Effects of Electrical Stimulation on the Peptides, Amino acids and ATP Related Compounds of Beef Muscle

M. MIKAMI, M. SEKIKAWA and H. MIURA

Laboratory of Meat Preservation, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Hokkaido, Japan

SUMMARY: Electrical stimulation (ES) was applied to Holstein cows at low voltage (40 V and 13.8 Hz) for 60 sec. Two days after slaughter, Biceps femoris muscles were prepared and stored at 1±1°C. From the result of HPLC (high performance liquid chromatography) of the supernatant of meat homogenate, area of protein peaks (RT-29 and 32) of sarcoplasmic proteins decreased in ES muscle on the HPLC patterns, whereas the ratio of peptide and amino acid peaks increased in ES muscle, as compared with control muscle. The large and small molecular peptides in ES muscle were more than in control, but middle sized peptides in control were more than in ES muscle.

The content of IMP (inosine-5'-monophosphate) at 2 days was the highest and it decreased gradually until 21 days. However, the content of IMP in ES muscle was higher than that of control at 10-21 days and the difference was about 0.6-1.0 μmols/g between control and ES muscles. These facts mean that ES contributes to conditioning of beef.

Introduction: ES hastens the onset of rigor mortis and prevents cold-shortening (CARSE,1973) and also causes disruption of the myofibrillar structure. So it has been recognized as a means of improving tenderness (FABIANSSON and LIBELIUS,1985). Muscle proteolytic enzymes, calpain (CANP) and lysosomal proteases (cathepsins B and L), appear to be involved in the conditioning process of meat, and there may be a cooperative mechanism between these different proteases (PENNY and FERGUSON-PRYCE,1979). DUTSON et al.(1980) have also reported that lysosomal membranes are disrupted due to ES and the activity of free lysosomal enzymes increases. Therefore, the possibility for increased tenderness of ES meat could be due to the increased rate of enzymes released from the lysosomes into the rapidly acidifying environment within the muscle fiber and the advance in the action of these enzymes at low pH. Proteolysis of myofibrillar proteins appears to be a major contributor to the tenderization process during post-mortem storage (ETHERINGTON,1984; GOLL et al.,1983). It is also thought that these proteases act on sarcoplasmic proteins to produce peptides and amino acids. However, there are few data on the effect of ES on sarcoplasmic proteins. In our preliminary experiment, it was found from HPLC patterns that the sarcoplasmic protein content decreased, and the peptide and amino acid fractions increased in ES muscle. In this paper we report on the contents of peptide, amino acid and ATP-related compound during storage in ES muscle.

Materials and Methods: 1. Preparation of ES muscle and storage condition Twelve Holstein cows, 6-7 year-old and 750-800 Kg liveweight, were slaughtered. ES of low voltage (40V, 13.8 Hz) was carried out on 6 of cows for 60 sec within 5 min after slaughter. Six cows were used as control. Two days (48 hr) after slaughter, samples of B. femoris muscle were obtained and a part of the muscle was homogenized with 3 times the volume of buffer (30 mM citrate-phosphate containing 0.1 M NaCl and 0.05% NaN_3 at pH 5.5) for the sample of sarcoplasmic proteins, peptides and amino acids. The homogenate was stored at 1±1°C and analyzed at 2, 7, 14 and 21 days after slaughter. The pH of these homogenates was near 5.6 in both samples. The muscles for ATP related compounds were vacuum-packed into small bags (about 10-15g) and stored as described above. Then they are

preserved at -90°C until required.

