

TITLE: ALBUMIN: AN ENDOGENOUS CALCIUM ANTAGONIST?

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Introduction. Intracellular calcium $[Ca]_i$ is important for regulating excitation-contraction coupling in myocardial cells. On the other hand, $[Ca]_i$ has been implicated as contributing to cellular damage during ischemia. Previous work suggests that cardioplegia containing blood (with albumin) causes less reperfusion injury than non-protein solutions, despite similar ionized calcium concentrations. Since albumin is a major carrier-protein for calcium, we postulated that albumin might modulate calcium entry into cells. This study was designed to determine whether different concentrations of extracellular albumin could alter calcium entry in isolated myocardial cells.

Methods. This study was approved by our university's Animal Care and Use Committee. Six male Sprague-Dawley rats were anesthetized with intraperitoneal sodium pentobarbital (75 mg/kg), the thoracic cage opened, and the heart excised and placed in a low-calcium buffer ($CaCl_2$ -0.25 mM). Heart ventricular tissue was minced and transferred to 0.1% collagenase solution and incubated at 37°C for 10 minutes. The supernatant was pipetted off and the tissue fragments stirred in buffer for an additional 30 minutes. During this time, the incubation medium becomes turbid and the supernatant contains free myocardial cells. Cell suspensions were centrifuged at low speed and the cell pellet resuspended in buffered solution. Cell viability was determined by microscopic morphology and by trypan blue exclusion.

In order to determine intracellular calcium levels, myocytes (5×10^5 /ml) were incubated with the fluorescent dye FURA-2/AM (3 μ M) in polyethylene tubes maintained at 37°C for 60 minutes. The cells were then washed by two-step centrifugation and resuspended in buffered solution containing saline (0.03 mM Ca) or 4 mM ionized calcium with one of three concentrations of albumin (Alb): zero albumin (control), 2 g albumin/dl, or 4 g albumin/dl. The free ionized calcium concentration (4 mM) was directly measured and was the same in all solutions. $[Ca]_i$ was determined by measuring 510 nm emission at 340 and 380 nm excitation wavelengths in an SLM 8000 C spectrofluorometer (1). Fluorescence ratios (340/380) were converted to free calcium $[Ca]_i$ using the equation: $[Ca]_i = KD [(R-R_{min})/(R_{max}-R)] B$ where KD is the dissociation constant of fura-2 for calcium, B is the ratio of fura-2 fluorescence at 380 nm in calcium-free and calcium replete solutions. R is the measured 340/380 ratio. R_{min} and R_{max} are the fluorescent ratios for solutions of (fura-2 + calcium) and fura-2, respectively. Data from 6 animals are presented as means \pm SEM. Differences between data points was determined by repeated measures ANOVA and Scheffe's multiple range test.

Results. Myocardial cells suspended in saline had a 38% increase in $[Ca]_i$ over two hours (see Table). Cells suspended in solution containing 4 mM

free calcium had an 80% increase in $[Ca]_i$ over time. Myocytes suspended in 4 mM ionized Ca solutions containing albumin had significantly smaller changes in $[Ca]_i$. In addition, myocytes exposed to the albumin solutions demonstrated an instantaneous reduction in $[Ca]_i$ (see Figure).

Discussion. Our data demonstrate a significant inhibition of calcium entry into isolated rat myocardial cells by extracellular albumin. This effect is evident while extracellular ionized calcium levels are held constant (i.e., calcium bound to albumin is taken into account). This is the first documentation of a modulating effect of extracellular albumin on calcium entry into cells. Our data also demonstrate an acute reduction in $[Ca]_i$ by albumin. This effect is too rapid to be explained by calcium efflux out of the cell and most likely results from increased sequestration of free intracellular calcium. An albumin receptor has previously been described in rat hepatic cells (2). We speculate that a "myocardial albumin receptor" may occur in rats which antagonizes the entry of extracellular calcium into the myocardial cell. Membrane interactions may also initiate processes which acutely lower $[Ca]_i$. The existence of such an interaction in man has obvious implications in the control of $[Ca]_i$ during acute operative, ischemic, and septic states and may provide further rationale for utilization of blood cardioplegia.

Reference.

1. Grynkiewicz G: J Biol Chem 1985;260:3440-3450.
2. Ockner RK et al: Am J Physiol 1983;245:G13-G18.

Table	0 min	60 min	120 min
Saline; .03 mM Ca	70.5 \pm 11.7	85.5 \pm 13.2*	97.2 \pm 14.8*
0% Alb; 4 mM Ca	98.1 \pm 17.6	144.7 \pm 27.5*	177.5 \pm 33.3*
2% Alb; 4 mM Ca	49.5 \pm 8.2§	59.1 \pm 10.1*§	65.3 \pm 10.1*§
4% Alb; 4 mM Ca	22.2 \pm 3.7§	26.9 \pm 4.9*§	29.3 \pm 5.3*§

*P<0.05 compared to time 0; *P<0.01 compared to time 0.
§P<0.01 2% and 4% ALB compared to 0% ALB

