THE EFFECT OF GRAM NEGATIVE ENDOTOXIN ON THE CALCIUM UPTAKE ACTIVITY OF SARCOPLASMIC RETICULUM ISOLATED FROM CANINE MYOCARDIUM

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SUMMARY

Gram negative endotoxin (Salmonella abortus equi) significantly inhibited the ATP dependent calcium uptake of canine myocardial sarcoplasmic reticulum (SR). The degree of inhibition was dependent upon the concentration of the endotoxin and its time of exposure to the SR. Significant inhibition was seen at endotoxin concentrations ranging between 0.024 mg/ml and 0.24 mg/ml. Inhibition, at a dose of 0.24 mg/ml, varied between 10.1 ± 0.1 percent with no preincubation to 61.0 ± 5.0 percent after fifteen minutes of preincubation. The relation between depression of the sarcotubular calcium pump and myocardial depression is discussed.

A growing body of data obtained from both *in vivo* (1,2,3) and *in vitro* (4,5,6) experiments demonstrate that gram negative endotoxin possesses a direct myocardial depressant action. The mechanism of this depression has, however, not been determined. It has been established that maintenance of venous return does not prevent ultimate ventricular failure (7) and that myocardial lactate extraction and redox potential differences provide no evidence of myocardial anoxia (8). Further, the electrocardiagram of the dog is unaltered until the preterminal phase of endotoxin shock (5). If excitation is not at fault, then some step further along in the excitation-contraction coupling system is suspect. Although this system is extremely complex, is appears that excitation, in the mammalian heart, initiates contraction by releasing calcium from the sarcoplasmic reticulum to the protein complexes re-

sponsible for contraction. A key step in the operation of this system is the uptake of calcium by an active pump of the sarcoplasmic reticulum. The activity of this pump is thought to be a determining factor in the amount of calcium released by an action potential. Failure of this pump would leave a relative deficit of calcium in the reticulum and less calcium would be released for the next contraction. This would lead directly to decreased contractility. Evidence for this view of the role of the sarcoplasmic reticulum calcium pump is based on the following observations. Gertz et al. (9) have demonstrated decreased rates of calcium uptake and depressed ATPase activity of the sarcoplasmic reticulum in the acutely failed heart-lung preparation. Various drugs and anesthetic agents known to produce myocardial depression have also been shown to depress the sarcotubular calcium pump (10,11,12). It is on this basis that we have studied the effects of a gram negative endotoxin (Salmonella abortus equi) on the ATP dependent calcium uptake of the sarcoplasmic reticulum isolated from canine myocardium.

METHODS

Healthy mongrel dogs (12-20 kg) were anesthetized with sodium pentobarbital (25 mg/kg) and the SR was isolated by a slight modification of the method we have described previously (12). The major difference was the forces used for centrifugation. The SR was isolated between 17,300 g-max for 20 min. and 34,800 g-max for 25 min. The protein concentration was determined by the method of Lowry et al.(13). The rate of calcium uptake by the SR was determined in the presence of oxalate using the Millipore filtration technique. All incubations were carried out at 37 °C. The standard incubation media contained 100 mM KCl, 18 mM imidazole (pH 7.0), 10 mM K-oxalate, 5 mM $MgCl_2$,

5 mM ATP, and 0.18 mM $CaCl_2$ with 0.05 μc/ml $^{45}CaCl_2$. The final volume was 5 mls. In experiments involving preincubation, the SR preparation was added to 4 mls of a solution containing 130 mM KCl, 12.5 mM K-oxalate, and 22.4 mM imidazole, pH 7.0 (Solution 1). The reaction was started after preincubation by the addition of 1 ml of a solution containing 25 mM $MgCl_2$, 25 mM ATP, 0.09 mM $CaCl_2$ with 0.25 μc/ml $^{45}CaCl_2$ (Solution 2). Endotoxin (Salmonella abortus equi, control number 541172, Difco Labs) was dissolved in Solution 1 to give a final concentration of 0.24 mg/ml. Aliquots taken at 0.5, 1.0, and 2.0 minutes after the start of the reaction with Solution 2, and filtered through Millipore filters of 0.45 μ pore diameter were counted in a Unilux III, Nuclear-Chicago liquid scintillation spectrometer.

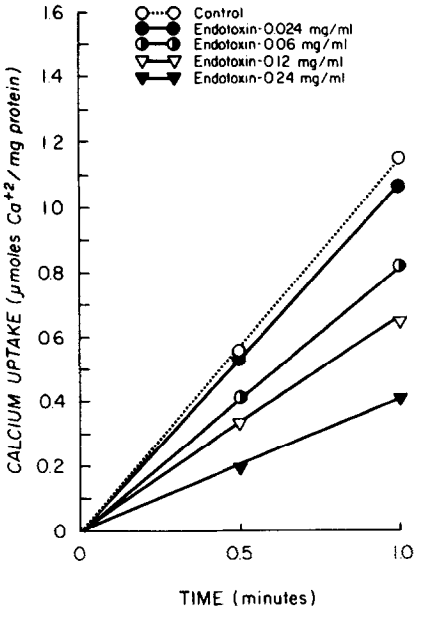

Figure 1. The effect of time and endotoxin on calcium uptake by cardiac sarcoplasmic reticulum. All experiments were done after fifteen minutes of preincubation. See Methods for experimental conditions.