2. Preparation of sarcoplasmic fraction After storage, 15 g of the homogenate was centrifuged at 11,000 × g for 20 min at 1°C. The supernatant was filtrated through a Toyo No. 5c filter paper and then passed through a 0.2 μm pore size filter. It was then used for the gel filtration. 3. HPLC analysis of sarcoplasmic fraction The HPLC apparatus used was Model CCPM-8000 system (Tosoh Co.) with a column of TSK-G2000SW (0.7 × 60 cm). The elution buffer was 0.25 M phosphate (pH 6.5) and pumped at a flow rate of 0.5 ml/min. Sample injection was at a constant volume of 10 μl, and proteins, peptides and amino acids were detected by absorbance at 280 nm. Apparent molecular weights were calculated from peak retention times using standard proteins, insulin and DNP-Gly. 4. Peptide and amino acid contents Samples for analysis of total peptides and amino acids were prepared from a portion of the supernatant obtained by the centrifuge at 11,000 × g as described above. The supernatants were mixed with an equal volume of 4% TCA (trichloroacetic acid) solution, incubated at 37°C for 30 min and centrifuged at 4,500 × g for 10 min. The filtrates were used for the determination of peptides by the method of LOWRY et al.(1951) with bovine serum albumin as a standard. Free amino acids and total amino acids containing peptides were determined by the ninhydrin method with Hitachi Model 835. 5. Preparation of ATP related compounds Meat (10g) was homogenized with 25 ml of 10 % PCA (perchloric acid) solution. Supernatant was obtained by the centrifugation at 4,500 × g for 10 min. Five ml of supernatant was adjusted to pH 6.5-7.0 using 5M KOH and filtrated by 0.2 μm pore size filter. Five μl of filtrate was analyzed by HPLC. 6. HPLC analysis of ATP-related compounds A column of TSK-ODS80TM was used. The elution buffer was 0.1M NaH_2PO_4(pH 4.1) and 0.05M NaH_2PO_4containing 20% acetonitrile and gradient method was applied (initial 98:2 and final 97:3). Flow rate was 1 ml/min.

Results and Discussion: 1. HPLC patterns and area ratio of sarcoplasmic fraction On the analysis of sarcoplasmic fraction by HPLC, we can observe the changes in the peaks of proteins, peptides and amino acids. Typical chromatogram of sarcoplasmic fraction on HPLC is shown in Fig.1. Myoglobin is eluted at the position of retention time (RT)-39 and DNP-Gly is RT-58, so peaks, RT-24, 32, 34, 37 and 39 are proteins, RT-47, 49 and 53 are peptides and RT-60 is amino acids (Fig 1). Table 1 shows the relative area (%) of each peak of sarcoplasmic fraction obtained from HPLC patterns. After ES treatment, some protein components decreased as shown by the reduction in peak size of RT-29, 32 and 34, and with a new peak of RT-37. The peaks, RT-29, 32 and 34, of protein in ES muscle decreased more than in control, but the peak of small molecular protein (RT-39) did not decrease. This suggests that protein fragments of similar size of RT-39 were eluted at this position. The peak of peptide (RT-49) was separated and increased in ES muscle at 2 days. The peaks, RT-49 and 53 of peptides, and RT-60 of amino acids, increased greatly during storage. On the other hand, the peak of the larger peptide fragment (RT-47) decreased, but the size of this peak remained larger in ES muscle than in control. The reason is thought to be that proteins or large peptides are degraded into small fragments and they are eluted to their position, respectively. Especially RT-53 with an estimated molecular weight of about 1-kDa was always larger in area for ES than for control muscle. The total area of peptides (RT-47, 49 and 53) and amino acids (RT-60) was also slightly larger in ES muscle than in control and this tendency was always observed each day.

Supernatant was also obtained from heated homogenate (75°C, 15min). In this case, proteins were almost all precipitated, and so peptides and amino acids were fractionated by gel filtration on HPLC. Relative area of each peak is shown in Table 2. Peptide peaks, RT-47, 49 and 53, were the main components and were about 90-91

% in control and ES muscle. At 2 days in ES muscle, RT 47 was smaller than in control and RT 49 was larger than in control, but these values were reversed at 7, 14 and 21 days, and this tendency was similar to the case of sarcoplasmic fraction (Table 1). These findings indicate that the proteolytic activity in ES muscle is higher than in control.

2. Peptide and amino acid contents Peptide and amino acid contents in the 2 % TCA soluble solution were determined at 2, 7, 14 and 21 days. Peptide contents were 128.5 mg and 116.7 mg per 100g meat at 2 days in control and ES muscle, respectively. After that these values increased became to 516.8 mg and 515.0 mg per 100 g meat at 21 days in control and ES muscles, respectively. However, these values were not significant between control and ES muscles during storage. From the HPLC patterns of sarcoplasmic fraction, it was observed that the area of each peptide fraction was different from control and ES muscles concerning molecular size. This reason may be that the Lowry method detects total peptides and there is no relation to molecular size.

Free amino acid contents were quite similar to both control and ES muscles. However, Arg, Phe, Thr and Gly in control were slightly larger than in ES muscle, but the content of glutamic acid related to favorable taste was not significant between control and ES muscles. Total amino acid containing peptides was also determined by ninhydrin method. It was 225.3 mg and 207.1 mg at 2 days, and 379.2 mg and 334.0 mg per 100 g meat at 21 days in control and ES muscles, respectively.

