The Relationship Between Plasma Epinephrine Concentration and the Activity of the Calpain Enzyme System in Porcine Longissimus Muscle

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ABSTRACT: The relationship between plasma epinephrine and postmortem activity of the calpain system in porcine longissimus muscle was investigated. Two groups of Large White pigs were continuously infused intravenously with either placebo (control) or epinephrine (treated) at a rate of .15 μg·kg⁻¹·min⁻¹ for a period of 1 wk before slaughter. Samples of longissimus muscle were taken at 0, 1, 2, 4, 8, 24, 48, and 192 h (t₀ to t₁₉₂) after slaughter and immediately snap-frozen in liquid nitrogen for subsequent analysis of μ-calpain, m-calpain, and calpastatin activity. Epinephrine infusion had no effect on the activities of μ- and m-calpain at t₀. Calpastatin activity at t₀ was increased (P < .01) in treated pigs by 97%. The ratio of total calpain:calpastatin activity at t₀ was reduced (P < .01) in treated pigs. The activity of μ-calpain decreased rapidly after slaughter, irrespective of treatment, dropping to less than 10% of the initial activity within 8 h. The activity of m-calpain also decreased over the first 8 h, although the rate of decrease was less (P < .05) in treated pigs. Consequently, m-calpain activity remained greater in treated pigs compared with controls throughout the period normally associated with tenderization. Postmortem values for calpastatin activity tended to be highly variable, with activities being similar between control and treated pigs within 1 h after slaughter. Over the entire 192 h sampling period, calpastatin activity decreased (P < .001), although the effect was independent of treatment. In general the results imply that variations in plasma epinephrine concentrations, which may naturally occur as part of the stress response, perturb the calpain enzyme system.

Key Words: Pigs, Calpain, Calpastatin, Skeletal Muscle, Beta-Adrenergic Agonists, Epinephrine

Introduction

The rate at which meat tenderizes depends in part on myofibrillar protein degradation in the postmortem period and is highly variable between carcasses, providing an economic problem for the meat industry. Currently there is no definitive method available to predict ultimate tenderness (Dransfield et al., 1994; Koohmaraie et al., 1995). Thus a better understanding of the underlying biochemical mechanisms responsible for postmortem muscle breakdown and their control is needed.

Much evidence suggests that myofibrillar degradation in postmortem muscle is dependent to a large extent on the activation or suppression of the calpain enzyme system (Koohmaraie, 1994; Dransfield, 1994). The major components of the system are μ-calpain and m-calpain, isoforms of the calcium-dependent thiol proteases capable of proteolytic activity at micromolar and millimolar concentrations of calcium, respectively, and their natural specific inhibitor, calpastatin. All three are present in skeletal muscle fibers giving them access to the myofibrillar substrate (Goll et al., 1992). Although there is some debate as to how effective the calpains are at the low pH and low temperature environment of postmortem muscle, their inability to hydrolyze either actin or myosin, neither of which are broken down during tenderization, sets them apart from other potential proteolytic systems such as the cathepsins and the multicatalytic proteinase or proteasome (Koohmaraie, 1994). The fact that the calpains are associated with an intrinsic specific inhibitor strongly suggests a
tightly regulated system and that myofibrillar breakdown could simply depend on the ratio of calpastatin to one or both calpain isoforms in the muscle. Consequently, if calpastatin activity were high or calpain activity low at the time of slaughter, there could be less proteolysis, resulting in tougher meat (Koohmaraie, 1994).

Calpastatin activity at slaughter has previously been shown to be increased in steers and sheep fed a diet supplemented by a β-adrenergic agent, such as cimaterol or clenbuterol (Higgins et al., 1988; Wang and Beermann, 1988; Kretchmar et al., 1990; Bardsley et al., 1992; Parr et al., 1992; Wheeler and Koohmaraie, 1992). Such treatment results in an increased incidence of tough meat (Fiems et al., 1990; Kretchmar et al., 1990; Warris et al., 1991; Geesink et al., 1990). The effects of β-agonists on μ-calpain and m-calpain activity and mRNA have been less striking (Bardsley et al., 1992), suggesting that regulation of the system is likely to be exerted primarily through calpastatin.

