Effect of androgens on concentration of LH and FSH in the peripheral plasma of anoestrous ewes

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Although testosterone induces behavioural oestrus (Lindsay & Robinson, 1964) and ovulation in anoestrous ewes (Radford & Wallace, 1971) and stimulates the synthesis and release of FSH in immature female rats (Naqvi & Johnson, 1969) and LH in lactating ewes (Restall, Radford & Wallace, 1972), its mechanism of action in the female has not yet been characterized. It may act either directly or by its conversion to dihydrotestosterone (Wilson & Gloyna, 1970) or oestrogens (Baird, Horton, Longcope & Tait, 1969), or by more than one of these mechanisms. This report evaluates the separate effects of testosterone propionate and dihydrotestosterone on the secretion of LH and FSH in intact anoestrous ewes.

Ten mature Clun Forest ewes were divided into four groups during the non-breeding season (July) and were given a single intramuscular injection of 0, 2.5, 5.0 or 10.0 mg testosterone propionate in arachis oil (Androject: Organon); the number of ewes in each of the treatment groups were 2, 2, 3 and 3 respectively. Another five ewes were injected i.m. with 5 mg (3 ewes) or 10 mg (2 ewes) 5 α -dihydrotestosterone (Sigma), in 2 ml arachis oil. Samples of up to 5 ml jugular venous blood were collected through indwelling catheters into heparinized syringes immediately before the injection of testosterone propionate or dihydrotestosterone and at 2-h intervals thereafter for the next 36 h.

Plasma LH and FSH were estimated by radioimmunoassay, using NIH-LH-S16 and NIH-FSH-S9 as standards, as described in detail by Pant, Hopkinson & Fitzpatrick (1977). Both antisera distinguished readily from prolactin, GH and TSH and there was <0.3% cross reaction between FSH and LH. Extracts of sheep pituitary glands, pooled sheep plasma and NIH standards gave assays which did not deviate from parallelism. For LH, over the range of concentrations 0.1–122 ng/ml plasma, recovery was $98.2 \pm 2.0\%$; coefficient of variation was 4.1–19.7%; precision was 7.2–10.5% and interassay variation was 9.7 and 19.0% (two estimates). For FSH, over the range 20–400 ng/ml plasma, recovery was $94.0\% \pm 1.77$; coefficient of variation was 2.1–9.6%; precision was 4.1–10.0% and interassay variation was 5.1% and 10.5% (two estimates).

Neither of the control ewes showed any major change in the concentration of LH or FSH (Text-fig. 1a); concentrations of LH ranged between 1.3 and 3.6 ng/ml (2.06 ± 0.08 , mean $\pm$ S.E.M.) and of FSH between 30.0 and 80.0 ng/ml plasma (51.3 ± 2.0). There were, however, significant increases in plasma concentrations of both gonadotrophins in each of the ewes given 2.5 mg testosterone propionate and in 2 of the 3 ewes treated with 5 and 10 mg respectively (Text-figs 1b–1d). Plasma LH began to rise between 16 and 24 h (18.6 ± 1.1) after injection of testosterone propionate, reached a maximum of 31.0–65.0 (41.2 ± 4.7) ng/ml between 20 and 30 h (24.6 ± 1.2) and returned to pretreatment values by 28–36 h (33.0 ± 3.0); the duration of rise changed between 10 and 18 h (14.3 ± 1.1). The changes in plasma FSH were very similar to those of LH and the maximum values ranged between 107 and 180 ng/ml (137.9 ± 10.4); only 2 ewes failed to show an FSH response and these were the same two in which LH failed to rise.

After the injection of dihydrotestosterone, there was no significant rise in the plasma concentration of either gonadotrophin in any ewe; LH concentration ranged between 0.9 and 3.2 ng/ml and FSH between 25.9 and 95.0 ng/ml (Text-fig. 2). This difference in the response of ewes to the two androgens was significant ($P < 0.02$; Student's *t* test).

These results demonstrate that injection of testosterone propionate in intact anoestrous ewes elicits release of LH and FSH from the pituitary, as does oestradiol-17 β (Pant, Fitzpatrick & Hop-

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Text-fig. 1. Plasma LH (—) and FSH (---) concentrations in 10 anoestrous ewes after the intramuscular administration of (a) arachis oil, (b) 2.5, (c) 5 or (d) 10 mg testosterone propionate. The responses of the individual animals are indicated by different symbols in each panel.

kinson, 1973). The observation that another potent but non-aromatizable androgen, dihydrotestosterone (Ito & Horton, 1971), proved ineffective in this respect suggests that if the sheep hypothalamus, like that of some other species (Naftolin, Ryan & Petro, 1972; Reddy, Naftolin & Ryan, 1973), is capable of aromatizing androgens then the aromatization of testosterone to oestradiol may be necessary for its observed stimulatory effect on gonadotrophin secretion in the ewe.

It is a matter of regret that testosterone was not available as the free alcohol or dihydrotestosterone as the propionate. Esterification usually prolongs the action of androgens. Nevertheless, the free dihydrotestosterone elicited no detectable response even though blood was sampled 2 h after administration and every 2 h thereafter: it is unlikely that a transient effect would have been totally missed. Furthermore, no effect was seen with the highest dose of dihydrotestosterone although the lowest dose of testosterone propionate elicited a definite increase in LH and FSH. The present

Text-fig. 2. Mean ($\pm$ S.E.M.) plasma concentrations of LH (—) and FSH (---) in 5 anoestrous ewes after the intramuscular administration of 5 or 10 mg dihydrotestosterone.

results do, however, require confirmation with a more direct comparison of the effects of the two esters and non-esterified alcohols.

The present study does also suggest a sex difference in the mechanism of action of testosterone on the regulation of gonadotrophin secretion in that testosterone is known to inhibit the synthesis and release of pituitary gonadotrophins in the male of several species (Ramirez & McCann, 1965; Pelletier, 1970). That aromatization of androgen to oestrogen is not involved in this inhibition was demonstrated in intact rats by Naftolin & Feder (1973).

It is well known that large amounts of androgens, mainly androstenedione and testosterone, are secreted by the ovary of the ewe (McCracken, Baird & Goding, 1971). However, in the absence of data on the concentration of testosterone in the peripheral circulation of ewes during the oestrous cycle it is not possible to indicate the physiological role, if any, of ovarian androgens in the regulation of gonadotrophin secretion and oestrous behaviour. Furthermore, investigations of the effect of lower doses of androgens are required before conclusions on the physiological importance of ovarian androgen secretion can be reached. It is tempting to speculate, however, that ovarian androgen secretion may be an additional mechanism for stimulating the expression of normal sexual behaviour and also for the surges of gonadotrophins at oestrus.

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