Effect of androstenedione on the oestrous cycle of the rat*

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Summary. The implantation of Silastic capsules containing androstenedione (release rate 63.2 ± 4.4 $\mu\text{g}/24$ h) into 4-day cyclic rats resulted in a prolongation of the cycle ($P < 0.001$), most rats showing 5-day cycles after the first, largely unaffected cycle. There was a reduction in ovulation rate ($P < 0.01$) and lower serum LH levels on the morning of oestrus ($P < 0.01$) but serum FSH levels were unaffected.

Introduction

Androstenedione, a weak androgen, is an important intermediate in the synthesis of oestrogens by the ovary. It is also secreted directly from the ovary as well as from the adrenal gland and serves as a major precursor for peripheral aromatization to oestrogens (Baird, Horton, Longcope & Tait, 1968), but very little is known about possible direct effects of the steroid on reproductive function. The administration of androstenedione can restore sexual receptivity in ovariectomized, oestrogen-treated, dexamethasone (0.5 mg/kg/day)-suppressed female rhesus monkeys (Everitt & Herbert, 1971), and elicit daily surges of luteinizing hormone (LH) in ovariectomized rats (Febres, Seron, Weiner & Siiteri, 1977). Active immunization of sheep against androstenedione results in an elevation of basal and episodic LH release (Martensz, Baird, Scaramuzzi & Van Look, 1976) and increases ovulation rate (Scaramuzzi, Davidson & Van Look, 1977; Van Look, Clarke, Davidson & Scaramuzzi, 1978). From these studies it was suggested (Martensz *et al.*, 1976) that androstenedione may facilitate the feedback effects of oestradiol-17 β on pituitary gonadotrophin secretion. The present work was undertaken to examine the effects of chronic administration of androstenedione on different aspects of the oestrous cycle in the rat.

Materials and Methods

Adult female rats were used from the Wistar-derived colony that is kept in the Department of Pharmacology under controlled conditions of light (from 05:00 to 19:00 h), temperature (22°C) and humidity (62–65%). Vaginal smears were taken each morning, and only those rats showing two consecutive 4-day cycles were included in the treatment groups. On the morning of the first day of dioestrus the rats were lightly anaesthetized with ether and capsules containing androstenedione (experimental group, N = 10), or cholesterol (control group, N = 12) were implanted subcutaneously in the neck region. The implants were prepared as described by Karsch *et al.* (1973) and incubated for 10 days in phosphate-buffered saline (pH 7.12) with constant agitation in a Julabo paratherm incubator at 37°C before implantation. Under these conditions, the androstenedione implants released 63.2 ± 4.4 μg (mean $\pm$ s.e.m.) steroid/24 h during the last 6 days of incubation. The cycle during which the capsules were implanted is referred to as the first cycle. After 4 cycles all the animals (except for 4 controls) were bled by aortic puncture under

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light ether anaesthesia on the morning of the day of oestrus. The ovulation rate for each animal was assessed from the number of ova present in the oviducts, and the ovaries were removed and weighed. Blood was centrifuged at 4°C and the serum was stored at -20°C until analysed.

Serum levels of follicle-stimulating hormone (FSH) and LH were measured by radioimmunoassay using NIAMD-rat-FSH-I1 and NIAMD-rat-LH-I3 for iodination and NIAMD-rat-FSH-RP1 and NIAMD-rat-LH-RP1 as standards. The sensitivity (defined as the amount of standard required to depress binding to 90% of that occurring in the absence of unlabelled hormone) was estimated as 1.0 ± 0.3 ng LH and 1.5 ± 0.5 ng FSH. The intra- and inter-assay coefficients of variation were between 10 and 15% for serum pools (measured amount of LH about 100 ng and FSH about 400 ng) (Welschen *et al.*, 1975). Student's *t* test was used for statistical analysis of all data except cycle length which was analysed by χ^2 test.

Results

The effect of androstenedione upon the length of the oestrous cycle is shown in Table 1. Most control animals continued to show regular 4-day cycles. In rats bearing an androstenedione implant the length of the first oestrous cycle was largely unaffected but thereafter cycles became significantly ($P < 0.001$) longer, most rats showing a 5-day cycle. This prolongation of the cycle was associated with a significant ($P < 0.01$) reduction in ovulation rate (Table 2). Serum levels of LH were also significantly lower in the experimental animals than in controls ($P < 0.01$), but levels of FSH were similar. There was no significant difference in ovarian weight between the two groups.

