MORPHOLOGY AND CALCIUM UPTAKE OF BOVINE SARCOPLASMIC RETICULUM AS AFFECTED BY ELECTRICAL STIMULATION AND TIME POSTMORTEM

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INTRODUCTION

IT HAS been proposed that one of the mechanisms by which electrical stimulation causes an increase in tenderness is by prevention of cold shortening (Bendall, 1976; Chrystall and Hagyard, 1975; Davey et al., 1976), even though most authors find no difference in sarcomere length between electrically stimulated and non-stimulated carcasses (Smith et al., 1977, 1979a; Savell et al., 1977, 1979; McKeith et al., 1979; Seideman et al., 1979; Will et al., 1980). However, at least in one instance, differences in sarcomere length between electrically stimulated and non-stimulated carcasses have been noted (Smith et al., 1979b). Thus, the possibility of cold shortening being a factor in tenderness differences between electrically stimulated and non-stimulated animals still may exist, particularly under certain conditions. Electrical stimulation, by inducing massive muscle contractions, causes the muscle to deplete glycogen and ATP. A reduced pH and a rapid rate of rigor mortis formation then ensues (Smith et al., 1979b; Bendall, 1976; Davey et al., 1976). The occurrence of rigor mortis at an earlier time postmortem, while the muscle temperature is still high, is most probably associated with the tenderization of electrically stimulated muscle, whether cold shortening has occurred or not (Dutson et al., 1977).

The increased glycolytic activity of electrically stimulated muscle is possibly caused by an increased concentration of Ca++ in the muscle sarcoplasm. The initial increase in Ca++ is probably a result of the stimulus received by the muscle. However, it is also possible that the electrical impulses might cause permanent alterations in the membranes of the sarcoplasmic reticulum which would maintain high levels of Ca++ in the sarcoplasm and accelerate the postmortem biochemical events even more.

Electrical stimulation has been shown to also accelerate the release of lysosomal enzymes from the lysosomal membrane (Dutson et al., 1980). Lysosomal enzymes might cause alterations in the sarcoplasmic reticulum (West et al., 1974) which would result in higher calcium levels in the muscle sarcoplasm during the critical period of accelerated postmortem glycolysis. Electrical stimulation has been shown to cause alterations in the ATPase of the sarcoplasmic reticulum (Tume, 1979), which may be related to postmortem calcium accumulating ability of these membranes.

The present study was conducted to determine if there might be an alteration in the Ca++ accumulating ability of the sarcoplasmic reticulum caused by electrical stimulation and if these alterations are brought about by the stimulating current itself or exposure to altered postmortem biochemical conditions.

MATERIALS AND METHODS

SIX MATURE (USDA E maturity carcasses) cattle were obtained from the Texas Agricultural Experiment Station, exsanguinated and split. The left sides of all carcasses were electrically stimulated (ES) with 18 impulses of approximately 500 volts at 45 min postmortem. The right side of each carcass served as the non-stimulated (NS) control. Samples (200 g) were excised from the longissimus dorsi muscle of both sides of the carcass (ES and NS) at 0 and 6 hr after stimulation of the ES sides for subsequent SR isolation using the procedure of Meissner and Fleischer (1971). Samples were identified as ES-0 and NS-0 for those excised immediately after stimulation of the left side (45 min postmortem) and ES-6 and NS-6 for samples excised 6 hr after stimulation of the left side.

The muscle samples were trimmed of excess fat and epimysial tissue and were diced. All subsequent procedures were performed in the cold (4°C). The diced muscle (150 g) was combined with 1.6 volumes (240 ml) of cold homogenization buffer (0.3 M sucrose and 0.1 M HEPES [N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid]) (pH 7.4) and was homogenized using a Waring blendor.

The homogenates from each treatment were placed in 50 ml centrifuge tubes and centrifuged for 20 min at 8000xg to remove myofibrillar and collagenous tissue. The supernatant yield from all treatments was measured. The crude fraction of SR (sarcoplasmic reticulum) vesicles was obtained by centrifugation of the supernatant for 75 min at 88,000xg. The pellets were resuspended in 22 ml of homogenization buffer (pH 7.4). The suspensions (ES and NS) were homogenized briefly (in the cold) using a Polytron homogenizer. The crude SR fractions (7.3 ml) were layered on centrifuge tubes (38.5 ml capacity) containing a discontinuous sucrose gradient and were centrifuged at 131,00xg for two hours. A 12 ml fraction was removed from the gradient and discarded. The next 11.3 ml of each tube containing the isolated SR was drawn from the gradient and was slowly (over 30 min to prevent osmotic shock) diluted with 2 volumes (68 ml) of 5 mM HEPES buffer (pH 7.4). The diluted suspensions were then centrifuged at 131,000xg for one hour. The resultant SR pellets were resuspended and pooled by treatment in 5 ml of 2.5 mM HEPES containing 0.3 M sucrose (pH 7.4). The SR samples from each treatment were divided into two, 2 ml vials for subsequent Ca++ accumulation determination and three, 0.4 ml centrifuge vials for electron microscope evaluation. All samples were frozen and stored in liquid nitrogen (Meissner and Fleisher, 1971).

