**Protein Turnover in Skeletal Muscle**

**II. EFFECTS OF DENERVATION AND CORTISONE ON PROTEIN CATABOLISM IN SKELETAL MUSCLE***

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**SUMMARY**

To determine the mechanism by which denervation leads to atrophy of skeletal muscle, the fate of previously labeled protein was studied in denervated and contralateral control muscles of hypophysectomized rats. To label muscle proteins, a single injection of $^3$H-leucine was given to the animals 2 days prior to section of the sciatic nerve. On subsequent days, the animals received unlabeled leucine and a high protein diet. Protein degradative rates were estimated from the disappearance of radioactivity from muscle proteins. Protein synthetic rates were estimated from the decrease in specific activity of these proteins, caused by the dilution of labeled proteins with newly synthesized (unlabeled) material.

Ten days after denervation, atrophic muscles contained less total radioactivity than contralateral controls. This result suggests increased protein degradation. Nevertheless, the specific activity of muscle proteins remained unchanged. Protein synthesis therefore must also have decreased following nerve section. The loss of muscle weight following denervation appeared to be directly proportional to the increase in protein degradation. The increase in catabolism of myofibrillar proteins was especially marked, and this finding accounts for the relative loss of myofibrillar material in the denervated muscles.

Analogous experiments were carried out to determine the mechanisms through which cortisone induces muscle atrophy. Two days after injection of labeled leucine, animals received daily injections of cortisone acetate (10 mg per day) or 0.9% sodium chloride solution. Cortisone caused marked atrophy of the plantaris muscle. Although the hormone decreased appreciably the amount of labeled proteins per muscle, it did not alter their specific activity. Thus cortisone both increased protein degradation and decreased protein synthesis. In contrast to denervation atrophy, cortisone-atrophy affected the breakdown of myofibrillar and soluble proteins similarly. Cortisone did not cause appreciable loss of weight of the soleus muscle, in which the hormone did not significantly increase protein degradation. However, upon denervation the soleus became susceptible to cortisone-induced atrophy, and in the denervated soleus cortisone further accelerated protein catabolism and reduced protein synthesis.

In contrast to the widespread interest in cellular growth mechanisms, there has been relatively little experimental study of the related process of tissue atrophy. Regression in the size of many organs occurs normally during development, while in the adult organism decrease in organ size is a reversible response to a variety of physiological stimuli, including organ disuse, starvation, or specific hormones. For example, denervation or simple disuse of skeletal muscle leads to marked atrophy. In addition, muscle wasting occurs in response to large doses of adrenal steroids, as is found in Cushing's syndrome.

For atrophy of any tissue to occur, protein catabolism must exceed protein synthesis. Thus a decrease in tissue protein theoretically could occur either by decreased protein synthesis, increased protein catabolism, or by some combination of the two. The present experiments were undertaken to determine which of these mechanisms is responsible for the atrophy of muscle which results from denervation and from treatment with cortisone.

Although numerous studies of denervated muscle have been carried out, there is little agreement on the cellular mechanisms underlying denervation atrophy. Several groups have suggested that protein catabolism is increased following denervation, although the supporting evidence has been largely indirect and difficult to interpret. In addition, there is disagreement as to whether protein synthesis is increased, decreased, or unchanged following nerve section. Denervation atrophy and work-induced growth of muscle may be closely related processes and may even represent a single reversible mechanism through which physiological demand influences muscle size. Earlier studies in this laboratory demonstrated that work-induced hypertrophy proceeds through increased protein synthesis and decreased protein degradation. The present experiments have utilized similar techniques to determine how synthetic and degradative rates in muscle are altered during denervation atrophy.

Analogous studies have also been carried out to examine the mechanism of muscle wasting in response to cortisone. The stimulation of net catabolism in muscle by the glucocorticoids...
serves physiologically to make amino acids available to the liver for gluconeogenesis (13-15). In promoting the net release of amino acids from muscle (16, 17), cortisone both inhibits the transport of amino acids into muscle (18) and their subsequent incorporation into protein (19, 20). In addition, it has frequently been assumed that glucocorticoids accelerate protein catabolism in muscle (14, 17), although hormonal effects on the degradative process are poorly documented. The early tracer studies of Hoberman (21) on intact rats suggested that cortisone both decreased protein synthesis and increased catabolism in muscle. In addition, the rapidity with which muscles can lose weight in response to adrenal steroids suggests that cortisone accelerates average rates of protein degradation in this tissue (3).

The present studies examined directly the effects of cortisone on catabolism of sarcoplasmic and myofibrillar proteins in different skeletal muscles of the rat.

