EXPRESSION OF CALCIUM CHANNELS AND APOPTOSIS-RELATED FACTORS IN PSE MEAT

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Abstract – It is well established that the calcium channels, including sarcoplasmic reticulum Ca^{2+}-ATPase 1 (SERCA1) as a Ca^{2+} recycling channel and inositol 1,4,5-trisphosphate receptor (IP3R) as a Ca^{2+} release channel, located on the sarcoplasmic reticulum (SR) play key roles in the calcium homeostasis in postmortem muscle. High sarcoplasmic calcium is associated with increased myofibrillar shrinkage and accelerated lactate production. It is also considered to be an important apoptosis inducer in skeletal muscle. In this study, eight PSE, (Pale, Soft and Exudative) and RFN (Reddish-pink, Firm and Non-exudative) samples (longissimus muscle) were used to determine the expression of calcium channels. SERCA1 was markedly down-regulated while IP3R was significantly up-regulated in PSE meat. The changed expression of apoptosis–related factors such as BAX, Bcl-2 and cytochrome C was also observed. BAX and cytochrome C were dramatically up-regulated while Bcl-2 was greatly reduced in PSE meat. This data suggests that the expression of calcium channels is changed in the favor of releasing calcium, and that apoptopic events are initiated in PSE meat.

Key Words – calcium imbalance, apoptosis appearance, PSE

I. INTRODUCTION

Calcium channels such as SERCA1, ryanodine receptor (RYR) and IP3R are generally used to infer the calcium status in sarcoplasm of skeletal muscle. High calcium has been observed when the protein expression of calcium release channels is up-regulated and the enzyme activity of recycling channels is down-regulated [1-3]. In PSE meat, high calcium status was well documented which could lead to the accelerated drop of pH in very short time by accelerating lactate production after slaughter [4]. Calcium has also been implicated as the mediator of apoptosis by affecting the integrity of the mitochondria and the expression of apoptosis-associated factors. When the calcium concentration is increased, mitochondria could be damaged by taking up the excess calcium for scavenging purposes if it exceeds its physiological threshold [5]. In this case, the expression of pro-apoptotic proteins, such as Bcl-2-associated X protein (BAX), which exerts its functions by inserting into mitochondrial membranes and forming pores to allow passage of diffusible pro-apoptotic proteins such as cytochrome C, would be raised [6]; while the expression of anti-apoptotic factor B-cell lymphoma 2 (Bcl-2), which is integrated into mitochondrial membranes to prevent the calcium release working as a pump additional to SERCA1 [7-9], has been found to be reduced [10].

High calcium has been demonstrated in PSE meat and it is strongly related to the apoptosis in muscle which has been discussed in various muscular diseases. However, only limited research has been conducted on the study of the expression of the calcium channels and the apoptosis appearance in PSE meat. The objective of this study was to determine the expression level of SERCA1 and IP3R and how the apoptosis-associated factors changed in PSE meat.

II. MATERIALS AND METHODS

2.1 Animals and meat samples

A total of 120 castrated Erhualian × Landrace × Yorkshire crossbred pigs weighing between 100 and 120 kg live weight were slaughtered in a commercial abattoir following electrical stunning (110 V, 4 s). The carcasses were quartered, and 12 suspected PSE loin (pH<5.8, L^*>50, 1 h postmortem) and 12 suspected RFN loin samples loin samples (pH > 6, L^*< 50, 1 h postmortem) were cooled to 0–4 °C (air velocity 1.0 m/s) for 24 h postmortem.

2.2 Physical measurements

The pH and temperature of the *M. longissimus dorsi* (LD) muscle was measured at 1 h and 24 h postmortem at the last rib using a portable pH meter (HI9025, HANNA, Co. Italy). The color of the meat surface was measured in triplicate on a freshly cut surface using a portable colorimeter (Minolta Cameras CR 400, Japan) (CIE LAB coordinates, light source D 65, 8-mm diameter) after allowing 10 min for blooming.

2.3 SDS-PAGE and Western blotting

Muscle samples were lysed in 50 mM Tris–HCl (pH 7.6) buffer containing 0.15 M NaCl, 1 mM EDTA, protease inhibitor cocktail, and 1% Triton X-100. Proteins in lysates were separated by SDS–PAGE using 5–12.5% gel and transferred to PVDF membranes. Membranes were blocked in 3% bovine serum albumin (BSA) in Tris-buffered saline (TBS)–Tween 20 (0.05%) and incubated overnight at 4 ºC with specific primary antibodies at 1:200–2000 dilutions followed by HRP-conjugated secondary antibodies at 1:5000 dilutions. Detection was performed using an enhanced chemiluminescence system.

