The localized elevation of cathepsins B and L in rat gastrocnemius muscle following tenotomy

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Tenotomy of the rat gastrocnemius muscle is commonly employed as a model of accelerated protein breakdown or as a system in which to follow the hypertrophy of the ipsilateral plantarius or soleus muscles [1]. We have studied changes in the activities of cathepsins B and L in rat gastrocnemius muscle over the period following tenotomy when the muscle atrophies.

Individually caged rats (160 g) were tenotomized under ether anaesthesia with a sham operation on the contralateral leg. Rats were killed 1 to 9 days thereafter and the gastrocnemius muscle or muscle segment was homogenized in 19 volumes of ice-cold phosphate buffered saline, pH 6.0 containing 2 mM-EDTA and 2 mM-cysteine, using a Polytron homogenizer at 30% of full speed for 15 s. The homogenate was further diluted and assayed for cathepsins B and L with Z-Phe-Arg-NMeC [2].

A time course of cathepsin B and L specific activities in the whole tenotomized gastrocnemius showed a maximum at day 5 (Fig. 1a). However, this increase in activity was not evenly distributed throughout the muscle. When the gastrocnemius was divided into three segments along its length, the changes in cathepsin activities were confined to the distal portion of the muscle, i.e. adjacent to the severed tendon (Fig. 1b; 3 days after tenotomy). The activity in the distal segment at day 2 was significantly greater than that in the contralateral control segment (data not shown). In contrast, catheptic activity in the proximal segment was similar to that in the comparable segment of the contralateral, sham-operated gastrocnemius at days 1, 2 or 3. The medial segment showed a significant increase in activity over its contralateral control segment only on day 3 (Fig. 1b).

Previously, such changes in enzyme activities have been ascribed to the muscle tissue alone, particularly when a whole muscle has been the source of a preparation. However, the differential changes in cathepsin activities following tenotomy (Fig. 1b) suggest either a localized response within the muscle fibres, a concept difficult to rationalize with the highly ordered structure of muscle, or a contribution from non-muscle cells. This latter view receives some support from the literature.

Sanoff et al. [3] showed by immunolocalization that cathepsins B, H and L were scarcely detectable in normal mouse muscle but all enzymes were readily demonstrated in macrophages resident in that muscle. Cathepsins B, H and L were present in all tissues of the rat but leucocytes and macrophages (from peripheral blood) also had appreciable levels of activity [4]. Significantly, when such macrophages were activated, the cathepsin B activity increased almost 10-fold [4].

That non-muscle cells show a metabolic response to tenotomy was demonstrated by Jablonski et al. [5] in a study of soleus hypertrophy following tenotomy of the synergistic gastrocnemius and plantaris muscles. Autoradiographic detection of RNA synthesis at 12, 24 and 48 h showed that all the activity was associated with the connective tissues (capillaries and fibroblasts) while muscle tissue showed no response.

Macrophages and other cells involved in the inflammatory response are likely to be present at the distal end of the gastrocnemius following surgical tenotomy. Armstrong et al. [6] and Kelso et al. [7], using surgical ablation of the gastrocnemius and soleus muscles in a study of hypertrophy of the synergistic plantaris, showed a short-term response of cells proliferation (at 7 days) that was attributed to inflammation-related leucocytes (mainly macrophages) and proliferating connective tissue cells (mainly fibroblasts). It is well documented that the cellular response to injury involves the sequential invasion of polymorphs, macrophages and fibroblasts, with macrophage infiltration reaching a maximum 3–5 days after injury [8].

It is suggested that the localized elevation of cathepsin B and L activities observed in the distal segments of tenotomized gastrocnemius muscle are caused, at least in part, by contributions of cathepsins from inflammation-related cell proliferation.

Pepsin 5 (gastricsin): atypical pH profile of mucolytic activity

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Eight zones of proteolytic activity have been identified in human gastric juice by agar-gel electrophoresis. Seven of these proteinase zones are pepsins numbered on the basis of electrophoretic mobility, the other zone is a cathepsin [1]. While pepsin 3 is the major pepsin in human gastric juice, pepsin 5 is also present in substantial amounts, being 70.3 ± 2.6% and 16.9 ± 2% (mean ± S.E.M., n = 8) of the total pepsin activity, respectively.

