Transaminase Affects Accumulation of Free Amino Acids in Electrically Stimulated Beef

M. Sekikawa, K. Seno, K. Shimada, M. Fukushima and M. Mikami

ABSTRACT

Electrical stimulation (ES) of a beef carcass increases the content of free amino acids, which affects meat flavor. Levels of glutamic acid (Glu)-aminopeptidase activity were similar in both control and ES meat but decreased during storage to about 65% at 14 days. When 2-ketoglutaric acid (a substrate of transaminase) was added to the sarcoplasm, the level of Glu increased. Transaminase activity was unaffected by storage and was about 20% greater in ES than in the control. Transaminase activity in beef was confirmed when radioactive 2-ketoglutaric acid was added to the sarcoplasm. Results suggest that transaminase contributes to accumulation of some amino acids such as Glu in beef during conditioning.

Key words: electrical stimulation, beef conditioning, aminopeptidase, transaminase, glutamic acid

INTRODUCTION

ELECTRICAL STIMULATION (ES) OF A BEEF CARCASS INCREASES the rates at which pH drops and glycogen levels decline. Because ES and hot boning may markedly affect processing, economics, and tenderness of meat, both treatments have been the subject of several studies (Cross and Seideman 1985). ES has the advantages of stabilizing color and increasing tenderness of meat (Devine et al., 1984; Chrystall and Devine, 1985; Uytterhaegen et al., 1992; Ho et al., 1996). It also increases the content of free amino acids during storage, which affects meat flavor (Mikami et al., 1994; Morris et al., 1997). Nishimura et al. (1994) considered the primary reason for this accumulation to be the action of protease, peptidase, or both. However, Sekikawa et al. (1996) found that the increase in each amino acid was not uniform: alanine (Ala) increased only slightly compared with glutamic acid (Glu). The objectives of our study were to compare the effects of ES on content of amino acids and to investigate another mechanism: contribution of transaminase to amino acid accumulation in beef during storage.

MATERIALS & METHODS

FOUR HOLSTEIN STEERS (21–23 MO OLD) WERE CONVENTIONALLY slaughtered and assigned to 1 of 2 groups: control (not stimulated) or electrically stimulated for 60s at 40V, 13.8 Hz, within 10 min of slaughter. Samples (30g) of ground quadriceps femoris muscle were taken 24h after slaughter and stored at 4°C in a refrigerator. Samples were then homogenized with 90g of 0.1M phosphate buffer (pH 7.2) at 1, 27, and 14 days. The homogenate was centrifuged (11000 × *g*, 20 min at 1°C, and the supernatant was filtered (0.45 µm) to produce the sarcoplasm. Free amino acids in the sarcoplasm were analyzed in a 2% trichloroacetic acid–soluble fraction with an amino acid analyzer (Hitachi 835, Tokyo).

Glutamic acid–aminopeptidase was measured by using glutamic acid–beta-naphthylamide as a substrate (Nakai et al., 1995). The sarcoplasm (0.1 mL) was incubated with 0.4 mL of 1.2 mM glutamyl-ß-naphthylamide in a 0.1M phosphate buffer (pH 7.2) containing 1 mM threo-1,4-dimercapto-2,3-butanediol at 37°C for 10 min. The released ß-naphthylamine was measured at 540 nm by using *p*-dimethylamino-cinnamaldehyde.

Transaminase (glutamic-pyruvic transaminase [GPT], E.C. 2.61.2) activity was measured with a GPT clinical test kit (Wako Co., Tokyo). The sarcoplasm (0.2 mL) was incubated with a 0.08M phosphate buffer (pH 7.4) containing 80 mM Ala, 18 mM 2-ketoglutaric acid, 0.12 mM ß-NADH, and 1400 u/mL LDH. The activity was measured as the decrease in absorbancy at 340 nm for 2 or 10 min. Results were recorded as change in absorption/min/g meat.

