Reduction of Corticosterone-Induced Growth Impairment by Testosterone and Its Mechanism

Kunioki Hayashi, A.G. Kayali and Yuichiro Tomita

Department of Biochemical Science and Technology
Faculty of Agriculture, Kagoshima University Kagoshima-shi 890

(Received March 6, 1992)

Abstract Two experiments were conducted to examine the effects of testosterone propionate (TP) on $\text{N}^\text{1}$-methylhistidine (MH) excretion as an index of the rate of muscle myofibrillar protein breakdown and the rate of muscle protein synthesis in young intact male rats treated with corticosterone (CTC). In both experiments, 6 animals were allotted to the 3 experimental groups (Control group, CTC group and CTC + TP group). CTC (10 mg/100 g BW/day) and TP (2 mg/100 g BW/day) were daily injected subcutaneously. Urine was collected every day for 6 days to measure MH excretion in Expt 1. In Expt 2, the rates of protein synthesis in the soleus and extensor digitorum longus muscle were measured by using the large-dose $[^3\text{H}]$ phenylalanine method after 4 and 8 days of hormone treatment. Growth ceased and MH excretion rose markedly with a peak at around 4 days in the group receiving CTC. Administration of TP minimized growth impairment and the rise in urinary MH excretion caused by CTC. The rates of protein synthesis in both muscles were significantly lowered by CTC at 4 and 8 days after treatment. The rates of muscle protein synthesis in the rats treated with both TP and CTC were similar to those in the CTC group. These results clearly show that testosterone can minimize the growth inhibiting effect of corticosteroids by reducing their effect on muscle protein breakdown.


Key words: growth, muscle protein breakdown, corticosterone, testosterone

Domestic animals are often subjected to different types of stress such as restraint, fasting, transport and exposure to high or low environmental temperature. During stress, glucocorticoid hormones are secreted, and the secretion of glucocorticoids is thought to be a central feature of the stress response. It is also known that stress induces a drastic fall of plasma testosterone. However, the role of testosterone in the stress response is little investigated.

Stress reduces the growth of animals, and a negative relation between blood glucocorticoid concentration and growth rate has been reported. It has been also reported that the overall effect of exogenous glucocorticoid on the body is usually catabolic, and the inhibitory effect of glucocorticoid on muscle growth has been suggested to arise from either a decreased rate of protein synthesis and an increased rate of protein breakdown. The mechanism of the regulation of muscle protein synthesis and breakdown is not well understood, but it is apparent that glucocorticoids have important roles in this process.
The catabolic effect of glucocorticoids is modulated by other hormones. That thyroid hormone accelerates the catabolic effect of glucocorticoid and insulin and testosterone minimize the catabolic effect of glucocorticoids have been reported. Genetic differences in thresholds for response to glucocorticoids have also been reported. In the present investigation, two experiments using rats were conducted to clarify the interaction of glucocorticoid and testosterone on the rates of muscle protein synthesis and breakdown.

**Materials and Methods**

In Expt 1, male Sprague-Dawley rats (Charles River, Atsugi, Kanagawa, Japan), fed on a commercial purified diet containing 24% casein (Oriental rat diet B, Oriental Yeast Co., Tokyo, Japan) ad libitum, were used. A total of 18 rats with about 210 g body weight (BW) each after 4 days of preliminary feeding of the experimental diet were divided into 3 groups (6 animals per group). The control (C) group received vehicle injection, and the corticosterone (CTC) group received 10 mg CTC/100 g BW. This level of CTC is usually used as a catabolic dose. The testosterone propionate (TP) group received 10 mg CTC plus 2 mg TP/100 g BW. Injections were done subcutaneously between 9:00 and 10:00 a.m. over a period of 6 days. The vehicle consisted of 0.2 ml corn oil used for dissolving the steroids. Food intake and body weight changes were recorded daily. On the final day of the experiment, rats were dissected to measure the weights of the fast twitch extensor digitorum longus muscle and the slow twitch soleus muscle as indexes of skeletal muscle growth.

A complete 24 hour-collection of urine was made in containers beginning at 10:00 a.m. throughout the experimental period, to measure MH excretion as an index of muscle proteolysis. The samples were stored at -20°C until analysis. The MH was analyzed by the method of NISHIZAWA et al., with a slight modification. The urine was treated with 2 M HCl in a boiling water bath for 2 hours to convert acetyl-MH to MH. The hydrolysates were then cooled and passed through filter paper. The hydrochloric acid was removed subsequently by evaporation under reduced pressure and the sample was redissolved in water and evaporated again in the presence of a small amount of sodium hydroxide to facilitate the removal of ammonia. The residue was solubilized in 0.2 M pyridine and made up to 5 ml. The solution was applied to a resin column (10 x 200 mm, Dewex 50-X8, pyridine form) to isolate MH, and the amino acid was measured photometrically by a minor modification of the method of WARD as reported previously.