MATERIALS AND METHODS

Hypophysectomized rats (96 to 115 g) were used in all studies in order to study the effect of hormones or nerve section independent of body growth (3, 22). Protein catabolism in the

![Graphs](Images)

**Fig. 1.** Effects of denervation on labeled proteins in soleus muscle. Values are the mean ± standard error obtained in seven animals 10 days after denervation. The findings with the denervated muscle are also expressed as percentage of contralateral innervated muscle (i.e. mean % ± S.E.M.). The decrease in muscle weight and in total counts per min per muscle following denervation are significant at p < 0.01, and indicate increased protein catabolism during denervation atrophy. No change in specific activity occurred (p > 0.1) suggesting decreased protein synthesis following denervation.

**Fig. 2.** Effects of denervation on labeled soluble and myofibrillar protein in plantaris. Values are the mean ± standard error obtained in the same seven animals studied in Fig. 1. The decrease in muscle weight, in radioactivity per muscle, and in the ratio of myofibrillar radioactivity to soluble radioactivity in the denervated muscle are all significant at p < 0.01. This data indicates increased protein catabolism with disproportionate loss of myofibrillar proteins following denervation.

soleus and plantaris muscles was studied by methods similar to those in the preceding article (12). Rats were injected initially with 25 μCi of [3H]-leucine to label muscle proteins and on subsequent days received subcutaneous injections of unlabeled leucine and were fed a high protein diet.

In experiments on denervation atrophy, the disappearance of labeled proteins was compared in contralateral denervated and innervated muscles. Two days after injection of the radioactive amino acid, the sciatic nerve on one limb was sectioned and a sham operation was performed on the contralateral side (3). At various times after denervation, the animals were killed, and the muscles were excised. Statistical comparisons of the contralateral muscles employed the method of paired analysis (23).

In studies of the effects of hormone therapy, rats were paired initially on the basis of weight, and received daily injections of cortisone acetate (100 mg per kg, Merck) or 0.9% sodium chloride solution. The animals were killed 7 or 10 days later.

RESULTS

Denervation Atrophy—Since the contralateral muscles contained equal amounts of radioactive proteins at the time of nerve section (12), the total amount of label in proteins retained by the denervated and control muscles at later times should re-
Fig. 3. Relationship of the loss of weight to the loss of labeled protein in denervated muscles. The animals were sacrificed 5, 10, or 15 days after nerve section. The solid line was drawn with a slope of -1.0 and does not differ from the best fit to this data, by the method of least squares. Also included are comparisons of contralateral denervated and innervated muscles in animals treated with growth hormone (1 mg per rat per day) or cortisone (10 mg per rat per day), both of which influence the absolute rates of denervation atrophy (3).
TABLE I
Effects of denervation on proteins labeled at different

times prior to denervation

<table>
<thead>
<tr>
<th></th>
<th>Weight denervated (muscle per control X 100)</th>
<th>Total labeled proteins (counts per min in denervated muscle per counts per min in control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% atrophy</td>
<td>%</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 days after 4H-leucine</td>
<td>67 ± 3</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>22 days after 4H-leucine</td>
<td>70 ± 2</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>Plantaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 days after 4H-leucine</td>
<td>70 ± 2</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>22 days after 4H-leucine</td>
<td>73 ± 4</td>
<td>73 ± 4</td>
</tr>
</tbody>
</table>

Fig. 4. Effects of cortisone on labeled proteins in plantaris. Each value is the mean ± standard error of eight animals after 7 days treatment with cortisone. The decrease in muscle size and total radioactivity are significant at p < 0.01, indicating increased protein catabolism in response to cortisone. The lack of change in the specific activity of either fraction (p > 0.1), further suggests decreased protein synthesis.

12 days increased the weight loss of the plantaris to approximately 50% and also caused greater loss of radioactivity from the muscle. Thus, as seen above with denervation atrophy (Fig. 3), the extent of loss of labeled proteins appears to be related to the loss of muscle weight. Unlike denervation (Fig. 2), however, cortisone increased catabolic rates of soluble and myofibrillar proteins to similar extents (Fig. 4). In the plantaris muscles as shown in Fig. 4, the ratio of soluble to myofibrillar radioactivity in control muscles was 24 ± 0.18, while in the cortisone-treated rats this ratio was 25 ± 0.16. Even after longer treatment with hormone, this ratio did not differ from that in controls. These findings suggest that average rate constants for degradation of soluble and myofibrillar proteins increased by the same amount.

Despite the increase in catabolism of previously labeled proteins in the plantaris in response to cortisone, the specific activity of soluble and myofibrillar proteins (counts per min per mg) (Fig. 4) did not change significantly. If protein synthesis were unaltered by cortisone, the specific activity of proteins in the hormone-treated animals should be significantly lower than that in the controls as a result of the more rapid loss of labeled material. The finding of no change in specific activity therefore indicates that protein synthesis must also have decreased in response to cortisone treatment.