2.4 Antibodies

Primary antibodies included polyclonal rabbit anti-BAX (SC-6236; Santa Cruz, USA; diluted 1:1,500), monoclonal mouse anti-Bcl-2 (SC-509; Santa Cruz, USA; diluted 1:1,000), monoclonal mouse anti-cytochrome C (SC-13560; Santa Cruz, USA; diluted 1:800), monoclonal mouse anti-SERCA1 (ab32445; Abcam, UK; diluted 1:4,000), and monoclonal goat anti-cytochrome C (SC-13560; Santa Cruz, USA; diluted 1:500). Secondary antibodies included goat anti-rabbit horseradish peroxidase (HRP) (AP132P; Millipore, USA; diluted 1:5000 for BAX), sheep anti-mouse (HRP) (ab97100; Abcam, UK; diluted 1:4000 for Bcl-2, cytochrome C and SERCA1), and rabbit anti-goat horseradish peroxidase (HRP) (ab97023; Abcam, UK; diluted 1:4000 for IP3R). BAX, Bcl-2, cytochrome C, SERCA1 and IP3R were indicated at the 25, 29, 12, 110 and 270kDa bands, respectively.

2.5 Statistical analysis

Data are presented as means ± SE of at least three independent experiments. Statistical analysis was performed by Duncan's test.

III. RESULTS AND DISCUSSION

3.1 Postmortem changes in muscle pH and color

The pH, temperature and color of RFN and PSE meat are shown in Table 1. Significant differences were observed between RFN and PSE meat in pH at 1 h. Compared with RFN meat, PSE meat had lower pH at 1 h ($p < 0.05$). Unexpectedly, the pH of PSE meat at 1 h almost attained its ultimate value (pH 24 h). The L* value of PSE meat was higher than 50 even at 1 h postmortem, which far exceeded ($p < 0.05$) values for RFN at 1 h and 24 h postmortem.

Table1 Comparison of pH values and color (mean ± SE) between RFN and PSE meat samples.

	RFN	PSE
$pH_{1\,h}$	5.92±0.10	5.29±0.02*
$pH_{24\,h}$	5.38±0.01	5.14±0.02
Color at 1 h postmortem		
*L**	36.51±0.19	51.26±1.78*
*a**	5.90±0.53	8.43±0.58*
Color at 24 h postmortem		
*L**	47.74±0.93	56.98±2.12*
*a**	6.49±0.82	7.37±0.64

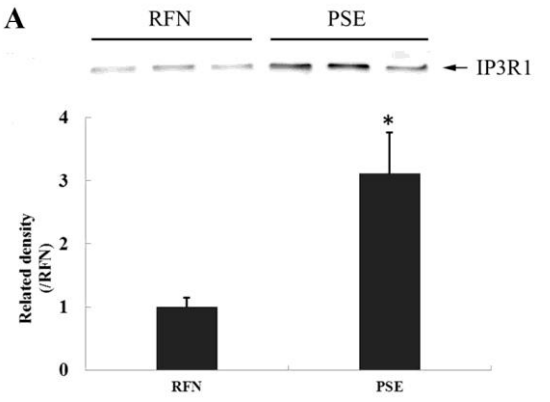
Note: RFN, Reddish-pink, Firm, Non-exudative; PSE, Pale, Soft, Exudative. * $p<0.05$

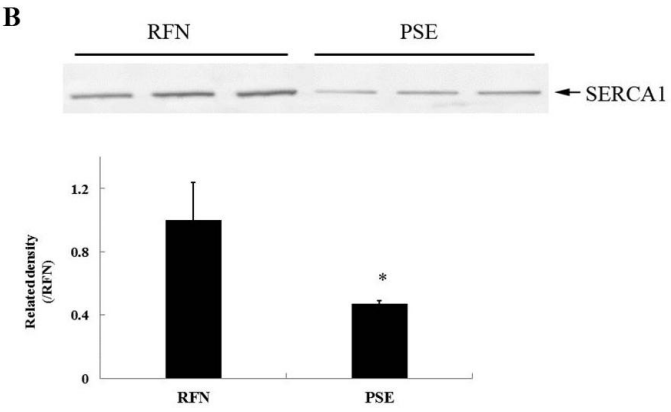
3.2 Expression of calcium channels and apoptotic factors

