very important for survival in septic shock. These findings are in agreement with both clinical and experimental studies on cardiac muscle function which indicate that the cardiac muscle becomes insufficient and damaged only at later stages [3, 18, 23, 24, 27].

The clinical picture of the initial phase of septic shock is characterized by a high CO induced by an enhanced myocardial contractility by β-adrenergic stimulation [21]. In our model we found both a hypodynamic and a hyperdynamic response. A decreased CO was the rule in the first 20–24 h in all animals. This behaviour is not attributable to the morphology of the heart muscle because no severe damage could be detected in the early phase and because the amount of morphological alterations does not differ between both groups. Therefore, functional disturbances seems to be more important for the function of the heart in septic shock. Some recent studies stressed relatively early myocardial dysfunction in septic shock without morphological changes [9]. These changes were attributed to a direct toxic effect of the endotoxin molecules on cardiac myocytes and also to functional impairment of enzymes mediated by the endotoxin [1, 19]. Examples of these effects are the inhibition of energy dependent ion transport processes across the myocardial cell membrane and the sodium/potassium-ATPase enzyme system of myocardiocyte plasma membranes [13, 16]. Furthermore, an uncoupling of sarcolemma β-adrenoreceptor from adenylate cyclase has been reported [10]. Endotoxins directly modify the molecular structure of cardiac myocyte membrane lipids and are able to activate phospholipases A1 and A2 [25]. Phospholipase A2 was shown to play a role in endotoxin-induced impairment of myocardial sodium/calcium exchange [10]. Reduced vesicular calcium uptake as a result of reduced ATPase activity caused by endotoxin has been reported [25]. Thus, endotoxins lead to a progressive energy deficit with decreased levels of ATP and creatine phosphate, and to electrophysiological and ionic disturbances. Hydrogen peroxide, released by granulocytes activated by complement factors 3a and 5a, was found to be a major mediator of depressed calcium uptake rates and impaired ATPase activity, affecting the activity of the excitation-contraction coupling system of cardiomyocytes directly [14].

It is evident that the heart is a target organ for endotoxins in septic shock. Marked leucocyte vascular adhesion, endothelial cell swelling and interstitial oedema are factors that contribute to myocardial cell damage. However, the alterations of cardiac endothelial cells are less dramatic when compared with those observed in skeletal muscle during septic shock. Thus, organ specific factors seem to be important in endothelial cell sensitivity to endotoxins. Moreover, because similar degrees of endothelial cell damage were accompanied by a very variable extent of myocardial cell injury in the present study, a direct toxic effect of the endotoxin on the cardiac muscle fibres appears likely.

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