Samples of sarcoplasm were treated at day 1 with 10 µmol radioactive 2-ketoglutaric acid (α-[^{14}CU]-; DuPont, France) and incubated for 48h at 4°C. Radioactive amino acids or other substrates were identified by thin-layer chromatography (TLC) with coated silica gels (Wako, Tokyo) and 2 solvents: (1): chloroform/methanol/ammonia (17%) in the ratio 40:40:20 and (2): phenol/water in the ratio 75:25. The radioactivity was recorded on an imaging plate with a Bio-Imaging Analyzer (Fuji Co., Tokyo), and the amino acids were detected by ninhydrin reaction.

Each analysis was done at least in duplicate. The significance of differences among treatment groups was determined by analysis of variance with Duncan's multiple-range test or Student's *t*-test (SAS Institute, Cary, NC). Significance of differences was defined at $P<0.05$.

RESULTS & DISCUSSION

OUR PREVIOUS STUDIES (MIKAMI ET AL., 1994; SEKIKAWA ET AL., 1996) and those of others have shown that almost all free amino acids in beef increase during storage (Field et al., 1971; Feidt et al., 1996). The mean values for each storage day indicated that Ala was present at the highest concentrations, and that hydroxyproline and cysteine were present at the lowest. In this study, the mean values for Ala were also the highest overall of the free amino acids analyzed, regardless of treatment (Table 1). Nishimura et al. (1988) stated that Ala was one of the major free amino acids in beef, pork, and chicken muscle tissue. This accumulation of free amino acids in meat may contribute to an improvement in flavor development during roasting (Morris et al., 1997). However, glutamine (Gln), which was not determined in our study or that of Nishimura et al.(1988), is the most abundant free amino acid in human plasma and muscle (Perriello et al., 1995). Gln can be synthesized by skeletal muscle, where high concentrations are found.

In our previous study (Mikami et al., 1994; Sekikawa et al., 1996), leucine and phenylalanine in the control increased more than Ala, and Ala in ES decreased slightly. We confirmed this decrease in the current study, but do not understand the cause. The increase in amino acid

Table 1—Effect of ES on Ala and Glu content during storage

	Control		ES	
Days	1	14	1	14
Glu	17.6[a]	66.8[b]	60.5[b]	85.0[b]
Ala	252.9[a]	340.4[b]	301.1[b]	272.4[a]

[a-b]Means with same letter not significantly different (p>0.05) within the same rows. (µmol/100g meat, n=2).

The authors are affiliated with the Dept. of Bioresource Science, Obihiro Univ. of Agriculture & Veterinary Medicine, Inada, Obihiro 080, Japan. Address inquiries to Dr. Mitsuo Sekikawa.

levels during storage has been thought to be due to the action of proteolytic enzymes located in cytoplasm or released partly from lysosomes, including aminopeptidase and cathepsins (Etherington et al., 1990; Koohmaraie, 1992; Nishimura et al., 1994; Dransfield, 1994; Sekikawa et al., 1998). The increase in ES meat was probably related to the action of lysosomal enzymes (Dutson et al., 1980) following disruption of the muscle structure (Savell et al., 1978; Will et al., 1980; Chrystall and Devine, 1985; Takahasi et al., 1987). It appears that the lysosomal enzymes released by ES acted more rapidly on the meat.

Daily means of Glu-aminopeptidase activity were similar in both treatments but decreased during storage to about 65% at 14 days (Fig. 1). Nakai et al. (1995) stated that aminopeptidase activity in beef decreased with storage. The levels of most amino acids identified in our studies increased almost linearly throughout storage, but the increase in individual amino acid levels was not uniform. Ala changed only slightly compared with Glu in ES meat. Therefore, aminopeptidase alone was not the explanation for the increases.

A possible reason for the increasing Glu vs decreasing Ala might be transaminase, which transfers the alpha-amino group of Ala to 2-ketoglutaric acid to yield Glu. GPT and glutamic-oxaloacetic transaminase are found in the liver, the heart, and skeletal muscles (Garber et al., 1976; Townsend and Davis, 1992). The transaminase activity we measured was unaffected by storage and was about 20% greater in ES than in the control (Fig. 1). Hamm et al. (1969) reported that the activity of transaminase in post mortem beef and pig muscles decreased only slightly during storage at 4° for a week.

