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Muscle proteinase activities during compensatory growth and atrophy

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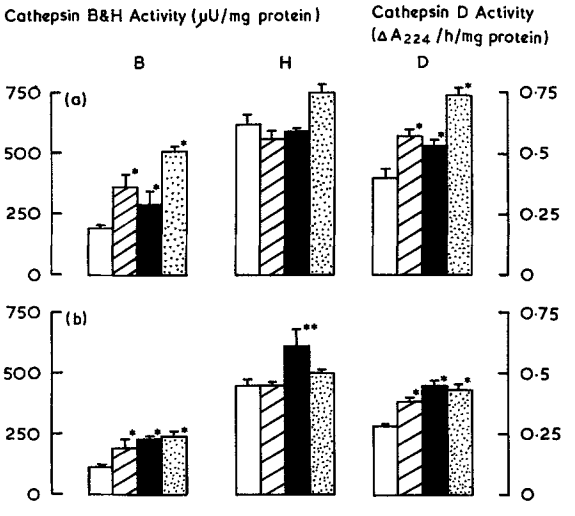
Summary. Specific activities of cathepsins B and D, but not H, increased in both the tenotomized gastrocnemius and functionally overloaded soleus muscles, thus correlating with previously reported increases in protein degradation. Subsequent denervation of the overloaded soleus caused an additive increase in proteolysis, suggesting a possible greater lability of proteins in this muscle.
Key words. Cathepsin B, D and H; skeletal muscle; tenotomy; denervation; protein degradation; compensatory growth; muscle atrophy.

Alterations in work demands are known to induce appreciable changes in the size of skeletal muscle(s); reduced activity generally leads to atrophy while increased functional demands evoke compensatory growth[1]. This plasticity of muscle clearly offers selective advantages to the organism, within a framework of biological efficiency and economy. Tenotomy of a lower hind limb muscle (e.g. gastrocnemius) is an experimental model which has been widely used, because it simultaneously induces muscle wasting and adaptive growth; the former is induced in the tenotomized muscle itself, while the latter arises in functional synergists (e.g. soleus[2]) which are left to perform proportionately more work if flexing the ankle. This form of compensatory growth has been extensively studied, with changes in fiber type proportions, metabolic enzymes and polymorphic forms of contractile proteins having been described[2-4]. In attempting to explain how the additional growth occurs good agreement exists, from a variety of approaches (both in vivo[2,5] and in vitro[4]), that protein synthesis is increased in such functionally overloaded muscles. The role played by protein degradation is however much less clear. Few studies have been undertaken in this area and the available information is contradictory; one study[2] has suggested a decrease in the degradative rates while another[5] has indicated an increase. These discrepencies clearly need to be resolved.

Surprisingly, much less overall information is available concerning the time related changes in the tenotomized muscle. Although it has only been possible to obtain indirect (i.e. calculated) rates of protein breakdown in this muscle[5], it has been suggested that increased proteolysis, rather than any appreciable change in protein synthesis, is primarily responsible for the observed muscle atrophy[5]. It is therefore important to establish precisely what role protein degradation plays in influencing muscle size in both experimental situations, for as yet this remains uncertain.

Results and discussion. The aim of this study is to resolve these uncertainties by measuring the activities of three proteinases believed to be involved in the catabolism of intracellular proteins. Two of these enzyme activities, i.e. cathepsin B and D, have recently been shown to change in accord with induced alterations in the rate of muscle protein degradation[6]. Such a correlation is further supported in the present study. That is, higher proteinase activities were found in the control soleus (fig. a), compared with the gastrocnemius (fig. b), which is in keeping with the higher rate of protein turnover in the former muscle[7,9]. In addition, the specific activities of both cathepsins B and D, were found to increase significantly in both the tenotomized gastrocnemius (fig. b) and the overloaded soleus (fig. a). In contrast, cathepsin H remained remarkably resistant to change.



Changes in cathepsin B, D and H activities in the soleus and gastrocnemius muscles after various experimental procedures. Total activities of cathepsin B, H and D were measured in homogenates (1:50 H_2O, w/v) of soleus (a) or gastrocnemius (b) muscles isolated from 55 g Charles River (CD strain) male rats, either 3 or 6 days after various operative procedures had been performed. Cathepsin D was assayed against denatured hemoglobin, at pH 3.5; activity is expressed (on right axis) as the change in absorbance of perchloric acid-soluble materials when measured at 224 nm[7]. Cathepsins B and H were measured against the synthetic substrates Z-Phe-Arg-NMec and Arg-NMe, at pH 6.0 and 6.8 respectively[8]; in both cases one unit of activity (left axis) represents the amount of enzyme capable of releasing 1 μmol of aminomethylcoumarin/min. Each value is the mean ± SEM of at least 5 muscles. Student's t-test was used to determine the level of statistical significance (* $p < 0.01$; ** $p < 0.025$), in all cases being compared to control muscle values of contralateral, sham-operated limbs. Soleus muscles (a) are denoted as control (□), 3-day functionally overloaded (▨), 3-day denervated (■), or 3-day overload followed by 3 further days of denervation (▩). Gastrocnemius muscles (b) are denoted as control (□), 3-day tenotomized (▨), 3-day denervated (■) or 3-day tenotomized followed by 3 additional days denervation (▩).

