Luteinizing Hormone, Testosterone, and Behavioral Response of Male-Oriented Rams to Estrous Ewes and Rams

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ABSTRACT: During the breeding season three experiments were conducted to evaluate the LH and testosterone (T) response of rams with male sexual orientation (e.g., male-oriented homosexual rams) to female sheep, to male sheep, and to treatment with LHRH. Male-oriented rams were identified through a series of sexual performance and sexual preference tests. Treatments included exposure to estrous females and to males for 15 min (Exp. 1) and exposure to estrous females and to males for 8 h (Exp. 2). Behavioral responses to stimulus animals were recorded. In Exp. 2 homosexual rams mounted males more than females (P < .02) and exhibited more flehmen (P < .002) and investigatory sniffs (P < .01) when exposed to males vs females. Acts of aggression (butting the stimulus animals) did not differ by gender (P > .1). Flehmen and butting were positively correlated to LH secretion (P < .02) of rams exposed to females but not to males. In Exp. 1, LH concentration determined every 15 min for 6 h was not affected (P > .05) by the gender of the stimulus animal. In Exp. 2, LH pulse frequency and concentration were similar (P > .05) by treatment. Lack of an LH response to sexual activity in homosexual rams was not a result of pituitary or gonadal insensitivity; within 1 h of a single injection of LHRH both LH and T increased (Exp. 3). Individual mean T concentrations were higher in five of seven homosexual rams when they were exposed to males vs females. As a group, mean T concentration during 8 h was higher (P < .1) when homosexual rams were exposed to males vs females. These data show that homosexual rams do not exhibit an endocrine response to estrous ewes. Sexual behaviors directed toward males do not influence LH secretion, but in some individuals T concentration increases.

Key Words: Rams, LH, Testosterone, Sexual Behavior

Introduction

Male homosexual behavior is widespread among mammals. In a summary of research on the subject Dagg (1984) found 63 mammalian species in which male homosexual behavior was reported. Homosexual behavior (e.g., male-male mounting) has been previously described in Bighorn and Dall sheep (Geist, 1971) and in domestic sheep (Hulet et al., 1975; Zenchak and Anderson, 1980; Price et al., 1988). These authors suggested that sexual behavior by rams directed toward other rams may represent dominance displays, acts of aggression, and/or atypical sexual orientation resulting from experimental factors during rearing. It is commonly accepted that male-male mounting of prepubertal animals is important in the development of normal rear orientation in mount interactions (Silver and Price, 1986). Although such hypotheses help us understand evolutionary and ontogenetic development of homosexual behavior, they do not explain the physiological basis for sexual orientation.
Sexually active heterosexual rams experience an increase in plasma LH and testosterone (T) concentrations with prolonged exposure to estrous females (Sanford, 1974; Perkins et al., unpublished observations). Sexually inactive rams (those lacking in libido or otherwise failing to perform sexually) show no change in LH or T concentration under the same conditions. Based on these observations, we hypothesized that LH and T concentrations of homosexual rams would increase with prolonged exposure to male sheep and remain at basal concentrations in response to female sheep.

It is generally agreed that LHRH stimulates synthesis and secretion of LH from the pituitary (Bennett and Whitehead, 1983). In rams, an increase in T concentration occurs 45 to 60 min after an LH pulse (D'Occhio et al., 1982). By administering LHRH to homosexual rams we determined whether plasma LH and T concentrations were limited by pituitary insensitivity to LHRH.