Luminal pepsin is an endogenous mucosal aggressor and the adherent mucus gel barrier is an important component of the gastroduodenal defence mechanisms against its auto-digestion of the mucosa [2]. Mucus gel is effectively a diffusion barrier to luminal pepsins preventing their access to the underlying mucosa. However, the adherent mucus layer is progressively, albeit slowly, penetrated by luminal pepsins as a consequence of mucolysis with the production of soluble degraded mucus. Normally, in vivo secretion of mucus is sufficient to outweigh mucolysis, thereby maintaining a continuous adherent mucus layer and preventing pepsin digestion of the underlying epithelium. However, the presence of excess gastric luminal pepsin in animal models leads to increased mucolysis producing mucosal damage and bleeding [2]. Increased mucolytic activity has been shown in gastric juice from peptic ulcer patients [3]. Previous studies have concentrated on pepsin 1 which has increased mucolytic activity relative to the major pepsin 3 and is elevated in peptic ulcer disease [3]. Here we report the mucolytic properties of pepsin 5.

Pepsin 3 and 5 were obtained from human gastric juice by ion-exchange chromatography on h.p.l.c. [4]. Purified pig gastric mucin was used for the mucolytic studies. Mucin gel scraped from the mucosal surface was solubilized by homogenization in 0.067 M-sodium phosphate buffer pH 6.7 containing a cocktail of proteinase inhibitors [5]. The glycoprotein was separated from contaminant protein by fractionation using Sepharose 4B gel filtration followed by equilibrium centrifugation in a CsCl density gradient. Mucolytic activity of the isolated pepsin 3 and 5 was assessed by measuring the fall in viscosity (η sp = η rel − 1) when incubated with a 5 mg/ml solution of purified mucin over 30 min at 37°C. The mucin solution was buffered over the pH range 1.2–3.5 by 0.1 M-glycine/HCl, 0.05 M-NaCl, 0.01% (w/v) sodium azide and over the pH range 2.2–7.0 by 0.1 M-citrate/sodium phosphate, 0.05 M-NaCl, 0.01% (w/v) sodium azide. The pepsins were incubated with the mucin solution as a 0.5% (w/w) (enzyme/glycoprotein). The viscosity results were expressed as a percentage drop in viscosity per 30 min corrected for any endogenous mucolytic activity and allowing for the residual viscosity of the totally proteolytically degraded subunit. Proteolytic activity was measured using a modification of the method of Lin et al. [6] using succinyl albumin as substrate.

Human pepsin 5 has a proteolytic activity profile on albumin substrate similar to that for human pepsin 3, in that it had a maximum activity below pH 2.5 with negligible activity above pH 3.5. In contrast the mucolytic activity of pepsin 5 for gastric mucin substrate was substantial over the pH range 1.5–5. The mucolytic activity (mucin substrate) of pepsin 3 closely mirrored its proteolytic activity (albumin substrate) and was minimal above pH 3.5. At pH 2.4, pepsin 5 had two-thirds the mucolytic activity of pepsin 3 (21% and 31% drop in specific viscosity over 30 min, respectively). At pH 4, pepsin 5 had 3.5 times the mucolytic activity of pepsin 3 (24% and 7% drop in gastric mucin specific viscosity over 30 min, respectively). Pepsin 5 demonstrated two peaks of mucolytic activity one at low pH between pH 1.7–2.5 and the other at higher pH between 3.5–4.3. Analysis of pepsin 5 showed it (1) to be a single peak when rechromatographed on h.p.l.c. (2) a single band on PAGE in SDS (M, 32,000), and (3) a single spot on agar-gel electrophoresis. Pepsin 5, like pepsin 3, contained no detectable carbohydrate. In contrast pepsin 1 had variable amounts of carbohydrate (42–58%). These results show that pepsin 5 has considerable mucolytic activity above pH 3.5. Therefore the mucolytic activity due to pepsin 5 in vivo will be present at the lower levels of pH attained in the duodenal bulb (down to pH 4), as well as over most of the pH range occurring in the stomach.

Therefore pepsin 5, in common with pepsin 1 (the ulcer-associated pepsin) [7], has the ability to degrade the mucus barrier at pH values of 4 and above. However pepsin 1 and 5 are different gene products and differ in molecular size and composition.

The levels of pepsin 5 in peptic ulcer disease compared to non-ulcer controls have not been investigated. However on the basis of the results reported here pepsin 5 in vivo may be an important factor in mucosal damage in disease.