Sexual behaviour and LH secretion in spayed androgenized ewes after a single injection of testosterone or oestradiol-17β

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Summary. The behavioural and endocrine responses to single injections of 50 or 500 µg oestradiol-17β or 5 mg testosterone were recorded in spayed (control) ewes and in spayed ewes exposed to testosterone between Days 30 and 80 or Days 50 and 100 of prenatal life. The control ewes showed oestrus after injections on 17/18 occasions. The androgenized ewes showed poorer oestrous responses to each hormone although rams showed interest in the ewes. Masculine sexual and aggressive behaviour was shown by the androgenized ewes given either steroid. Both steroids caused a reduction in the plasma LH levels of all the ewes (negative feedback), followed by a preovulatory-type surge (positive feedback). The peak LH values were significantly lower ($P < 0.05$) in the Day 50–100 androgenized ewes than in the controls.

It is concluded that prenatal androgenization causes a qualitative shift in the sexual behaviour of ewes from the female type to the male type and affects the sensitivity of the brain to ‘positive feedback’ by steroids.

Introduction

Androgenized female rats (Gerall & Ward, 1966), guinea-pigs (Phoenix, Goy, Gerall & Young, 1959), hamsters (Johnson, 1975), mice (Manning & McGill, 1974) and dogs (Beach, Kuehn, Sprague & Anisko, 1972) all show enhanced masculine behaviour, compared to normal females, after ovariectomy and chronic steroid administration. This prolonged steroid therapy is an unphysiological situation as normal females will also show enhanced masculine behaviour if given such treatment. For example, female sheep rarely show any component of male sexual or aggressive behaviour (Clarke, 1977), but if they are given repeated injections of 50 mg testosterone their behaviour becomes masculine within 14 days (Johnson, Hudson, Bogart, Oliver & McKenzie, 1956). In contrast, a single injection of testosterone will induce normal oestrous behaviour in ewes (Lindsay & Robinson, 1961, 1964). The emphasis in the present work has therefore been on the acute administration of small amounts of testosterone or oestradiol-17β to spayed androgenized and normal ewes. Masculine and feminine behaviour were then measured to see if the type of sexual behaviour displayed was dependent on the steroid injected, or on sexual differentiation of the brain.

Prenatally androgenized ewes show masculine behaviour and a reduction in oestrous behaviour during the mating season, although some of these ewes experience regular ovulation (Clarke, 1977; Clarke, Scaramuzzi & Short, 1977). In the absence of regular overt oestrous cycles, however, it is difficult to determine the extent to which the sexual behaviour is controlled by ovarian steroids. The animals used in the present studies were spayed so that their sexual and aggressive behaviour could be observed after the injection of a standard dose of oestradiol-17β or testosterone.

The hypothalamo-pituitary responses to oestradiol-17β and testosterone were also measured. The preovulatory type of LH release after oestrogen administration is a sexually dimorphic character in sheep (Short, 1974; Karsch & Foster, 1975). Failure of positive feedback in prenatally androgenized ewes may be regarded as evidence of brain masculinization. It has been reported that androgenized ewes are less likely to show positive feedback during anoestrus (Short, 1974; Clarke, Scaramuzzi & Short, 1976a) but a seasonal component may have been partly responsible for this result. To study

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positive feedback in response to steroid injections during the mating season spayed animals are essential. This paper compares the LH release and the masculine and feminine behaviour of spayed androgenized and normal ewes after the injection of oestradiol-17β or testosterone during the mating season.

Materials and Methods

Animals

Androgenized Finnish Landrace × Dorset Horn ewes were used; their mothers had received 1 g testosterone implants (s.c.) between Days 30 and 80 (D30–80 group) or Days 50 and 100 (D50–100 group) of the 145-day pregnancy period (Clarke, Scaramuzzi & Short, 1976b). Masculinization of the external genitalia was complete in the D30–80 ewes and partial in the D50–100 ewes. Six androgenized ewes from each of these groups and 6 normal ewes were spayed during December 1975. One month was allowed for recovery, and experiments were begun in January 1976.