### Materials and Methods

**Classification of Rams.** Rams with male sexual orientation were identified from a population (n = 94) of 1- to 2-yr-old sexually inexperienced males at the U.S. Sheep Experiment Station, Dubois, ID. Breeds of rams represented were Rambouillet, Targhee, Columbia, Polypay, and 1/4 Finnish Landrace crosses of the standard breeds. Between September 1 and October 31, 1988 (autumn breeding season) rams were repeatedly tested with three or four estrous ewes. After a minimum of three 30-min tests and one overnight exposure to estrous ewes, rams that had not mounted females were subjected to a sexual preference test similar to that used by Price et al. (1988). Rams were first isolated for 48 to 72 h to prevent mounting and courtship activity with male penmates. Rams were then individually exposed for 30 min to two males and two estrous females restrained in a four-way stanchion (Figure 1). Stimulus rams used for the choice test were selected on the basis of approximately 18 h of preliminary home pen observations. During the preliminary observations two rams were observed being mounted repeatedly (receivers) by penmates. These receivers usually did not attempt to prevent other rams from mounting them. The two receivers were used as the stimulus males during the choice test. If a test ram did not mount females or either of the receivers during its first choice test, it was observed for 2 h in its home pen. If it mounted another male in its home pen, the male penmate that was mounted was used as one of the stimulus males during a second choice test. Rams that chose to mount males in preference to females were classified as male-oriented (homosexuals). These rams mounted only males and were never observed mounting females during either sexual preference tests or sexual performance tests. A total of eight homosexual rams was identified by this procedure. Six rams mounted one of the receivers and two rams (Numbers 50 and 74) mounted each other exclusively. Rams were maintained in pens with both non-homosexual and homosexual male penmates and fed alfalfa hay daily with salt and water available ad libitum. Three separate experiments were conducted during autumn between September 15 and December 1.

**Experiment 1.** The objective of this study was to evaluate the plasma LH concentration of homosexual rams after a 15-min exposure to estrous females and to males. Six homosexual rams were fitted with jugular cannulas and placed in individual pens. The next day, blood samples were drawn at 15-min intervals for 6 h beginning at 0800. After 2 h of sampling, rams were removed from their individual pens and placed in an observation pen with either two receptive females or two restrained T.9

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Figure 1. Four-way stanchion used during the sexual preference test to restrain the estrous females and stimulus males. Estrous females (and stimulus males) were restrained on opposite sides of the apparatus facing the center. This limited the approach of the test rams to the side or rear and minimized aggressive interactions directed at the stimulus animals.
males for 15 min. The experiment was repeated the following week in a switchback design by exposing rams to sheep of the opposite gender. The frequencies of vocalizations, foreleg kicks, investigatory sniffs, flehmen responses, mounts, and ejaculations of the homosexual rams were recorded. After returning the rams to their individual pens, blood samples were collected every 15 min for 4 h. Blood plasma was harvested and stored at -20°C until samples were assayed for LH.

Experiment 2. The objective of this experiment was to evaluate the plasma LH and T concentration of homosexual rams exposed to males and estrous females during an 8-h period. Eight homosexual rams were exposed to restrained estrous females and to restrained males. Rams were removed from their original home pens and placed with four or five new male penmates 3 wk before the experiment to segregate the test subjects (homosexual rams) from each other and to allow them to find new sexual partners that could be used as stimulus animals during the experiment. One week before testing, the experimental rams were placed in individual, adjacent observation pens to prevent sexual activity before the experiment and to allow rams to become familiar with the test environment. Each observation pen contained a two-way stanchion for restraining the stimulus animals. During the experiment blood samples were drawn from jugular cannulas at 20-min intervals for 10 h (0700 to 1700). No stimulus animals were provided for the rams during the first 2 h of sample collection. Immediately after the sixth sample was drawn, two receptive ewes were placed into the stanchions of four of the test pens and two males were restrained in each of the remaining four test pens. Rams tested with females had no visual contact with rams being tested with males. To sustain sexual interest, stimulus animals within each gender were exchanged among pens every 40 min. One week later rams were retested with sheep of the opposite gender. All occurrences of mounts, ejaculations, flehmen responses, and aggression (butting of stimulus animals) were recorded. Blood samples were processed as described for Exp. 1. Luteinizing hormone concentration was determined in all samples collected. Testosterone concentration was determined in alternate samples (every 40 min).

