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Myofilament calcium sensitivity is decreased in skinned cardiac fibres of endotoxin-treated rabbits

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Abstract

Objective: The aim was to determine whether myofilament Ca^{2+} sensitivity is altered in skinned cardiac fibres from endotoxin-treated rabbits. **Methods:** Endotoxin was injected i.v. in conscious New-Zealand White rabbits at a dose of 0.5 mg/kg (groups I, II, and III) or 1 mg/kg (group IV). A fifth group was used as control. Hearts were excised 4 h (groups I and IV), 24 h (group II), or 5 days (Group III) after injection. Skinned fibres were obtained with chemical (EGTA + Brij 58) treatment of bundles isolated from papillary muscles. **Results:** The maximal Ca^{2+}-activated force (F_0) of skinned cardiac fibres was not different between groups. However, $[Ca^{2+}]$ required to evoke 50% of F_0 (Ca_{50}) was higher in the fibres from group I than in controls (1.78 ± 0.05 vs. 1.53 ± 0.03 μM; $P < 0.05$). This effect was dose-dependent (group IV: $Ca_{50} = 2.08 \pm 0.12$ μM; $P < 0.05$ vs. group I), larger after 24 h (group II: $Ca_{50} = 2.12 \pm 0.05$ μM; $P < 0.05$ vs. group I). By 5 days, myofilament Ca^{2+} sensitivity had returned to normal (group III: $Ca_{50} = 1.54 \pm 0.05$ μM). **Conclusion:** Myofilament Ca^{2+} sensitivity is decreased in skinned fibres taken from rabbit myocardium after i.v. endotoxin injection. This effect is dose- and time-dependent, and reversible with time.

Keywords: Endotoxin; Cardiac function; Contractile apparatus; Calcium ion; Rabbit

1. Introduction

Depression in myocardial contractility is a common feature of septic shock. It is manifested by a reduced left and right ventricular ejection fraction and increased end-diastolic volume indices of both ventricles [1]. This myocardial depression contributes to cardiovascular insufficiency, progressive hypotension, and death. However, in patients who survive, it is reversible 5 to 10 days after its onset [1]. Lipopolysaccharide endotoxin injection mimics the haemodynamic changes seen in human septic shock, and represents a valid experimental model in animals and in healthy humans [2]. Endotoxaemia decreases myocardial contractility in vivo and in isolated heart and cardiac muscle preparations as well [3]. However, exact mechanisms of endotoxin action are not yet fully understood. At the subcellular level, changes in myocardial contractility result either from an alteration in intracellular Ca^{2+} transient or from changes in myofilament sensitivity to Ca^{2+} or both. Available data suggest that myoplasmic Ca^{2+} transient is decreased in hearts removed from endotoxaemic animals. This effect involves alterations in Ca^{2+} transport by the sarcolemma [4–7] and/or impairment in sarcoplasmic reticulum function [8,9]. In addition, in ventricular cells from endotoxin-treated rabbits, myocyte shortening is reduced over a wide range of extracellular Ca^{2+} concentration values, suggesting that endotoxin may reduce myofilament sensitivity to Ca^{2+} and/or decrease the maximal Ca^{2+}-activated force [4]. Recent work has shown that high concentrations of tumor necrosis factor-alpha (TNFα), a cytokine that has been implicated in the pathogenesis of septic shock, result

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in rapid and reversible decline in contractile function of adult rabbit ventricular myocytes [10]. This effect is unaccompanied by a significant change in $[Ca^{2+}]_i$ activity and is abrogated by nitric oxide synthase (NOS) inhibitors, suggesting a NO-mediated decrease in the sensitivity of the myofilaments to Ca^{2+} [10]. NOS, induced by in vitro lipopolysaccharide exposure, negatively modulates the contractile function of cultured myocytes, with a decreased Ca^{2+} sensitivity of myofilaments contributing to this effect [11]. However, whether Ca^{2+} sensitivity of myofilaments is reduced in myocardium of endotoxaemic animals remains unknown. To test this hypothesis, we investigated directly the relationship between force and Ca^{2+} concentration at the myofilament level in skinned papillary muscle bundles from endotoxin-challenged rabbits. Our results show that the Ca^{2+} sensitivity of tension, assessed by the Ca^{2+} concentration for half-maximal activation of tension, is significantly depressed in a dose- and time-dependent fashion in skinned myocardium of endotoxaemic rabbits. This occurs without any change in maximal Ca^{2+}-activated tension.

