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Inhibition of Corticosterone-induced Muscle Atrophy and Reduction in Muscle Protein Synthesis by the Anabolic Steroid Nandrolone Phenylpropionate in Female Rats

Choo, Jong Jae

Department of Foods and Nutrition, Kunsan National University, Kunsan, Korea

ABSTRACT

This study was undertaken to determine whether the anabolic steroid nandrolone phenylpropionate (NPP) can inhibit the muscle atrophy and reduction in muscle protein synthesis caused by glucocorticoids in female rats. Daily injections of 50mg/kg of corticosterone for eight days induced significant reductions in body weight gain and protein without affecting food intake. The mass, protein and RNA content, ratio of RNA to protein, and fractional rate of protein synthesis, measured *in vivo*, of gastrocnemius muscle were all significantly reduced by corticosterone treatment. Simultaneous administration of NPP at a dose of 10mg/kg with corticosteorne (50mg/kg) fully inhibited the reductions in the mass, protein and RNA content of gastrocnemius muscle, and body weight gain and protein with no alteration in food intake but the reduction in fractional rate of muscle protein syntheis was only partially prevented. The results indicate that the anabolic steroid nandrolone phenylpropionate is capable of preventing muscle atrophy in female rats treated with excess corticosterone. (*Korean J Nutrition* 29(8) : 867~873, 1996)

KEY WORDS : anabolic steroid · nandrolone phenylpropionate · corticosterone · muscle · protein synthesis.

Introduction

Although it has been known for over 40 years that synthetic derivatives of testosterone, commonly called anabolic steroids, possess anabolic effects on muscle protein, the mechanism(s) through which anabolic steroids increase mucle protein content are not fully understood.

They may have a direct effect on muscle[1,2] or modulate endogenous hormone patterns such as growth hormone[3]. In addition, it might be expected that anabolic steroids exert their anabolic effects by suppressing the action of glucocorticoids since anabolic steroids have been shown to be effective in improving nitrogen balance and body protein in human patients suffering from muscle wasting associated with increase in catabolic effects of glucocorticoids in a number of clinical conditions such as accidental injury[4], surgical trauma[5,6,7] and myotonic dystrophy[8].

However, the mechanism of anabolic steroid action as anticatabolic agents remains unresolved. On the one hand, Mayer and Rosen[9] (1975) have demonstrated that anabolic steroids are able to compete with glucocorticoids for their cytoplasmic receptors. On the other hand, several studies[1,10] show little binding specificity by anabolic steroids for the glucocorticoid receptor. Therefore, the present study has been developed to determine whether an anabolic steroid nandrolone phenylpropionate(NPP, Durabolin, Δ^4-estren-17β-ol-3-one phenylpropionate) can prevent muscle a-

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trophy and suppression of muscle protein synthesis caused by corticosterone treatment.

Materials and Methods

1. Animals

Female Sprague-Dawley rats weighing between 140 and 150g were housed singly at 24℃ with a 12-hour light/12-hour dark cycle, and fed a semi-synthetic diet(see table 1 for composition) for 3 days before the commencement of the experiments. At the begining of the experiments, animals were divided into three groups of six animals according to body weight gain during the period of adaptation. Two groups received daily subcutaneous injections of 50mg/kg body weight of corticosterone suspended in carboxy-methyl-cellulose(CMC) vehicle[11] for eight days. One of these groups also received daily subcutaneous injections of 10mg/kg body weight of nandrolone phenyl-propionate(NPP, Organon Ltd) while the other received CMC vehicle. The remaining group served as a control and received twice daily injections of CMC vehicle. Animals were weighed everyday and daily food intake was recorded, taking into account spillage.

At the end of the treatments animals were injected with L-[2,3-^{3}H]phenylalanine (150μmol and 50μCi/100g body weight; Amersham International, UK) via the lateral tail vein in order to determine the fractional rate of muscle protein synthesis. Animals were killed after 10min by decapitation, rapidly cooled in ice water, and gastrocnemius muscle, which is a representative skeletal muscle, was removed, frozen in liquid nitrogen and stored at -20℃ until analysis.

