INCREASED ACTIVITY OF INTRAMUSCULAR PROTEASES IN THE HYPERTHYROID STATE

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1. Introduction

Hyperthyroidism can induce skeletal muscle weakness and atrophy [1,2]. These clinical signs could be explained by increased proteolysis in hyperthyroid muscle [3,4], and it might be reasonable to assume that the protein degradation is due to increased activities of proteases present in muscle tissue.

However, relatively few proteases have been defined which have the property of degrading myofibrillar proteins. Here we report increased activities in hyperthyroid skeletal muscle of calcium-activated neutral protease (CANP), cathepsin B and D. CANP is included in the cytosol fraction [5], is activated by Ca^{2+} at neutral pH [6,7] and is inhibited with leupeptin or antipain [8]. Both cathepsin B and D are contained in the lysosome fraction [9,10] and are active at acidic pH [9–11].

These proteases are important, since CANP has been shown to degrade both tropomyosin and troponin [6–8] and cathepsin B and D have been demonstrated to destroy both myosin and actin among myofibrillar proteins [11]. The mechanism of increased activity of these proteases and observed difference of protein degradation between the skeletal and the cardiac muscle are discussed.

2. Materials and methods

Eight hyperthyroid and 9 normal male New Zealand white rabbits (1.0–1.5 kg body wt) received daily injections of either L-thyroxine (200 μg/kg body wt) or saline only for 2 weeks. After sacrificing the animals, paravertebral skeletal muscle and cardiac muscle from both ventricles was rinsed with cold saline briefly, and the connective tissue was dissected away in ice-cold glass ware. All subsequent procedures were performed below 4°C. The muscle was minced, weighed, and lysosomal and cytosol fractions were prepared as in [12]. Cathepsin B and D reside mainly in the lysosomal fraction [9,10], and the homogenate was divided into two parts. The supernatant fraction, which could not be sedimented with the centrifugation at 40 000 × *g* for 30 min in 0.25 M sucrose and 10 mM K-phosphate buffer (pH 6.8), contained both the free cytosol fraction in vivo and the fraction which leaked from lysosome in the course of homogenization. Here, this fraction is termed 'supernatant fraction'. The precipitate was suspended in 3 vol. 0.1% Triton X-100 to disrupt lysosome, and centrifuged at 100 000 × *g* for 2 h. The resultant supernatant was termed 'precipitate fraction'. Both fraction were used for the assay of cathepsin B and D activity, by the methods in [10,13].

One unit of cathepsin B activity was defined as 1 nmol β-naphthylamine released from synthetic substrate, *N*-α-benzoxyl L-arginine β-naphthylamide (Sigma), per minute at 37°C. One unit of cathepsin D activity/min at 37°C was defined as an increase of 0.001 in A_{750} after color development in the trichloroacetic acid-soluble fraction by Lowry's method [14].

Another sample of muscle tissue was used for the assay of CANP. Muscle mince (3.0 g) was homogenized with a Virtis homogenizer at medium speed for 15 s, with 4 bursts in 3 vol. 0.03 M KCl, 5 mM neutralized EDTA and 20 mM Na-phosphate buffer (pH 7.0), and centrifuged at 8000 × *g* for 30 min. The supernatant was acidified to pH 4.7 by adding

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1 N acetic acid, an essential step for separating the endogenous protease inhibitor from CANP [6,7,15]. The precipitate was suspended in equal volume of 0.4 M Tris–HCl buffer (pH 7.4) and 1 mM EDTA, and centrifuged at 100 000 × *g* for 1 h. The resultant supernatant was used for the assay of CANP, following the method in [7].

Protein concentration was determined by the Biuret method with crystalline bovine serum albumin as a standard. Statistical analyses were performed by Student's unpaied *t*-test.

3. Results and discussion

The whole body weight and weight of both ventricles were compared in the hyperthyroid and euthyroid group. After the administration of L-thyroxine for 2 weeks, the whole body weight was reduced from 1.28 ± 0.08 (SEM) kg to 1.05 ± 0.07 kg, while euthyroid rabbits gained body weight from 1.45 ± 0.13 kg to 1.65 ± 0.12 kg. These changes between two groups were significant ($p < 0.01$). Heart weight was not different in the 2 groups (4.33 ± 0.12 g in hyperthyroid group and 4.01 ± 0.21 g in euthyroid group).

3.1. *Lysosomal proteases activity in skeletal muscle*

As summarized in table 1, in skeletal muscle from the hyperthyroid group, the supernatant fraction showed 1.7-times higher cathepsin B activity than the control group ($p < 0.001$). The precipitate fraction of hyperthyroid rabbits demonstrated 3.1-times higher activity ($p < 0.001$), and the total cathepsin B activity per gram muscle was 2.5-times higher in hyperthyroid group ($p < 0.001$).

Relative to protein concentrations, cathepsin B activity in the hyperthyroid group also revealed higher values than the control group both in supernatant and precipitate fractions (both $p < 0.001$).

Cathepsin D activity was measured in the same fraction as cathepsin B. In the supernatant fraction, the activity was 1.5-times higher in hyperthyroid rabbits ($p < 0.001$, table 1), whereas in the precipitate fraction, the activity increased 2.5-times in hyperthyroid group ($p < 0.001$). Therefore, total cathepsin D activity per gram muscle weight was increased ~2-fold in the hyperthyroid group ($p < 0.005$).

Relative to protein concentrations, the hyperthyroid rabbits demonstrated higher cathepsin D activity both in the supernatant and precipitate fractions ($p < 0.001$).