Earlier studies indicated that the soleus muscle of the rat was relatively resistant to cortisone-induced atrophy (3). In response to cortisone, the soleus of the same animals studied in Fig. 4 decreased in size by only about 10% (Fig. 5). In the soleus, in contrast to the plantaris, cortisone failed to reduce significantly the amount of labeled proteins (p > 0.05) or to change their specific activity (p > 0.05). Treatment with cortisone for longer periods (12 days) did not cause further atrophy of the soleus and did not significantly increase catabolism of labeled proteins. Thus the resistance of the soleus to

Fig. 5. Effects of cortisone on labeled proteins in soleus. Each value is the mean ± standard error of eight animals (the same ones studied in Fig. 4). The decrease in size of the soleus was significant at p < 0.05. Total radioactivity and specific activity of the fractions of cortisone-treated and control muscles were not significantly different (p > 0.05).
cortisone-induced wasting is correlated with a failure of the hormone to increase protein catabolism in this muscle.

Earlier experiments also showed that denervation increased sensitivity of muscles to cortisone-induced wasting. Fig. 6 compares the loss of labeled proteins during denervation atrophy in control and cortisone-treated rats. The denervated soleus of the hormone-treated animals atrophied more and lost more of the labeled proteins than the denervated muscle of controls. Even though the denervated muscles of cortisone-treated rats contained much less total radioactivity, the specific activity of proteins was not lower than that in contralateral muscles. Thus cortisone must have also further reduced the rates of synthesis of unlabelled proteins in the denervated muscle.

**Discussion**

These experiments have shown that the loss of muscle weight following denervation or treatment with cortisone result both from increased protein catabolism and decreased protein synthesis. Thus both processes of muscle atrophy appear, at least formally, to be opposite to work-induced hypertrophy, in which protein synthesis is increased, while protein degradation is decreased (12). The importance of this increase in catabolism for the process of denervation atrophy is evident in Fig. 3, which shows that the increased degradation of labeled proteins is directly proportional to the decrease in muscle weight. In cortisone-induced atrophy, the loss of labeled proteins from the muscle was also related to the extent of muscle wasting. In response to cortisone, the plantaris underwent marked atrophy and a marked acceleration of protein catabolism (Fig. 4). The soleus, on the other hand, did not show appreciable loss of weight or a significant increase in catabolism (Fig. 5). Denervation of the soleus made it susceptible to cortisone-induced atrophy and to a further acceleration of catabolic rates (Fig. 6). Together these findings clearly emphasize the importance of protein catabolism in the regulation of organ size.

Greater protein catabolism following denervation of muscle has also been indicated by the recent studies of Pearlstein and Kohn (5), Paton and Kohn (8), and Dreyfus (4). However, the present conclusion that protein synthesis is also reduced in denervated muscle contradicts earlier conclusions based upon measurements of amino acid incorporation into protein (1, 8, 10). Because denervation profoundly alters the transport of amino acids (11), the validity of such measurements of protein synthetic activity is questionable. Although indirect, the present evidence circumvents such difficulties inherent in studies of amino acid incorporation.

The extent of the decrease in protein synthesis in the denervated muscle appears somehow related to the net increase in protein catabolism. As shown in Fig. 1, the specific activity of labeled proteins in denervated and control muscles did not differ, even though the total amount of radioactivity differed appreciably. The lack of scatter in Fig. 3 further indicates this relationship obtained at different stages of denervation atrophy. In response to cortisone protein synthesis was also found to decrease whenever protein catabolism was accelerated (Figs. 4 to 6). The reduction in synthetic rates in both types of atrophy may result either from some feedback system coordinating synthetic and degradative processes or from accelerated degradation of some protein (e.g. a ribosomal protein) required for protein synthesis. Either mechanism would ensure that the changes in protein breakdown and synthesis are synergistic in promoting loss of weight.

 Normally protein turnover in muscle is a relatively slow process (0, 12, 25, 26). Therefore only by accelerating protein degradation in muscle, can cortisone cause the rapid mobilisation of protein reserves for gluconeogenesis (3). Whatever mechanism is involved in this mobilisation of amino acids from muscle, the net loss of muscle protein may have significant effects on muscle composition and function. For example, if cortisone only blocked protein synthesis, there would occur a selective decrease in those proteins with very short half-lives and a relative increase in the more stable components (e.g. actin and myosin). It would appear important to the organism that the loss of protein caused by glucocorticoids affect the physiological capacity of the muscle as little as possible. As shown in Fig. 4, cortisone did not change the relative proportions of labeled soluble and myofibrillar proteins. Acceleration of catabolism in this manner would result in greater absolute loss of myofibrillar than soluble proteins since the former are more abundant, but would not by itself affect the composition of the muscle fibers.