Autolysis in rabbit muscle was shown by OKITANI et al.(1977) and nonprotein nitrogenous compounds (NPNC) in muscle homogenate with the addition of EDTA is higher at pH 3 and 4.5 than that of control. They reported that these 2 peaks around pH 3 and 4.5 were the action of cathepsins D and B, respectively, but at pH 5.5 (similar pH in this experiment), NPNC was almost the same in both control and EDTA-treated homogenates. Cathepsins and calpain are endogenous endopeptidase and generate peptides during storage. A high content of peptides ensures adequate substrate for aminopeptidases such as aminopeptidase C and hydrolase H (NISHIMURA et al.,1988). However, in this experiment amino acid contents were also not significant between control and ES muscles.

3. ATP related compound content: Contents of ATP (adenosine-5'-triphosphate) related compound is shown in Fig. 2. ATP disappeared at 2 days after slaughter. On the contrary, IMP was the highest, 6.4 μmoles and 6.8 μmoles per g in control and ES muscles decreasing gradually during storage. IMP content at 21 days was 1.3 μmoles and 2.1 μmoles per g in control and ES muscles, respectively. Although it takes about 10-14 days for conditioning of beef and IMP content decrease during storage, the difference was 0.6-1.0 μmoles/g higher in ES muscle than in control at 10-21 days.

In a previous paper (MIKAMI et al.,1990) apparent changes of myofibrillar proteins found in ES muscle were the degradation of troponin T and the production of 26, 28, 30, 32 and 33-kDa components. These findings suggest that ES is effective in promoting early proteolysis of myofibrillar proteins. PENNY and FERGUSON-PRYCE (1979) proposed that cathepsin B had degraded proteins in the meat homogenate at pH 5.0-6.0 and containing EDTA. The optimum pH of cathepsin B is 5.2 (SCHWARTZ AND BIRD,1977) and cathepsin L is near 5.5-6.0 (Mason et al.,1984). ES treatment causes a rapid fall in muscle pH and a more rapid release of lysosomal enzymes (DUTSON, 1980). The low pH environment is assumed to provide a favorable condition for acidic proteases such as cathepsins B and L. From these facts and the results of this experiment, it is assumed that proteolysis in muscle is hastened by ES, although free amino acid contents were similar to control and ES muscles.

Conclusion: ES hastened the proteolysis in meat and produced many small molecular peptides, although total peptides and free amino acids were not significant. IMP contributing to favorable taste as well as glutamic

acid was higher in ES muscle than in control.

References: CARSE, W.A. (1973): Meat quality and the acceleration of post-mortem glycolysis by electrical stimulation. J. Food Technol. 8: 163-166.

DUTSON, T.R., SMITH, G.C. and CARPENTER, Z.L. (1980): Lysosomal enzyme distribution in electrically stimulated ovine muscle. J. Food Sci. 45: 1097-1098.

ETHERINGTON, D.J. (1984): The contribution of proteolytic enzymes to postmortem changes in muscle. J. Anim. Sci. 59: 1644-1650.

FABIANSSON, S. and LIBELIUS, R. (1985): Structural changes in beef longissimus dorsi induced by postmortem low voltage electrical stimulation. J. Food Sci. 50: 39-44.

GOLL, D.E., OTSUKA, Y., NAGAINIS, P.A., SHANNON, J.D., SATHE, S.K. and MUGURUMA, M. (1983): Role of muscle proteinases in maintenance of muscle integrity and mass. J. Food Biochem. 7: 137-177.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

MASON, R.W., TAYLOR, M.A.J. and ETHERINGTON, D.J.(1984): The purification and properties of cathepsin L from rabbit liver. Biochem. J. 217: 209-217.

MIKAMI, M., KUDOH, T., HAYASHI, A., HIRUTA, E. and MIURA, H. (1990): Effects of electrical stimulation on myofibrillar proteins and tenderness of beef muscle. Agric. Biol. Chem. 54: 531-532.

NISHIMURA, T., OKITANI, A. and KATO, H. (1988): Identification of neutral aminopeptidases responsible for peptidolysis in postmortem rabbit skeletal muscle. Agri. Biol. Chem. 52: 2183-2190.

