EFFECT OF AN ANABOLIC STEROID (METANDIENON) ON PLASMA LH, FSH, AND TESTOSTERONE AND ON THE RESPONSE TO INTRAVENOUS ADMINISTRATION OF LRH

By

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ABSTRACT

Plasma levels of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) as well as the response of LH and FSH to the intravenous administration of 100 µg of luteinizing hormone releasing hormone (LRH) were measured in 16 well-trained athletes (mean age 30 years) before and after 2 months of daily oral intake of 15 mg of metandienon, an anabolic steroid (Anabolin®, 17α-methyl-17β-hydroxy-1,4-androstadien-3-one, Medica, Finland). All athletes continued to train regularly, just as they had done for several years.

During administration of metandienon the mean plasma testosterone level fell 69%, from 29.4 ± 11.6 nmol/l to 9.1 ± 7.5 nmol/l. The mean plasma levels of LH and FSH also fell significantly (P < 0.001 and P < 0.01, respectively), both about 50%. Because LH and FSH levels were low after administration of the steroid the maximum stimulation values after LRH administration were also lower than pre-treatment values although the mean increments did not differ significantly before and after administration of the anabolic steroid. However, after treatment, the FSH response curve had a biphasic pattern in most subjects, with peaks at 10 to 20 and 50 to 60 min after the iv injection of LRH. Administration of LRH after the treatment period had no effect on FSH secretion in two subjects and no effect on LH secretion in one. Our results show that administration of an anabolic steroid causes a pronounced lowering of
plasma levels of testosterone, LH and FSH but causes no gross alteration in the response of LH secretion to stimulation by LRH. The reason for the biphasic response pattern of FSH to LRH administration in most subjects is not known.

There is evidence that both in animals and in man the secretion of gonadotrophins by the pituitary gland is influenced by sex steroids. These steroids may have both a direct effect on the pituitary gland and a feedback effect through the hypothalamus and the releasing hormones (Wynn et al. 1962; Yen & Tsai 1971; Debeljuk et al. 1972; Schally et al. 1972, 1973; Cargille et al. 1973; Debeljuk 1973; von zur Mühlen & Köberling 1973; Negro-Vilar 1973; Aakvaag & Strømme 1974; Jones & Boyns 1974; Keye & Jaffe 1974; de Kretser 1974; Robyn et al. 1974; Galloway & Pelletier 1975; Shaw et al. 1975).

The wide-spread and frequently uncontrolled use of high doses of anabolic steroids among athletes has necessitated studies on their possible effects on endogenous hormones. In addition, the recent interest in the use of these steroids for the control of male fertility (Brenner et al. 1975; Holma, to be published) calls for an investigation of their effect on the hypothalamic-hypophyseal-gonadal axis during long-term use. A review of the literature showed that few studies on the effects of the administration of "pure" anabolic steroids on endogenous hormone levels have yet been carried out. Liddle & Burke (1960) and Wynn et al. (1962) found a marked suppression of the urinary excretion of 17-ketosteroids and 17-hydroxycorticosteroids in subjects who had used metandienon (17α-methyl-17β-hydroxy-1,4-androstadien-3-one) for a long time. Wynn et al. (1962) concluded that metandienon retards the rate of production of adrenocortical steroids by inhibiting either the production or release of corticotrophin (ACTH). One man given large doses of metandienon exhibited a decrease in the urinary level of pituitary gonadotrophin (Liddle & Burke 1960).

Aakvaag & Strømme (1974) recently reported on the effect of mesterolone (1α-methyl-17β-hydroxy-5α-androstan-3-one) on plasma levels of testosterone, LH and FSH. Mesterolone, however, has only slight anabolic effects and the authors, in fact, observed no significant change in muscle strength, in aerobic power or in the plasma levels of LH and FSH as a result of its use. A slight decrease (23%) in plasma testosterone levels was, however, noted. Further analysis revealed that during treatment with mesterolone the binding of testosterone by plasma proteins was significantly reduced, whereas the plasma level of free testosterone appeared to remain unchanged.