2. Measurement of body composition

The carcasses were dried at 105℃ to constant weight. Body protein and fat contents were determined on dried homogenized carcasses by the Kjeldahl method (N×6.25) and petroleum ether extraction, respectively.

3. Measurement of fractional rate of muscle protein synthesis

The fractional rate of muscle protein synthesis was determined by the method described by Garlick et al. [12]. The whole gastrocnemius muscle was homogenized in ice-cold 2% perchloric acid and the free amino acid fraction was separated as the supernatant after centrifugation. The precipitated protein was washed 3 times then hydrolysed with 6M-HCl. The hydrolysates as well as the free amino acid fractions were then incubated with L-tyrosine decarboxylase(EC 4.1.1.25) to convert phenylalanine to β-phenylethylamine. Phenylethylamine was extracted at alkaline pH, after adding 3M-NaOH into a chloroform:heptane(1 : 3) mixture, then back-extraction at acid pH into 0.01M-H_2SO_4. Aliquots were counted for ^{3}H radioactivity in a Beckman LS 1800 scintillation counter and β-phenylethylamine content was measured by the fluorimetric method of Suzuki and Yagi. The fractional rate of protein synthesis(Ks : % per day) was calculated as follows :

$$Ks = \frac{Sb}{Sa \times t} \times 100$$

where Sa and Sb are the specific radioactivies of free and protein-bound phenylalanine, respectively and t is the incorporation time in days.

4. Measurement of protein and RNA content

Tissue protein content was measured by the method of Lowry et al.[13] using bovine serum albumin as a standard and tissue RNA content by the UV method as described by Munro and Fleck[14].

5. Measurement of plasma corticosterone

Plasma corticosterone was measured by the methods of Lambert et al.[15] and Scott et al.[16] using high performance liquid chromatography(HPLC). Corticol was used as an internal standard and isocratic elution was performed on a C_{18} reversed-phase column(Nova Pak C_{18}, Waters) at room temperature using a mixture of methanol and water(60 : 40, v/v). The flow rate was 0.7ml/min. The absorbance of the colume effluent was monitored at 254nm.

6. Statistical analysis

The data are expressed as mean values with their standard errors. Statistical significance was analysed by one-way ANOVA. Significance of the differences between two groups was determined by least significant difference(LSD) at a probability level of 0.05. Analysis was carried out using Minitab(Minitab Corporation, State College, PA, USA).

Results

The administration of corticosterone at a dose of 50mg/kg body weight caused a marked reduction in body weight gain of 34% which was associated with a reduction in body protein but body fat was increased (Table 2). Food intake was not changed by corticosterone(Table 2). The mass, protein and RNA content, ratio of RNA to protein, and fractional rate of protein synthesis of gastrocnemius muscle were all significantly reduced by corticosterone(Table 3 and Fig. 1).

When a dose of 10mg/kg body weight was administered concurrently with corticosterone, nandrolone phenylpropionate(NPP) appeared to be anticatabolic. NPP completely restored the mass, protein and RNA content of gastocnemius muscle, and body wegith gain and protein to control values with no alteration in food intake(Tables 2 and 3). The increases in protein and RNA content of gastrocnemius muscle were in proportion, thus leaving the ratio of RNA to protein being unchanged. NPP also inhibited much of the corticosterone-induced reduction in fractional rate of muscle protein synthesis(Fig. 1). However, the fractional rate of gastrocnemius muscle protein synthesis was still significantly lower in the rats treated with NPP plus corticosterone than in the control rats treated with CMC vehicle only(Fig. 1).