Experiment 3. The objective of this study was to challenge the pituitary and gonads by treating homosexual rams with a single injection of LHRH. The same eight homosexual rams from Exp. 2 were equipped with jugular cannulas and placed in individual pens. Blood samples were drawn at 15-min intervals for 4 h. After 2 h each ram was given 500 ng of LHRH (NIADDK) in physiological saline via the jugular cannulas. Blood samples were collected every 15 min for another 2 h. Plasma LH concentration was determined. Testosterone concentration was evaluated in samples taken 45 and 60 min before and 45 and 60 min after treatment with LHRH.

Hormone Assays. Plasma LH was determined by RIA (Fitzgerald and Stellflug, 1991). The primary antibody (NIADDK-anti-o-LH) and o-LH standard (NIADDK-o-LH-13) were provided by NIADDK. The o-LH used for iodination was provided by Leo Reichert (LER-1056-C2). The low (90%) and high (5%) limits of detection of the assay were .25 and 7.0 ng, with a 50% intercept at 1.2 ng. Plasma samples run at different volumes showed parallel displacement to the standard curve. Absolute values were determined from the mean of samples run in duplicate. Within-assay CV was < 10% for all assays. Between-assay CV was < 15% for samples assayed for the three experiments.

Plasma T was determined by RIA using a commercial assay kit provided by Cambridge Medical, Billerica, MA. Plasma from castrated rams was used to dilute T standards. Limits of assay detection were .1 and 20 ng. The 50% intercept was 1.0 ng. Testosterone measured in plasma from ovarietomized ewes was below detection limits. All T samples were run in one assay. Intraassay CV was < 10%.

Statistical Analysis. Luteinizing hormone pulsatile activity was determined as an increment of LH in a sample that exceeded the previous and subsequent sample by twice the assay variation (Fitzgerald et al., 1982). Plasma LH values below the level of sensitivity of the assay were assigned a value of 25 ng/mL. During Exp. 2, one ram had T values above the limits of detection and was omitted from analysis of T. The effects of treatment (gender of stimulus animals, or alone) on LH and T over time were evaluated using the GLM procedure (SAS, 1988) for repeated measures analysis of variance (Gill, 1978). Ram within treatment was used as the error term to test effect of sex of stimulus animal. An ANOVA was used to compare the total number of each sexual behavior (e.g., mounts, ejaculations, flehmen, sniffs, etc.) exhibited by test rams toward female vs male stimulus animals. The degree of association between behaviors in response to females or males and LH concentration over time was tested using Spearman rank correlation coefficients (Siegel and Castellan, 1988). The rams were ranked during each 20-min interval for behaviors and for LH concentration. Data from two rams that were sexually inactive were not included for the Spearman rank analysis.
Table 1. Mean (± SE) of behavioral events exhibited by rams (n = 8) during separate exposures to males and estrous females

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mounts</td>
<td>7.5 ± 3.7</td>
<td>0.8 ± 2.1</td>
</tr>
<tr>
<td>Ejaculations</td>
<td>0</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Flehmen responses</td>
<td>1.2 ± 1.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Sniffs</td>
<td>2.3 ± 0.9</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Vocalizations</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Foreleg kicks</td>
<td>1.5 ± 1.2</td>
<td>1.2 ± 1.2</td>
</tr>
<tr>
<td>Butts</td>
<td>0</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Pacing or vocalizations</td>
<td>0</td>
<td>2.3 ± 1.1</td>
</tr>
</tbody>
</table>

*Two of the rams were sexually inactive during testing.

*No treatment differences were observed (P > .05).

**Results**

*Experiment 1.* Table 1 summarizes the behaviors of rams during the exposure period to both genders. During the 15-min exposure period, four of the eight test rams were sexually responsive to males. None of the test rams mounted, ejaculated, or exhibited flehmen in response to females. Two rams (Numbers 50 and 74) exhibited loud vocalizations and repeatedly paced during testing with females but not with males. Data were analyzed with and without sexually inactive rams. Sexually active rams mounted males an average of 11.3 ± 4.4 times during testing. No ejaculations were observed.