If synthetic β-agonists have such striking potential to modify the extent of postmortem proteolysis, it is important to ascertain what effects variable levels of the endogenous β-adrenergic agonist epinephrine could have on the system. If such a link can be established, an understanding into why tenderization rates vary among animals may arise, because plasma epinephrine concentrations are likely to be highly variable between individual animals, depending on a number of factors associated with transport and lairage during the period before slaughter and on factors associated with earlier handling and housing regimens.

In this study, the effects of raising the levels of epinephrine by infusion for a period of 7 d before slaughter on the activity of the calpain system in longissimus muscle were investigated in samples taken from pigs immediately after slaughter and during the period associated with tenderization.

**Materials and Methods**

**Animals**

Twelve female Landrace × Large White pigs, consisting of six pairs of siblings, were used in the trial. Pigs were transferred to individual pens 1 wk before surgery for acclimatization. Food and water were freely available throughout, with food being withdrawn only for the 12 h before surgery.

Surgery was performed when the pigs reached 71 ± 1.1 kg. Following intramuscular premedication with 3 mL of Stresnil (40 mg/mL of azaperone, Janssen Animal Health, Grove, U.K.), sodium thiopentone (.5 mL/kg of Intraval, May & Baker Ltd., Dagenham, U.K.) was injected intravenously and anaesthesia was maintained with 2 to 4% halothane plus a 2:1 mixture of oxygen and nitrous oxide. An incision was made along the right side of the trachea. The right carotid artery was located by blunt dissection. A polyvinyl catheter (Microflex Infusion Set, external diameter .5 mm, Vygon, France) was inserted into the artery so that the tip was positioned close to the heart. The catheter was secured and exteriorized at the back of the neck. The incision was closed in three layers with coated vicryl suture (Ethicon, Edinburgh, U.K.), and Triobiotic spray (bacitracin, polymyxin and neomycin aerosol, 3M HealthCare, Loughborough, U.K.) was applied between each layer to prevent infection. A second incision was made between the right forelimb and chest wall, allowing the cephalic vein to be visualized. A polyvinyl catheter (.3 mm external diameter, Norfolk Medical Products, Skokie, IL) attached to a pre-prepared osmotic minipump (model 2ML1, Alzet, Palo Alto, CA) containing either placebo (2 mL saline + 40 μg/mL of ascorbic acid [Sigma Chemical, Poole, U.K.]) or epinephrine (placebo + 135 mg of epinephrine bitartrate, Sigma Chemical) was inserted into the vein toward the heart and secured in place. The minipump was implanted subcutaneously, and the incision was closed in a single layer. In this way, epinephrine was infused continuously for a period of 1 wk at approximately .15 μg·kg⁻¹·min⁻¹. The sibling of each epinephrine-infused animal received placebo. At the end of surgery each animal received 2 mL of Depocillin (300 mg of procaine penicillin/mL, Mycofarm U.K., Cambridge, U.K.). The arterial catheters were flushed with heparinized saline (100 U/mL) daily, although this did not completely prevent partial blockage of the catheters from occurring periodically. Sufficient blood (4 to 5 mL) could be collected from only six placebo and four epinephrine-infused pigs on d 1 after surgery, from four pigs in each group on d 6 after surgery and from six placebo and two epinephrine-infused pigs immediately before slaughter at the end of the infusion period (d 7). At least one blood sample was obtained from each pig in the trial. The collected blood was centrifuged at 3,000 × g for 10 min in tubes containing 50 μL of 250 mM EGTA and 195 mM glutathione. The plasma was collected and stored at −20°C for subsequent catecholamine analysis by HPLC with electrochemical detection (MacDonald and Lake, 1985).