The administration of corticosterone caused a several fold increase in plasma concentrtion of cor-

Table 1. Composition of diet

Component	g/kg
Casein	250
DL-methionine	2
Sucrose	280
Corn starch	280
Corn oil	100
α-cellulose	30
Vitamin mix[1)]	20
Mineral mix[2)]	40

1) The vitamin mix provides(per kg of diet) retinol acetate 10mg ; cholecalciferol 1mg ; tocopherol acetate 75mg ; menadione 1mg ; thiamin HCl 10mg; pyridoxine HCl 10mg ; riboflavin 10mg ; nicotinic acid 60mg ; calcium pantothenate 40mg ; folic acid 5mg ; biotin 1mg ; cyanocobalamine 0.05mg ; ascorbic acid 75mg ; choline bitartrate 1.8g
2) The mineral mix provides(per kg of diet) $CaHPO_4$ 13g ; $CaCO_3$ 8g ; KCl 8g ; Na_2HPO4 7.5g ; $MgSO_4 \cdot H_2O$ 180mg ; $C_8H_5O_7Fe \cdot 3H_2O$ 174mg ; $CuSO_4$ 15mg ; $ZnCO_3$ 30mg ; KIO_3 1mg

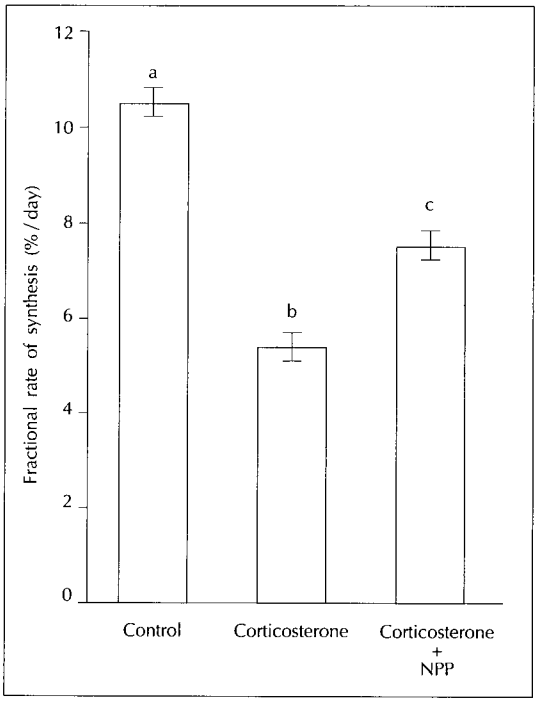

Fig. 1. The effects of nandrolone phenylpropionate(NPP) on fractional rates of protein synthesis of gastrocnemius muscle in corticosterone-treated female rats. Values are means±SE for six rats. Columns bearing different letters, indicated on the top of the error bars, were significantly different($P<0.05$).

Table 2. The effects of nandrolone phenylpropionate(NPP) on food intake, weight gain, and body protein and fat body in corticosterone-treated female rats

	Food intake (g/8d)	Weight gain (g/8d)	Body protein (g)	Body fat (g)
Control	147 ± 4^a	42.0 ± 2.5^a	34.8 ± 0.6^a	27.3 ± 0.8^a
Corticosterone	155 ± 6^a	26.7 ± 4.9^b	29.5 ± 1.3^b	35.1 ± 2.9^b
Corticosterone+NPP	166 ± 5^a	46.0 ± 3.8^a	34.7 ± 0.4^b	$30.1 \pm 2.4^{a,b}$

Values are means±SE for six rats. Values within a column with different superscript letters were significantly different ($P<0.05$)

Table 3. The effects of nandrolone phenylpropionate(NPP) on protein and RNA content of gastrocnemius muscle in corticosterone-treated female rats

	Muscle weight (g)	Protein content (mg)	RNA content (mg)	RNA/Protein (mg/mg×10³)
Control	0.91±0.03[a]	153±2[a]	1.66±0.05[a]	10.7±0.2[a]
Corticosterone	0.76±0.02[b]	129±5[b]	1.08±0.06[b]	8.2±0.3[b]
Corticosterone+NPP	0.87±0.02[a]	148±4[a]	1.54±0.06[a]	10.1±0.3[a]

Values are means±SE for six rats. Values within a column with different superscript letters were significantly different ($P<0.05$)

