THE EFFECT OF ENDOGENOUS NITRIC OXIDE ON TENDERNESS CHANGES OF MEAT

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INTRODUCTION

Although m-calpain appears to be the main protease responsible for meat tenderisation (Utterhaegen et al, 1994) there are some unexplained aspects such as tenderness variability, the quadratic relationship of tenderness and ultimate pH which appear to affect the rate of tenderisation (Watanabe et al, 1996), partial inactivation of calpains at temperatures above or below 15°C (Simmons et al, 1996) and non enzymic mechanisms (Takahashi et al 1987; Takashi, 1992). Nitric oxide (NO) a gaseous intercellular messenger has recently been proposed in ubiquitous roles, including those within normal functioning skeletal muscle. NO has not, however, been considered as a component of changes in post mortem muscle, yet because of its ubiquitous nature, and its triggering by energy compromisation situations such as anoxia is likely to be involved.

The enzyme responsible for the synthesis of NO, NO synthase (NOS), has been localised in skeletal muscle, concentrated mostly in fast twitch fibres (Kobzik et al, 1994) so would be available for synthesis of NO in muscle. The inhibition of NOS, or the provision of NO enhancers or donors suggests that, physiologically, NO may be involved in muscle relaxation, a role mediated by cyclic GMP. However, under conditions of muscular exertion, NO also may be responsible for muscle damage via modulation of free radical activity. At low levels of NO, and in the presence of superoxides, NO can bind to and inactivate them, and recent work suggests that in uncontracted skeletal muscles, this occurs. However, at higher concentrations of either superoxide or NO, peryoxynitrite is formed which decomposes to extremely reactive free radicals capable of breaking down fibre structure or its integrity.

NO can be modulated by stress, by β-adrenergic activation, shows a strong relationship to calcium levels and its regulation, calmodulin and a number of other enzymes and thus could have an important role in postmortem muscle biochemistry. By modifying changes of the enzymes responsible for meat tenderisation, it could affect meat quality. Because of its transient nature, its effects are best studied by, the effect of the addition of NO enhancers and inhibitors on post mortem muscle.

MATERIALS AND METHODS

Five striploins (*m. longissimus lumborum*), with an ultimate pH 5.5-5.6, to avoid other confounding variables, were obtained within two hours of slaughter, from 24 month-old bulls. These striploins were cut into three cm-long strips, pricked with 20 G needles (to facilitate penetration of solutions) and randomly divided equally into the following test solutions.

These solutions were: (a), saline containing L-arginine (control), (b), saline containing N-nitro-L-arginine and N-nitro-L-argininemethyl ester hydrochloride (NOS inhibitors) or (c), saline containing diethylenetriamine/nitric oxide adduct and S-nitroso-N-acetylpenicillamine (NO enhancers).

All chemicals were obtained from Research Biochemicals International (Natick, MA 011760-2447, USA) and all solutions were made up to 0.1 mol l^{-1} concentrations. The meat samples, placed in each solution at room temperature (18°C) for 15 hours to allow rigor, were completely covered, and were separated from each other and were stored at 2°C for the duration of the experiment.

On day 1, 3, 6 and 8 after rigor mortis, samples were removed for tenderness measurements. The samples were placed in individual Tuflex bags (Trigon Packaging Systems, Hamilton, New Zealand) and cooked in a water bath at 100°C until they reached an internal temperature of 75°C. The cooked samples were immediately placed in an ice-water slurry. From the chilled samples, ten 1 cm x 1 cm samples, approximately 3 cm long were cut parallel to the fibre direction and the force required to shear each portion perpendicular to the meat fibre was measured using a MIRINZ tenderometer and calculated as the mean shear force value in kgF for ten bites. Student t test (2-tail) was used either paired or non paired to follow the day-to-day changes within or between treatment groups respectively.

RESULTS AND DISCUSSION

All treatments (Fig. 1) showed a significant decrease in shearforce values over time. At days 3 and 6, shear force values were significantly ($p <= 0.01$) lower in the NO enhanced group than in other groups, whereas with the NO inhibited group on day 6, the shear force values were significantly higher. As the duration of storage increased further to 6 days, there was no significant difference in shear force values between the control group and the NO enhanced group. With the control group and the NO enhanced group, the shear force values were significantly lower than the NO inhibited group. However at the end of the period of storage at 8 days, the tenderness values tended to become similar and all treatment groups were not ($p <= 0.05$) significantly different.

