J. steroid Biochem. Vol. 35, No. 1, pp. 127–132, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

PITUITARY AND GONADAL FUNCTION DURING PHYSICAL EXERCISE IN THE MALE RAT

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(*Received* 7 *April* 1989)

Summary—The effects of training and acute exercise on serum testosterone, luteinizing hormone (LH) and corticosterone levels and on testicular endocrine function in male rats were studied. In the first part of the study, the rats were trained progressively on a treadmill, over 8 weeks. Training did not change the basal levels of serum testosterone, LH and corticosterone, or the testicular concentrations of testosterone and its precursors progesterone and androstenedione. The levels of testicular LH (30.3 ± 2.6 ng/g wet wt, mean ± SEM) and lactogen (150 ± 14 pg/g) receptors were unchanged after training. However, the capacity of testicular interstitial cell suspensions to produce cAMP and testosterone increased by 20–30% during *in vitro* gonadotropin stimulation.

In the second part, the trained and untrained control animals underwent acute exhaustive exercise. Serum testosterone levels decreased by 74 and 42% in trained and untrained rats, respectively ($P < 0.02$), and corticosterone rose by 182% in trained and 146% in untrained rats ($P < 0.01$), whereas the LH level was unchanged. Testicular levels of testosterone and its precursors decreased, with the exception of unchanged androstenedione, in trained rats; the cAMP concentration was unchanged. In both trained and untrained rats, acute exercise decreased the capacity of interstitial cell suspensions to produce cAMP, whereas there were no consistent effects on testosterone production. Acute exercise had no effect on LH or lactogen receptors in testis tissue.

In conclusion, training had no effect on serum or testicular androgen concentrations, but increased Leydig cell capacity to produce testosterone and cAMP. Acute exercise decreased serum and testicular testosterone concentrations without affecting serum LH. A direct inhibitory effect of the increased serum corticosterone level on the hypothalamic–pituitary level and/or testis may be the explanation for this finding.

INTRODUCTION

Hormones have an important role in regulating energy and protein metabolism. The endocrine system reacts to physical exercise. The general finding has been that plasma testosterone rises during physical exercise [1–3]. However, some hours after intense short-term exercise, or at the end of long-term exercise, low serum testosterone and high cortisol concentrations were regularly observed in athletes; the more severe the strain the greater and longer-lasting were these changes [2, 4–8]. Aakvaag *et al.*[9] observed that intense physical and mental stress over several days decreased plasma testosterone in men, eventually almost to female levels. Plasma sex hormone binding capacity was also found to increase markedly during this period, indicating a decrease in biologically active free testosterone concentration.

To clarify the mechanism of decreased plasma androgen levels in stress, we studied the effect of acute exercise and progressive training on pituitary and gonadal function in rats to elucidate possible changes in androgen synthesis and/or metabolism at the testicular level.

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EXPERIMENTAL

Rats and exercise

Fifty-six 2–3-month-old male rats of the Sprague–Dawley strain were used in the experiments. Sixteen rats were trained progressively on a treadmill for 5 days per week over an 8 week period. The speed of the treadmill was 10 m/min, and the rats ran 15 min per day during the first week whereafter the running time was progressively increased by 15 min per day so that the rats ran for 5 h during the last week. The control rats were also taught to run in a treadmill twice a week for 15 min.

To study the effect of acute exercise, the trained and untrained rats were exposed to exhaustive treadmill exercise. The rats ran at a speed of 20 m/min until completely exhausted. This took 0.5–1 h for untrained rats and 2–3 h for the trained animals. The rats were killed by decapitation immediately after exhaustion. Due to the diurnal variation of plasma

hormone levels the changes in hormones were always compared in pairs between values of a trained animal and a control rat killed at the same time. The trunk blood was collected and both testes excised. The right testis was frozen in liquid N_2 and the left testis was used for the preparation of interstitial cell suspensions.