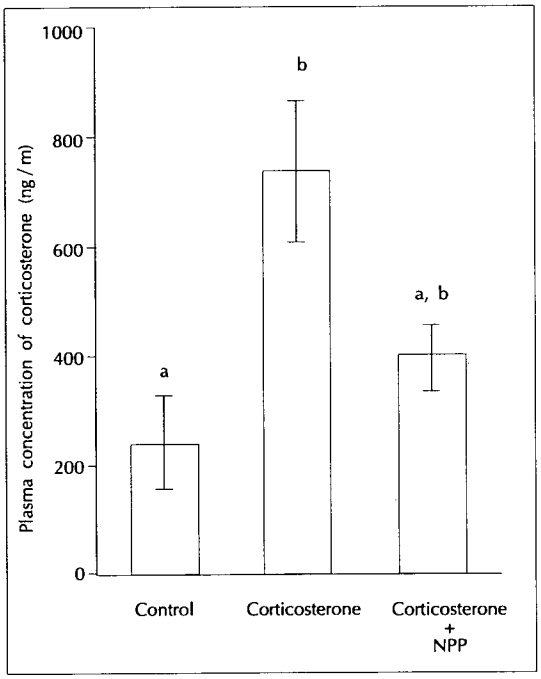

Fig. 2. The effects of nandrolone phenylpropionate(NPP) on fractional rates of protein syncorticosterone in corticosterone-treated female rats. Results are means±SE for six rats. Columns bearing different letters, indicated on the top of the error bars, were significantly different($P<0.05$).

ticosterone(Fig. 2). NPP suppressed much of this raised plasma corticosterone concentration, but the suppression was not statistically significant(Fig. 2).

Discussion

Glucocorticoids are generally considered to be potent catabolic hormones on muscle protein. The overall effects of excess amounts of glucocorticoids, whether from endogenous(e.g., Cushing's syndrome) or exogenous sources[17], are widely recognized. Growth retardation and marked atrophy of skeletal muscles after glucocorticoid administration have been consistently observed in a variety of animals[18,19,20]. Glucocorticoids certainly exert their catabolic effects on muscle protein through suppressing muscle protein synthesis(see Kettelhut et al.[21] 1988 for a review). However their effects on muscle protein degradation are equivocal. For example, Tomas et al.[11] reported a large increase in muscle protein degradation, judged by 3-methylhistidine excretion, whereas Odedra and Millward[22] and Odedra et al.[18] did not confirm this observation, muscle protein degradation being estimated as the difference between synthesis and net change in protein mass. It seems likely that the divergence of opinion may have been a function of the different dietary regimes and methods to estimate protein degradation. Muscle protein degradation does not change in fed animals or only transiently increases at large doses whereas glucocorticoids clearly stimulate muscle protein degradation in food deprived animals, coupled with an activation of the ATP-ubiquitin dependent proteolytic pathway[21,23]. Furthermore, glucocorticoids appear to exert a relatively specific effect on actomyosin turnover and have little effect on sarcoplasmic protein degradation[24]. Therefore, measurement of muscle protein degradation based on total protein after glucocorticoid treatment can be quite differnt from that based on actomyosin degradation estimated by the excretion of 3-methylhistidine.

Consistent with previous findings, the results of the present study indicate potent catabolic effects of glucocorticoids on skeletal muscle. In the present study, corticosterone completely arrested growth of gastrocnemius muscle, without affecting food intake, which was associated with 50% reduction in fractional rate of protein synthesis. Protein and RNA content were also reduced by corticosterone but the reduction in RNA content was greater than in protein content

so that there was a significant decrease in the ratio of RNA to protein which provides an estimate of translation capacity, and is an indicative of the rate of protein synthesis[25]. Therefore, the reduction in the ratio of RNA to protein in gastrocnemius muscle is in a good agreement with the reduction in fractional rate of protein systhesis. However, the reduction in fractional rate of protein synthesis was greater than in the ratio of RNA to protein which implies a reduction in translation efficiency(protein synthesized/RNA). Therefore it appears that glucocorticoids suppress not only translation capacity but also translation efficiency of skeletal muscle.