Despite the earlier report[2] to the contrary the increases in cathepsin B and D (fig. a), together with other lysosomal enzymes[10], in the functionally overloaded soleus would appear to support the view that protein degradation is accelerated[5]. Hence this form of compensatory growth involves an increase in both the protein synthetic and degradative rates[5]. However, since the former rate exceeds the latter, the net effect must be growth. The similarity of these changes in both functionally overloaded and immobilized (stretched[11]) muscles serves to strengthen the concept that passive stretch may be a common factor promoting growth in both experimental systems[5,12].

Previous studies[5,13] have also suggested that the newly synthesized proteins in the overloaded soleus are of a more 'labile' nature and are therefore more susceptible to degradation when this tissue is suddenly rendered inactive e.g. after nerve section. Denervation alone certainly causes cathepsin B and D activities to increase (fig. a). This is in agreement with other earlier studies[7] and previously reported increases in protein breakdown[14]. However, when inactivity was imposed upon the formerly overloaded muscle by denervation a roughly additive increase in proteolysis was observed (fig. a). Once again this supports the earlier observations of an additional increase in protein degradation[5], and possible greater lability of proteins[5,13], when this form of compensatory growth is arrested by denervation.

Denervation alone also increased the proteinase activities in the gastrocnemius (fig. b). However no additive effect was found in this muscle when tenotomy and denervation were combined. The latter is not particularly surprising since, unlike the overloaded soleus, there is less reason to predict any change in the lability of the proteins still being synthesized in a wasting tenotomized muscle.

Throughout the above studies the induced alterations in cathepsins B and D consistently changed in parallel with reported changes in the rates of protein breakdown under identical experimental situations[5]. On the basis of these (fig.), and our earlier[6], findings a good correlation appears to exist in skeletal muscle between these two endopeptidases and protein degradative rates. Interestingly here (fig.), as in previous studies[6], cathepsin H activity appeared very resistant to change. It is not immediately obvious why this should be, especially since all three proteinases are probably lysosomal in origin. The failure of this enzyme to respond in like manner may not become apparent until we obtain a better understanding of the mechanical and chemical events which link the changes in muscle activity and protein turnover.

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Inbreeding considerations in a REM sleep model for rat swimming activity

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Summary. Over the past few years, our laboratory group has elaborated a repeated measures rat swimming test. It provides an animal base for showing that the REM sleep mechanism is important to both emotional responsiveness and environmental adaptations. All of that work has been done with Sprague-Dawley rats obtained from a local supplier. Work done with two European rat stocks (by researchers in France and The Netherlands) shows general agreement with our own. In this presentation, we directly compare rats derived from an English vendor's Sprague-Dawley stock with the U.S. based Sprague-Dawley stock which we have been using. We also make strain comparisons via the F344 and the Long Evans strains. Although the literature has numerous examples of swimming test differences between inbred and wild rat stocks, strain difference effects have not been reported. We report that there are significant differences attributable to inbred strain but not to vendor on this measure.
Key words. Adaptation; animal models; animal vendor effects; evolutionary mechanisms; rat strain effects; rat swimming; sleep.

We have previously presented a serial, week-long, procedure known as the rat swimming test[2]. The present report is based on the key outcome from that protocol: After an initial period of vigorous swimming activity, rats in a swimming cylinder gradually learn to adopt a characteristic posture. An experienced rat simply lies quietly with only its eyes and nose unsubmerged in the water. This postural stillness is known as swimming immobility[3,4]. Since it conserves energy, we consider this behavioral change to be an adaptive response to a stressful situation[4]. Of theoretical interest is the finding that rats which have been REM sleep deprived, do not show improvement with regard to such immobility[2]. Instead, REM sleep deprived rats become progressively more active with succeding days of REM sleep deprivation treatment. The effect is reversable. Just 24 h after undisturbed sleep is allowed, the REM sleep deprived animals also show a high level of the immobility response. It is important to note that the procedure does not affect all of an animal's behavioral repertoire. Two concomitant measures (diving and headshaking) show no changes with REM sleep deprivation treatment. We conclude, therefore, that rat swimming immobility is a behavior which is selectively affected by REM sleep deprivation[2].

Long term REM status is important to such adaptive responsiveness. We infer this from ontogenetic work involving environmental enrichment[5,6]. A number of polygraphic studies have compared rats or mice which had been raised in enriched as compared with impoverished laboratory environments[7-9]. The results were mutually supportive: In development, the enriched