Transaminase activity in beef was confirmed when 2-ketoglutaric acid (a substrate of transaminase) was added to the sarcoplasm. The level of Glu increased (Table 2) but remained unaffected upon addition of maleic acid (an inhibitor of transaminase; Garber et al., 1976)

Table 2—Glu and Ala content of sarcoplasm with (1) H_2O, (2) 2-ketoglutaric acid, or (3) maleic acid

	0 hr	Incubated for 48h with		
		(1)	(2)	(3)
Glu	22.9[a]	32.6[a]	113.6[b]	33.7[a]
Ala	207.1[a]	209.5[a]	120.0[b]	210.6[a]

[a-b]The sarcoplasm from 1 day after slaughter incubated with (1) H_2), 50 µM of (2) 2-ketoglutaric acid or (3) maleic acid for 48h at 4°C. Means with same letter not significantly different (p>0.05) with the same rows. (µmol/100g meat, n=2).

Moreover, when radioactive 2-ketoglutaric acid was added to the sarcoplasm, the hot spot corresponding to Glu was detected on the TLC plate (Fig. 2). However, because there were a few hot spots of other amino acids on the TLC, more reliable, detailed analyses are needed.

As well as providing movement and locomotion, skeletal muscle also generates heat, helps to maintain body temperature, and generates amino acids to maintain a homeostatic level. Muscle lacks glucose-6-phosphatase and is therefore unable to produce glucose. During fasting it serves as an energy reservoir, and its proteins are degraded to amino acids, many of which are converted to pyruvate, which is then transaminated to alanine. The alanine is transported in the bloodstream to the liver, where it is transaminated back to pyruvate, a glucose precursor (Voet and Voet, 1995).

CONCLUSION

OUR DATA INDICATE THAT TRANSAMINASE CONTRIBUTES TO the accumulation of amino acids such as Glu during beef conditioning. We presumed that ES had an effect not only on proteases but also on other enzymes such as transaminase. The mechanism by which

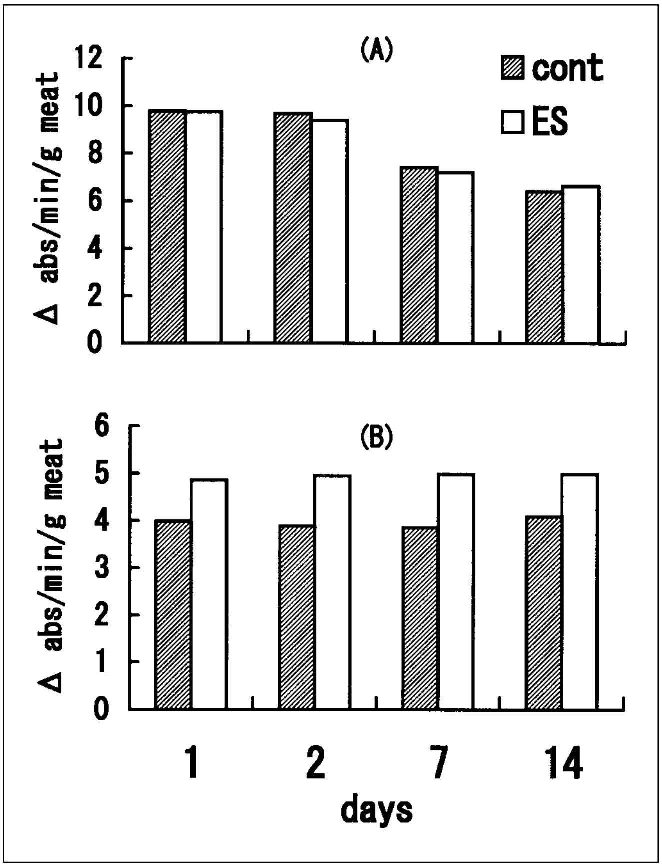

Fig. 1—Mean values of glutamic acid-aminopeptidase (A) and GPT (B) activities in ES and control samples. Mean differences between ES and control were not significant (A) and significant (B) using Student's t-test (p<0.05).

