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Effects of the Anabolic Steroid, Nandrolone Phenylpropionate, on Growth and Muscle Protein Metabolism in ACTH-treated Rats

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ABSTRACT

The effects of an anabolic steroid, nandrolone phenylpropionate(NPP), on body weight gain and body protein, and muscle protein metabolism were investigated in adrenocorticotrophic hormone(ACTH)-treated male and female rats. Daily injections of 100μg/day of ACTH for 7 – 8 days caused a cessation of growth in females and a net loss of body weight in males which were associated with significant reductions in body protein content. However, food intake was not affected by ACTH in either sex. The weight, protein content and fractional rate of protein synthesis, measured *in vivo,* of gastrocnemius muscle were all significantly reduced in both sexes. NPP at a dose of 4mg/kg body weight prevented the reduction in body weight gain in ACTH-treated females but not in males. However, body protein content was increased by NPP in both sexes which was associated with increases in the weight, protein content and fractional rate of protein synthesis of gastrocnemius muscle. ACTH treatment caused a marked increase in plasma concentrations of corticosterone in both sexes. NPP suppressed much of the increases in corticosterone concentrations in both sexes. The results of the present study suggest that NPP exerts at least part of its anabolic effect by reducing plasma concentrations of catabolic glucocorticoid hormones, through suppressing the response of the adrenals to ACTH. (*Korean J Nutrition* 29(8) : 874~880, 1996)

KEY WORDS : anabolic steroids · nandrolone phenylpropionate · ACTH · muscle · protein synthesis.

Introduction

Because in most species, males have larger body size and greater musculature than females, it is assumed that androgens secreted by the testes are responsible for this anabolic action. Indeed, the principal androgen testosterone has been shown to increase muscle and body protein in various animals[1)2)3)4)] and also in man[5)6)]. However, androgens also possess androgenic activities such as the development of male characteristics and accessory sex organs. These effects of androgens are certainly undesirable when administered to women or children, or even men because of their potential effect on aggressiveness, libido etc[7)]. The realization of this fact had initiated the attempt of the pharmaceutical industry to modify the structure of testosterone to minimize the androgenic activity while retaining or increasing the anabolic activity. As a consequence, a number of synthetic derivatives and analogues of testosterone, the so-called anabolic steroids, have been introduced and shown to stimulate growth and carcass protein in animal production[8)9)], and to improve nitrogen balance and body protein in patients suffering from muscle wasting[10)11)12)]

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[13]. However, the mechanism by which anabolic steroids exerts their anabolic effects on muscle protein is not fully understood.

There are several mechanisms which have been suggested to be involved in the action of anabolic steroids. These can be largely divided into two categories: direct action, through binding to androgen receptors[14], and indirect action, through suppressing the effects of catabolic hormones, glucocorticoids, or altering plasma concentrations of anabolic hormones such as growth hormone[15].

With regard to the suppression of the catabolic effects of glucocorticoids by anabolic steroids, Mayer and Rosen[16] suggested that anabolic steroids might exert their effects on muscle protein through competing with glucocorticoids for their receptors. In support of this mechanism, the anabolic steroid nandrolone phenylpropionate has been reported to prevent muscle atrophy induced by corticosterone treatment in the rat[17]. In addition, it has been suggested that anabolic steroids lower plasma concentrations of glucocorticoids, possibly through suppressing the adrenal activity[18][19]. It was therefore decided to investigate the effects of an anabolic steroid, nandrolone phenylpropionate(NPP, Durabolin, Δ^4-estren-17β-ol-3-one phenylpropionate), on muscle atrophy and decrease in muscle protein synthesis induced by adrenocorticotrophic hormone(ACTH) treatment in rats.

Materials and Methods

1. Animals

Eighteen female and eighteen male Sprague-Dawley rats were used. The weights of rats were about 200g and 150g for males and females, respectively. They were housed under controlled conditions, with constant temperature(23 – 25℃) and humidity(50 – 60%), and with a 12-h light-darkness cycle. The animals were given tap-water and a semi-synthetic diet[17]. Rats of each sex were randomly assigned to three groups. Two groups(6 rats in each) received daily subcutaneous injections of 100μg synthetic ACTH (Synacthen, Ciba). One of these group also received daily subcutaneous injections of 4mg/kg body weight of nandrolone phenylpropionate(NPP, Organon Ltd) suspended in carboxymethylcellulose(CMC) vehicle[20] and the other received CMC vehicle. The remaining one group served as control and received 0.9% saline and CMC vehicle. All injections were done separately at 16 : 00 – 17 : 00. The periods of treatments were 8 days for males and 7 days for females.