The pigs were transferred individually to a slaughter pen within the facility 7 d after surgery. As soon as the final blood sample had been taken the pigs were slaughtered by electrical stunning and severance of the carotid arteries. The total time between leaving their pens and slaughter was less than 3 min, thereby minimizing pre-slaughter stress effects. Following slaughter the carcasses were hung and split down the length of the back. Cores (2 g) of longissimus muscle were taken from the area of the last rib at 0, 1, 2, 4, 8,
24, and 48 h after slaughter, the carcasses were being hung at ambient room temperature for the first 3 h before transfer to a chiller held at 2°C. The samples were immediately snap-frozen in liquid nitrogen for subsequent enzyme analysis. Carcasses were processed 48 h after death. A single chop in the region proximal to the sampling area was vacuum-packed (Jolly Vacuum Packer, Scobie & Junor, Glasgow, U.K.) and conditioned at 1°C for a further 6 d at which time a core was snap-frozen in liquid nitrogen.

The pH of the longissimus muscle was measured in triplicate 45 min after slaughter (pH45) in the region of the last rib using a combination pH puncture electrode (Ingold, Mettler-Toledo Ltd., Leicester, U.K.).

Determination of Calpain and Calpastatin Activity

All cores were analyzed for calpastatin, μ-calpain, and m-calpain activity between 1 and 4 wk after collection, thereby minimizing effects of sample storage time.

The three components of the system were extracted from the longissimus muscle essentially as described previously (Higgins et al., 1988; Parr et al., 1992), using hydrophobic chromatography on a phenyl-Sepharose column (Sigma Chemical) to separate a calpastatin pool from the calpain isoforms and then performing fast protein liquid chromatography using a monoQ column (FPLC, Pharmacia LKB Biotechnology, Cambridge, U.K.) to separate the two calpains. Briefly, each core was pulverized in liquid nitrogen and homogenized in 3 volumes of ice-cold buffer A (20 mM Tris HCl, 5 mM EDTA, 2 mM dithiothreitol, .5 mM phenylmethylsulphonyl fluoride, pH 7.5). The homogenate was centrifuged at 30,000 x g for 20 min at 4°C. The salt concentration and pH of the supernatant were adjusted to .3 M NaCl and pH 7.5, respectively, before loading onto a 2-mL phenyl-Sepharose column equilibrated with .3 M NaCl in buffer A. An equal volume of buffer A containing .3 M NaCl was loaded onto the column to elute the calpastatin fraction (calpastatin pool). Both calpain isoforms were eluted together using a fixed volume of 50% ethanediol diluted in buffer A. The eluant was diluted with 4 volumes of buffer A to reduce the ionic strength and filtered through a .22-μm filter before being loaded onto a 1-mL monoQ column equilibrated with buffer A. Fractions (1 mL) were collected over a rising salt gradient (0 to .6 M). Fractions collected between salt concentrations of .13 M and .55 M were assayed for calpain activity using a fluorimetric technique adapted from Sasaki et al. (1984).

Calpain Assay. Assay mixture (125 μL) containing 30 mM imidazole buffer (pH 8.0), 5 mM L-cysteine, 2.5 mM β-mercaptoethanol (MCE), 4% DMSO and either 5 mM CaCl2 or 5 mM EDTA (final assay concentrations) was added to individual wells on a 96-well microtiter plate. An aliquot (25 μL) of fluorescent substrate, N-succinyl-Leu-Tyr-7-amido-4-methylcoumarin (Suc-Leu-Tyr-AMC) (Bachem, Switzerland), was added to each well, giving a final substrate concentration of .3 mM, and the reaction was started by the addition of 100-μL aliquots taken from individual fractions collected from the FPLC fractionation. Fluorescence was measured after 15 min of incubation at 30°C using a CytoFluor 2350 Fluorescence Measurement System (Millipore, Watford, U.K.) using excitation at 360 nm and measuring the emission at 460 nm. Differences between fluorescence in wells containing CaCl2 or EDTA were plotted for each fraction, allowing calpain activity to be calculated in terms of fluorescence units per kilogram of muscle. One unit of μ- or m-calpain activity was defined as the amount of enzyme that catalyzed an increase of 1 fluorescence unit. In preliminary studies (data not shown), we found that the fluorescence signal was linear with concentration of either calpain isoform and that 107 units of fluorescence activity was equal to 138 caseinolytic units as defined by Higgins et al. (1988).