2. Methods

2.1. Animal model

Protocols were approved by the French Agricultural Office in conformity with European legislation for research involving animals. Experiments were carried out on New-Zealand White rabbits of either sex, weighing 2.6–3.4 kg. All rabbits were obtained from the same source. They were assigned to five different groups (Table 1) and studied in a random order. Conscious animals were injected intravenously with 0.5 mg/kg (groups I, II, and III) or 1 mg/kg (group IV) body weight of purified endotoxin (lipopolysaccharide *Escherichia coli* 055:B5 from a single batch from Sigma Chemical Co., St. Louis, MO, USA) or equivalent sterile saline (control group). In all rabbits, the injectate was administered rapidly, within 1 min. Animals were maintained throughout on a standard rabbit chow diet with food and water available ad libitum. Arterial blood gases were measured after 4 h in all groups. Four hours (groups I, IV, and control), 24 h (group II), or 5 days (group III) after endotoxin injection, animals were weighed and then anaesthetised with thiopentone 20–30 mg/kg i.v. Hearts were removed, rinsed and placed into Krebs buffer gassed with 5% CO_2 in O_2 at room temperature. Segments of papillary muscle from right and left ventricles were dissected free in the Krebs buffer, cut into small fibre bundles, and immediately placed in a relaxing solution containing EGTA (ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid) at 4°C. In preliminary experiments, skinned fibres obtained from each side of the heart of endotoxinic animals behaved identically, and thus results obtained with fibres from both ventricles were pooled.

In a separate set of rabbits ($n = 5$), an aortic catheter was inserted via carotid artery under anaesthesia (thiopentone 20–30 mg/kg i.v.). Once animals were wide awake (at least 8 h after anaesthesia), arterial blood pressure and arterial blood gases (PaO_2, $PaCO_2$, and pH) were obtained during a steady state 10 min before intravenous injection of endotoxin 0.5 mg/kg. Measurements were then repeated every 20–30 min for a total of 4 h.

Table 1
Characteristics of the rabbits

Group	*n*	Endotoxin dose (mg/kg)	Time	Body weight (kg)
Control	5	–	4 h	2.95 ± 0.06
I	5	0.5	4 h	3.08 ± 0.07
II	5	0.5	24 h	2.92 ± 0.05
III	5	0.5	5 days	2.92 ± 0.06
IV	5	1.0	4 h	2.94 ± 0.06

n, number of rabbits; time, time between endotoxin injection and heart excision, expressed in hours or days; body weight measured before endotoxin injection. Values are mean ± s.e.m.

2.2. Skinned fibre preparation

Chemically skinned fibres were prepared in EGTA at 4°C for 24 h as previously described [12]. Chemical skinning with EGTA renders the muscle fibre sarcolemma freely permeable to external solutes. The skinning solution was replaced after 4 and 12 h with fresh solution. After 24 h, the segments were transferred to a skinning storage solution that was identical to the relaxing solution except for the addition of 50% glycerol and stored at −20°C until used (1–2 weeks). No change in skinned fibres properties could be noticed after 2–3 weeks of storage.

Bundles of cardiac muscle were isolated from the main fascicle under a ×25 binocular microscope (model Zeiss Technival 2). Each skinned cardiac bundle was mounted horizontally between two clamps in a muscle bath (0.8 ml) filled with a relaxing solution. One clamp was attached to a Grass Model FT-03C force displacement transducer. The muscle contracture was amplified on a differential amplifier (model AM 502, Tektronix) and recorded on a Gould TA240 recorder. The preparation was bathed for 30 min in a relaxing solution containing the non-ionic detergent Brij 58 (2%), which irreversibly eliminates the capability of the sarcoplasmic reticulum to sequester Ca^{2+} and to release it under appropriate stimulation, but does not affect the contractile proteins [13]. The length and diameter of the preparations were measured under a ×400 Zeiss ID02 lens. Fibres were assumed to be cylindrical. Each cardiac bundle was straightened to a length at which an increase in resting tension was first detected, and the preparation was then stretched a further 20% of the initial length of the bundle, as previously described by Maughan et al. [14]. The sarcomere length in our setup was verified by a

calibrated micrometer for several bundles in each experimental group under a ×400 Zeiss lens. Values ranged between 2.2 and 2.4 μm for all bundles tested.