No treatment differences were observed in LH concentrations (P > .05). There was no change in LH activity after 15-min exposures to either male or female stimulus animals. With the exception of one ram, there were no detectable LH pulses observed either before or after the exposure period. One ram exhibited a single LH pulse within 45 min after exposure to males. However, he was not sexually active during the exposure period.

*Experiment 2.* During the test period, four of eight rams (Numbers 6, 28, 81, and 169) were sexually active in response to males only. Two rams (Numbers 50 and 74) were sexually active with both males and females. The remaining two rams (Numbers 59 and 75) responded to both genders but showed a preference for males over females. Ram Number 59 was exposed to females for 3 h and 40 min before he began mounting. Ram Number 75 mounted and ejaculated upon first introduction to females but showed no further sexual interest until his 7th h of exposure to estrous ewes. Both of these rams were sexually active when exposed to male stimulus animals, averaging 1.2 ± 1.8 and 3.6 ± 5.6 mounts per 20-min interval. Ejaculations by rams when they were exposed to male partners were followed by the classic head thrust and refractory period of sexual inactivity. Seminal fluid was observed under and around the tail of stimulus males. One individual mounted a male several times and then ejaculated after dismounting. Although homosexual rams mounted males more often than females (P < .02), they did not achieve more ejaculations with males (P > .05; Table 2). Rams exhibited more flehmen responses (P < .002) and investigatory sniffs (P < .01) when exposed to males than when exposed to females. Incidence of aggression toward the stimulus animals and bouts of pacing and/or loud vocalizations were not different by gender (P > .01).

Neither LH pulse frequency (P > .05) nor concentration differed (P > .05) by treatment. The secretion of LH was episodic and occurred before and after stimulus animals were introduced (Figure 2). There was an effect of time of exposure (P < .01) to stimulus animals on mean T concentration. The greatest difference between male and female exposure occurred between 4 h and 6 h 40 min after exposure (Figure 3). Individual mean plasma T concentration was greater (P < .05) in five of seven test rams when they were exposed to male vs female stimulus animals. Two rams (Numbers 81 and 50) exhibited increases of plasma T when they were exposed to males but did not have concomitant changes in LH secretion. Both incidence of flehmen and acts of aggression were positively correlated to LH secretion (P < .02) when rams were exposed to females but not when they were exposed to males. Mounting and flehmen were positively correlated (P < .002) for both experiments.

*Experiment 3.* The concentration of LH increased (P < .05) in all rams within 15 min after treatment with LHRH (Figure 4). The concentration of T increased nearly twofold (3.1 ng/mL ± 1.5 ng/mL vs 8.9 ng/mL ± 1.1 ng/mL) after the treatment in all rams.

Table 2. Mean (± SE) number of behavioral events and LH pulse responses exhibited by homosexual rams (n = 8) during separate 8-hour exposures to males and estrous females

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mounts</td>
<td>68.5 ± 24.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ejaculations</td>
<td>2.8 ± 1.0</td>
<td>1.1 ± 0.8</td>
</tr>
<tr>
<td>Flehmen responses</td>
<td>19.5 ± 6.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sniffs</td>
<td>137.0 ± 40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.6 ± 11.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butts</td>
<td>11.5 ± 6.3</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Pacing or vocalizations</td>
<td>.1 ± .1</td>
<td>.7 ± .6</td>
</tr>
<tr>
<td>directed out of pen</td>
<td>.1 ± .1</td>
<td>.4 ± .3</td>
</tr>
</tbody>
</table>

*<sup>a,b</sup>Row means with different superscripts differ (P < .05).*
Discussion

Short-term (e.g., 15 min) exposure to estrous females does not result in an immediate elevation of LH in sheep (Perkins et al., unpublished observations), as has been reported in other species. Therefore, it was not surprising that homosexual rams showed no change in LH secretion when they were briefly exposed to males. In contrast, prolonged sexual activity (e.g., 8 h) increases LH secretion patterns in heterosexual rams (Sanford et al., 1974; Gonzalez and Signoret, 1988; Perkins et al., unpublished observations). If homosexual behavior had the same physiological consequences as heterosexual behavior (with the only difference being the gender of the preferred partner), we would expect to find similar elevations of LH and T concentrations in male-oriented rams.