Calpastatin Assay. Assay mixture (125 μL) containing 30 mM imidazole buffer (pH 8.0), 5 mM L-cysteine, 2.5 mM β-MCE, 4% DMSO and 5 mM CaCl2 (final assay concentrations) was pipetted into each well on a 96-well microtiter plate. An aliquot (25 μl) of active or heat-inactivated porcine m-calpain, followed immediately by different dilutions of the calpastatin pool, was added to the wells, and the reaction was started by the rapid addition of 25 μl of Suc-Leu-Tyr-AMC (.3 mM final assay concentration), giving a final assay volume of 250 μL. The fluorescence in each well was read after 15 min of incubation at 30°C as above. For a fixed level of m-calpain activity a linear relationship was seen between increasing volume of calpastatin and reduction of the difference in fluorescent signal over controls incubated with inactive m-calpain. One unit of calpastatin activity was defined as the amount that inhibits 1 unit of calpain activity.

Statistical Analysis

Unless otherwise stated, the data were analyzed using two-way analysis of variance to determine the effect of time after death on enzyme activity and to determine the effect of treatment on any changes identified. Single point values, such as pH45 and enzyme activities at t0, were compared using Student’s t-test for paired data.

Results

Animal Performance. Epinephrine infusion had no effect on changes in the body weight of the pigs; placebo-infused pigs (controls) gained 4.6 ± 1.9 kg
Table 1. Changes in arterial plasma epinephrine concentrations (nmol/L) in pigs during infusion with placebo or epinephrine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of infusion</th>
<th>Day 1</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SE</td>
<td>n</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Placebo</td>
<td>6</td>
<td>3.47 ± .72</td>
<td>4</td>
<td>1.20 ± .27</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>4</td>
<td>11.3 ± 5.79</td>
<td>4</td>
<td>14.4 ± 2.40</td>
</tr>
<tr>
<td>P-value&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>&lt; .001</td>
<td></td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on Student's t-test for unpaired data (two-tailed). NS = not statistically significant.

Table 2. Calpain and calpastatin activities and the proteolytic potential of the system in porcine longissimus muscle at slaughter following intravenous infusion of placebo or epinephrine for 7 days before slaughter

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>µ-Calpain × 10&lt;sup&gt;7&lt;/sup&gt; fluorescence units/kg</th>
<th>m-Calpain × 10&lt;sup&gt;7&lt;/sup&gt; fluorescence units/kg</th>
<th>Calpastatin × 10&lt;sup&gt;7&lt;/sup&gt; fluorescence units/kg</th>
<th>Proteolytic potential&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>6</td>
<td>5.02 ± .53</td>
<td>2.48 ± .24</td>
<td>7.30 ± 2.13</td>
<td>1.27 ± .19</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>6</td>
<td>4.82 ± .54</td>
<td>2.62 ± .31</td>
<td>12.9 ± 2.92</td>
<td>.71 ± .15</td>
</tr>
<tr>
<td>P-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>&lt; .01</td>
<td>&lt; .01</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated as the ratio between total calpain activity (µ- + m-) and calpastatin activity.

<sup>b</sup>Based on Student's t-test for paired data (two-tailed). NS = not statistically significant.
Figure 1. Postmortem changes in μ-calpain activity in porcine longissimus muscle taken from pigs infused with placebo (□) or epinephrine (■) for 7 d before slaughter. Activity is expressed as $1 \times 10^7$ fluorescence units/kg of muscle. Each data point is the mean of six pigs, sampled repeatedly from 0 to 192 h. The curve represents the predicted inactivation of μ-calpain using the model proposed by Dransfield (1994) for the pooled data. The bar represents the standard error of the differences of the means.