For all experiments described below, the length of the fibres was kept constant to avoid sarcomere length-dependent changes in Ca^{2+} sensitivity. All experiments were performed at room temperature (20 ± 1°C).

2.3. Solutions

The relaxing solution contained (in mM) MOPS (3-[*N*-morpholino]propanesulfonic acid) 10, K-propionate 170, Mg-acetate 2.5, and K_2-EGTA 5. Activating solutions had the same composition as the relaxing solution except that Ca^{2+}-EGTA was substituted for K_2-EGTA at various ratios. The concentrations of the different components in the solutions were calculated using program 3 of Fabiato and Fabiato [15] to keep the ionic strength at 200 mM. The stability constants of Orentlicher et al. [1,3] were used in the calculations: K_{CaEGTA} 1.919×10^6/M, K_{CaATP} 5.0×10^3/M, K_{MgEGTA} 40/M, and K_{MgATP} 1.0×10^4/M. Each solution contained ATP (2.5 mM), and pH was 7.00 ± 0.01. Free Ca^{2+} concentrations of activating solutions ranged from pCa 6.4 ($[Ca^{2+}] = 0.3$ μM) to pCa 4.8 ($[Ca^{2+}] = 15.8$ μM, maximally activating solution), where $pCa = -\log_{10}[Ca^{2+}]$. All chemicals were obtained from Sigma.

2.4. Experimental procedure

Maximal Ca^{2+}-activated force and Ca^{2+} sensitivity of isometric tension were examined by exposing the skinned fibres to various activating solutions until a plateau in force was reached. Each bundle was exposed to solutions in the following manner. First, a maximal contraction was induced by activating the preparation in a solution of pCa 4.8. After a steady tension had developed, the preparation was returned to relaxing solution. Thereafter, a pCa–tension curve was obtained by stepwise exposure of the preparation to solutions with increasing Ca^{2+} concentration (from pCa 6.4 to 4.8) and measurements of developed tension. The fibre was allowed to fully relax before a final contraction was done in a solution of pCa 4.8 to determine whether the maximal tension generating capability of the preparation had declined during the measurement protocol. Typically, tension declined by less than 5% between the initial and final contractions at pCa 4.8. For analysis of data, the average of the initial and final contractions evoked by pCa 4.8 was taken as the maximal Ca^{2+}-activated force (F_0) of the skinned myocardial bundle and was normalised to cross-sectional area of the bundle. Tension data from preparations in which maximal tension declined by more than 10% between the initial and final concentration at pCa 4.8 were not analysed and are not included in the results reported in this study. To analyse Ca^{2+} sensitivity of isometric tension, intermediate tensions were expressed as a percentage of the maximal tension obtained with each pCa–tension curve. Data were fitted using non-linear regression analysis (Enzfitter, Elsevier Biosoft) to the modified form of Hill's equation: $F/F_0 = [Ca^{2+}]^{nH}/(Ca_{50}^{nH} + [Ca^{2+}]^{nH})$, where F/F_0 is the relative tension, nH (Hill coefficient) is a measure of the slope of the relationship, and Ca_{50} is the Ca^{2+} concentration (expressed in μM) that yields 50% of the maximal Ca^{2+}-activated force.

2.5. Statistical analysis

Six to eight skinned cardiac fibres were studied for each animal in all groups. In order to avoid to bias the analysis with a high number of values, Ca_{50} (the Ca^{2+} concentration giving half-maximal tension), nH (the Hill coefficient), and F_0/cross-sectional area values obtained from each animal were averaged and considered representative of that animal. All *n* values thus indicate the number of animals. Statistical analysis was made by a one-way analysis of variance with Fisher's protected least significant difference post hoc test. Haemodynamic and biological data from the five instrumented animals were analysed by a one-way repeated-measures analysis of variance. Differences between baseline and post-endotoxin results were assessed using Dunnett's post hoc test. Values of $P < 0.05$ were considered significant. All results are expressed as mean ± standard error of the mean (s.e.m.).