Because information on this subject is scanty and because the effects of "pure" anabolic steroids have not been studied at all, we decided to investigate, in 16 well-trained athletes, the effects of a daily intake of 15 mg of
methandienon 1) on several physiological parameters (Holma, to be published), and 2) on plasma levels of testosterone, LH, FSH and on the response of LH and FSH secretion to the iv administration of LRH. A preliminary report of our findings with regard to the hormones has already been published (Holma & Adlercreutz 1975).

**MATERIAL AND METHODS**

**Subjects**

The subjects were 16 well-trained and well-informed male athletes, whose mean age was 30 years (range 19–48 years), and who volunteered for this study. All the men continued to do their training exercises 6 or 7 times a week just as they had done for at least 3 years before the study.

On the first day of the study the athletes came to the laboratory at 6.30 a.m. after having fasted for 8 h. Basal blood samples for testosterone, LH and FSH measurements were drawn three times within 40 min starting at 7.00 a.m. After the rapid intravenous injection of 100 µg of synthetic LRH (a generous gift from Dr. Sheldon Segal, Biomedical Division, Population Council, New York, N.Y.), blood samples were drawn every 10 min for 1 h. After these pre-treatment assays the test subjects began taking metandienon (Anabolin®, Medica, Finland) orally 15 mg/day in three equal doses. The diet of the athletes remained unchanged and they used no other drugs. All athletes remained healthy throughout the study. After metandienon had been taken regularly for 2 months all measurements were made again in exactly the same manner as described above. In one subject only testosterone measurement were carried out.

**Hormone assays**

All hormone assays were carried out in duplicate.

Plasma testosterone levels were determined by a modification of the method of Ismail et al. (1972). After extraction of the steroids from the ammonium sulphate precipitate and evaporation of the organic solvent, the residue was taken up in 0.133 mol/l borate buffer, pH 8.0, that contained 0.02% gelatin and 0.00001% Tween 20. The sample was incubated overnight at 4°C with antiserum (raised in rabbits against testosterone-3-bovine serum albumin, Searle Diagnostics, High Wycombe, Bucks, England) and [1,2,6,7-3H]testosterone (The Radiochemical Centre, Amersham, England). Bound and unbound testosterone were separated with dextran-coated charcoal in borate buffer (2.5 g Norit-A and 2.5 g Dextran T 70 in 1 l of the borate buffer).

Plasma luteinizing hormone (LH) levels were determined by a double antibody solid-phase technique (DASP) performed largely as described by den Hollander & Schuurs (1971). The LH-antiserum and purified human LH were gifts from the National Institute of Arthritis and Metabolic Diseases (National Institute of Health, Bethesda, Maryland, USA). The LH was labelled with 125I by coupling of the lactoperoxidase to a cross-linked co-polymer of maleic anhydride and butanediol divinyl ether (E. Merck, Darmstadt, Germany) and the coupled enzyme was used for iodination (Karomen et al. 1975). MRC reference material of human pituitary hormone (68/40) (World Health Organization by the International Laboratory for Biological
Standards, Mill Hill, London, England) was used for the standard curves (assuming a value of 40 U/ampoule). Antigen (unknown or standard), labelled antigen and the first antibody were incubated at 4°C for 48 h in a volume of 550 μl. Insolubilized second antibody (sheep antiserum to rabbit gamma-globulin) (immunosorbet 5.5 ml diluted in 50 ml of 0.02 mol/l sodium phosphate buffer, pH 7, containing 0.02 mol/l NaCl, 0.005 mol/l Na-EDTA and 0.1% w/v merthiolate) was added (500 μl), after which the tubes were rotated at room temperature for 6 h and then centrifuged. The supernatant was discarded and the solid phase washed and counted. The inter-assay coefficient of variation for a pooled sample from which aliquots were analysed 17 times over 18 months was 14.2%. Sixty-seven analyses of a human LH/FSH reference preparation (LER-907) during a 2-year period gave a coefficient of variation of 9.6%.