Figure 2. Early postmortem changes in m-calpain activity in porcine longissimus muscle taken from pigs infused with placebo (□) or epinephrine (■) for 7 d before slaughter. Activity is expressed as $1 \times 10^7$ fluorescence units/kg of muscle. Nonlinear regression curves of the form $y = a + br^x$ are shown for each treatment group. Each data point is the mean of six pigs, sampled repeatedly from 0 to 8 h. The bar represents the standard error of the differences of the means.

tem changes in activity ($P < .05$). The interaction was most marked over the first 8 h after slaughter ($P < .01$), during which time activity decreased by 81% in control pigs and by 44% in treated pigs (Figure 2). Half-lives ($t_{1/2}$) for the reduction in m-calpain activity were calculated to provide an index of the effect of epinephrine. The $t_{1/2}$ calculated from the model applied to data obtained from control samples was 1.65 h. This was considerably shorter than the $t_{1/2}$ of 8.03 h calculated similarly for treated pigs. The $t_{1/2}$ of m-calpain decay in individual pigs was found to correlate positively with both the epinephrine concentration measured immediately before slaughter ($r^2 = .72, P < .01$) and the pH45 of the muscle ($r^2 = .82, P < .001$).

The extractable activity of calpastatin during the postmortem period was found to be highly variable in the first few hours (Table 3). Overall, there was a significant linear decrease in activity over the sampling period of 192 h ($P < .001$) that was independent of pre-slaughter treatment. No significant effects of epinephrine were observed at any time after slaughter, and activity tended to be equalized within 1 h of death.

Discussion

Variability in the rate of meat tenderization may arise as the result of stress-induced activation or suppression of key proteolytic enzymes involved in myofibrillar protein turnover. Evidence suggests that the potential interaction between adrenergic stimulation and the calcium-activated proteolytic (calpain) system in skeletal muscle may be an important mechanism by which this variability in postmortem proteolysis arises (Koohmaraie, 1992; Dransfield, 1993, 1994). It seems that the inclusion of a synthetic β-agonist in the diet for several weeks not only induces hypertrophy in some muscle types in sheep, cattle, and pigs but can also have a negative effect on sensory meat quality (Fiems et al., 1990; Kretchmar et al., 1990; Warris et al., 1991; Geesink et al., 1993). In addition, the activity of the components of the calpain system measured at slaughter in sheep and steers fed a diet supplemented with a β-agonist have shown a significant response, especially in calpastatin activity (Higgins et al., 1988; Kretchmar et al., 1990; Bardsley et al., 1992; Wheeler and Koohmaraie, 1992; Pringle et al., 1993; Speck et al., 1993). Effects on μ-calpain and m-calpain have been variable (Bardsley et al., 1992), although significant increases in m-calpain and significant decreases in μ-calpain in sheep have been recorded (Higgins et al., 1988; Kretchmar et al., 1990; Pringle et al., 1993). Overall, the proteolytic potential of the calpain system, as calculated by the ratio of calpain:calpastatin, tends to be reduced in animals treated with synthetic β-adrenergic agents. Such a distinct effect on an enzyme system...
Table 3. Effects of epinephrine infusion for 7 days before slaughter on postmortem changes in the activity of the calpain/calpastatin system in porcine longissimus muscle