3. Results

Endotoxin-treated rabbits appeared depressed over a period of 24–36 h following endotoxin injection. They developed moderate acidosis with arterial pH of 7.33 ± 0.01, arterial bicarbonate concentration (HCO_3^-) 12 ± 0.1 mmol/l, and $PaCO_2$ 15 ± 1 mmHg 4 h after endotoxin injection. There was a body weight loss of 15 ± 2% and 12 ± 1% in groups II (0.5 mg/kg; 24 h) and III (0.5 mg/kg; 5 days), respectively. The collective mortality for all groups combined was 25%. However, the mortality rate varied between different groups (0/10 for group I + instrumented rabbits, and 1/6 for group IV, but 4/9 and 3/8 for groups II and III, respectively). Mortality thus appeared to be primarily dependent upon the duration of endotoxaemia, with most deaths occurring between the 6th and 24th hour post-endotoxin injection. The results of the 4-h study in the five instrumented endotoxin-treated rabbits are illustrated in Fig. 1. pH remained close to the normal range throughout the study (lowest value: 7.35 ± 0.01, 4 h after injection, $P < 0.05$ vs. baseline) although arterial bicarbonate concentration rapidly decreased (HCO_3^-: 14.4 ± 1.2 mmol · l^{-1} 1 h after injection, vs. 21.6 ± 0.8 mmol · l^{-1} at baseline, $P < 0.05$). There was a modest reduction in PaO_2 over this period (lowest value: 87 ± 7 mmHg, 4 h after injection). Mean arterial blood pressure (MABP) remained close to baseline value during

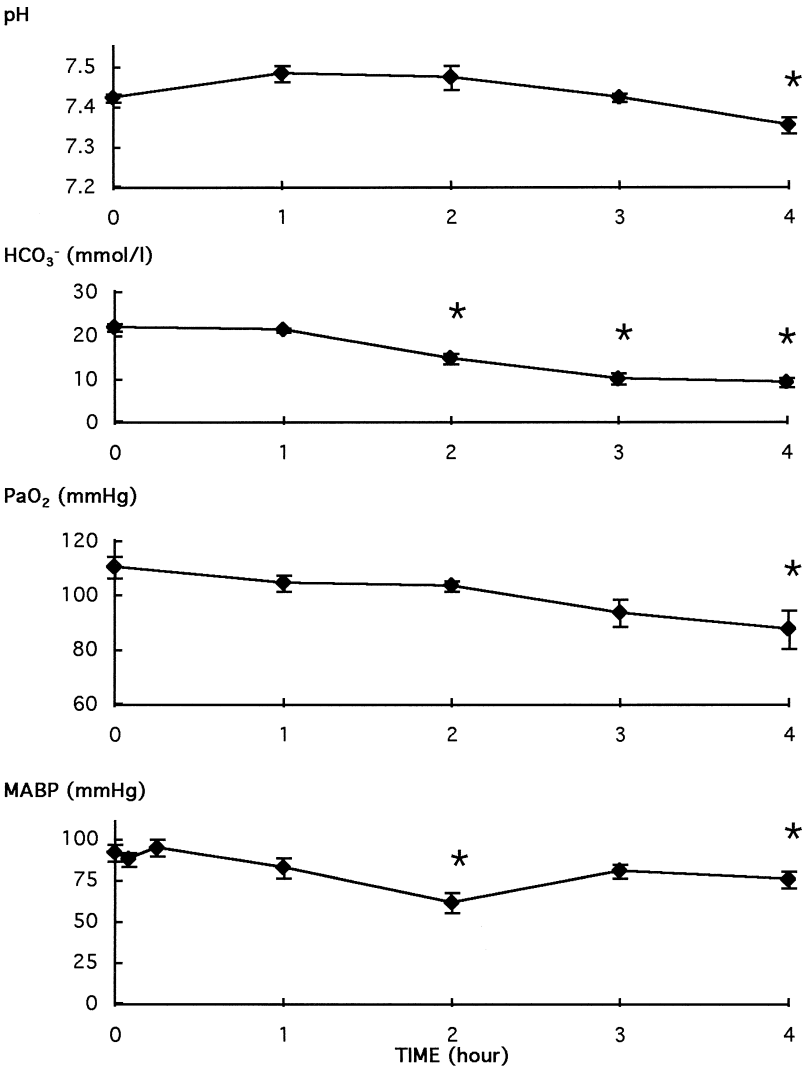

Fig. 1. Mean (±s.e.m.) values for arterial blood gases (pH, bicarbonates concentration [HCO_3^-], and PaO_2) and mean arterial blood pressure (MABP) at baseline and during 4 h after intravenous injection of 0.5 mg/kg endotoxin in five instrumented conscious rabbits. * $P < 0.05$ vs. baseline.

the first hour observation period. This was followed by a decrease in MABP that reached statistical significance at 2 and 4 h after injection (Fig. 1).

