The effect of pre-slaughter events on the expression of small heat shock proteins in the muscle

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Abstract

Heat shock proteins (HSP) 20 and 27 are inducible chaperone proteins produced by cells in response to metabolic and environmental changes. These small HSP's are produced rapidly in response to various events and could therefore be used as a method to measure pre-slaughter stress. To evaluate this, HSP 20 and 27 were measured in muscle samples collected early after slaughter from cattle that had undergone different pre-slaughter experiences. Three distinct procedures were targeted: direct to the abattoir from a local farm; overnight transport from a distant producer; and travel from undefined sources via two local saleyards. A total of 100 *M.longisimus dorsi* samples were collected within 20 minutes of slaughter. HSP20 expression was lowest in consignments from local farms (21.0±2.48 ng HSP 20/mg muscle), followed by consignments from saleyards (27.29 ± 5.64 ng/m) while higher levels (36.91 ± 5.64 ng/mg, $P<0.01$) were measured in consignments subjected to overnight transport. These results demonstrate that HSPs are induced in response to transport and lairaging, and are potential markers for quantifying stress associated with physical exertion and therefore likely to have an effect on meat quality.

Introduction

The production of heat shock proteins is a protective response of cells to stressors such as ischemia, toxins, heat and oxidation (Golenhofen et al, 2004). Pre-slaughter stress caused by factors such as transport distance, cattle mixing, temperature changes and physical injury (Gregory, 1996) is well known to have a detrimental effect on meat quality. Van Laack et al, (1993) did not obtain a relationship between HSP 70 and meat quality in pigs but more recent work has shown that this HSP 27 may be linked with tenderness in a breed of beef (Morzel et al, 2007).

HSP 20 and 27 are part of the small heat shock protein family and are highly constitutively expressed in skeletal muscle tissues, along with other heat shock proteins including alpha B crystallin and HSP 70 (Golenhofen et al, 2004). HSP 20 and 27 form macromolecular associations (Kato et al, 1994) and localise from the sarcoplasm to myofibrils within 30 minutes after the onset of stress (Golenhofen et al, 2004; Huot et al, 1996). In addition, the expression of HSP 27 increases in the muscle after stressful events such as exercise (Thompson et al, 2002), and HSP 20 produces a similar response in cardiac muscle (Boluyt et al, 2006). The rapidity of HSP induction makes these proteins ideal candidates for markers of preslaughter stress. Additionally, because HSP 27 and 20 bind with and potentially influence the degradation of myofibrillar proteins, elevated levels may have further consequence on meat quality attributes.

The objective of this study was to measure the expression of HSP 20 and 27 in muscle samples collected early after slaughter from cattle that had undergone a range of pre-slaughter experiences. Three distinct preslaughter procedures were targeted: direct consignment to the abattoir from a local farm; overnight transport from a more distant producer; and consignment from undefined sources via two local sale yards.

Materials and methods

Samples from the *M.longisimus dorsi* were collected at the end of the dressing operation within 20 minutes of slaughter. Each collection included at least two separate treatments, and 10 samples were collected from each treatment on each collection. A total of 100 samples were collected. After excision from the carcass, the samples (~10g) were snap frozen in liquid nitrogen and held at -25°C for a maximum of three weeks prior to analysis.

HSP 20 ELISA.

Briefly, 2g of meat was homogenised in 10mL of 50 mM Tris-MES, pH 5.5, 100 mM KCl, 10 mM EDTA, 1 mM 2-mercaptoethanol, 0.1 mM PMSF using an Ultraturrax homogeniser (13500 rpm, 20 sec). The sample was diluted 1/20 and pipetted into a 96 well microplate (Maxisorp, Nunc) that had previously been coated in mouse anti-HSP 20 (Hytest), then blocked with 5% PBS-milk. Purified Hsp 20 recombinant protein (Hytest) was used to generate a standard curve. After incubation (1 h, 37 ^{o}C) the samples were removed, the wells

washed three times with PBST, and rabbit anti-HSP 20 (Stressgen) added. After 1 h further incubation, followed by washing, secondary antibody was added (mouse anti-rabbit horseradish peroxidase, Pierce). After 1 h incubation, the wells were washed out and OPD solution was added to allow colorimetric measurement at 490nm.