Six vasectomized and sexually experienced rams of the same breed were maintained for the detection of oestrus and behavioural observations.

Treatments

The animals were randomly allocated to three treatment groups, each containing two D30–80, two D50–100 and two control ewes. Before each observation period, all the ewes received 10 daily injections (i.m.) of 10 mg progesterone in oil (Robinson, 1959) (Intervet Laboratories Ltd, Bar Hill, Cambs, U.K.). At 04:00 h on the 2nd day after the last progesterone injection, the 6 ewes within a group were given a single i.m. injection of 50 µg oestradiol-17β, 500 µg oestradiol-17β or 5 mg testosterone (Sigma Chemical Co., Kingston-upon-Thames, Surrey, U.K.) in 1 ml peanut oil. This procedure was repeated for 3 observation periods so that each ewe received each dose of steroid, and two ewes from each of the D30–80, D50–100 and control groups were given the same dose at each period. After the first and second observation periods 2 days elapsed between oestradiol-17β and testosterone injections and the start of the following progesterone therapy.

Behavioural observations

After the oestradiol-17β or testosterone injections, the ewes were tested for oestrus at 4-h intervals by placing a vasectomized ram in the pen. Ewes were regarded as showing oestrus if they stood for the ram at two consecutive tests. The time of onset of oestrus was taken as 2 h before the first test for oestrus at which a ewe stood for the ram (Scaramuzzi, Lindsay & Shelton, 1971).

Detailed behavioural observations were carried out on each ewe for a 5-min period as follows.

Test 1. Each ewe was paired with a ram and the sexual behaviour of both animals was recorded (see definitions below and Clarke, 1977). One of six rams was randomly assigned to each test so that each ewe was exposed to any one ram on only one occasion. No efforts were made to analyze between-ram variation. The tests were conducted 24 h before and 24–36 h after the steroid injections.

Test 2. Each ewe was paired with an oestrous ewe 24 h before as well as 24–36 h after the oestradiol-17β or testosterone injections. The same behavioural events were recorded as in Test 1. Test 1 was always carried out before Test 2.

The behaviour of the 6 vasectomized rams during a 5-min period with an oestrous ewe (Test 1) was also recorded and the male-like behaviour of androgenized ewes was compared to this.

Male and female sexual behaviour was recorded according to the definitions described elsewhere (Clarke, 1977).

Plasma LH assay

Blood samples were collected at 4-h intervals from all the ewes by jugular venepuncture, from 12 h before to 44 h after steroid injection. The blood was centrifuged immediately and the plasma removed.
and stored at -20°C until assay. Plasma LH concentrations were measured by the radioimmunoassay described by Martensz, Baird, Scaramuzzi & Van Look (1976): the limit of detection was 0.05-0.075 ng LH/tube and the intra- and inter-assay coefficients of variation were 8 and 12% respectively. The results are expressed as ng equivalents of NIH-LH-S14/ml.

Results

Induction of oestrus in the spayed ewe

The control ewes showed oestrus in 17 out of 18 tests; the time from injection of oestradiol-17β or testosterone to the onset of oestrus was 12.0 ± 1.6 (s.e.m.) h and was unaffected by the dose or type of steroid. One D30-80 ewe given 50 μg oestradiol-17β came into oestrus 28 h after injection and had an oestrous period lasting more than 12 h. One D50-100 ewe given 500 μg oestradiol-17β showed oestrus 18 h after injection and also remained in oestrus for more than 12 h. Both of these ewes displayed female courtship behaviour which was similar to that of the control ewes. Six other D30-80 ewes and one D50-100 ewe showed signs of oestrus for less than 8 h.