Characteristics of the chemically skinned myocardial bundles from each group are shown in Table 2. Maximal activation of the bundles revealed no change in the maximal tension-generating capability of myocardium of endotoxaemic animals from either group compared with control (Table 3). Fig. 2 shows an example of the changes in

Table 2
Characteristics of the skinned fibres

Group	*n*	Length (mm)	Diameter (μm)
Control	5 (34)	1.54±0.10	191±12
I ($E_{0.5}$; H_4)	5 (36)	1.39±0.11	180± 9
II ($E_{0.5}$; H_{24})	5 (35)	1.71±0.22	173± 7
III ($E_{0.5}$; D_5)	5 (34)	1.50±0.08	186± 7
IV (E_1; H_4)	5 (32)	1.42±0.07	177± 5

($E_{0.5}$; H_4) expresses results obtained in hearts excised 4 h after injection of 0.5 mg/kg endotoxin.
n, number of rabbits; total number of fibres in parentheses. Values are mean±s.e.m.

Table 3
Calcium sensitivity and maximal tension of the skinned cardiac fibres

Group	Ca_{50} (μM)	nH	F_0 (mN/mm^2)
Control	1.53±0.03	3.39±0.29	28.6±1.6
I ($E_{0.5}$; H_4)	1.78±0.05 *	3.83±0.39	28.9±2.3
II ($E_{0.5}$; H_{24})	2.12±0.05 *†	3.41±0.23	27.3±4.4
III ($E_{0.5}$; D_5)	1.54±0.05	3.23±0.25	23.4±2.0
IV (E_1; H_4)	2.08±0.12 *†	3.36±0.28	24.3±2.6

($E_{0.5}$; H_4) expresses results obtained in hearts excised 4 h after injection of 0.5 mg/kg endotoxin. $Ca_{50} = Ca^{2+}$ concentration for half-maximal tension; nH = the Hill coefficient; F_0 = maximal Ca^{2+}-activated tension normalised to cross-sectional area of the preparation. Values are mean±s.e.m.
* $P < 0.05$ vs. control.
† $P < 0.05$ vs. group I.

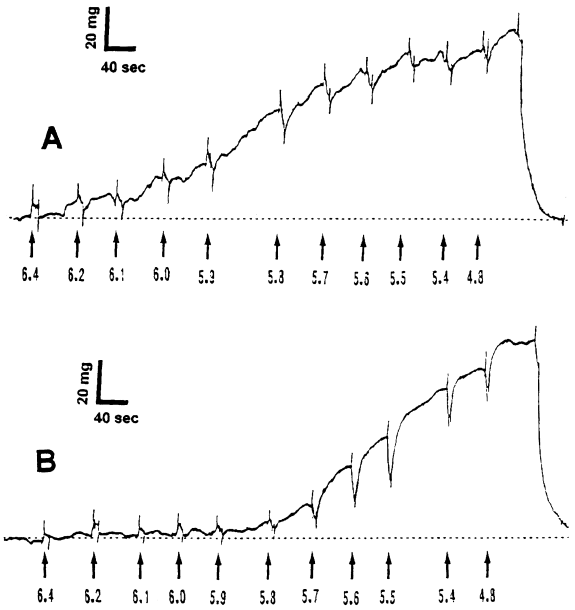

Fig. 2. Changes in tension related to increasing Ca^{2+} concentrations, expressed as pCa (where $pCa = -\log_{10}[Ca^{2+}]$). (A) The changes in tension obtained in a control rabbit. (B) The changes in tension obtained in an endotoxin-treated rabbit (group IV; endotoxin 1 mg/kg, heart excised 4 h after injection). Arrows indicate changes in solutions.

tension in response to increasing Ca^{2+} concentrations observed in control group (Fig. 2A) and in group IV (endotoxin 1 mg/kg, 4 h) (Fig. 2B). The relationship between force production and free Ca^{2+} concentration in skinned myocardial preparations of control group, group I (endotoxin 0.5 mg/kg, 4 h) and group IV (endotoxin 1 mg/kg, 4 h) are shown in Fig. 3. As can be seen in Fig. 2 and Fig.