Plasma follicle-stimulating hormone (FSH) levels were measured with the same technique used for LH determinations with only minor alterations. The antiserum and purified human FSH were gifts from the National Institute of Arthritis and Metabolic Diseases (National Institutes of Health, Bethesda, Maryland, USA). The FSH was labelled in exactly the same manner as was described for LH. MRC reference material of Human Pituitary Follicle-Stimulating Hormone (68/39) was used for the standard curves (assuming a value of 32.8 U/ampoule). Antigen (unknown or standard), labelled antigen and the first antibody were incubated at 4°C for 24 h in a volume of 550 μl. Insolubilized second antibody (sheep antiserum to rabbit gamma-globulin) (immunosorbet 5.5 ml diluted in 40 ml of 0.02 mol/l sodium phosphate buffer, pH 7, containing 0.02 mol/l NaCl, 0.005 mol/l Na-EDTA and 0.1% w/v merthiolate) was added (500 μl), and the tubes were rotated for 4 h and then centrifuged. The supernatant was discarded and the solid phase washed and counted. Twenty-one analyses of a human LH/FSH reference preparation (LER-907) during a 2-month period gave a coefficient of variation of 8.9%.

All results were analysed statistically with the t-test according to de Jonge (1964).

**RESULTS**

**Plasma testosterone**

During treatment there was a highly significant ($P < 0.001$) decrease in the basal secretion of testosterone. The decrease was 69%, from $29.4 \pm 11.6$ to $9.1 \pm 7.5$ nmol/l.

**Plasma luteinizing hormone**

Treatment with metandienon also caused a highly significant ($P < 0.001$) decrease in the basal plasma level of LH. This decrease was 53%, from $11.4 \pm 3.3$ to $5.4 \pm 1.9$ U/l. Because LH levels were low after the treatment, the maximum stimulation values after administration of LRH were also lower (Fig. 1) than pre-treatment values. However, the mean maximum increments before and after treatment, $11.4 \pm 3.3$ and $8.9 \pm 5.9$, did not differ significantly ($0.05 < P < 0.1$). The response of plasma LH to LRH was slightly delayed after metandienon treatment.
The response of LH secretion to iv administration of 100 μg of LRH before and after treatment with metandienon. Mean value and standard error. Levels of significance:

\[** = P < 0.01, \ *** = P < 0.001, \ * = P < 0.05.\]

**Plasma follicle-stimulating hormone**

Treatment with metandienon also caused the mean basal plasma level of FSH to fall significantly \((P < 0.01)\). The decrease was 47 %, from 7.0 ± 4.1 to 3.7 ± 2.8 U/l. As was observed for LH the maximum increments in FSH
levels after administration of LRH before and after metandienon treatment, 5.5 ± 4.6 and 4.3 ± 3.9 U/l respectively, did not differ significantly. When given after metandienon treatment, the LRH caused two peaks in FSH levels, the first 10 to 20 min and the second 50 to 60 min after the injection of LRH (Fig. 2). Hence the pre-treatment and post-treatment plasma levels of FSH recorded after LRH administration differed significantly at 30, 40 and 50 min after the injection of LRH but not at 10, 20 and 60 min. In two subjects LRH administration after the use of metandienon had no effect on plasma FSH levels; in two others the response was very slight. In one subject basal FSH secretion did not differ significantly from zero after metandienon therapy, and in another the basal level was very low.

**DISCUSSION**

Metandienon is a powerful anabolic agent that affects haemodynamics, spermatogenesis (*Holma*, to be published), and the development of muscular strength (*Johnson & O'Shea* 1969). The most profound effect of this steroid on the parameters we investigated was a statistically significant drop in the basal levels of plasma testosterone, LH and FSH. The percentage decrease was highest for testosterone (69%) and nearly the same for LH and FSH (about 50%). The influence of metandienon on the response of plasma LH and FSH to the administration of LRH was less pronounced. Because LH and FSH levels were low at the end of the treatment period, the maximum stimulation values after the administration of LRH were lower than pre-treatment values, although the mean maximum increments before and after treatment with the anabolic steroid did not differ significantly. However, after treatment the response curve of FSH to LRH administration showed a peculiar biphasic pattern in most subjects, the curve peaking at 10 to 20 and 50 to 60 min. Present knowledge about the regulation of FSH secretion does not offer any explanation for such a response pattern. Treatment with metandienon had no effect on FSH secretion in two subjects and no effect on LH secretion in a third.