Results are presented as means ± SE, and the significance of differences between the means was assessed by the Duncan’s multiple range test.

Experiment 2 was conducted in the same manner as Expt 1 using 36 male rats each weighing about 130 g. The groups were consisted of 3 treatment groups and 2 time-course groups. The rates of muscle protein synthesis were measured on the 4th and 8th day of experiment by large-dose [3H]phenylalanine method, but MH excretion was not measured. L-[4-3H]phenylalanine, purchased from Amersham International (Amersham, U.K.) was combined with unlabeled phenylalanine (150 mM in water) to give 25 μCi/ml. The rats were injected with 1.0 ml/100 g BW via the tail vein and killed 10 min. after injection by decapitation. The soleus and extensor digitorum longus muscle were rapidly excised and frozen in liquid N2. The time from the beginning of injection to soaking the muscle into liquid N2 was measured precisely. Frozen tissue was homogenized with cold 2% (W/V) HClO4 by a glass homogenizer and centrifuged at 3000 g for 15 min. The supernatant was adjusted to pH 6.3 with saturated potassium citrate and centrifuged at 3000 G for 15 min. to
remove KClO₄ before being used to determine the specific radioactivity of free phenylalanine. After washing carefully, the pellet was hydrolyzed in 6M HCl at 110°C for 20 hr with subsequent evaporation and resuspension in 1.5 ml of 0.5 M sodium citrate (pH 6.3) to determine the specific radioactivity of protein-bound phenylalanine. A 1 ml portion of the supernatant or hydrolysate was incubated with 5 ml of a suspension of L-tyrosine decarboxylase (acetone dried powder from *Streptococcus faecalis*, Type 1, Sigma) overnight at 50°C. The enzyme was suspended in 0.5 M sodium citrate, pH 6.3 (0.7 unit/ml for supernatants, 1.4 unit/ml for hydrolysates) containing 0.5 mg of pyridoxal phosphate/ml. After the addition of 1 ml of 3 M sodium hydroxide, β-phenylethylamine was extracted into 10 ml of n-heptane/chloroform (3:1, V/V). The organic layer was removed and to it was added 5 ml of chloroform and 4 ml of 0.01 M sulfuric acid and shaken. A sample of the sulfuric acid layer was used to determine radioactivity in a commercial scintillant, ACS (Amersham, Illinois, U.S.A.), with Packard TRI-CARB Liquid Scintillation Spectrometer Model 2002. Another sample was used to determine the amount of β-phenylethylamine fluorimetrically by the method of Suzuki and Yagi20). The fractional rate of protein synthesis was calculated from the equation, \( K_S = 100 \times S_A / S_B t \), where \( K_S \) is the fractional synthesis rate (in %/day) and \( S_B \) is the specific radioactivity of phenylalanine in protein at the time the tissue was frozen (about 13 min). \( S_A \) is the specific radioactivity of free phenylalanine. \( t \) is the time of incorporation including the time needed to remove the tissues, expressed in days.

**Results**

**Expt 1**: Body weight changes of the groups of rats during the 8-day experimental period are shown in Fig. 1. Growth ceased due to CTC treatment, and the growth impairment tended to be minimized by TP treatment. Body weight gain during the experimental period of 6 days were 40±2, -26±5 and -15±6 g, respectively, and differences between these groups were significant (P<0.05) except those between CTC and CTC + TP groups. Food intake and weights of the extensor digitorum longus muscle and soleus muscle are summarized in Table 1. Absolute food intake was not changed by CTC treatment, but it was significantly decreased when rats were treated with both CTC and TP. Food intake per unit of body weight tended to be increased by CTC treatment, but treatment with CTC + TP had no effect. Weights of the muscles were decreased significantly by CTC treatment, and the effects of CTC were minimized by TP treatment. However, the TP effect was significant only on the soleus muscle. The differences of the weights of the muscles relative to the body weight were not significant between groups except that
Table 1. Effects of treatments with corticosterone (CTC) and both CTC and testosterone (TP) on food intake and weights of extensor digitorum longus (EDL) muscle and soleus muscle at 6 days after the treatment (Expt 1)