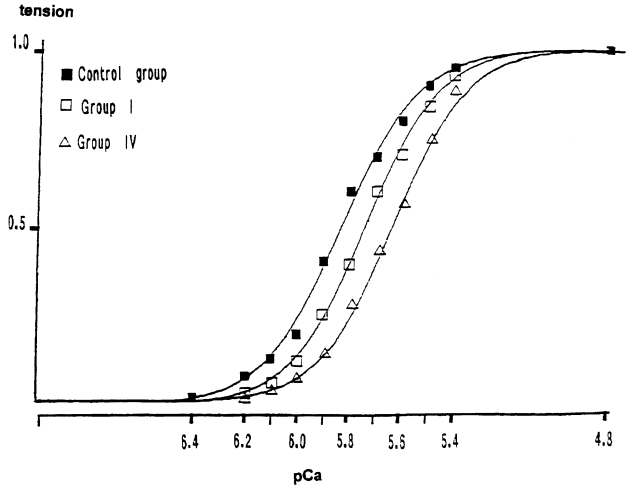

Fig. 3. Mean pCa–tension relations (where $pCa = -\log_{10}[Ca^{2+}]$) obtained in skinned cardiac fibres from control group, group I (endotoxin 0.5 mg/kg, heart excised 4 h after injection), and group IV (endotoxin 1 mg/kg, heart excised 4 h after injection). The curves of the fibres from endotoxaemic rabbits are significantly shifted, in a dose-dependent fashion, to higher Ca^{2+} concentrations. This indicates a decrease in the Ca^{2+} sensitivity of the contractile proteins with increasing doses of endotoxin. For clarity, error bars have been omitted.

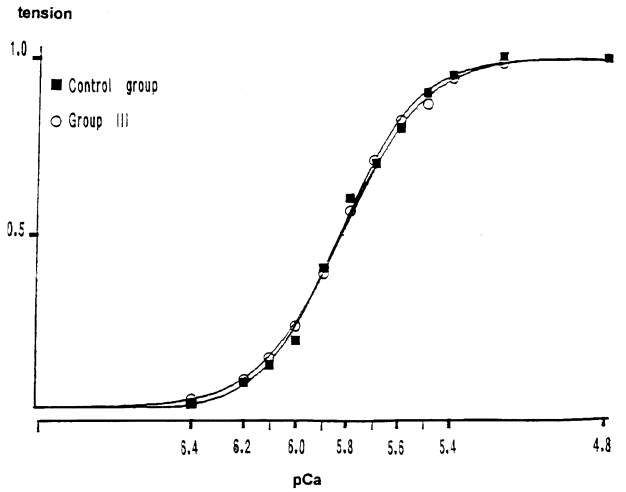

Fig. 4. Mean pCa–tension relations (where $pCa = -\log_{10}[Ca^{2+}]$) obtained in skinned cardiac fibres from control group and group III (endotoxin 0.5 mg/kg, heart excised 5 days after injection). The pCa–tension relation of the fibres from endotoxaemic rabbits is not altered compared with control curve. For clarity, error bars have been omitted.