Serum hormone determinations

Serum testosterone was measured as described by Ismail *et al.*[10] using an RIA kit supplied by Farmos Diagnostica (Turku, Finland). Serum LH was measured with an RIA method according to Karonen *et al.*[11] using anti-rat-LH-serum (rabbit) (NIAMDD-anti-RAT LHS-3, National Pituitary Agency, Baltimore, Md) and a rat luteinizing hormone standard (NIAMDD-RAT LH-RP-1, N.P.A., Baltimore, Md).

Serum corticosterone was determined using an RIA kit of Inter Sci Diagnostics (Los Angeles, Calif.). However, before the RIA, serum samples were diluted with 0.05 mol/l borate buffer, pH 8.0 containing 0.25% BSA (1:60) and then incubated for 30 min at 60°C (according to plasma corticosterone RIA procedure of Endocrine Sciences Products, Tarzana, Calif.). The exercise samples were thereafter further diluted 1:2 with the same borate buffer.

Testicular steroid determinations

The testosterone concentrations in the testes were measured by homogenizing a 10-mg piece of tissue in 50 μl of 0.13 mol/l borate buffer, pH 8.0, containing 0.02% gelatine. The homogenate was frozen and thawed 3 times with "Vortex" mixing between freezings. For recovery correction 185 Bq (0.005 μCi) of [^{3}H]testosterone (Amersham, Bucks., England) were added to the homogenate. The homogenate was extracted with 1 ml of an ethyl acetate-ether solution (1:9; v:v). 200 μl of this solution were evaporated to dryness under a N_2-stream, whereafter 3 ml of ACS-solution (Amersham, Bucks., England) were added and the activity counted in a liquid scintillation counter LKB-Wallac 81000 (LKB-Wallac Ltd, Turku, Finland). The recovery was regularly 90–100%. For testosterone determination 100 μl of extract was used and assayed as described for the serum testosterone RIA.

The androstenedione concentration was determined by homogenizing a 25 mg piece of testis in 500 μl of 0.1 mol/l phosphate buffer, pH 7.0, containing 0.1% gelatine. For recovery correction 74 Bq (0.002 μCi) of [^{3}H]androstenedione (Amersham, Bucks., England) were added to the homogenate. The homogenate was extracted twice with 2 ml of petroleum ether (35–60°C, Mallinckcrodt, Diezenbach, F.R.G.), and the combined extracts were evaporated to dryness under N_2. The residue was dissolved in 20 μl of absolute ethanol, whereafter 500 μl of 0.1 mol/l phosphate buffer, pH 7.0, were added. 250 μl of this solution were transferred to counting vials, 3 ml of ACS-solution added, and the samples counted for determination of recovery. This was between 80 and 95%. For androstenedione determination, 50 μl of the extract were used as described previously by us for serum measurements of this steroid with RIA [2].

The progesterone concentration was measured in the same way as androstenedione. The recovery was between 75 and 90%.

A control serum was included in every series of determinations and the quality control chart plotted. The coefficients of variation (CV) between assays were: for serum testosterone 7%; serum LH 8%; serum corticosterone 10%; testicular testosterone 7%; testicular androstenedione 17%; and for testicular progesterone 15%. The CV within assays was less than 5% for each hormone measured.

Preparation of radioiodinated hCG, hFSH, hGH and GnRH-A

Highly purified hCG and hGH were radioiodinated by the lactoperoxidase-glucose oxidase method (Enzymobeads, Bio-Rad, Richmond, Calif.) according to the instructions of the manufacturer, and purified by chromatography on a 1.5 × 90 cm column of Sephadex G-100 [12]. The specific activity of each batch of labelled hormone was determined by self-displacement analysis in a radioligand-receptor assay employing rat testis homogenate [12]. A correction was also made for the fraction of radioactivity associated with biologically active hormone, as measured in each batch of labelled hormone preparation by determining the maximum specific binding of a sample of the tracer to an excess of rat testis receptors [12]. The fraction varied between 50 and 70% for [^{125}I]hCG and 35–50% for [^{125}I]hGH. The corrected specific activities of the two hormones were 2.59–2.96 TBq/g (70–80 Ci/g) and 1.85–2.59 TBq/g (50–70 Ci/g), respectively.