OKITANI, A., MATSUKURA, U., OTSUKA, Y., WATANABE, M. and FUJIMAKI, M. (1977): Removal of Ca-sensitivity from myofibril and breakdown of actin by the action of an antipain-inhibited protease in rabbit muscle. Agric. Biol. Chem. 41: 1821-1823.

PENNY, I.F. and FERGUSON-PRYCE, R. (1979): Measurement of autolysis in beef muscle homogenates. Meat Sci. 3: 121-134.

SCHWARTZ, W.N. and BIRD, J.W.C. (1977): Degradation of myofibrillar proteins by cathepsins B and D. Biochem. J. 167: 811-820.

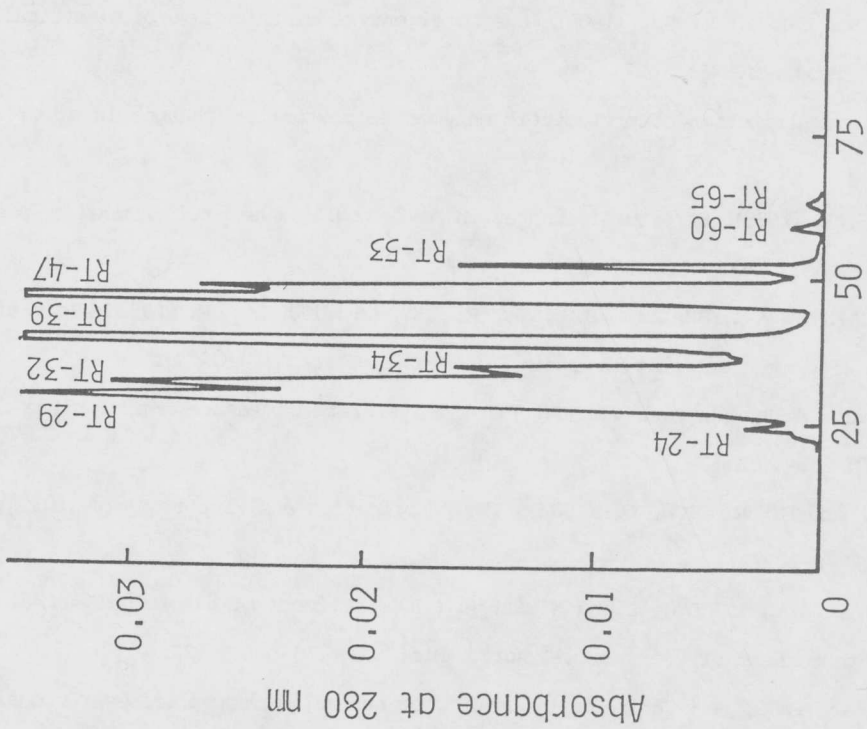

Fig. 1. HPLC pattern of sarcoplasmic fraction obtained from B. femoris. The 10 µl (control at 2 days) was applied to the column of TSK-G2000SW. Elution buffer used was 0.25 M phosphate (pH 6.5) at flow rate of 0.5 ml/min.