3, the relationship between force and Ca^{2+} concentration in skinned fibres from endotoxin-treated rabbits was shifted to higher Ca^{2+} concentrations. In the fibres of group I (endotoxin 0.5 mg/kg, 4 h), the Ca_{50} was significantly higher than that of the control fibres (1.78 ± 0.05 vs. 1.53 ± 0.03 μM, respectively; $P < 0.05$), without significant change in the slope of the tension–pCa relationship, quantified as the Hill coefficient (Table 3). This indicates a decrease in Ca^{2+} sensitivity of isometric tension in skinned cardiac fibres of endotoxaemic rabbits. In addition, the relationship between force and Ca^{2+} concentration in skinned fibres of group IV (endotoxin 1 mg/kg, 4 h) shows that endotoxaemia decreased the Ca^{2+} sensitivity in a dose-dependent fashion (Fig. 3). This was attested by the significant increase in Ca_{50} values with increasing endotoxin doses to 1 mg/kg (1.78 ± 0.05 vs. 2.08 ± 0.12 μM, for group I and group IV, respectively; $P < 0.05$) without differences in the Hill coefficient (Table 3). This effect was also time-dependent, as the decrease in Ca^{2+} sensitivity was more important 24 h after injection of 0.5 mg/kg of endotoxin (group II) than in group I (Table 3). Finally, the decrease in Ca^{2+} sensitivity appeared to be reversible with time, as pCa–tension curves in group III (0.5 mg/kg endotoxin, 5 days) were not shifted compared with control curves (Fig. 4). Hence, no significant differences were observed between Ca_{50} or nH of fibres from group III and control group (Table 3).

4. Discussion

Endotoxic shock is characterised by a depression in myocardial contractility, but the cellular mechanisms of this cardiac dysfunction are not clearly understood. The

present study demonstrates that Ca^{2+} sensitivity of skinned myocardial fibres is decreased after endotoxin injection in rabbits. This effect is dose- and time-dependent, and spontaneously reversible with time.

The functional consequence of reduced sensitivity to Ca^{2+} is that isometric tension at any given submaximal concentration of myoplasmic Ca^{2+} is decreased. Reduced Ca^{2+} sensitivity, alone or in concert with other factors may thus contribute to reduced contractile capabilities as well as increased end-diastolic ventricular dimensions that have been observed in endotoxic shock. However, the relative importance of the decreased Ca^{2+} sensitivity compared with other effects leading to a decrease in myoplasmic Ca^{2+} transient cannot be assessed from the present study. A series of experimental works in dogs suggest that endotoxin administration impairs ATP-dependent Ca^{2+} uptake by sarcoplasmic reticulum [9] and leads to a reduction in calcium-induced calcium release mechanism [8]. Endotoxin administration in dogs impaired the ATP-dependent calcium transport by the sarcolemma as well as the sodium–calcium exchanger in some studies [6,7], whereas others found a normal sodium–calcium exchange and sarcolemmal Ca^{2+} transport [16]. More recently, Hung and Lew evidenced in myocytes from rabbit hearts a decrease in the action potential duration, with little or no endotoxin-induced alteration in sarcoplasmic reticulum function or resting membrane potential [4]. Lew et al. subsequently attributed this effect to a decrease in functional L-type calcium channels, as reflected by the number of dihydropyridine receptors [5]. Interestingly, they showed reduced myocyte shortening over a wide range of Ca^{2+} concentration values, suggesting that endotoxin may also reduce myofilament sensitivity to Ca^{2+} and/or decrease the maximal Ca^{2+}-activated force [4]. The existence of the first mechanism (i.e. a reduced Ca^{2+} sensitivity of myofilaments) in endotoxic shock is demonstrated in the present study. Moreover, the characteristic features of the myocardial dysfunction of the endotoxic shock (i.e. a dose- and time-dependent effect, spontaneously reversible with time) have also been found in this study. No attempt was made to assess the myocardial function, but several studies have shown that maximal contractile depression occurs 12–24 h after intravenous injection of sublethal doses of endotoxin in conscious animals [17,18].

The skinned fibre preparation has been widely used to study contractile apparatus itself. However, the skinned fibre techniques were demonstrated to be highly sensitive to experimental conditions such as temperature, intracellular pH, or changes in surrounding substrate concentrations [19]. The technique allows the diffusion outside the cell of low molecular weight proteins, and may induce also inadvertent proteolysis, alterations in myosin light chain phosphorylation, and changes in cross-bridge kinetics [20]. A possible concern is that the skinning procedure may modify the preparations, thereby inducing artifactual changes in the mechanical properties of the preparations, which finally may account for the effects observed in this study. This is unlikely, however, as the shift in Ca^{2+} sensitivity found in this study occurred only in myocardial bundles from hearts excised 4 or 24 h after endotoxin injection (groups I, II, and IV) and not in hearts obtained 5 days after injection (group III). No ATP regenerating system was present in our solutions. In addition, tension was measured only in bundles isolated from papillary muscles. However, myofilament Ca^{2+} sensitivity was also decreased in separate experiments recently performed in skinned fibres obtained from the left ventricular free wall of endotoxaemic rabbit hearts, using solutions where creatine kinase was added (unpublished results).