<table>
<thead>
<tr>
<th></th>
<th>Food intake</th>
<th>EDL muscle weight</th>
<th>Soleus muscle weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/6 days</td>
<td>g/6 days /100 g BW</td>
<td>mg</td>
</tr>
<tr>
<td>Control</td>
<td>114±7.8 a</td>
<td>46±2.9</td>
<td>187±15.1 a</td>
</tr>
<tr>
<td>CTC</td>
<td>113±10.2 ab</td>
<td>61±5.7</td>
<td>130±11.8 b</td>
</tr>
<tr>
<td>CTC+TP</td>
<td>86±8.6 b</td>
<td>46±4.1</td>
<td>159±15.9 ab</td>
</tr>
</tbody>
</table>

1) Values are means±SE of 6 observations, and means in the same column bearing different superscripts are significantly different (P<0.05).

soleus muscle weight was increased significantly by CTC+TP. Time-course changes in MH excretion due to the treatments are illustrated in Fig. 2. The MH excretion was increased, with its peak at around Day 4 by CTC treatment, and it was about 7-times that of the control group at the peak. The increase in urinary MH excretion due to CTC treatment was reduced by TP. However, since daily urine samples of each respective group were combined, MH excretion could not be evaluated statistically.

Expt 2: Effects of the treatments on body weight change were illustrated in Fig. 3, and those on food intake and weights of muscles are shown in Table 2. Effects of CTC and CTC+TP on body weight change and muscle weight were similar to those observed in Expt

Fig. 2. Effects of treatments with corticosterone (CTC) and both CTC and testosterone propionate (TP) on MH excretion in rats (Expt 1)

Values are means of 6 rats in each of the following treatment groups: Control (○); CTC (◼); CTC+TP (●).

Fig. 3. Effects of treatments with corticosterone (CTC) and both CTC and testosterone propionate (TP) on growth in rats (Expt 2)

Values are means of 6 rats in each of the following treatment groups: Control (○); CTC (◼); CTC+TP (●). Bars indicate SE.
Testosterone on Corticosterone-Induced Muscle Growth Impairment

**Table 2.** Effects of treatments with corticosterone (CTC) and both CTC and testosterone on food intake and weights of extensor digitorum longus (EDL) muscle and soleus muscle at 8 days after the treatment (Expt 2)

<table>
<thead>
<tr>
<th></th>
<th>Food intake</th>
<th>EDL muscle weight</th>
<th>Soleus muscle weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/8 days</td>
<td>g/8 days/100 g BW</td>
<td>mg</td>
</tr>
<tr>
<td>Control</td>
<td>143±3.3</td>
<td>71±1.2</td>
<td>91±2.3</td>
</tr>
<tr>
<td>CTC</td>
<td>129±9.8</td>
<td>94±4.5</td>
<td>68±2.7</td>
</tr>
<tr>
<td>CTC+TP</td>
<td>137±9.8</td>
<td>99±5.7</td>
<td>61±2.6</td>
</tr>
</tbody>
</table>

1) Values are means±SE of 6 observations, and means in the same column bearing different superscripts are significantly different (P<0.05).

**Table 3.** Effects of treatments with corticosterone (CTC) and both CTC and testosterone propionate (TP) on the rates of protein synthesis in extensor digitorum longus (EDL) muscle and soleus muscle at 4 and 8 days after the treatments (Expt 2)

<table>
<thead>
<tr>
<th></th>
<th>EDL</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 days</td>
<td>8 days</td>
</tr>
<tr>
<td>Control</td>
<td>%/day</td>
<td>%/day</td>
</tr>
<tr>
<td>CTC</td>
<td>7.3±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CTC+TP</td>
<td>4.4±0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.1±0.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1) Values are means±SE of 6 observations, and means in the same column bearing different superscripts are significantly different (P<0.05).

However, growth retardation due to CTC treatment was less intense in Expt 2 than in Expt 1. This difference is thought to be derived from the developmental stage of the animals as we have recently observed (unpublished data). The body weight gain during the experimental period was 70±5, -2±2 and 11±3 g for Control, CTC and CTC+TP group, respectively, and the differences between groups were all significant (P<0.05). Absolute food intake tended to be decreased by both the treatments, but the relative values were significantly higher in the treatment groups than that in the control group. Both the absolute and the relative weights of the muscles were decreased significantly by CTC, but the muscle growth retardation due to CTC was not minimized by TP, although TP was effective in minimizing growth impairment and in reducing MH excretion. This indicates that skeletal muscles other than those used in the present study contributed to minimize growth retardation. Effects of CTC and CTC + TP on the rates of muscle protein synthesis are summarized in Table 3. The rates of synthesis in the muscles were significantly decreased by the treatment with either CTC or CTC + TP on both Day 4 and Day 8, and there was little difference between these two treatment groups.