Highly purified hCG (CR-121, 13,500 IU/mg by bioassay) was prepared by Dr R. Canfield (Columbia University, New York) and supplied by the Center for Population Research, NICHD (Bethesda, Md.). Partially purified hCG (Pregnyl, 3000 IU/mg) was purchased from Organon (Oss, Holland). Purified human growth hormone (hGH, NIADDK-I-1) was donated by the National Pituitary Agency, Baltimore, Md. and NIADDK.

Measurement of testicular LH and prolactin receptors

The decapsulated testes were homogenized in 10 ml/g tissue of Dulbecco's phosphate buffered saline containing 0.1% bovine serum albumin (PBS-BSA, pH 7.4), and the homogenates were used as such for LH and prolactin receptor measurements. 100 or 200 μl aliquots of the homogenate were equilibrated (overnight at 23°C) with 1.67 kBq (0.045 μCi) of [^{125}I]hCG and 4 ng/tube of nonradioactive hCG or 2.5 kBq (0.067 μCi) of [^{125}I]hGH (total volume 250 μl). Nonspecific binding was measured in

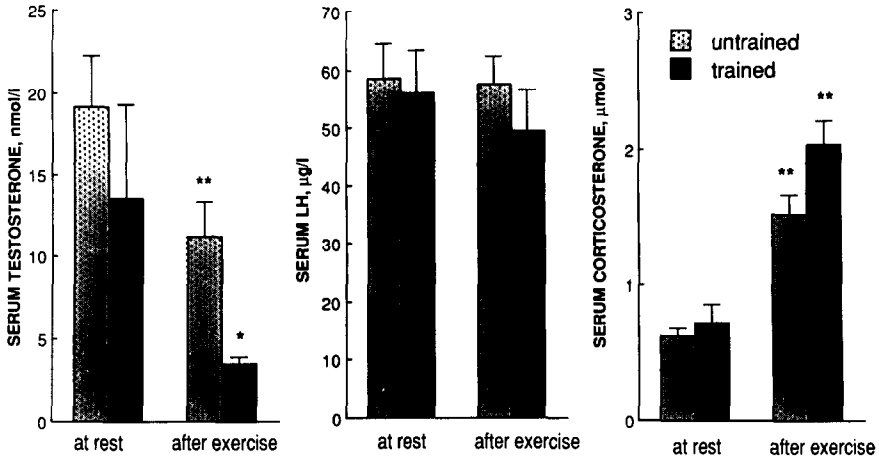

Fig. 1. Serum testosterone, LH and corticosterone concentrations before and after treadmill running in untrained and trained rats. The values are expressed as means ± SEM. $*P < 0.02$, $**P < 0.01$ for significance of differences from the pre-exercise values.

matched samples in the presence of 50 IU hCG (Pregnyl, Organon, Oss, Holland) or 5 μg ovine prolactin (Sigma, St. Louis, Mo.), respectively. Bound and free hormone were separated after 15-fold dilution of the samples with ice-cold PBS, and by centrifugation. The hCG concentration used (0.7 nmol/l) results in near-maximal saturation of the LH receptor sites [12], and the binding measured can be considered to be equal to the number of LH receptor sites in the tissue. The concentrations of hGH (0.4 nmol/l) were below those needed for receptor saturation [13], but since the K_a of hGH binding did not differ between the treatment groups (results not shown), the binding is proportional to, though not identical with, the number of prolactin receptors in the tissues.