Behaviour of rams towards the ewes

The rams showed considerable interest in the androgenized ewes although many of them were not in oestrus. The number of tests (Test 1) in which the rams displayed investigatory, courtship and mating patterns is given in Table 1. The most common precopulatory display of rams is 'kicking' and 'nudging' (Clarke, 1977) and these two behavioural patterns commonly occur together (Banks, 1964). The occurrence of 'kicking' and 'nudging' was therefore used as an index of courtship behaviour. The proportion of tests in which investigatory ('scenting' and 'Flehmen') and courtship ('kicking' and 'nudging') patterns occurred was similar when the rams were paired with control or androgenized ewes. The frequency of courtship display by the rams but not their investigatory behaviour increased above the preinjection levels when ewes were injected with oestradiol-17β or testosterone. The increase was statistically significant only for the rams paired with the D30-80 ewes. The rams made significantly (P < 0.05) more unsuccessful attempts to mount the androgenized ewes than they did the controls (Table 1). The rams' behaviour did not vary with the type or dose of hormone given to the ewes.

Table 1. The results of tests (Test 1) in which rams displayed various types of precopulatory behaviour towards spayed androgenized ewes and spayed controls injected with oestradiol-17β or testosterone†

<table>
<thead>
<tr>
<th></th>
<th>Control ewes</th>
<th>D30-80 ewes</th>
<th>D50-100 ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tests</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>No. of tests in which rams showed:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenting</td>
<td>14</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Flehmen</td>
<td>7</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Kicking and nudging</td>
<td>18</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Attempted mounting (unsuccessful)</td>
<td>1</td>
<td>7*</td>
<td>7*</td>
</tr>
<tr>
<td>Mounting</td>
<td>18</td>
<td>5*</td>
<td>2*</td>
</tr>
<tr>
<td>Ejaculation</td>
<td>17</td>
<td>0**</td>
<td>0**</td>
</tr>
</tbody>
</table>

Compared to behaviour of rams with control ewes, *P < 0.05; **P < 0.01 (Fisher's Exact test).

† There were no significant differences in the behaviour of rams towards ewes given either steroid and the results for all ewes in a group were pooled.
Table 2. The masculine sexual behaviour shown by androgenized ewes during a 5-min exposure to an oestrous ewe (Test 2), before and 24–36 h after a single injection of oestradiol-17β (E₂, 50 or 500 µg) or testosterone (T, 5 mg)

<table>
<thead>
<tr>
<th></th>
<th>D30–80 ewes</th>
<th></th>
<th>D50–100 ewes</th>
<th></th>
<th>Untreated normal rams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Oestradiol-17β</td>
<td>Before</td>
<td>Oestradiol-17β</td>
<td>Untrated normal rams</td>
</tr>
<tr>
<td></td>
<td>injection</td>
<td>50 µg</td>
<td>500 µg</td>
<td>T</td>
<td>E₂ + T†</td>
</tr>
<tr>
<td>No. of tests</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>No. of tests in which ewes displayed:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenting</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Flehmen</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Kicking and nudging</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>15**</td>
</tr>
<tr>
<td>Mounting</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>10**</td>
</tr>
<tr>
<td>Mounting and thrusting</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

Compared to preinjection response, *P < 0.05; **P < 0.01 (Fisher’s Exact test).
† Data pooled for the 3 hormone treatments.
Masculine behaviour in the ewes

Aggression was observed in the androgenized ewes but never in the controls. Before steroid treatment only one D50–100 ewe displayed aggression towards an isolated ram. After the injections fighting occurred in 3/18 tests with the D30–80 ewes (not significant compared to the preinjection level) and 7/18 tests with the D50–100 ewes (P < 0.01 compared to preinjection response) and 0/18 tests with control ewes. Fighting was much more prevalent when the rams were introduced to pens of ewes to detect oestrus. Five of the 6 D30–80 and all 6 of the D50–100 ewes fought with the rams during these group encounters.