HSP27 Western analysis

A sample of ELISA homogenate was solubilised 1/10 into 30 mM Tris-HCl, pH 8.0, 3% SDS, 10 mM EDTA and heated at 37 ^{o}C for 10 min. The solubilised sample was mixed with E-PAGE loading buffer, and then loaded onto 96 well 6% E-PAGE gels (Invitrogen) in triplicate. Heat stressed HeLa cell lysate (Stressgen) was loaded as a positive control. Electrophoresis was carried out according to the instructions provided. The separated proteins were semi-dry blotted onto PVDF membrane, then blocked in 5% skim milk - TBS overnight at 4 ^{o}C. Primary incubation was 1:5000 anti-HSP27 (Hytest) in TBS for 2 h at room temperature, the membrane was washed in TBST, then secondary incubation was 1:3000 goat anti-mouse horseradish peroxidase in TBST for 2 h at room temperature. Immunoreactive bands were visualised by ECL detection.

Results and discussion

A clear variation in the HSP20 and 27 expression was evident in the sample population (Figure 1). The mean values for the HSP expression according to the preslaughter treatment is shown in Table 1.

The HSP20 expression was lowest in the consignments from local farms. In contrast, consignments subjected to overnight transport produced significantly higher concentrations ($P<0.01$). The three sample groups from local sale yards did not differ significantly from cattle delivered directly from local farms although these values tended to be slightly higher. It was also evident that the variability in the HSP concentrations was greater when they had been subjected to sale yards or overnight long-distance transport compared to those that arrived at the plant direct from local farms. Although the results of HSP27 showed similar trends, the differences were not significant.

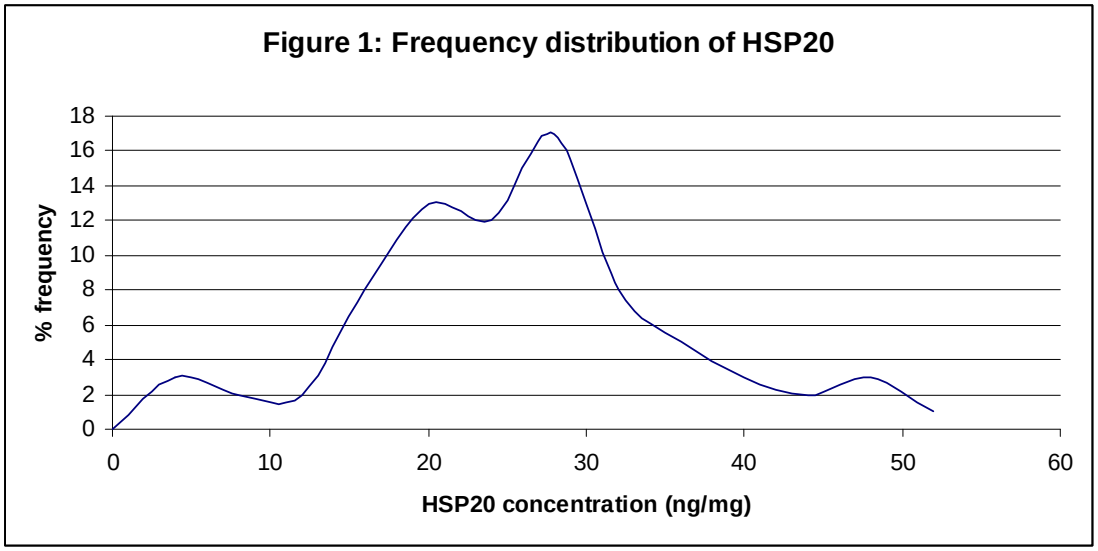

Table 1. Results of pre-slaughter treatments on HSP20 and HSP27 expression

Consignment source	HSP20 ng/mg (SEM)	HSP27 absorbance units (SEM)
Local farm	21.0[a] (2.48)	2484.8 (534.7)
Overnight transport	36.91[b] (5.64)	3174 (1253.4)
Local sale yard	27.29[a] (5.64)	2497.1 (834.7)
Significance	**	ns

Values with different superscripts within a row are significantly different

Conclusions

Both assays demonstrated a significant range of HSP response in the post mortem muscle. This identifies that there are considerable individual differences in the induced expression of the HSP proteins and this is particularly marked when they have been subjected to potentially stressful pre-slaughter treatments. Intuitively, the short transport distances and rapid slaughter of cattle produced local to the slaughter plant would be expected to show the lowest physiological responses during the pre-slaughter experience, and this expectation is confirmed in the HSP20 measurements. Increasing the travel distances to require overnight transport produced a significant increase in HSP20 expression. Perhaps surprisingly, the sale yards had little effect on HSP expression. However, the sale yards were local to the slaughter-plant and the cattle were also produced locally and travelled only limited distances. One implication is that the HSP20 responds to physical exertion rather than any emotional stress associated with the novel environment.

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