Interstitial cell production of cAMP and testosterone

Interstitial cell suspensions of two separate running experiments were prepared by collagenase dispersion, as previously described [14]. The cells were prepared from six testes pooled from six animals per treatment group, and finally diluted in Medium 199 (containing 0.1% BSA, Gibco, Renfrewshire, Scotland) to give a cell count of about 10^6/ml. Leydig cell concentrations were determined in the suspensions using a Bürker counting chamber, after staining of the cells for 3β-hydroxysteroid dehydrogenase (3β-HSD) [14, 15]. The purity of the interstitial cell suspensions prepared from each treatment group was similar (25–30% 3β-HSD positive cells). Aliquots of the cell suspensions (350 μl) were incubated in duplicate in the presence of 0–10^{-9} mol/l hCG (CR-121) and 0.125 mmol/l 1-methyl-3-isobutylxanthine at 34°C for 3 h in an atmosphere of 95% O_2/5% CO_2. After incubation, the media were separated by centrifugation and aliquoted for cAMP and testosterone measurements. cAMP was measured with RIA

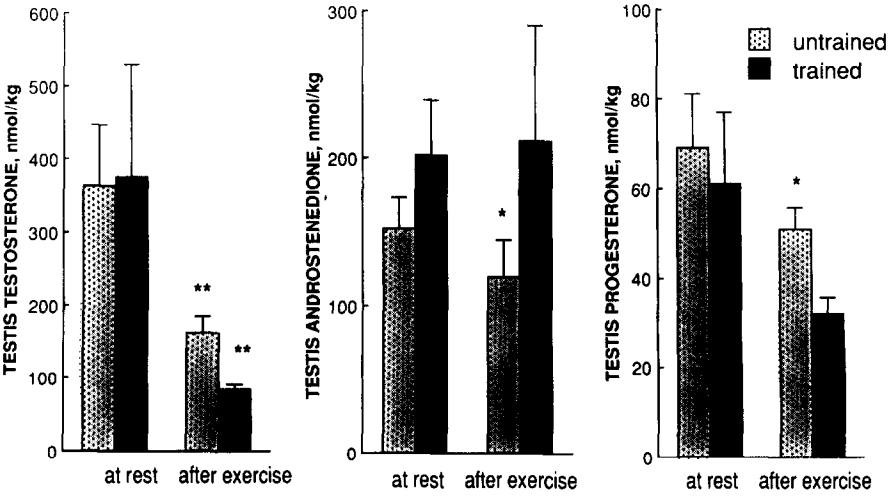

Fig. 2. Testis testosterone, androstenedione and progesterone concentrations before and after exercise. For details see text to Fig. 1.

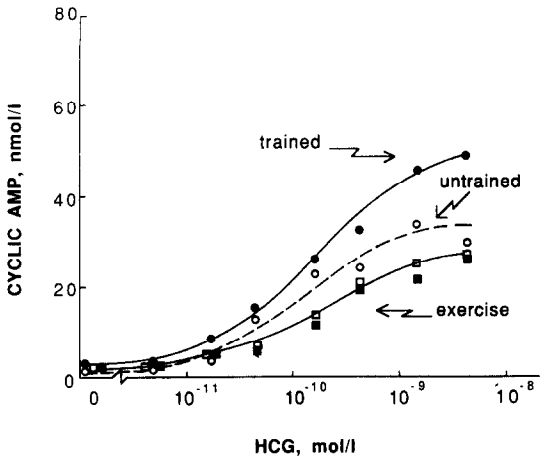

Fig. 3. Testicular interstitial cell production of cAMP during hCG stimulation *in vitro* in trained and untrained rats and after treadmill running. The rate of cAMP production is given as cAMP concentration in the medium at the end of incubation.

essentially as described by [16], and testosterone as described above for the serum samples.

Statistical Analysis

Statistical analyses were performed using the Wilcoxon matched-pair signed-rank test and the Mann–Whitney U-test [17].

RESULTS

Progressive training

Eight weeks training on a treadmill had no effect on basal serum testosterone, LH or corticosterone levels of male rats (Fig. 1). Likewise, the basal levels of testosterone and its precursors, progesterone and androstenedione in testis tissue were unaffected by training (Fig. 2).