Figure 2. Plasma luteinizing hormone (LH) concentration (ng/mL) measured at 20-min intervals for eight homosexual rams. Samples were collected for 10 h with 8-h exposure periods to males and estrous females in separate experiments. Arrow indicates the introduction of stimulus animals.
rams when they direct sexual behaviors toward males. In spite of apparent sexual arousal and clearly defined sexual activity, there were no increases in either LH pulse frequency or basal concentration when homosexual rams were exposed to male partners for 8 h. Although sexual behaviors directed toward males or females are outwardly similar, these results demonstrate that physiological responses are not the same.

Based on these observations we reject the hypothesis that rams exhibiting sexual responses to males experience the same physiological changes as rams responding to females. These data suggest that stimuli from ewes, perhaps in the form of a pheromone (Knight, 1983), are necessary to drive the LH secretion of sexually active rams. Rams that are homosexual in orientation seem to be inherently different in their ability to secrete LH in response to females compared with non-homosexual males. However, a single injection of LHRH (Exp. 3) stimulated LH and T in homosexual rams. Therefore, lack of an effect of sexual arousal on plasma LH in homosexual rams is not attributed to pituitary insensitivity to LHRH or to inability to secrete LH. These data are similar to previous observations on low-libido and sexually inactive rams (Perkins et al., unpublished observations).

In this study we found that average T concentration in five of seven homosexual rams was greater after exposure to males vs females for 8 h. It would be premature to conclude that T concentration in homosexual rams is influenced by homosexual behavior. The dynamics of LHRH-induced T secretion of homosexual rams (Exp. 3) are similar to previous observations noted with heterosexual rams (Perkins et al., unpublished observations). For some rams in this study, differences in T concentration when exposed to males vs females are associated with pulsatile LH activity. However, patterns of increased T concentration without noticeable changes in LH were also observed in two of the homosexual rams exposed to males (Exp. 2). Elevations in blood T independent of changes in LH concentration have been reported in men (Sutton et al., 1973; Rowe et al., 1975) and monkeys (Goodman et al., 1974).

In the landmark paper by Phoenix et al. (1959), the early organization and subsequent activation effects of T on neural tissue was demonstrated in guinea pigs. Since then, numerous papers have addressed neurobehavioral differentiation between the sexes. The predisposition of animals to display mainly masculine or feminine sexual behavior is related to early exposure of the central nervous system and genitalia to androgen and estrogen (Hart and Leedy, 1985). Homosexual rams in this study exhibited typical male motor patterns, including foreleg kicks, investigatory sniffs, flehmen responses, mounts, pelvic thrusting, and ejaculations. Rather than exhibiting feminine postures and behaviors, or lacking in sexual behaviors, they exhibited typical sexual behaviors of the species with the exception of
sexual preference. Hence, lack of organizational or activational effects of androgens on neural structures mediating male motor patterns is not likely the cause of homosexual orientation in these rams.

The increased concentrations of T observed when male-oriented rams were exposed to males could result from events important in establishing social status. Sympathetic catecholamines (epinephrine and norepinephrine) acting peripherally lead to increases in T concentration with no concomitant elevations in LH in baboons (Sapolsky, 1986). Mendoza and Mason (personal communication) suggest that rapid hormonal differentiation occurs during the formation of status relationships. This brings about a prompt change in behavior dispositions, which allows individuals to establish stable relationships quickly and economically. Well-established groups of primates show no evidence of hormonal differentiation associated with dominance status (Mendoza and Mason, 1984). During a stressful encounter, however, T concentration in high-ranking male baboons increases, whereas in low-ranking males it declines (Salpolsky, 1986). The increased T concentration in rams that we classified as male-oriented may have resulted from a period of hormonal differentiation that corresponds to a dynamic phase of social organization during which individuals are establishing relationships (social rank) similar to those reported by Sapolsky (1986) in primates.