Several studies have shown that postischaemic myocardial dysfunction, or stunning, is associated with a reduction in myofilament sensitivity to Ca^{2+} (see for review Ref. [21]). This may potentially explain the findings of the present study since a prominent feature of endotoxin shock is hypotension which may cause coronary hypoperfusion. However, this is an unlikely explanation for the following reasons. Firstly, following endotoxin injection, myocardial blood flow usually increases as a result of a decrease in coronary vascular resistance [22,23]. Secondly, the decrease in Ca^{2+} sensitivity described in experimental stunning is a phenomenon specifically associated with reperfusion [24] following severe (very low-flow or no-flow) myocardial ischaemia [25,26]. However, in the present study, the mean arterial blood pressure in catheterised rabbits remained at its control level during the first hour following endotoxin injection and then progressively decreased to values ranging between 60 and 85 mmHg. Such a blood pressure profile is inconsistent with the occurrence of severe ischaemia–reperfusion. In addition, we have failed to detect any of the arrhythmias characteristically associated with myocardial reperfusion phenomena in our catheterised rabbits.

The cellular mechanisms by which Ca^{2+} sensitivity of the contractile proteins is decreased during endotoxin shock are not known. The specific mechanism may involve reduced affinity of troponin C (the Ca^{2+}-binding subunit of the thin-filament regulatory protein troponin) for Ca^{2+}. This could be the result of a direct effect on troponin C or an indirect result of an effect on other regulatory proteins within the thin filament, such as possible alterations in phosphorylation of either myosin light chain-2, troponin I, or troponin T. The reduced Ca^{2+} sensitivity found in the present study is independent of a direct effect of ions or metabolites, as the changes persist in skinned cardiac muscle preparations where buffered (pH 7.0) and energy-rich solutions are used. Recent reports suggest that NO, released during endotoxic shock by endothelial cells or within myocytes, modifies myocardial contraction by raising cGMP [27]. This latter reduces the myofilament response to Ca^{2+} in intact cardiac myocytes [28], probably via the phosphorylation of troponin I by cGMP-dependent protein kinase [29]. Accordingly, high concentration of

TNFα as well as endotoxin in vitro exposure induce a NO-dependent decrease in the myofilament response to Ca^{2+} of isolated myocytes [10,11]. However, the role of NO in the in vivo endotoxin-induced myocardial dysfunction is not clearly determined [30]. Alternatively, isoformic changes of contractile proteins may be involved, as contractile proteins mRNAs have been shown to be modified as early as several hours after a pressure overload [31]. Several studies have suggested that TNFα, interleukin-1β and supernatants of activated macrophages also cause a delayed and prolonged depressant effect on in vitro myocardial tissue beginning hours after exposure and persisting for days [32–34]. These effects may involve de novo protein synthesis and may represent a somewhat different biochemical pathway than the early depression described in other studies. Finally, the finding that the maximal Ca^{2+}-activated tension of myocardial bundles were unchanged during endotoxic shock suggests that this condition has no significant effects on myosin or actin or on the chemical equilibrium between tension-generating and non-tension-generating crossbridges states.

The dose of endotoxin used in our model was relatively high and was probably the major contributory factor for the high mortality rate observed between the 4th and the 24th hour following endotoxin injection. The choice of such a high dose of endotoxin was deliberate since our aim was to produce a model of septic shock rather than sepsis. It is thus possible that our results may not be relevant to mild forms of sepsis, but we believe them to be relevant to septic shock, which is associated with a severe cardiovascular dysfunction and a mortality rate approaching 50% despite treatment.

In summary, the results of these experiments show that myofilament Ca^{2+} sensitivity is decreased in skinned fibres taken from rabbit myocardium excised 4 and 24 h after i.v. injection of endotoxin. This effect is dose- and time-dependent, and spontaneously reversible with time. These results strongly support the hypothesis that sensitivity of cardiac myofilaments to Ca^{2+} is reduced in endotoxic shock. The exact contribution of this response to sepsis-associated myocardial dysfunction remains to be determined.

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