That gonadal steroids have a negative feedback effect on the secretion of pituitary LH and FSH is well documented (*Yen & Tsai* 1971; *Schally et al.* 1972; *Cargille et al.* 1973; *von zur Mühlen & Köberling* 1973; *Negro-Vilar* 1973; *de Kretser* 1974; *Brenner et al.* 1975). However, in post-menopausal women a relatively brief treatment with ethinyl oestradiol caused an initial marked suppression in the secretion of LH and FSH followed by a positive feedback effect that was quantitatively much greater for LH than for FSH (*Yen & Tsai* 1971). Gonadal steroids are also known to be able to modify the pituitary response to LRH (*Schally et al.* 1972; *Debeljuk et al.* 1972;
Schally et al. 1973; Debeljuk 1973; von zur Mühlen & Köbberling 1973; Negro-Vilar 1973; Jones & Boyns 1974; Robyn et al. 1974; Galloway & Pelletier 1975; Shaw et al. 1975). von zur Mühlen & Köbberling (1973) reported that, when administered for a short time testosterone seemed to block only the basal secretion of LH, whereas when administered in the same doses over a longer period of time testosterone also blocked LRH-induced LH increase. These same investigators observed that the negative feedback of testosterone was more rapid and more pronounced on FSH than on LH, whereas most other studies have shown that oestrogen and testosterone suppress LH more rapidly and markedly than they do FSH.

According to Aakvaag & Strømme (1974) plasma testosterone levels fall after the administration of mesterolone because the binding of testosterone by plasma proteins is reduced. In the method used to determine the plasma concentration of testosterone, the amount of radioactive internal standard recovered gives a measure of the percentage of radioactive testosterone bound to the globulin fraction (mainly sex hormone-binding globulin) at 4°C in vitro in the presence of endogenous steroids. This, in turn, gives a rough estimate of the amount of specific binding protein present in plasma. Our results showed, however, that the pre-treatment percentage of testosterone bound to the globulin fraction was comparatively low in this group of well-trained athletes, and that it did not change significantly during treatment with metandienon. In 9 out of 16 subjects this percentage fell slightly during treatment, but in others it rose. Hence it seems that in well-trained athletes the decrease in plasma testosterone levels caused by treatment with metandienon is due only slightly, if at all, to a decrease in the level of the binding protein. In addition to causing a drop in the plasma level of testosterone by inhibiting LH secretion, metandienon might also either accelerate the metabolic clearance rate of testosterone or inhibit its biosynthesis by direct action on the gonads; these possibilities were not, however, studied in the present investigation. From our knowledge, such a considerable decrease in plasma testosterone as recorded in our subjects cannot result solely from the observed moderate decrease in the secretion of gonadotrophins.

Whether the significant deviations from the normal pattern of plasma testosterone, LH or FSH levels reported here have any physiological consequences cannot be determined from our data. There was no correlation between the basal levels of these three hormones before and after treatment. Nor was there any correlation either between the decrease in the levels of testosterone, LH and FSH caused by treatment or between the effect of LRH administration on LH and FSH levels before and after treatment.

The low basal levels of LH and FSH recorded after metandienon treatment were most likely due to a suppression of the hypothalamic function, because maximum increments in LH and FSH levels after LRH administration did
not differ significantly before and after treatment. However, the peculiar biphasic FSH response after treatment might have been due to a direct effect of metandienon on the storage of FSH in the pituitary.

No attempt was made in this study to measure duration of the effects we observed. A recent study by Remes & Adlercreutz (to be published) has shown, however, that it takes several weeks for the decrease in plasma testosterone levels to return to normal.

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