Multicatalytic proteinase activity in skeletal muscle from starving rat

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While intracellular protein catabolism occurs within all cell compartments, the steps proceeding extralysosomally may be rate limiting for the degradation of many proteins (Kay, 1978; Mayer & Doherty, 1986; Beynon & Bond, 1986). From skeletal muscle tissue, we have isolated a non-lysosomal, high-molecular mass proteinase that has been designated 'multicatalytic proteinase' (MCP), since its activities are differently or even antagonistically affected by various inhibitors (Dahlmann et al., 1985a). This neutral proteinase has been identified in a variety of rat tissues (Kuehn et al., 1986). A characteristic feature of the purified enzyme is its latent state of activity that is considerably stimulated by long-chain free fatty acids (Dahlmann et al., 1985b). The serum-concentration of free fatty acids rises severalfold during starvation and diabetes mellitus (Goodman et al., 1980; Starsins et al., 1979) and increased levels of free fatty acids have been measured within skeletal muscle tissue under these insulin-deficient conditions (Garland & Randle, 1963, 1964). We therefore investigated whether hyperlipidaemia resulting from prolonged starvation affects the activities of the MCP in skeletal muscle tissue.

From a group of 24 male rats with a body wt. of 121 ± 2 g, eight animals were killed and their gastrocnemius muscles were analysed (Kuehn et al., 1986). The remaining 16 rats were restricted in their food intake but received water ‘ad libitum’. At each of the following 4 days, a group of four rats was killed and their gastrocnemius muscles were analysed as specified above. Within the period of starvation the body wt. of the animals significantly decreased to 85.8 ± 4.2 g (day 4) and the wet wt. of the pooled pair of gastrocnemius muscles fell from 1282 ± 50 mg at day 0 of the experimental period to 1080 ± 74 mg at day 4. Total protein contents in the muscle extract at day 0 and day 4 were 33.8 ± 2 and 29.5 ± 1.5 mg, respectively; values that are statistically not significantly different.

As shown in Table 1, a significant decrease was observed in the overall activity catalysing the hydrolysis of Bz-Val-Gly-Arg-NMec and Suc-Ala-Ala-Phe-NMec, two substrates that are split by various muscle proteinases including MCP. Since the total amount of immunoprecipitable MCP in gastrocnemius muscle, as quantified by rocket immunoelectrophoresis, remained constant during the experimental period, we tested whether these immunodetectable MCP molecules were responsible for the drop in total proteolytic activities against the peptide substrates. This was done by immunoinhibition tests. As shown in Table 1, the amount of anti-(MCP)-IgG required for a 50% inhibition of the proteolytic activities, that is, hydrolysis of 1 pmol/min of peptide substrate, was not significantly different in muscle extracts of fasted rats when compared to those of fed rats. Thus, the loss of total activities hydrolysing the substrates Suc-Ala-Ala-Phe-NMec and Bz-Val-Gly-Arg-NMec is not due to alterations in the activities of the MCP, but rather results from changes of other proteolytic enzymes from the muscle extract.

In conclusion, the amount and activity of muscle MCP is not affected by starvation-induced hyperlipidaemia.

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Abbreviations used: MCP, multicatalytic proteinase; NMec, 4-methyl-7-coumarylamide.

Table 1. Multicatalytic proteinase α-gastrocnemius muscle from starving rats

<table>
<thead>
<tr>
<th>Day of last</th>
<th>MCP protein (µg)</th>
<th>Total activity (pmol/min)</th>
<th>Immunoinhibition of MCP activity (ng IgG/pmol - min)</th>
<th>Total activity (pmol/min)</th>
<th>Immunoinhibition of MCP activity (ng IgG/pmol - min)</th>
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</thead>
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<tr>
<td>0</td>
<td>26.3 ± 1.0</td>
<td>351 ± 58</td>
<td>388 ± 49</td>
<td>316 ± 45</td>
<td>230 ± 23</td>
</tr>
<tr>
<td></td>
<td>26.7 ± 1.8</td>
<td>167 ± 28</td>
<td>592 ± 46</td>
<td>166 ± 31</td>
<td>314 ± 65</td>
</tr>
<tr>
<td>(N.S.)</td>
<td>(&lt; 0.02)</td>
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<tr>
<td>2</td>
<td>27.5 ± 1.6</td>
<td>194 ± 20</td>
<td>549 ± 111</td>
<td>177 ± 18</td>
<td>320 ± 32</td>
</tr>
<tr>
<td>(N.S.)</td>
<td>(&lt; 0.05)</td>
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<tr>
<td>3</td>
<td>27.8 ± 0.8</td>
<td>180 ± 22</td>
<td>551 ± 87</td>
<td>189 ± 18</td>
<td>314 ± 10</td>
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<td>(N.S.)</td>
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<tr>
<td>4</td>
<td>27.7 ± 2.1</td>
<td>161 ± 8</td>
<td>540 ± 68</td>
<td>173 ± 22</td>
<td>218 ± 41</td>
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<td>(&lt; 0.02)</td>
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Convergence of autophagic and endocytic pathways at the level of the lysosome