It has often been suggested that anabolic steroids can reverse glucocorticoid-induced muscle atrophy[17,26,27], a property referred to as anticatatolic. In the present study, the anabolic steroid nandrolone phyenylpropionate(NPP) prevented the reductions in body protein, and protein and RNA content, and fractional rate of protein synthesis of gastrocnemius muscle caused by exogenous corticosterone. These observations support the suggestion that anabolic steroids act on muscle protein as anticatabolic agents presumablly through displacing the glucocorticoid molecules, thus diminishing their effects. However, it should be noted that while reductions in protein and RNA content were completely prevented by NPP, reduction in fractional rate of protein synthesis was only partially prevented. This means that more than the antagonisim of the catabolic activity of glucocorticoids was involved in the improvement of muscle protein by NPP.

The prevention of muscle atrophy by NPP might have resulted from a direct effect of NPP on muscle protein through their own receptor. Snochowski et al.[1] and Snochowski et al.[28] observed distinct receptors for androgens in muscle and many anabolic steroids bind to the same androgen receptor as testosterone[2]. Alternatively, NPP might have exerted their influence on muscle protein by the secondary action of other anabolic hormones such as growth hormone[3]. However, from the limited number of variables measured in the present study, it is hard to draw any conclusion on relative importance of each effect.

It is interesting to note that NPP reduced plasma concentration of corticosterone by 30% in corticosterone-treated female rats. Though the reduction did not reach statistical significanc due to a considerable variation, it might indicate that anabolic steroids increase the hepatic clearance of glucocorticoids. However, this implication is in contrast to previous findings. Kitay[29] reported a decrease in the biological half-life and an increase in hepatic inactivation of corticosterone in castrated male rats, suggesting that the presence of androgens increases the half-life of glucocorticoids. James et al.[30] also showed delayed catabolism of infused cortisol in human subjects after the administration of an anabolic steroid, methandienone. This considerable disparity remains to be resolved.

In conclusion, the results of the present study show that the anabolic steroid nandrolone phenylpropionate is capable of preventing the muscle atrophy in rats treated with exogenous corticosterone. This anticatabolic effect of anabolic steroids might be useful in counteracting muscle wasting associated with excess glucocorticoids.

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=국 문 초 록=

아나보릭스테로이드인 Nandrolone Phenylpropionate가 암컷 쥐에서 코티코스테론에 의해 야기된 근육단백질 쇠퇴와 근육단백질 합성율 감소에 미치는 영향

주 종 재
군산대학교 식품영양학과

본 연구는 아나보릭스테로이드인 nandrolone phenylpropionate(NPP)가 암컷 쥐에서 catabolic hormone인 corticosterone 처리에 의해 야기된 근육단백질 쇠퇴(muscle atrophy)와 근육단백질 합성율 감소를 저해하는지를 알아보기 위해 수행되었다. Corticosterone을 8일 동안 매일 체중 1kg당 50mg을 투여하였을 때 식이섭취량은 변화하지 않았으나 체중 및 체단백질량은 대조군에 비하여 유의적으로 감소하였다. Gastrocnemius muscle의 무게, 단백질 함량, RNA 함량, RNA/protein 그리고 단백질 합성율 모두 corticosterone에 의해 유의적으로 감소하였다. NPP(10mg/kg)를 corticosterone과 동시에 투여하였을 때 NPP는 식이섭취량은 변화시키지 않으면서 corticosterone에 의해 야기된 gastrocnemius muscle의 무게, 단백질 함량, RNA 함량, 단백질 합성율의 감소 등을 유의적으로 저해하였다. 이러한 결과들을 통해 아나보릭스테로이드인 NPP는 corticosterone의 과다로 인해 야기되는 근육단백질 쇠퇴를 방지하는데 효과가 있으며 anti-catabolic 물질로 작용한다는 것을 알 수 있다.