<table>
<thead>
<tr>
<th>Time after slaughter, h</th>
<th>Treatment</th>
<th>n</th>
<th>Activity, $\times 10^7$ fluorescence units/kg</th>
<th>$\mu$-Calpain</th>
<th>m-Calpain</th>
<th>Calpastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Placebo</td>
<td>6</td>
<td>5.02$^a$</td>
<td>2.48</td>
<td>7.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>6</td>
<td>4.82</td>
<td>2.62</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Placebo</td>
<td>6</td>
<td>3.32</td>
<td>1.78</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>6</td>
<td>3.64</td>
<td>2.51</td>
<td>10.1</td>
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</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td>6</td>
<td>2.21</td>
<td>1.14</td>
<td>9.51</td>
<td></td>
</tr>
<tr>
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<td>3.52</td>
<td>1.89</td>
<td>8.74</td>
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</tr>
<tr>
<td>4</td>
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<td>6</td>
<td>1.21</td>
<td>.36</td>
<td>9.00</td>
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</tr>
<tr>
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<td>Epinephrine</td>
<td>6</td>
<td>2.00</td>
<td>1.39</td>
<td>12.0</td>
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<tr>
<td>8</td>
<td>Placebo</td>
<td>6</td>
<td>.63</td>
<td>.47</td>
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<td>Epinephrine</td>
<td>6</td>
<td>1.95</td>
<td>1.48</td>
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<tr>
<td>24</td>
<td>Placebo</td>
<td>6</td>
<td>.24</td>
<td>.31</td>
<td>8.70</td>
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<td>Epinephrine</td>
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<td>.33</td>
<td>.72</td>
<td>8.49</td>
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<tr>
<td>48</td>
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<td>6</td>
<td>.15</td>
<td>.28</td>
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<td>.93</td>
<td>6.77</td>
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<tr>
<td>192</td>
<td>Placebo</td>
<td>6</td>
<td>0</td>
<td>.14</td>
<td>3.73</td>
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<tr>
<td></td>
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<td>.05</td>
<td>.54</td>
<td>4.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SED$^b$</td>
<td></td>
<td>.45</td>
<td>.30</td>
<td>4.02</td>
<td></td>
</tr>
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</table>

$^a$Mean values.  
$^b$Standard error of the difference of the means.  
$^c$Linear effect.  
$^d$Quadratic effect.  
$^e$F-probability.  
$^f$NS = not statistically significant.  
$^g$Nonlinear regression.  
$^h$Nonlinear regression between 0 and 8 h ($P < .01$).

The effect of epinephrine on the activity of the calpain system during the period associated with postmortem tenderization is less well defined. The activities of $\mu$-calpain and m-calpain both fell rapidly during the first 8 h after slaughter. Epinephrine infusion had no effect on the rate of $\mu$-calpain inactivation, but markedly reduced the inactivation rate of m-calpain, the activity of which remained greater than in controls during the first 192 h after death. This observation is not consistent with the idea that adrenergic stimulation leads to reduced calpain-mediated proteolysis. However, the true relationship between in situ and extractable activity remains to be clarified. Activities may seem to be greater in epinephrine-treated animals due to the detection of calpain that would be inactive in situ, whereas in controls the assay may detect mainly residual activity remaining after autolytic activation followed by deactivation of the enzyme (Dransfield, 1994).

On the basis of data accumulated from calpain and calpastatin activities in bovine and ovine muscle types (Koohmaraie, 1992, 1994; Dransfield, 1993, 1994), decreases in $\mu$-calpain have been observed that closely resemble those found in the current study with pigs. One study in steers in which activity was measured 1, 24, 48, 72, and 144 h after slaughter reported a similar rapid decrease in $\mu$-calpain activity in longissimus muscle but slower inactivation rates in other muscles (Geesink et al., 1992). A single injection of epinephrine to deplete glycogen stores had little effect on the activity in longissimus muscle, as seen in the present study, but increased the rate of inactivation in other muscles.