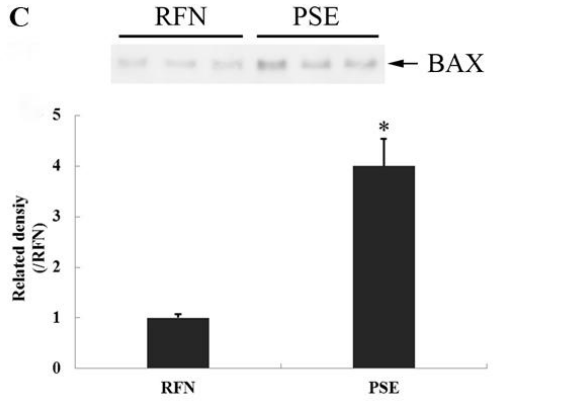
To identify the possible molecular mechanisms of calcium status in sarcoplasm and the tendency for apoptosis in PSE meat, we tested the expression of calcium channels including SERCA1 and IP3R located in SR. As shown in Fig. 1 A and B, there was a significant increase in protein expression of IP3R ($p< 0.05$) and decrease in protein expression of SERCA1 ($p< 0.05$) in PSE meat. These data show that the expression of calcium releasing channels rose and the recycling channels was reduced, which had the potential to increase calcium

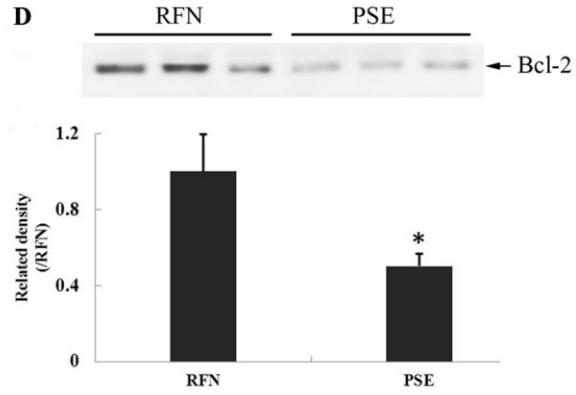
concentration in PSE meat. Next we examined the protein fluctuation of BAX, Bcl-2 and cytochrome C which play a central role in apoptotic cell death. As shown in Fig.1, the protein expression of BAX and cytochrome C was up-regulated ($p < 0.05$) while that of Bcl-2 was down-regulated ($p < 0.05$), leading to the increased ratio of pro-apoptotic BAX/Bcl-2.

Calcium overload has been suggested to be the final common pathway of most types of cell death, including apoptosis [10]. The imbalance of calcium homeostasis in SR has been suggested to result in structural damage of the SR and an increase in cytoplasmic calcium, leading to further activation of proteases and lipases which finally destroy the structure of the cell membrane and ensues apoptosis [11]. It is well known that the expression of many apoptosis-related factors, such as BAX, Bcl-2 and cytochrome C, are influenced by cytoplasmic calcium overload [12]. In addition, these factors are not isolated; BAX and Bcl-2 have been shown to exert an enhanced effect on calcium homeostasis. It has been reported that BAX may favor IP3R by binding it, or oligomerizes itself to assist calcium leakage in the SR membrane [6] while Bcl-2 is also found in the SR membrane where it may work as an additional pump to the SERCAs or to prevent IP3 channel opening to block the calcium leaking from SR [9]. This means apoptosis-associated factors may be involved in regulating sarcoplasmic calcium concentration. According the mechanism and the results above, we suggest that all these factors show a positive trend for calcium overload in PSE meat and contributes to PSE occurrence by enhancing calcium releasing from SR.









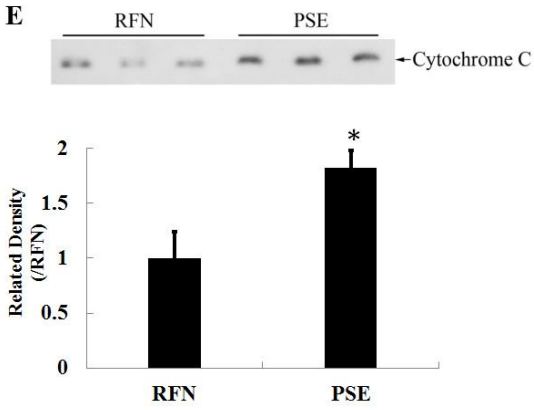


Fig 1. The detection of SR calcium channel (SERCA1 and IP3R1) (A and B) and apoptosis-associated factors (BAX, Bcl-2 and cytochrome C) (C, D and E) protein expression by Western blotting from total protein extracted from PSE and RFN samples, respectively. The density of each protein band was calculated by comparison with the corresponding GAPDH band.

IV. CONCLUSION

In this study, we have shown different expressions of calcium channels in PSE meat. The expression of the apoptosis-associated factors such as BAX, Bcl-2 and cytochrome C were significantly modified in PSE meat. These results suggest that these factors contribute to the calcium imbalance which may implicate apoptosis in PSE meat.

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