RESULTS

The yield of SR averaged 0.22 mg/g of myocardium. The control calcium uptake rate at 37°, zero time preincubation, averaged 1.34 $\pm$ 0.19 μmoles/mg-min. Uptake rate was consistently linear for 1.0 minute. A typical experiment is shown in Figure 1. Uptake rates after one minute of preincubation averaged 1.31 $\pm$ 0.19 μmoles/mg-min., after 5 minutes of preincubation 1.26 μmoles/mg-min., after 10 minutes of preincubation 1.05 $\pm$ 0.20 μmoles/mg-min., after 15 minutes of preincubation, 0.972 $\pm$ 0.17 μmoles/mg-min.

Gram negative endotoxin produced a significant inhibition of ATP dependent Ca^{+2} uptake by the sarcoplasmic reticulum (Fig. 2). The dose of 0.24 mg/ml was chosen by assuming that the LD_{50}, 10 mg/kg total body weight, is distributed throughout the extra cellular fluid compartment. It was later found that this dose is identical to that used by DePalma et al. (14) in their study of the effects of endotoxin on liver mitochondria. Figure 2 also demonstrates the progressive increase in endotoxin activity with incubation times. It is of interest to note that this curve seems to have two phases, indicating that endotoxin may influence SR function at two different sites.

Figure 3 summarizes the effect of endotoxin concentration on the ATP dependent calcium uptake rate. Even at the lowest dose, 0.024 mg/ml, there was significant ($p < 0.05$) inhibition of calcium uptake. A search of the literature revealed no previous reports on the interaction of endotoxin and SR; however, these doses are comparable to those of Moss (15), DePalma (14), and Mela (16) who studied the effect of gram negative endotoxin on liver mitochondria.

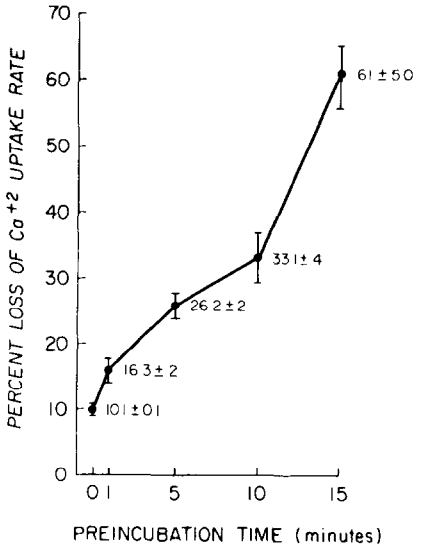

Figure 2. The effect of endotoxin-SR preincubation time on calcium uptake rate. The final concentration of endotoxin was 0.24 mg/ml. Control and experimental SR preparations were subjected to identical periods of preincubation. Each point represents the mean of six experiments, and the solid bars indicate plus and minus one standard error of the mean.

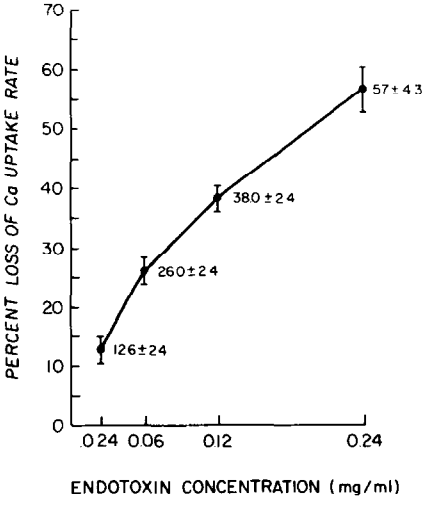

Figure 3. The effect of endotoxin concentration on cardiac SR calcium uptake. All experiments were done after 15 minutes of preincubation. Each point represents the mean of six experiments with the solid bars representing plus and minus one standard error of the mean.

DISCUSSION

The present study indicates that gram negative endotoxin can inhibit ATP dependent calcium uptake by cardiac SR preparations. If this same effect takes place within the heart, there would be a consequent depression in the calcium content of the SR and less calcium would be available to activate contraction. This would depress the contractility of the heart. Although there is agreement that endotoxin shows its greatest effects after a lag period (5,6,16,17), it is less clear whether there is also an immediate effect of the toxin. Our studies, however, did show a slight but immediate effect. Like other studies, however, the effectiveness of the endotoxin, even when exposed directly to an isolated membrane system, increased with time. This suggests that the lag period is not due to the time required for the endotoxin to get to its site of action, at least if one defines as the site of action the SR membrane. It is possible that the large size of the molecule (0.6-2.0) x 10^6 daltons prevents its ready penetration into the membrane, and that the lag period is related to this problem.

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