The social organization of wild mountain sheep was studied in detail by Geist (1971). Rams continue to mature for 5 or 6 yr after puberty. They segregate into groups of their own away from females and juveniles. Rams behaving like juveniles are allowed (by the females) to remain within the flock. Those acting like "males" form all-male societies in which dominant rams act the role of courting males and subordinate rams behave like estrous females. Geist (1971) considers mountain sheep societies to be basically homosexual. The dominant ram treats all sheep smaller than it, regardless of sex and age, like females. A subordinate ram may act aggressively toward a dominant ram that is mounting it, or it may behave like a receptive female by standing still. Young males that mimic female behavior are allowed to live side-by-side with larger males.

Based on this information, rams identified as receivers in this study should be subordinate and those mounting other rams should be dominant. We did not systematically identify social rank of the rams in this project. The receivers seemed to tolerate being mounted by both male-oriented and heterosexual rams. Homosexual rams (those that successfully and persistently mounted only other males) did not seem to be subordinate and may have been the more dominant-ranking individuals within the pen. These observations are consistent with Geist's (1971) observations of mountain sheep. The nature of social relationships is a plausible factor influencing the sexual behaviors exhibited by rams. Some homosexual rams would mount any restrained ram, whereas others would mount only preferred individuals (e.g., a receiver or a particular penmate). Yet, the social status and/or relationships of individuals do not account for the failure of these rams (homosexuals) to respond either sexually or physiologically to estrous females. Heterosexual rams show little interest in males if they have an opportunity to copulate with females. Therefore, we must also speculate that homosexual orientation has a biological basis.

Gladue et al. (1984) reported that estrogen injection differentially affected LH and T secretion of homosexual men compared with heterosexual men. These authors suggested that there may be physiological as well as psychological components in the sexual orientation of some homosexual men. Yet, there is no evidence to indicate that the medial preoptic area of the hypothalamus, which mediates libido in males (Sachs and Meisel, 1988), plays a role in sexual partner preference.

Several studies have supported the hypothesis that early sexual experience with males inhibits subsequent interest in females. Zenchak et al. (1981) hypothesized that male-male sexual activity (e.g., mounting) during prepuberal development conditions rams to seek out male stimuli toward which to direct their sexual behaviors. Katz et al. (1988) reported that the sexual performance of rams reared with females was better than that of rams reared without females when tested at 10 mo of age. The extent of male-male mounting was more than 10-fold greater for rams reared in all-male groups than for rams reared with exposure to females. Sexual imprinting has been reported in birds (Bateson, 1978). Such imprinting begins at a specific stage in development when the animal is particularly sensitive to certain classes of stimuli such as adult plumage coloration. Collectively, the latter studies provide evidence that prepubertal sexual experiences are important for "priming" hypothalamic structures. Such experiences in rams may mediate differences in LH secretion due to partner preference. Although experiential factors are important, genetic predisposition must be considered. Katz et al. (1988) observed that 2 of 24 rams were male-oriented when tested as mature rams despite continuous exposure to females during prepuberal development.

In summary, this study provides evidence that physiological consequences of homosexual behavior in rams are not analogous to heterosexual
experiences. Homosexual rams do not exhibit an endocrine (LH secretion) or behavioral (courtship) response to estrous females. Sexual activity of homosexual rams with male partners is not associated with increased LH secretion. Perhaps events during a sensitive stage of development such as lack of interaction with females or excessive male-male sex play, interacting with genetic factors, are involved in priming the hypothalamus or higher brain centers. This may ultimately modify the LH secretory response to female or male sexual stimuli.

Implications

This study supports the premise that there is a physiological basis for the sexual orientation of male sheep. Full knowledge and understanding of ram sexual performance, social status, and orientation is a necessary prerequisite to studies of hypothalamic, pituitary, or gonadal function and sexual behavior.

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