The protein concentration of the isolated SR vesicles was determined using the method of Lowry et al. (1951). The calcium accumulating ability of the isolated SR vesicles was determined using the procedure of Martinosi et al. (1968) using ^{45}Ca to quantitate the amount of calcium accumulated.

RESULTS AND DISCUSSION

A TREND was noted for ES and NS muscles at 6 hr post-stimulation to yield lower average quantities of SR protein than the same treatments at 0 hr. Similarily, a trend was noted for lower yields of SR protein from ES-6 muscle when compared to NS-6 muscle (Table 1) which may indicate SR disruption by electrical stimulation and postmortem time.

The overall average yields obtained in the present study from longissimus dorsi (L.D.) muscles (50.6 µg/g of muscle) are within the range of SR yields obtained from beef sternomandibularis muscle by Pearson (1977), 42.2 µg/g, Cornforth (1978), 62.2 µg/g and Kanda (1975), 42.3 µg/g. The E-maturity cow (USDA) L.D. muscle used in the present study probably contained substantial amount of cross-linked connective tissue which may be associated with lowered SR protein yields (Kanda, 1975).

The calcium accumulation values for SR vesicles isolated from ES and NS muscle at 0 and 6 hours post-stimulation are presented in Table 2. Sarcoplasmic reticulum vesicles isolated at 6 hr postmortem from electrically stimulated muscle exhibited a significant ($P<0.05$) reduction (45-55%) in Ca++ uptake ability when compared to the Ca++ uptake by the SR vesicles from ES-0, NS-0, and NS-6 samples (Table 2 and Figure 1). Sarcoplasmic reticulum vesicles isolated from electrically stimulated muscle immediately following electrical stimulation tended to have a greater ability to accumulate calcium than either the NS-0 or NS-6 samples which is evident in both Table 2 and Figure 1 and is in agreement with the data of Tume (1979); however, these differences were not significant ($P>.05$).

From the data of the present study it appears that the only effect electrical stimulation itself has on the SR membrane is to slightly activate its calcium accumulating ability. However when the SR membranes have been exposed to the altered biochemical conditions of electrically stimulated muscle for a period of six hours, a marked decrease in the Ca++ accumulating ability of these membranes occurs. It appears that the biochemical conditions present in non-stimulated muscle may also cause slight alterations in the SR membrane at 6 hours, but not significantly so (Table 2, Figure 1).

Greaser et al. (1969) observed a reduction in Ca++ accumulating ability by SR vesicles isolated from pale, soft and exudative (PSE) muscle within 1 hr postmortem. This effect was attributed to the rapid decline in pH characteristic of PSE muscle. Savell et al. (1979) reported that the pH of muscle from electrically stimulated sides of beef carcasses declined to pH 5.8 at 6 hr postmortem while the control (NS) paired sides were approximately pH 6.1 at the same time postmortem. Berman et al. (1977) reported that exposure of SR vesicles to pH values of 5.5-6.0 causes a rapid and irreversible inactivation of Ca++ accumulation. The low pH at 6 hr postmortem may also activate lysosomal enzymes, released from the lysosomes, as a result of electrical stimulation (Moeller et al. 1976; 1977; Dutson et al., 1980). Fields (1976) and West et al. (1974) found that catheptic proteolysis also causes a reduction in Ca++ uptake by impairing the SR ATPase. It is possible that electrical stimulation may also cause a disordering of the ATPase in the membrane of intact muscle, which may render the ATPase labile to proteolytic cleavage (Tume, 1979). Proteolysis of the SR ATPase may also cause an uncoupling of the Ca++ pump through its partial unfolding (Berman et al., 1977) or may alter the integrity or ion permeability of the membrane. Fields (1976) suggested that a portion of the SR ATPase may be exposed to catheptic digestion while the remainder of the protein is embedded within the membrane and is more resistant to proteolysis.