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Introduction
Radioactive sucrose may be readily introduced into the cytosol of isolated rat hepatocytes by means of electropermeabilization (Gordon & Seglen, 1982; Seglen & Gordon, 1984; Gordon & Seglen, 1986). A significant portion of the electroinjected [14C]sucrose eventually accumulates in lysosomes having entered them via autophagy, i.e. the way in which intracellular substances destined for autophagic lysosomal degradation are initially sequestered by membranous entities termed phagophores (Seglen et al., 1985) into non-digestive vesicles (autophagosomes) and later delivered to lysosomes for digestion, by a process involving fusion between these two types of vesicle.

It is well established that certain substances enter the lysosomal compartment as a result of heterophagy, i.e. the process by which extracellular substances are taken up into the cell via endosomes which eventually deliver their contents to lysosomes, probably after fusing with them.

The question has arisen whether there exist separate populations of lysosomes whose function is to receive material from these distinct pathways or whether there is a common population of lysosomes capable of receiving material from both pathways. We investigated this question by following the fate of autophagically sequestered sucrose in hepatocytes which were allowed to endocytose the sucrose-degrading enzyme invertase.

Results and discussion
The net accumulation of autophagically sequestered sucrose was practically abolished in the presence of invertase (Fig. 1). The very small amount of sucrose accumulating above the background may possibly represent a steady-state level of sequestered sucrose present in autophagosomes in transit to the lysosomes. It should be noted that the experiment shown in Fig. 1 does not display the initial, transient accumulation of sequestered sucrose previously demonstrated in the presence of invertase (Seglen et al., 1986), probably because of the higher concentration of enzyme presently used.

The fact that no net accumulation of sucrose occurred beyond 20 min indicated that all autophagically sequestered sucrose eventually reached invertase-containing lysosomes, in other words that a complete intermixing of autophagically and endocytically delivered material occurred. However, we needed to exclude the possibility that invertase might affect the autophagic process per se. Hepatocytes were therefore allowed to autophagically sequester the radioactive sugar [14C]lactose (which is degraded within lysosomes) under conditions in which it could not reach the lysosomes, i.e. in the presence of vinblastine (0.05 mm). During such experiments, the sequestered [14C]lactose accumulated in pre-lysosomal vesicles (autophagosomes) at exactly the same rate in the absence or presence of invertase, showing that the enzyme did not interfere with autophagy per se.

To see whether the autophagic and endocytic pathways converged at the lysosomal level or pre-lysosomally, the lysosomes were loaded autophagically with [14C]sucrose, and then the autophagic pathway was shut off before the cells were allowed to endocytose invertase. This was achieved by allowing the hepatocytes to sequester sucrose for 1 h before their autophagic activity was blocked using the specific inhibitor 3-methyladenine (Seglen & Gordon, 1982). As shown in Fig. 2, 3-methyladenine stopped all further sequestration of sucrose, and the pre-sequestered radioactivity (known to reside in lysosomes) remained at a constant level in the absence of invertase. In the presence of invertase, on the other hand, the remaining amount of pre-sequestered sucrose fell gradually until all radioactivity had disappeared. This latter result would tend to indicate that the endocytic pathway delivers invertase directly to the lysosome, independently of whether the autophagic pathway is open or shut (active or inactive). Thus, autophagic-endocytic rendezvous would seem to take place at the level of the lysosome.

In view of the fact that our data point to a rather complete

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Abbreviations used: 3-MA, 3-methyladenine; INV, invertase.