2. Measurement of fractional rate of muscle protein synthesis

This was done by the method of Gralick et al.[21]. Briefly, at the end of the treatments conscious rats were injected intravenously via the lateral tail vein with a solution of L-[2,3-^{3}H]phenylalanine(150μmol and 50μCi/100g body weight ; Amersham International, UK). 10 min(t) later the animals were killed by decapitation and placed in a mixture of ice and water. The heart and gastrocnemius muscle were rapidly removed and frozen in liquid nitrogen before measurement of the specific radioactivity of free(Sa) and protein-bound(Sb) phenylalanine. Muscle Ks (fractional rate of protein synthesis, % per day) was calculated from the equation.

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

3. Measurement of body protein

After the content of gastrointestinal tract was removed, the carcass was dried in an oven at 105℃. Normally, it took 24 hours/100g body weight. Dired carcass was homogenized by grinding in an electrical grinder and then homogenized carcass was subjected to the measurement of protein content by the Kjelhahl method.

4. Measurement of plasma corticosterone

For determination of plasma corticosterone concentrations, animals were decapitated and blood was collected in heparinized tubes. After centrifugation, plasma was stored at -70℃ until analysis. Plasma corticosterone concentrations were measured by adapting the HPLC(high performance liquid chromatography) mothods of Lambert et al.[22] and Scott et al.[23] as described previously[17].

5. Statistical analysis

Results are presented as mean values and standard errors. Data were subjected to one-way analysis of vari-

Table 1. The effects of nandrolone phenylpropionate(NPP) on food intake, weight gain, body protein, and weight and protein content of gastrocnemius muscle in ACTH-treated male rats

	Control	ACTH	ACTH + NPP
Body weight gain (g/8d)	49.8 ±3.9[a]	−18.2±4.6[b]	−5.8±7.7[b]
Food intake (g/8d)	177 ±8[a]	170±13[a]	139±9[a]
Body protein (g)	49.0 ±1.0[a]	34.9±0.7[b]	39.1±1.3[c]
Gastrocnemius muscle			
Weight (g)	1.41±0.05[a]	0.76±0.01[b]	0.90±0.04[c]
Protein content (mg)	242 ±5[a]	128±4[b]	154±4[c]

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

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

	Control	ACTH	ACTH + NPP
Body weight gain (g/7d)	34.0±4.4[a]	1.3±3.3[b]	27.2±2.8[a]
Food intake (g/7d)	116±8[a]	109±4 [a]	121±5[a]
Body protein (g)	33.0±0.7[a]	26.6±0.7[b]	31.4±0.4[a]
Gastrocnemius muscle			
Weight (g)	0.97±0.04[a]	0.69±0.04[b]	0.83±0.02[c]
Protein content (mg)	164±4[a]	128±6[b]	148±3[c]

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

ance before the evluations were performed with least significant difference(LSD) between different groups using the SAS/PC software program. A comparison was considered to be statistically significant when $P<0.05$.

Results

When ACTH was given at a dose of 100 μg/day for 7 - 8 days, the growth was almost stopped in female rats(Table 2) while in male rats, there was a net loss of body weight(Table 1). These reductions in weight gain were associated with significant reductions in body protein content(Tables 1 and 2). However, food intake was not affected by ACTH in either sex. The weight, protein content and fractional rate of protein synthesis of gastrocnemius muscle were all significantly reduced in both sexes(Tables 1 and 2, Fig. 1).

The reduction in body weight gain by ACTH was prevented by NPP in female rats(Table 2) but NPP had no effect on body weight gain in ACTH-treated male rats(Table 1). However, body protein content was increased by NPP in both sexes which was associated with increases in the weight, protein content and fractional rate of protein synthesis of gastrocnemius muscle(Tables 1 and 2, Fig. 1). In males, food intake tended to be reduced but was no statistically significant.

ACTH treatment caused a marked increase in plasma levels of corticosterone in both sexes(Fig. 2). NPP suppressed much of the increases in corticosterone levels in both sexes but in males, the suppression was

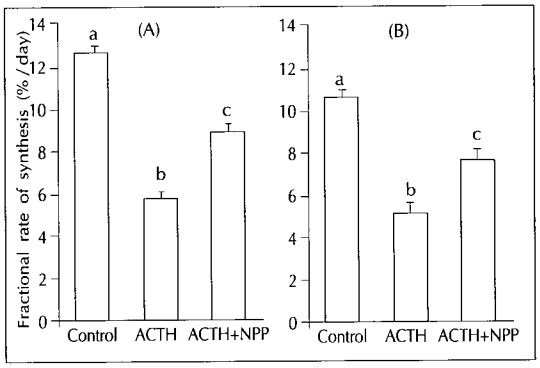

Fig. 1. The effects of nandrolone phenylpropionate(NPP) on fractional rates of protein synthesis of gastrocnemius muscle in ACTH-treated male (A) and female (B) rats. Values are means±SE for six rats. Columns bearing different letters, indicated on the top of the error bars, were significantly different($P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