All components of masculine sexual behaviour were observed in the androgenized ewes when they were paired with oestrous ewes (Test 2: see Table 2). The control ewes never showed masculine behaviour during these tests and are therefore excluded from Table 2. The number of androgenized ewes displaying masculine courtship and mounting behaviour was significantly increased by the hormonal treatments. The frequency of mounting by the androgenized ewes was also increased by steroid treatments. Before injection the mean mounting frequency/5-min test was 0.1 mounts/ewe by the D30–80 ewes and 0.3 mounts/ewe by the D50–100 ewes. Injections of 50 µg oestradiol-17β, 500 µg oestradiol-17β or 5 mg testosterone increased the mounting frequencies of the D30–80 ewes to 0.8, 1.3 and 1.3 respectively; the mounting frequencies of the D50–100 ewes rose to 1.7, 1.0 and 2.7 respectively. The frequencies of mounting observed in the testosterone-treated animals were significantly (P < 0.05) greater than the preinjection frequencies (Mann–Whitney U Test). The frequency with which the androgenized ewes showed masculine courtship patterns and mounting after injection of oestradiol-17β or testosterone did not differ significantly with the type or dose of hormone received.

Plasma LH concentrations

These are shown in Table 3. A significant depression in plasma LH occurred in all the ewes by 4 h after injection; the magnitude of this depression was similar in all the ewes irrespective of the type or dose of hormone received.

In the control and the androgenized ewes the post-injection depression in plasma LH levels was followed by a marked increase. No statistically significant trends could be attributed to the dose or type of hormone injected and the time from injection to the LH peak was similar in all cases. Compared to preinjection levels, the LH peaks were consistently lower in the D50–100 ewes than in the controls (Table 3).

Table 3. The negative and positive feedback effect of oestradiol-17β (E₂) and testosterone (T) on plasma LH levels (mean ± s.e.m.) of spayed androgenized and control ewes

<table>
<thead>
<tr>
<th>Ewes</th>
<th>Treatment</th>
<th>No. of ewes</th>
<th>Plasma LH concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before injection</td>
</tr>
<tr>
<td>D30–80</td>
<td>50 µg E₂</td>
<td>6</td>
<td>7.1 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>500 µg E₂</td>
<td>6</td>
<td>6.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>5 mg T</td>
<td>6</td>
<td>6.8 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>18</td>
<td>6.8 ± 1.2</td>
</tr>
<tr>
<td>D50–100</td>
<td>50 µg E₂</td>
<td>6</td>
<td>14.2 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>500 µg E₂</td>
<td>6</td>
<td>11.9 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>5 mg T</td>
<td>6</td>
<td>17.9 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>18</td>
<td>14.8 ± 2.0</td>
</tr>
<tr>
<td>Control</td>
<td>50 µg E₂</td>
<td>6</td>
<td>9.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>500 µg E₂</td>
<td>6</td>
<td>11.0 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>5 mg T</td>
<td>6</td>
<td>12.0 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>18</td>
<td>10.4 ± 1.8</td>
</tr>
</tbody>
</table>

The values in parentheses are the percentages of the preinjection values.
* This value is significantly different from that for control ewes, P < 0.05 (analysis of variance).
† Represents negative feedback effect.
‡ Represents positive feedback effect.
Discussion

The results of this study confirm and extend the previous finding (Clarke, 1977) that prenatal exposure to testosterone promotes masculine behaviour in ewes as well as inhibiting oestrus. The display of masculine mating behaviour by the androgenized ewes was clearly enhanced by injections of oestradiol-17β or testosterone. The behaviour of the androgenized ewes included the complete repertoire of the mating patterns seen in normal rams. It is therefore apparent that the masculine behaviour of these animals before ovariectomy (Clarke, 1977) was maintained by ovarian steroids. The ovary of the ewe secretes oestrogens and androgens (Baird, McCracken & Goding, 1974), and the behaviour of the intact androgenized ewes (Clarke, 1977) could have been influenced by these hormones. As in androgenized female rats (Sodersten, 1973), the masculine behaviour of the androgenized ewes was reduced but not eliminated by ovariectomy (I. J. Clarke, unpublished), further indicating the involvement of ovarian hormones in this behaviour.