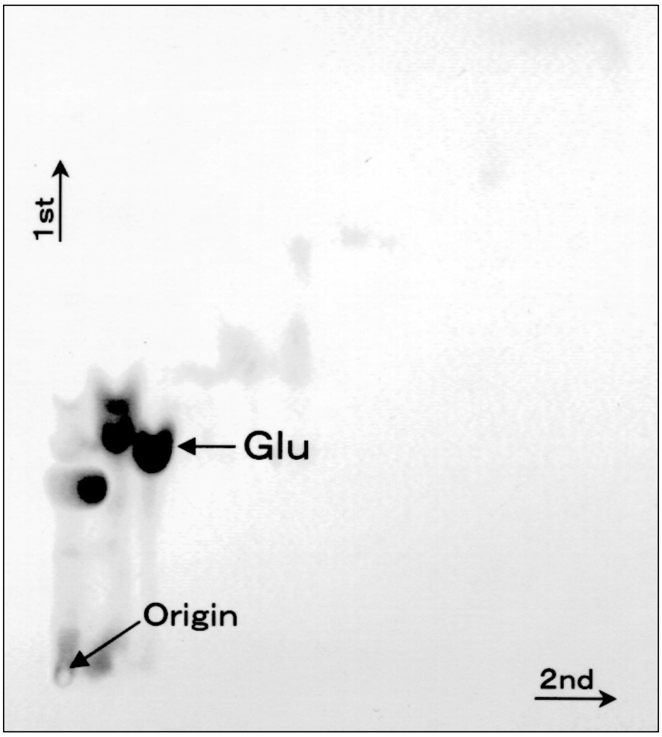

Fig. 2—RI-TLC chromatogram of sarcoplasma incubated with 2-ketoglutaric acid (α-[[14]CU]-).

amino acids accumulate in meat is important to meat scientists and biochemists, especially those investigating ischemia of muscle cells. To elucidate the role of various enzymatic systems such as transaminase during meat conditioning, further experiments are needed.

REFERENCES

Chrystall, B.B. and Devine, C.V. 1985. Electrical stimulation: Its early development in New Zealand. In *Advances in Meat Research, Electrical Stimulation*, A.M. Pearson and T.R. Dutson (Ed.), p. 73-115. AVI Publishing Co., Westport, CT.

Cross, H.R. and Seideman, S.C. 1985. Use of electrical stimulation for hot boning of meat. In *Advances in Meat Research. Electrical Stimulation.* A.M. Pearson and T.R. Dutson (Ed.), p. 159-183. AVI Publishing Co., Westport, CT.

Devine, C.E., Ellery, S., and Averill, S. 1984. Response of different types of ox muscle to electrical stimulation. Meat Sci. 10: 35-51.

Dransfield, E. 1994. Optimisation of tenderisation, ageing and tenderness. Meat Sci. 36: 105-121.

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., Taylor, M.A.J., Wakefield, D.K., Cousins, A., and Dransfield, E. 1990. Proteinase (cathepsin B, D, L and calpains) levels and conditioning rates in normal, electrically stimulated and high-ultimate-pH chicken muscle. Meat Sci. 28: 99-109.

Feidt, C., Petit, A., Bruas-Reignier, F., and Brun-Bellut, J. 1996. Release of free amino acids during ageing in bovine meat. Meat Sci. 44: 19-25.

Field, R.A., Riley, M.L., and Chang, Y. 1971. Free amino acid changes in different aged bovine muscle and their relationship to shear values. J. Food Sci. 36: 611-612.

Garber, A.J., Karl, I.E., and Kipnis, D.M. 1976. Alanine and glutamine synthesis and release from skeletal muscle. I. Glycolysis and amino acid release. J. Biol. Chem. 251: 826-835.