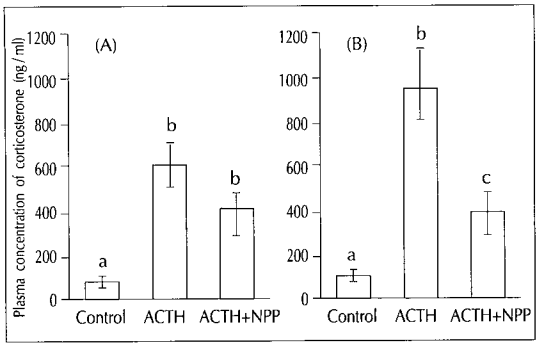

Fig. 2. The effects of nandrolone phenylpropionate(NPP) on plasma concentration of corticosterone in ACTH-treated male (A) and female (B) rats. Results are means±SE for six rats. Columns bearing different letters, indicated on the top of the error bars, were significantly different($P<0.05$).

not statistically significant(Fig. 2).

Discussion

Glucocorticoids exert potent catabolic effects on body and muscle protein mainly through reducing the rate of protein synthesis[24]. Their release from the adrenal cortex is triggered by a variety of conditions imposed by physical, psychological and nutritional damands and is mediated by the hypothalamus-anterior pituitary axis. Afferent input to the hypothalamus induces secretion of corticotrophin-releasing factor(CRF), which is transported to the anterior pituitary and stimulates the secretion of adrenocorticotrophic hormone(ACTH). The ACTH, in turn, is carried to the adrenal and stimulates the secretion of glucocorticoids. Reaching the target tissues, glucocorticoids form a complex with the cytoplasmic receptors which alters protein metabolism. Therefore, there are various sites at which the effects of glucocorticoids can be regulated.

It is now apparent that female rats show a greater adrenal cortical secretory response to stress than do males. Malendoricz and Mlynarczyk[25] found higher adrenal concentrations of corticosterone in females than in males and in the present study it was observed that when the secretion of glucocorticoids was stimulated by exogenous ACTH, female rats had 50% higher plasma concentrations of corticosterone than males. Together, these findings implicate that sex differences in the growth of the rat might be reflected by a difference in pituitary-adrenal function and anaboic steroids might exert their protein-anabolic effects by reducing plasma concentrations of glucocorticoids.

Thomas and Rodway[19] have suggested the involvement of anabolic steroids in the response of the adrenal to ACTH. They observed significant reductions in plasma glucocorticoid concentrations in female rats and lambs treated with an anabolic steroid, trenbolone acetate(TBA), 1 and 2 hours after ACTH administration, respectively. The same research group also showed lower activity of peak hepatic tyrosine aminotransferase, an enzyme induced by glucocorticoids, and peak plasma corticostereone concentrations associated with an improvement in growth rate in normal female rats after administration of TBA[28][29]. In normal male rats, plasma corticosterone concentrations tended to be reduced by TBA though it was not statistically significant. It has also been shown that in vitro, androgens have been shown to inhibit adrenal steroidogenesis by inhibition of the 11β-hydroxylase activities[30]. On the other hand, in human studies, Carter et al.[31] observed that an anabolic steroid, 17α-ethyl-19-nortestosterone, had no effect on the adrenocortical response to ACTH, as assessed by changes in plasma levels of 17-hydroxycorticosteroids. Wynn et al.[32] also reported no changes in urinary excretion of 17-hydroxycorticosteroids in the anabolic steroid methandienone-treated patients following ACTH administration.

In the present study, the effects of an anabolic steroid, nandrolone phenylpropionate(NPP), on the adrenal activities in ACTH-treated rats were assessed by measuring plasma concentrations of corticosterone and also changes in growth, protein content and protein synthetic rate of gastrocnemius muscle, and body protein. NPP prevented much of the reductions in body and muscle protein content, in both sexes, caused by exogenous ACTH. The prevention of muscle atrophy was associated with improvements in fractional rate of muscle protein synthesis and reductions in plasma corticosterone concentrations. Though the reduction in males did not reach statistical significance these observations strongly suggest that NPP might exert its protein-anabolic effects by suppressing the adrenal response to ACTH.