Testes of the control animals (without training or acute exercise) had an LH receptor level of 30.3 ± 2.6 ng/g wet wt (mean $\pm$ SEM, $n = 15$), when the data of three independent experiments were pooled. The amount of lactogen receptors in the same animals was 150 ± 14 pg/g wet wt. No consistent effects of the progressive training were observed for these parameters.

The dose–response curves of hCG-stimulated cAMP and testosterone production in Leydig cell suspension prepared from testicles of untrained and trained animals showed that the maximal capacities of cAMP (Fig. 3) and testosterone (Fig. 4) production were increased by about 20–30% in the trained animals.

Acute exercise

The serum testosterone level decreased significantly ($P < 0.02$) during acute exercise, by 74% in trained and by 42% in untrained rats. The decrease tended to be greater in trained compared with untrained rats. However, the serum LH level was unchanged (Fig. 1). The serum corticosterone level rose ($P < 0.01$) by 146% in untrained and by 182% in trained rats.

The concentration of testosterone in testis tissue of exercised rats decreased by 78 and 55% in trained and untrained rats, respectively (Fig. 2). As for serum testosterone, the testicular testosterone concentration tended to decrease more in trained rats.

The decreases in the testicular concentrations of testosterone precursors, progesterone and androstenedione, in untrained rats were 26 and 21% ($P < 0.02$), respectively. In trained rats the level of testicular androstenedione did not change whereas the concentration of progesterone decreased by 48% (Fig. 2).

The acute exercise had no effect on testicular cAMP concentration. In untrained rats testicular cAMP concentration was 0.77 ± 0.03 μmol/kg wet wt at rest and 0.76 ± 0.04 μmol/kg wet wt at exhaustion.

Acute exercise had no effect on LH or lactogen receptor levels in testis tissue of untrained or trained animals (results not shown). Acute exercise decreased hCG-stimulated cAMP production in Leydig cell suspensions by about 30% (Fig. 3). This decrease in cAMP production occurred both in untrained and trained animals. However, acute exercise had no consistent effect on testosterone production in the same Leydig cell suspensions.

DISCUSSION

The main route of testicular steroidogenesis in the rat is the 4-ene pathway from pregnenolone via progesterone, 17-hydroxyprogesterone and andros-

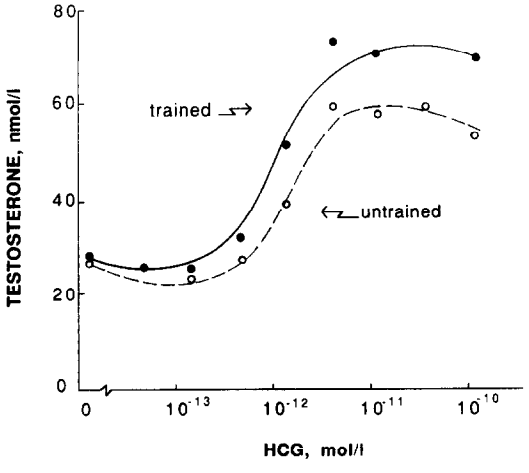

Fig. 4. Testicular interstitial cell production of testosterone during hCG stimulation *in vitro* in trained and untrained rats. The rate of testosterone production is given as the concentration of testosterone in the medium at the end of incubation.

tenedione to testosterone, whereas in man, the 5-ene route predominates [18]. The concentrations of testicular testosterone, androstenedione and progesterone in this study agree well with those obtained in the study of Eriksson *et al.*[19] when Lipidex-5000 chromatography was used for purification of testicular steroids before radioimmunoassay. Therefore, simple extraction and recovery estimation is suitable for the determination of these steroids in rat testis for the purpose of this study.

In trained and untrained animals there were no differences between steady-state levels of testosterone and its precursors. This does not necessarily mean that long-term training has no effect on the rate of testosterone biosynthesis since the capacity of isolated Leydig cells to produce cAMP and testosterone was greater in the trained animals. While the mechanism of this change remains to be clarified, it may represent a compensatory response of the Leydig cells to the short-term stress that was repeated in the animals during the progressive training period. Since the increased cAMP and testosterone production capacities were only observed under submaximal to maximal gonadotropin stimulation, it is concordant with previous observations in man that training does not increase basal circulating testosterone levels [6]. Indeed the serum testosterone level may decrease [20].