Electron microscopy of isolated SR vesicles revealed no differences in the observable morphology due to the electrical stimulation treatment or to holding muscle for six hours postmortem. However, slight alterations were shown to occur in the intact SR of fixed and embedded electrically stimulated muscle. Electron microscopy also revealed that SR preparations were relatively free of mitochondria, indicating that any alterations observed were due to changes in the SR and not mitochondria.

CONCLUSION

THE ACTUAL process of electrical stimulation causes very little alteration in the calcium accumulating ability of the sarcoplasmic reticulum of bovine muscle. However, the exposure of sarcoplasmic reticular membranes to the altered biochemical conditions of electrically stimulated muscle, for periods of up to six hours, causes a marked reduction in the ability of the SR to accumulate and hold Ca++. The alterations in the SR caused by electrical stimulation could be due to the effect of pH or to a combined effect of pH and proteolysis.

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Table 1. Yield of Sarcoplasmic Protein from Stimulated and Non-stimulated Muscle at 0 and 6 Hours Post-stimulation[a]

	NS-0	NS-6	ES-0	ES-6
SR protein (μg per g muscle)	54.6	49.2	56.3	42.8

[a]Each value represents a mean of six samples.

Table 2. Mean Difference Values and Paired Comparisons for Calcium Uptake by Isolated Sarcoplasmic Reticulum Vesicles

Ca++ Uptake Reaction	Paired Comparison[a]					
	(AB)	(AC)	(AD)	(BC)	(BD)	(CD)
1	13.7[b]	-8.4	35.5*	-9.4	21.9*	43.9**
2	8.9	-9.8	39.1*	-16.6	30.2**	46.8*
3	14.3	-3.2	45.5**	-17.6	31.2*	48.7**
5	12.6	-6.9	43.0**	-12.0	30.4**	40.8*
7	0.1	-8.5	41.0**	-17.9	31.5*	49.4**
10	1.8	-7.1	33.6*	-9.0	31.8*	40.6**

* Significant t value at $P<0.05$.

** Significant t value at $P<0.005$.

[a]A=Non-stimulated, 0 hour; B=Non-stimulated, 6 hour; C=Electrically stimulated, 0 hour; D=Electrically stimulated, 6 hour.

[b]Values are mean differences between ES and NS treatments. Those values with no asterisk were not significant ($P>.05$).

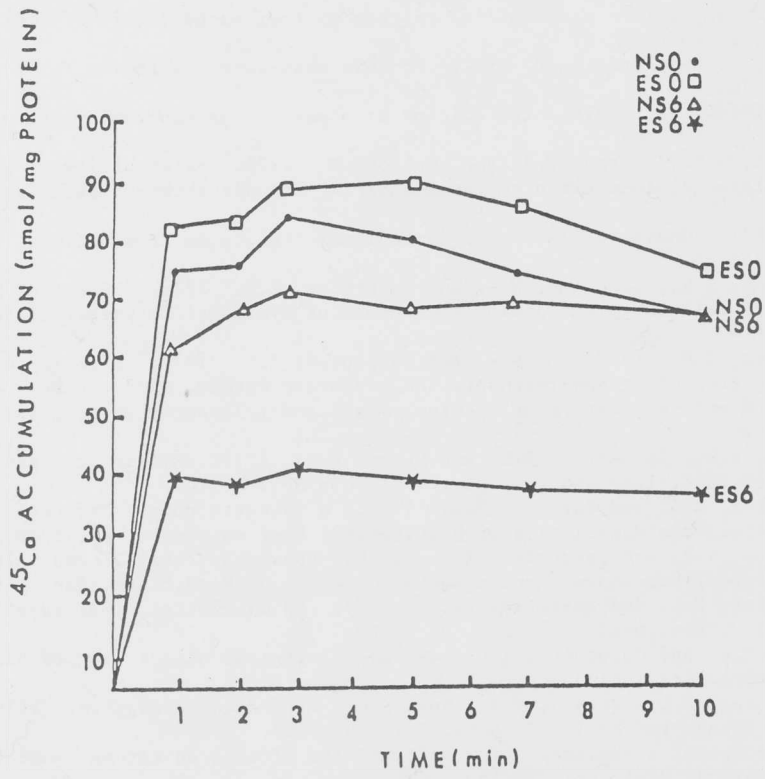

Fig. 1-Calcium accumulation by sarcoplasmic reticulum vesicles isolated from electrically stimulated (ES) and control (NS) muscle at 0 and 6 hr (0.01 mM Ca^{++}).