Intravenous infusion of epinephrine or placebo for 7 d before slaughter produced two groups of pigs with significantly different arterial plasma epinephrine concentrations. This correlated to a reduction in the rate of pH fall after death, with pH45 values of 6.2 and 5.8 being measured in epinephrine-treated and control animals, respectively. Such a differential in the rate of pH decline following epinephrine administration has been reported in previous studies (Geesink et al., 1992) and is likely to influence the stability of the calpain system (Dransfield, 1993).
In contrast to previous studies on ruminant species that indicate that m-calpain activity remains stable during postmortem tenderization (Kretchmar et al., 1990; Koohmaraie et al., 1991a; Geesink et al., 1992; Wheeler and Koohmaraie, 1992), our study found that m-calpain activity significantly decreased after slaughter in porcine longissimus muscle. To our knowledge, the present study is the first in which postmortem changes in calpain and calpastatin have been studied in this species in this way. Thus, m-calpain is apparently much less stable in porcine longissimus muscle than in tissues of ruminants. Furthermore, clear differences were observed in the stability of m-calpain in the epinephrine-treated pigs. Geesink et al. (1992) found that the m-calpain stability in bovine skeletal muscle was unaffected by a single pre-slaughter injection of epinephrine. However, in lambs and steers fed a diet supplemented with β-adrenergic agonists, the m-calpain activity was significantly greater in treated animals after conditioning, which is consistent with the present study in pigs (Kretchmar et al., 1990; Koohmaraie et al., 1991a; Wheeler and Koohmaraie, 1992).

On the basis of bovine and ovine data, calpastatin activity decreases during postmortem conditioning, although in most studies activity was measured only at slaughter and at a single time point 4 or 7 d after death (Kretchmar et al., 1990; Koohmaraie et al., 1991a; Wheeler and Koohmaraie, 1992). In the current study in pigs, the calpastatin activity remaining at the end of the conditioning period, i.e., at 192 h, had declined by a similar amount in the control group, compared with activity measured immediately after slaughter. However, in the epinephrine-treated group there was a rapid initial decline of calpastatin activity during the first hour of conditioning, after which the rate of decrease in calpastatin activity did not differ significantly between control and treated groups. Thus, there seems to be an epinephrine-related difference in calpastatin activity in the immediate postmortem period in porcine longissimus muscle. In previous studies in ruminants the activity of calpastatin several days after slaughter has been shown to remain greater in animals treated with β-adrenergic agonists (Kretchmar et al., 1990; Koohmaraie et al., 1991a; Wheeler and Koohmaraie, 1992). This was not evident in porcine longissimus muscle or in epinephrine-treated steer longissimus muscle in which activity was measured in samples taken at multiple time points after slaughter (Geesink et al., 1992).

An important factor that may partially explain the differences seen between the present study and previous reports is the species studied. There is clear evidence that the activity of the calpain system differs in cattle, sheep and pigs; much higher calpastatin activities generally prevail in ruminant species (Koohmaraie et al., 1991b). Furthermore, the rate of tenderization of pork is considerably more rapid than that of lamb or beef (Dransfield et al., 1981; Etherington et al., 1987), and it seems highly likely that these observations are not unconnected. In the present study, the most striking effect of the epinephrine treatment seemed to be an initial elevation of the recoverable calpastatin activity to double the activity in controls. However, this differential rapidly disappeared within 1 h. If this initial elevation is related to meat toughness in pigs undergoing increased adrenergic stimulation, then this influence must be exerted immediately after slaughter, even if the full repercussions of any proteolytic events are likely to take longer to develop. At present there is no way to relate true in situ activity to the activity recovered in biochemical assays. It seems likely that targeting or localization of the calpain system components may be a contributory factor to meat quality determination.

**Implications**

The natural β-adrenergic agonist epinephrine is capable of perturbing the calpain system in a fashion similar to that involving synthetic agents. Thus, variations in epinephrine concentrations, which may arise due to long-term handling or short-term stress before slaughter, may partly account for variability in tenderization rates mediated through the calpain system. However, although the proteolytic potential of the calpain system measured at slaughter might provide a valuable potential predictor of ultimate meat quality, to further understand the biochemical origins of variability in the rate of meat tenderization, determination of the in situ activity of the calpain system is required.

**Literature Cited**