Similarly, Komadel *et al.*[21] did not find any difference in plasma testosterone concentrations between athletes (strength training) and control men. However, after giving choriogonadotropin for three consecutive days plasma testosterone rose significantly more in athletes. This also indicates that the Leydig cell capacity of athletes to synthesize testosterone was increased by training.

After acute exercise an initial small increase is followed by a decrease in plasma testosterone in man, 1–3 h after stopping the exercise, its degree depending on the intensity of the exercise [4]. In this experiment, testosterone in rat plasma was already less than half of the starting value at the end of the exercise. This may depend on the type of exercise, which was exhaustive, and the rapid kinetic behavior of rat plasma testosterone which is not affected by sex hormone binding globulin (SHBG) in this species, as in man. This low serum testosterone is probably associated with decreased testicular secretion, since testosterone and its precursors were also lowered in the testis. Low plasma and testicular testosterone was also seen in rats after swimming for 1 h [22].

No changes in serum LH were found after running in this study which is in agreement with previous studies in man [7, 23, 24]. However, in rats, prolonged physical exercise has been shown to decrease both plasma testosterone and LH and a large dose of hCG was shown to return plasma testosterone to a normal level [25]. Since no change in testicular gonadotropin or prolactin receptor levels was observed, the impairment of LH secretion or the amount of LH receptors cannot be the reason for the decreased testicular synthesis and secretion of testosterone during and after acute exercise.

Since the lowered serum testosterone seemed to be primarily caused by decreased testosterone synthesis one would expect a compensatory rise in the serum LH level. The absence of this feedback response may be explained by a stress associated increase in endorphin activity in the hypothalamus, which can lead to depressed LHRH release [26–28].

Serum testosterone decreased even though LH levels did not change significantly. On the other hand, isolated Leydig cells respond normally to LH indicating that inhibition of testicular steroid production was not inhibited *in vitro*. It is possible that inhibitory factors may have been eliminated during the preparation of Leydig cell suspensions for incubation. This concept about reversible inhibition of Leydig cells due to some other influence than a change in gonadotropin stimulation is in agreement with the study of Charpenet *et al.*[29] where it was suggested that an unknown pituitary factor is responsible for a decrease in rat testicular sensitivity to gonadotropic stimulation upon immobilization stress. The stress-induced drop in serum testosterone was prevented in that study by hypophysectomy, which gives reason to propose the pituitary as the origin of this putative effector.

On the other hand, experiments with cultured Leydig cells have shown that glucocorticoids directly suppress Leydig cell steroidogenesis by decreasing gonadotropin-stimulated cAMP production and the activity of 17α-hydroxylase [30]. In our study decreased serum and testicular testosterone concentrations after exercise were associated with highly elevated serum corticosterone levels. Therefore, this unknown pituitary factor could be ACTH, which by increasing glucocorticoid concentration in plasma indirectly causes decreased testosterone synthesis. It is also possible that the energy stores of the testes are exhausted during the exercise, which could explain why the capacity to produce cAMP, a metabolite of ATP, was impaired in the Leydig cells of the exercised animals. That this was not followed by decreased testosterone production could be explained by the fact that only a minute fraction of total cellular cAMP production is needed for maximal stimulation of steroidogenesis [14].

The results of this study show that the decreased serum testosterone level after acute exercise is associated with impaired testicular testosterone synthesis. This may be due to a direct inhibitory effect of corticosterone on testicular steroidogenesis with unchanged serum LH level. Training seems to have no effect on basal or exercise testosterone levels, but increases the capacity of interstitial cells to produce testosterone and cAMP during *in vitro* gonadotropin stimulation.

Acknowledgements—This study was supported by grants from the Ministry of Education and the Finnish Olympic Committee.

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