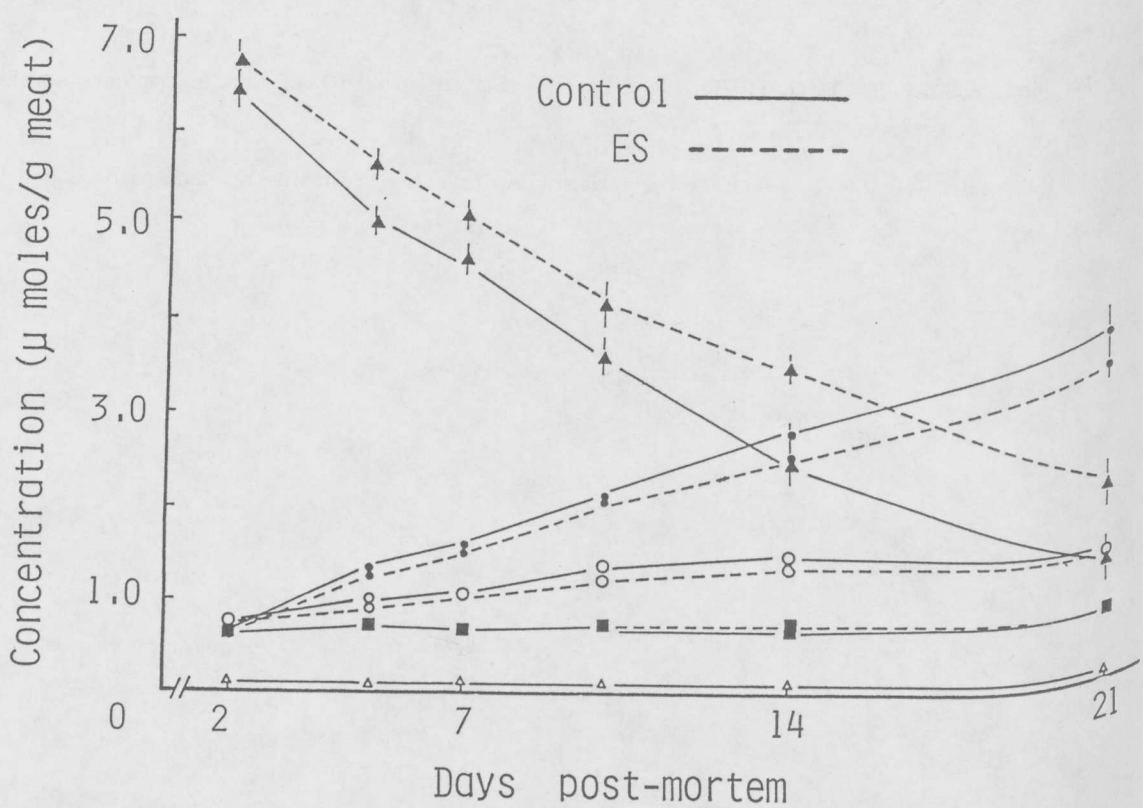

Fig. 2. Changes in ATP related compounds obtained from B. femoris during storage at 1 ℃.
ATP was not detected in the meat at 2 days post-mortem.
■ ; ADP, △ ; AMP, ▲ ; IMP, ○ ; HxR, ● ; Hx.

Table 1. Relative area (%) of sarcoplasmic fraction from B. femoris analyzed by HPLC

Peak	Days Post-mortem 2		7		14		21	
	Control	ES	Control	ES	Control	ES	Control	ES
RT-24	0.8%	0.7%	1.2%	1.3%	0.6%	0.8%	0.8%	1.1%
RT-29	22.5	21.5	17.9	17.4	17.7	17.2	18.0	17.0
RT-32	17.1	15.0	15.8	14.1	14.3	13.4	13.9	12.9
RT-34	6.3	5.7	6.5	5.9	6.2	5.6	6.2	5.4
RT-37	.....	1.5	.....	.....	.....	.....	.....	.....
RT-39[1]	18.4	18.5	20.0	21.3	20.2	21.0	19.5	20.2
RT-47	30.4[2]	19.2	16.8	17.5	11.2	13.2	4.2	7.7
RT-49	.....	12.4	12.0	11.9	16.0	13.7	17.9	14.4
RT-53	4.0	4.9	8.4	9.3	11.8	13.4	17.4	19.8
RT-60	0.3	0.3	0.8	0.7	1.3	1.1	1.5	1.1
RT-65	0.3	0.3	0.6	0.6	0.7	0.6	0.6	0.4

Homogenate was stored at 1℃ for up to 21 days after slaughter. The supernatant from homogenate was analyzed by the same method as in Fig. 1.
1), Retention time of myoglobin. 2), This value contains RT-49.

Table 2. Relative area (%) of supernatant from cooked homogenate analyzed by HPLC

Peak	Days Post-mortem 2		7		14		21	
	Control	ES	Control	ES	Control	ES	Control	ES
RT-24	0.4%	0.4%	0.3%	0.3%	0.3%	0.2%	0.3%	0.2%
RT-32	0.2	----	0.4	--	--	--	0.1	0.4
RT-39[1]	1.7	1.9	1.2	0.9	0.3	0.2	1.1	1.7
RT-47	63.8	57.8	37.1	43.7	28.9	31.5	21.5	23.3
RT-49	28.0	38.3[2]	38.7	32.2	24.2	20.5	31.0	27.3
RT-53	3.2	----	18.7	19.5	41.4	44.2	38.7	39.4
RT-60	2.0	1.4	2.7	2.5	3.8	3.1	5.8	5.8
RT-65	0.7	0.2	0.9	0.9	1.1	0.3	1.5	1.9

Homogenate was stored at 1℃ for up to 21 days after slaughter. The supernatant from cooked homogenate(75℃, 15 min) was analyzed by the same method as in Fig. 1.
1), Retention time of myoglobin. 2), This value contains RT-53.