**Discussion**

A decreased rate of protein synthesis and an increased rate of protein breakdown similarly result in an inhibition of muscle growth. The present study clearly shows that accelerating effect of CTC on muscle protein breakdown is minimized by the TP treatment, resulting in a modification of the growth of the CTC-treated rats.

It has been reported that testosterone increases both the rates of muscle protein synthesis and breakdown<sup>10</sup>, and it has also
been reported that testosterone reduces a catabolic effect of glucocorticoid\textsuperscript{17}. However, whether testosterone interferes with the ability of glucocorticoids to stimulate myofibrillar protein breakdown and/or inhibit myofibrillar protein synthesis has not been established. It was shown in the present experiment that growth retardation of the rats treated with CTC were minimized by the administration of TP without increasing food consumption. Urinary MH excretion was extremely increased by CTC and it was almost halved by the TP treatment, showing that the CTC induced proteolysis was reduced by the TP treatment. However, the rates of protein synthesis in the slow–twitch soleus and fast–twitch extensor digitorum longus muscles of the rat treated simultaneously with CTC and TP were almost same as those of the rats treated with only CTC. This shows that inhibition of muscle protein synthesis due to CTC cannot be modulated by TP treatment.

It is reported that plasma insulin is elevated\textsuperscript{23} and testosterone is lowered\textsuperscript{2} by CTC administration. These two hormones may have important roles in the regulation of muscle protein metabolism. The secondary changes in the secretion of these hormones might be responsible for the changes in the muscle protein metabolism of the CTC-treated animals. Insulin was not studied in the present experiment. However, it is thought that insulin is the most influential factor for maintaining positive protein balance in skeletal muscles. The secondary rise in plasma insulin concentration after corticosterone treatment of intact rats has been shown to decrease the catabolic response\textsuperscript{20}. Testosterone has an anabolic effect on muscle protein metabolism, and the synthesis of testosterone is decreased by CTC treatment as mentioned previously. So, growth retardation due to CTC treatment might partially be resulted from the secondary change in plasma testosterone concentration. Likewise, in the stressed animals, acceleration of muscle protein breakdown may be induced since plasma glucocorticoid is increased and testosterone is decreased. Furthermore, this study suggests that the difference of the growth rate between male and female may be explained similarly.

The glucocorticoid effect on target cells is mediated via a cytosolic glucocorticoid receptor, and the steroid–receptor complex binds to the cell nucleus to exert its effect\textsuperscript{40}. Thus, the role of testosterone in minimizing the growth retardation of the CTC-treated animal might result from the reduction in receptor sites for glucocorticoid in skeletal muscles.

References

8) Kelly, F.J. and D.F. Goldspink, The dif-
Testosterone on Corticosterone-Induced Muscle Growth Impairment


テストステロンによるコルチコステロン誘発性
成長阻害の抑制およびその機構

林 國興・A.G. KAYALI・冨田裕一郎
鹿児島大学農学部、鹿児島市 890

動物がストレスを受けると副腎皮質ホルモン分泌が増加するので、骨格筋蛋白質の合成抑制および分解亢進が生じ、成長が阻害される。本研究では、2 回の実験を行なって、副腎皮質ホルモン（コルチコステロン、CTC）による成長阻害がテストステロンプロピオネイト（TP）によって緩和されることならびにそのメカニズムの一端を示した。実験 1 では体重約 210 g の SD 系雄ラット 18 頭を対照（C）区、CTC区および CTC+TP 区の 3 区に分けた。体重 100 g あたり CTC は 10 mg、TP は 2 mg を毎日皮下注射し、6 日間、骨格筋蛋白質分解速度の指標である N¹-メチルヒステジン（MH）排泄量を測定した。実験 2 では、実験 1 と同様の処理を施したラット 36 頭を用いて、[²H]フェニルアラニンの大量投与法により実験開始後 4 日目と 8 日目の骨格筋蛋白質の合成速度を測定した。その結果、CTC により、成長は著しく阻害され、MH 排泄は 4 日目まで顕著に増加し、以後、減少した。また、CTC による成長阻害ならびに MH 排泄増加は TP により、抑制された。一方、合成速度は、CTC により著しく低下したが、TP にはこれを緩和する作用はなかった。以上の結果より、テストステロンは、コルチコステロンによる骨格筋蛋白質の合成阻害を抑制せず、分解促進を抑制することによって、成長阻害を緩和すると考えられる。また、この結果は、雄と雌の成長の違いが副腎皮質ホルモンとテストステロンの関係に基づく可能性を示唆している。

日畜会報，63 (10): 1001-1008，1992