Behavioural defeminization in androgenized ewes may be partly explained by a loss of brain sensitivity to oestrogen because such ewes are refractory to the oestrus-inducing properties of the hormone. This, however, does not explain the behavioural masculinization of these ewes. The most simple explanation of the behavioural effects of prenatal androgenization in ewes is that there is a qualitative shift in behavioural potential which involves a loss of the potential to display feminine sexual behaviour and an acquisition of the potential to display masculine sexual behaviour. Testosterone or oestrogen therefore cause feminine behaviour in ewes with a female brain, and masculine behaviour in ewes with a masculine brain. The ‘brain sex’ of the ewes also determined the degree of their aggressive behaviour, irrespective of the type of hormone that they were given. The masculinized ewes showed more aggressive behaviour than the normal ewes, conforming to the fact that, as in most species, males are more aggressive than females (Bronson & Desjardins, 1971; Moyer, 1974; but see Payne & Swanson, 1970).

After the steroid injections the rams showed an obvious interest in the androgenized ewes even though the ewes were unreceptive, and regardless of the fact that D30–80 ewes had male external genitalia. This was evident from the amount of courtship the rams showed and also their attempted mounting of unreceptive ewes. The steroid injections may have resulted in the production of pheromones by the androgenized ewes so that they were attractive, but not receptive, to the rams. It was notable that the rams often scented the end of the penis of the D30–80 ewes. The origin of such a pheromone is unlikely to be the vagina because the urine from these ewes is voided from the bladder directly into the penile urethra (I. J. Clarke, unpublished data).

The pattern of LH release in the spayed control ewes after the steroid injections was similar to that described by other workers (Scaramuzzi et al., 1971; Symons, Cunningham & Saba, 1973; Martensz et al., 1976). Although a single injection of testosterone has been shown to cause ovulation in anoestrous ewes (Radford & Wallace, 1971) and induce oestrus in ovariectomized ewes (Lindsay & Robinson, 1961, 1964), the pattern of LH secretion following an injection of testosterone has not previously been reported. At the dose employed in the present study, testosterone elicited a response similar to that of the control ewes to oestradiol-17β.

Although the LH secretion patterns in the spayed androgenized ewes following the progesterone + oestradiol-17β or progesterone + testosterone treatments were essentially similar to the responses observed in the controls, LH values in the D50–100 ewes were significantly lower than in the control ewes. The D50–100 ewes were less likely to ovulate during the mating season than were the D30–80 ewes or the controls (Clarke et al., 1977) and failure of ovulation may therefore have been due to a partial impairment of the positive feedback mechanism in the androgenized ewes. The effect of prenatal androgen on brain centres controlling positive feedback should thus be viewed in quantitative rather than in qualitative (masculine versus feminine) terms. Prenatal androgenization of the female brain reduced but did not abolish positive feedback. The anovulatory condition in androgenized ewes (Clarke, Scaramuzzi & Short, 1977) may be due to their reduced hypothalamic sensitivity to oestrogen rather than to masculinization and therefore differs from the situation in rats in which perinatal androgenization of females abolishes positive feedback (Brown-Grant, 1974). In monkeys, prenatal androgenization of females does not prevent ovulation during adulthood (Goy & Resko, 1972), but
castrated males treated chronically with oestrogen will also show positive feedback (Knobil, 1974). It therefore appears that sheep are intermediate between rats and monkeys with respect to brain mechanisms that regulate positive feedback and ovulation. Early androgen treatment abolishes positive feedback in female rats, has a partial effect in ewes and is ineffective in monkeys. Ewes exposed to testosterone from Day 20 of fetal life until just before term are completely anovulatory (Short, 1974), whereas androgenization at Day 50 of life results in only partial impairment of the ovulatory process.

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