The increased activities of cathepsin B and D did not result from increased fragility of muscle lysosomes, since the activity retained in the lysosome fraction was higher in hyperthyroid group ($p < 0.005$).

The observed increases in protease activity are not due to decreased non-muscle weight, or to a selective decrease of proteins other than proteases, since activi-

Table 1
Effect of L-thyroxine administration on lysosomal protease activities in skeletal muscle

Protease		Euthyroid (n = 6)	Hyperthyroid (n = 5)	
Cathepsin B				
Cell fraction	Supernatant	3.91 ± 0.12	6.52 ± 0.35[b]	unit/g muscle
	Precipitate	4.82 ± 0.47	15.02 ± 1.50[b]	
	Total	8.73 ± 0.53	21.54 ± 1.49[b]	
% Retained in lysosome		54.6 ± 2.20	69.3 ± 2.44[a]	
Cell fraction	Supernatant	0.238 ± 0.009	0.367 ± 0.023[b]	unit/mg protein
	Precipitate	0.272 ± 0.026	0.844 ± 0.043[b]	
Cathepsin D				
Cell fraction	Supernatant	41.0 ± 3.8	64.8 ± 2.4[b]	unit/g muscle
	Precipitate	69.0 ± 8.3	171.6 ± 5.0[b]	
	Total	110.0 ± 10.4	236.4 ± 7.1[b]	
% Retained in lysosome		60.9 ± 2.52	72.6 ± 0.42[a]	
Cell fraction	Supernatant	2.52 ± 0.23	3.62 ± 0.12[b]	unit/mg protein
	Precipitate	3.88 ± 0.24	9.20 ± 0.24[b]	

Mean ± SEM; [a] $p < 0.005$; [b] $p < 0.001$

Table 2
Effect of L-thyroxine administration on calcium-activated neutral protease activity in either skeletal or cardiac muscle

Muscle	Euthyroid ($n = 9$)	Hyperthyroid ($n = 8$)	
Skeletal	10.1 ± 1.6	27.1 ± 7.5[a]	unit/g muscle
	4.53 ± 0.64	9.13 ± 2.07[a]	unit/mg protein
Cardiac	10.8 ± 2.8	14.6 ± 2.4	unit/g muscle
	3.55 ± 0.84	3.27 ± 0.55	unit/mg protein

Mean ± SEM; [a] $p < 0.05$

ties expressed both as per muscle weight and per mg protein increased. It is reasonable to assume that the increased activity is due to increased amount of enzymes.

3.2. *Calcium-activated neutral protease (CANP) activity in skeletal and cardiac muscle*

Table 2 summarizes the data of CANP activity. In skeletal muscle, CANP activity in hyperthyroid rabbits was 2.7-times higher ($p < 0.05$), and the animal which showed the highest activity also had the greatest decrease in body weight (40%). In cardiac muscle, however, there was no difference between the two groups in CANP activity.

Relative to protein concentrations, the hyperthyroid skeletal muscle showed 2-fold higher CANP activity than the control group ($p < 0.05$). Again, the activity in cardiac muscle was not different in the 2 groups, and there was no significant difference between the skeletal and the cardiac muscle in the euthyroid rabbits.

This finding agrees well with other data showing that lysosomal protease activity in heart was not changed after the administration of T_4 to the rat, even when it was increased in skeletal muscle [4]. Thus, the mechanism of thyroid hormone on protease synthesis and protein turnover appears different between the skeletal and cardiac muscle [16].

We suggest that thyroid hormones may regulate myofibrillar protein degradation by altering the amount of proteases in skeletal muscle and not in cardiac muscle, and that the activities of endogenous proteases, which degrade either contractile proteins (i.e., myosin and actin) or the regulatory proteins, (i.e., tropomyosin and troponin) [6,7,11], were increased in the skeletal muscle, resulting in muscle atrophy or muscle weakness.

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References

[1] Thorn, G. W. and Eder, H. A. (1946) Am. J. Med. 1, 583–601.
[2] Ramsey, I. D. (1966) Lancet ii, 931–934.
[3] Goldberg, A. L. and Griffin, G. E. (1977) J. Physiol. abst. 270, 51–52.
[4] DeMartino, G. N. and Goldberg, A. L. (1978) Proc. Natl. Acad. Sci. USA 15, 1369–1373.
[5] Reville, W. J., Goll, D. E., Stromer, M. H., Robson, R. M. and Dayton, W. R. (1976) J. Cell Biol. 70, 1–8.
[6] Dayton, W. R., Reville, W. J., Goll, D. E. and Stromer, M. H. (1976) Biochemistry 15, 2159–2167.
[7] Toyo-oka, T. and Masaki, T. (1979) J. Mol. Cell. Cardiol. 11, 769–786.
[8] Toyo-oka, T., Shimizu, T. and Masaki, T. (1979) Biochem. Biophys. Res. Commun. 82, 484–491.
[9] Barrett, A. J. (1973) Biochem. J. 131, 809–822.
[10] Barrett, A. J. (1970) Biochem. J. 117, 601–617.
[11] Schwarz, W. N. and Bird, J. W. C. (1979) Biochem. J. 167, 811–820.
[12] Ruth, R. C., Kennett, F. F. and Weglicki, W. B. (1978) J. Mol. Cell. Cardiol. 10, 739–751.
[13] Barrett, A. J. (1972) Analy. Biochem. 47, 280–293.
[14] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[15] Drummond, G. I. and Duncan, L. (1966) J. Biol. Chem. 241, 3097–3103.
[16] Toyo-oka, T. and Ross, J. jr (1980) Biochim. Biophys. Acta 590, 407–410.