Hamm, R., Kormendy, L., and Gantner, G. 1969. Transaminases of skeletal muscle. The activity of transaminases in post-mortem bovine and porcine muscles. J. Food Sci. 34: 446-448.

Ho, C.Y., Stromer, M.H., and Robson, R.M. 1996. Effect of electrical stimulation on post mortem titin, nebulin, desmin, and troponin-T degradation and ultrastructural changes in bovine longissimus muscle. J. Anim. Sci. 74: 1563-1575.

Koohmaraie, M. 1992. The role of $Ca^{(2+)}$-dependent proteases (calpains) in post mortem proteolysis and meat tenderness. Biochemie 74: 239-245.

Mikami, M., Nagao, M. Sekikawa, Miura, H., and Hongo, Y. 1994. Effects of electrical stimulation on the peptide and free amino acid contents of beef homogenate and sarcoplasma during storage. Anim. Sci. & Technol. 65: 1034-1043.

Morris, C.A., Theis, R.L., Miller, R.K., Acuff, G.R., and Savell, J.W. 1997. Improving the flavor of calcium chloride and lactic acid injected mature beef top round steaks. Meat Sci. 45: 531-537.

Nakai, Y., Nishimura, T., Shimizu, M., and Arai, S. 1995. Effects of freezing on the proteolysis of beef during storage at 4°C. Biosci. Biotech. Biochem. 59: 2255-2258.

Nishimura, T., Rhue, M.R., Okitani, A., and Kato, H. 1988. Components contributing to the improvement of meat taste during storage. Agric. & Biol. Chem. 52: 2323-2330.

Nishimura, T., Rhue, M.R., Kato, T., and Arai, S. 1994. Purification and properties of aminopeptidase H from bovine skeletal muscle. J. Agric. Food Chem. 42: 2709-2712.

Perriello, G., Jorde, R., Nurjhan, N., Stumvoll, M., Dailey, G., Jenssen, T., Bier, D.M., and Gerich, J.E. 1995. Estimation of glucose-alanine-lactate-glutamine cycles in postabsorptive humans: role of skeletal muscle. Am. J. Physiol. 269: E443-450.

Savell, J.W., Dutson, T.M., Smith, G.C., and Carpenter, Z.L. 1978. Structural changes in electrically stimulated beef muscle. J. Food Sci. 43: 1606-1609.

Sekikawa, M., Seno, K., and Mikami, M. 1996. Accumulation of free amino acids in electrically stimulated beef. Proceedings of the 8th Animal Science Congress of the Asian-Australasian Association of Animal Production Societies. Japanese Society of Zootechnical Science (Tokyo) 2: 1008-1009.

Sekikawa, M., Seno, K., and Mikami, M. 1998. Degradation of ubiquitin in beef during storage. Meat Sci. 48: 201-204.

Takahasi, G., Wang, S.M., Lochner, J.V., and Marsh, B.B. 1987. Effects of 2-Hz and 60-Hz electrical stimulation on the microstructure of beef. Meat Sci. 19: 65-76.

Townsend, W.E. and Davis, C.E. 1992. Transaminase (AST/GOT and ALT/GPT) activity in ground beef as a means of determining end-point temperature. J. Food Sci. 57: 555-557.

Uytterhaegen L, Claeys, E., and Demeyer, D. 1992. The effect of electrical stimulation on beef tenderness, protease activity and myofibrillar protein fragmentation. Biochemie 74: 275-281.

Voet, D. and Voet, J.G. 1995. *Biochemistry*, 2nd ed. John Wiley & Sons, Inc., New York.

Will, P.A., Ownby, C.L., and Henrickson, R.L. 1980. Ultrastructural post mortem changes in electrical stimulated bovine muscle. J. Food Sci. 45: 21-25.

Ms received 10/2/98; revised 12/28/98; accepted 1/11/99.

We are indebted to Dr. M. Kroger, Dept. of Food Science, The Pennsylvania State Univ. for revision of this manuscript. This research was supported by a Grant-in Aid for Science Research from the Ministry of Education, Science and Culture, Japan (MS: #10660253).