The nonsignificant reduction in plasma con-

centrations of corticosterone in males was probably due to the small reduction relative to that in females and to the large variation within the group. The seemingly contradictory observations in animals and man suggest a species difference in the effects of anabolic steroids on adrenal activity. However, it should be noted that in most cases of human studies, the doses of ACTH used were relatively much lower than those used in animal studies. Therefore, the possibility of a suppressive effect of anabolic steroids, had the stimulation on adrenal activity been greater, could not be discounted.

We have no direct evidence to suggest that the prevention of muscle atrophy by NPP was entirely due to the suppression of the adrenal response to ACTH. It is likely that at least part of the prevention was a result of suppression of catabolic effects of glucocorticoids by NPP competing with glucocorticoids for their receptors. Anabolic steroids have been shown to inhibit the binding of glucocorticoids to their receptors in muscle[16] and to prevent muscle atrophy associated with excess glucocorticoids[17]. Alternatively, the improvements might have resulted from a direct effect of NPP on muscle through their own receptors. In this aspect, it is interesting to note that reductions in blood levels of testosterone have been shown to occur with raised levels of glucocorticoids in such clinical conditions as traumatized patients[33] or glucocorticoid-administered subjects[34]. In animal studies, we have observed that the administration of corticosterone significantly reduced plasma concentrations of testosterone+dihydrotestosterone(4.1 ± 0.7 and 1.1 ± 0.1ng/ml, respectively, for control and corticosterone-treated male rats, $P<0.01$). Therefore it might be expected that in the present study, reductions in endogenous androgen levels in corticosterone-treated males occured and this reduction was partly responsible for muscle atrophy. Consequently, the prevention of muscle atrophy by NPP in males can be interpreted as a result of the replacement of reduced endogenous androgens by NPP. However, it is unlikely since we have found that in corticosterone-treated male rats, although NPP increased the weight of seminal vesicle, a secondary sex organ, to a value even two-fold higher than that of normal control, it had no effect on either reduction in body protein or muscle atrophy(data not shown). The concentration of androgen receptors in muscle had been shown to be much lower than those in sex tissues. For example, the sex tissue prostate contains 70 times the number of androgen receptors as in muscle[35]. Therefore, it is possible that in ACTH-treated males, the androgen receptors in muscle were already saturated despite reduction in androgen levels in blood.

In conclusion, NPP prevents muscle atrophy induced by exogenous ACTH administration in rats regardless of sex. This effect appears to be mediated primarily through the suppression of adrenal activity.

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=국 문 초 록=

ACTH를 투여한 흰쥐에서 아나보릭스테로이드인 Nandrolone Phenylpropionate가 성장과 근육단백질 대사에 미치는 영향

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본 연구는 아나보릭스테로이드인 nandrolone phenylpropionate(NPP)가 adrenocorticotrophic hormone(ACTH)을 투여한 흰쥐에서 체중, 체단백질 함량 그리고 근육 단백질 대사에 미치는 영향을 조사하기 위하여 수행되었다. ACTH를 매일 100㎍씩 7~8일간 투여하였을 때 암컷의 경우에는 성장이 중지되었고 수컷에서는 체중 감소 현상이 일어났다. 체단백질 함량은 암컷과 수컷 모두에서 유의적으로 감소하였으며 gastrocnemius muscle의 무게, 단백질 함량 그리고 단백질 합성율도 유의적인 감소를 보였다. 그러나 식이섭취량은 암컷에서도 수컷에서도 ACTH에 의해 영향을 받지 않았다.

NPP(4mg/kg body weight)를 ACTH와 동시에 투여하였을 때 NPP는 암컷에서는 ACTH에 의한 체중 감소를 방지하였으나 수컷에서는 체중에 영향을 미치지 않았다. 그러나 ACTH에 의한 체단백질 감소는 암컷, 수컷 모두에서 NPP에 의해 어느정도 방지되었으며 아울러 gastrocnemius muscle의 무게, 단백질 함량 그리고 단백질 합성율의 감소도 NPP에 의해 부분적으로 억제되었다. 한편 ACTH 투여는 암컷과 수컷 모두에서 corticosterone의 혈장 농도를 유의적으로 상승시켰는데 NPP는 ACTH에 의한 이러한 상승을 일부 억제하였다(수컷에서는 이러한 억제현상이 유의적이지는 못했다). 이러한 결과들로부터 ACTH에 의한 근육단백질 쇠퇴는 NPP에 의해 억제될 수 있으며 이는 NPP가 ACTH에 의한 부신의 활성을 감소시켜 혈중 glucocorticoids의 농도를 낮춤으로써 발휘된다는 결론을 얻었다.