The experiments suggest that NO can affect the rate of meat tenderisation as the NO enhanced group were more tender than the NO inhibited group in the early stages of tenderisation, but the differences became less with storage duration (eventually all muscles reached the same tenderness). This begets the question of whether in normal preslaughter or post mortem conditions, situations arise that ensure NO is active in altering rates of tenderisation.

The mechanism of NO in tenderisation is not known, but NO can mediate its effects by free radicals and calcium changes which in turn affect proteolytic enzymes. The effects of NO inhibitors however, suggest roles additional to those mediated via free radicals. The initial change in tenderness was lower in control samples compared to samples treated with NO inhibitors and speculatively, this possibly could be attributed to effects on endogenous calpains (Utterhaegen et al, 1994). It may be that NO is needed to trigger calpain action, either directly or through secondary actions. Both NO and calpain are influenced by calcium.

By day 8, when ageing has almost been completed, the shearforce values for all groups were now similar, suggesting that there is tenderisation from other causes. This could arise from a delayed activity, or a removal of inhibition by calpains, or alternatively it could arise from the emergence of a second enzyme system such as cathepsins which are not involved in initial tenderisation (Utterhaegen et al, 1994). Given that penetration into the muscles is unlikely to be complete, the effects of NO on post mortem muscle may be more dramatic than shown here. Effects of inhibitors implies that effects of NO are normally present, but situations that either enhance it or inhibit it need to be identified

Prior to death and during subsequent periods post mortem, low levels of NO seem unlikely as post mortem changes would ensure considerable synthesis. NO elevation also could be a consequence of preslaughter stress. Fast twitch fibres which have the highest NOS activity are extremely prone to stress effects. The nonenzymic tenderisation of meat proposed by Takashi (Takahashi et al 1987; Takashi, 1992) and involving calcium ions could, in part, be explained by the actions of NO. While the mechanisms of action of either NO enhancers or inhibitors in meat tenderisation are not yet clear, their effect may explain the variability in meat tenderness from previously unexplained causes and in particular the role of stressors immediately before slaughter.

Conclusion.

This experiment suggests that endogenous NO could under certain circumstances influence processes involved in meat tenderisation.

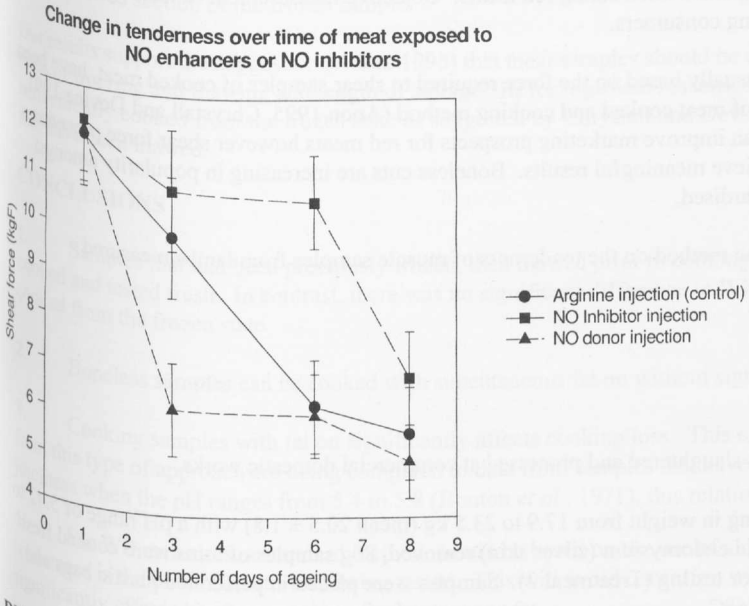

Figure 1. The time course of tenderness changes in meat that has been exposed to saline containing L-arginine (filled circles; control), saline containing N-nitro-L-arginine and N-nitro-L-argininemethyl ester hydrochloride (filled squares; NOS inhibitors) and saline containing diethylenetriamine/nitric oxide adduct and S-nitroso-N-acetylpenicillamine (filled triangles; NO enhancers). The error bars show the standard deviation.

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