In denervation atrophy, on the other hand, myofibrillar proteins appear to be degraded selectively. More pronounced catabolism of this fraction would be expected to cause an increase in the proportion of soluble proteins in the denervated muscle. This result has been reported frequently (27-29). Thus, although cortisone and denervation both lead to increased catabolism, each affects different fractions of the cell in a characteristic manner. The rates of degradation of soluble and myo-
fibrillar proteins also were found to be altered in distinct manners, during work-induced hypertrophy (12). Together these findings make it likely that catabolism of these cellular fractions are regulated independently. Intracellular degradation must therefore be a precisely controlled and highly specific process.

Little is known at present about the turnover of subcellular components such as myofibrils, although it appears likely that the virtually crystalline array of myofilaments are laid down and degraded in some ordered fashion. For example, in the electron microscope, the myofilaments of denervated muscles appear to decrease in diameter by progressive loss of peripheral myofilaments (30, 31). However, the present observation that radioactive proteins were lost by the denervated muscle at similar rates as total protein (Fig. 3) suggests that muscle proteins are degraded randomly. This view is further supported by the similar results obtained when the muscle proteins were labeled 2 or 12 days prior to denervation (Table I). Schapira, Dreyfus, and Kruh (9, 24) have claimed that myosin is not lost by exponential decay but instead has a definite life-span like that of the red cell. The experimental evidence cited in support of this conclusion, however, is insufficient to distinguish between a long half-life and a specific life-span. In addition, it appears likely that the data of Dreyfus et al. (9, 24) reflect appreciable recycling of labeled amino acids into myosin. When these workers performed similar studies on animals receiving a high protein diet (9, 24), myosin was found to decay more rapidly with apparently first order kinetics. Although widely quoted, the conclusion of a life-span for myosin has not been confirmed by others, who have, however, reported long half-lives for these contractile proteins (25, 26). The present findings with denervated muscle (Fig. 3 and Table I) further suggest that the insoluble proteins are degraded with first order kinetics like other cellular proteins.

The biochemical systems responsible for protein turnover in cells are completely unknown at present. It is hoped that the finding of experimental conditions during which protein catabolism in specific muscles is accelerated may serve as a useful tool in the identification of these enzymes. The best studied hydrolytic systems in cells are the lysosomes (32, 33) which have been shown to be important in digestion of phagocytized material as well as in the "autophagic" destruction of intracellular components in viable cells (34). Several groups have observed increased cathepsins and other hydrolytic enzymes of lysosomal origin (35-39) in extracts of denervated muscle and therefore suggested an increase in protein degradation. Increased lysosomal enzymes in muscle extracts have also been reported in genetic muscular dystrophy and in muscles wasting because of vitamin E deficiency (39). In these conditions, it was suggested that the lysosomes originate not in the muscle fibers but in the invasive macrophages (37). Although acid hydrolases are normally present in muscle extracts (40), lysosomes cannot be demonstrated morphologically in normal muscle fibers but appear to be localized within the connective tissue cells of the muscle (41). In the fibers of denervated muscle, Pellegrino and Franzini (41) observed vesicular structures resembling lysosomes. Unfortunately in this study, histochemical identification was not attempted. With the light microscope, Romanul and Hogan (42) observed increased amounts of acid phosphatase, a lysosomal enzyme, within the fibers of denervated muscle. Thus the increase in protein degradation following denervation may result from release of lysosomal enzymes, although these hydrolases may also serve functions other than intracellular catabolism in the muscle cells (e.g. in phagocytic reactions of the macrophages or in the dissolution of peripheral nerves).

Treatment with cortisone however does not increase the activity of the acid hydrolases in skeletal muscles (43). On the contrary, these enzymes are increased in muscle extracts immediately following adrenalectomy (44), when some muscle growth occurs (19). This finding is in accord with results on other tissues, in which cortisone appears to protect against the rupture of the lysosomal membrane and release of hydrolytic enzymes (45). It thus appears quite unlikely that lysosomes play a role in cortisone-induced atrophy. Other peptidases and proteolytic enzymes have been identified in muscle, but are poorly characterized (46, 47). In diaphragm muscle, dinitrophenylated amino peptidase activities increase in response to cortisone (48), although the physiological significance of this interesting observation is not clear. Although further studies are necessary, these observations suggest that the increased protein catabolism induced by denervation and by cortisone may be mediated by different proteolytic systems.

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