Table 1. Length of the oestrous cycle in adult rats (no. in parentheses) treated with implants of androstenedione or cholesterol

Treatment	Cycle after implantation	Length of cycle			
		4 days	5 days	6 days	7 days
Cholesterol (N = 12)	1	10	2	—	—
	2	11	1	—	—
	3	11	1	—	—
	4	11	1	—	—
Androstenedione (N = 10)	1	7	3	—	—
	2	2	7	1	—
	3	3	6	—	1
	4	2	8	—	—

Table 2. The effect of androstenedione implants on ovulation rate, ovarian weight and gonadotrophin levels on the morning of oestrus of the 4th cycle after treatment in the rat

	Treatment	
	Cholesterol	Androstenedione
No. of rats	8	10
No. of tubal ova	12.4 ± 0.4	$9.8 \pm 0.6^*$
Ovarian weight (mg)	70.8 (50.0–101.3)	83.7 (65.8–121.2)
FSH (ng/ml)	417.0 ± 88.1	438.0 ± 55.4
LH (ng/ml)	36.1 ± 4.4	$20.4 \pm 3.2^*$

Values are mean $\pm$ s.e.m. or mean and range.

* Significantly different from control value, $P < 0.01$.

Discussion

The data reported here indicate that continuous administration of androstenedione to adult female rats prolongs significantly the length of the oestrous cycle from a median value of 4 days to one of 5 days. This prolongation was due to an increase in the length of the dioestrous period, since in these prolonged cycles there was still only one day of oestrus and one of pro-oestrus as indicated by the vaginal smears. These effects are quite different from those obtained after continued administration of oestrogens (see Neill, 1974, for review), testosterone (Mark & Biskind, 1941) or dehydroepiandrosterone (Ward, Costoff & Mahesh, 1978) since administration of these steroids results in suppression of ovulation with either ovarian atrophy (oestrogens, testosterone) or the formation of polycystic ovaries (dehydroepiandrosterone).

The decrease in ovulation rate and serum levels of LH on the morning of oestrus confirms earlier observations (Martensz *et al.*, 1976; Scaramuzzi *et al.*, 1977; Van Look *et al.*, 1978) showing that active immunization of sheep against androstenedione produces the opposite effects, i.e. an increase in ovulation rate and pituitary LH secretion. Since androstenedione treatment of ovariectomized rats (Febres *et al.*, 1977), sheep (P. F. A. Van Look & R. J. Scaramuzzi, unpublished) and women (Yen, Vandenberg, Tsai & Parker, 1974) has no effect on the elevated tonic secretion of LH, it seems likely that the lower LH levels observed in the rats with an androstenedione implant are the result of a permissive action of the administered hormone on the negative-feedback effect exerted by endogenous oestrogen in these animals (Martensz *et al.*, 1976). The exact site at which androstenedione exerts this effect is uncertain. It has been shown that the hypothalamus has the capacity to convert androstenedione to oestrone (Reddy, Naftolin & Ryan, 1974) and that implantation of oestrogen in the median eminence region lowers tissue content of LH-RH and plasma levels of LH (Chowers & McCann, 1965). Alternatively, androstenedione may influence gonadotrophin secretion directly through an action on the pituitary gonadotrophs. Drouin & Labrie (1976) have demonstrated that addition of androstenedione to rat pituitary cells in culture reduces the secretion of LH in response to LH-RH. The absence of a decrease in serum levels of FSH in the animals treated with androstenedione may be related to the greater sensitivity of LH release than of FSH release to androgens (Swerdloff, Walsh & Odell, 1972). The increase in cycle length in androstenedione-treated rats was associated with a significant decrease in ovulation rate. It is unlikely that this reduced ovulation rate is a direct consequence of the increase in cycle length *per se* since the ovulation rate in spontaneous 5-day cyclic rats is similar to that in 4-day cyclic rats in our colony of animals. The mechanism underlying this decreased ovulation rate is uncertain. The stimulus for ovulation may be inadequate as a result of the central effect of the steroid. Alternatively, the reduction of ovulation rate may be due to a reduction in the number of pre-ovulatory follicles, since androgens have been shown to induce follicular atresia through a direct action on the ovary (Louvet, Harman, Schreiber & Ross, 1975).

In conclusion, the present results indicate a possible role of androstenedione in the regulation of the oestrous cycle and the process of ovulation in the rat. Further studies are required to determine if the observed effects are exerted by androstenedione itself or by oestrogens, formed through peripheral or central aromatization.

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