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An Investigation of the Requirement for Increased LH Secretion During the Compensatory Response in Androgen Secretion After Unilateral Castration of the Adult Rat

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ABSTRACT: The endocrine mechanisms underlying the response to unilateral castration were examined by determining systemic androgen and luteinizing hormone (LH) concentrations, as well as testicular vein androgen at 3, 6, 12, and 24 hours after sham surgery and castration. Systemic androgen was significantly depressed 3 hours after unilateral castration, but had recovered to concentrations observed in sham operated rats at 6, 12, and 24 hours. The recovery of serum androgen after castration was apparently due to increased testicular secretion of androgen, seen as a significant increase in testicular vein androgen. Systemic concentrations of bioactive and immunoactive LH were significantly increased only at 6 hours after castration. The authors next examined whether the increase in LH was necessary for the compensatory secretion of androgen seen after castration. This was accomplished by examining the response to castration when circulating LH was prevented from changing by suppressing endogenous LH secretion with subcutaneous steroid implants and maintaining circulating LH with subcutaneous osmotic pumps containing ovine LH. The compensatory increase in testicular vein androgen was observed 1 and 7 days after castration in rats bearing sham implants. When circulating LH was prevented from changing by using the combination of steroids and LH, however, compensatory secretion of androgen did not occur 1 and 7 days after castration. These results suggest that: 1) compensation in androgen secretion in response to unilateral castration occurs as early as 6 hours; 2) the increase in LH 6 hours after castration may provide the stimulus for the increase in androgen secretion by the testis at this time; 3) the lack of an increase in testicular vein androgen 1 and 7 days after castration in rats whose endogenous LH is prevented from changing indicates that the change seen 6 hours after castration may be an important component of the short- and long-term compensatory response.

Key words: Hormone implants, immunoactive, bioactive.
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A classic physiologic technique for studying the mechanisms regulating growth and secretion is observation of the responses to the removal of one member of a pair of endocrine organs. Compensatory growth of the contralateral organ, termed compensatory hypertrophy, often occurs after such a perturbation, as in the case for follicle-stimulating hormone (FSH)–dependent ovarian hypertrophy after unilateral ovariectomy (Bast and Greenwald, 1977; Butcher, 1977), or neurally mediated adrenal hypertrophy after unilateral adrenalectomy (England and Dallman, 1976). Compensatory hypertrophy occurs in the remaining testis of the neonatal rat after unilateral castration (UC) (Ojeda and Ramirez, 1972a; Ojeda and Ramirez, 1972b; Stewart et al, 1973; Cunningham et al, 1978), but does not occur in the postpubertal rat (Howland and Skinner, 1975; Gomes and Jain, 1976; Robaire, 1979; Furuya, 1990). Circulating testosterone in adult rats that have undergone UC, however, is comparable to that of control animals 24 hours after surgery (Frankel and Wright, 1982; Mock and Frankel, 1982; Moger and Anakwe, 1986), indicating that a compensatory secretory response has occurred. This compensatory response to UC is rapid and long lasting, first occurring within 8 hours (Frankel and Wright, 1982; Frankel and Mock, 1982), and present for at least 5 weeks (Gomes and Jain, 1976; Robaire, 1979; Furuya, 1990). The maintenance of circulating testosterone can be attributed to the increased output of testosterone by the remaining testis. Increased output has been observed as a doubling in testosterone concentration in testicular vein effluent as early as 24 hours after UC (Mock and Frankel, 1982; Frankel et al, 1984; Moger and Anakwe, 1986; Frankel et al, 1989a; Frankel et al, 1989b), and also as a doubling in intratesticular testosterone concentration in the remaining testis seen 20 days after UC (Lindgren et al, 1976).

It is unclear what role gonadotropins play in the rapid compensatory increase in testosterone secretion after UC. Circulating FSH increases 2 to 16 days after UC, well after the compensatory testosterone response has been estab-

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lished (Gomes and Jain, 1976; Frankel and Wright, 1982; Mizunuma et al, 1983). Nance and Moger (1982) suggested that this FSH increase was neurally mediated after demonstrating differential FSH release after ipsilateral or contralateral UC in rats with hypothalamic hemi-islands. Schanbacher (1988) observed that the increase in FSH after UC of the postpubertal ram was associated with decreased serum inhibin, suggesting a possible role for inhibin in the increase in FSH. Some reports have associated the normalization of circulating testosterone after UC with an increase in serum luteinizing hormone (LH) (England, 1972; Howland and Skinner, 1975), but most have not (Gomes and Jain, 1976; Robaire, 1979; Frankel and Wright, 1982; Mock and Frankel, 1982; Mizunuma et al, 1983; Furuya, 1990).

Many of the cited studies used protocols that were initiated after the compensatory increase in testosterone secretion had occurred, and thus provide little information about the mechanism responsible for initiating the increase in androgen secretion. Therefore, we have executed a group of studies in which the increase in androgen secretion was monitored at frequent intervals and the concomitant change in LH bioactivity and immunoactivity in plasma was determined, to better define this compensatory response to UC. Having noted a change in serum LH, we investigated the requirement for this LH change in eliciting the compensatory response by examining the ability of the remaining gonad to compensate when circulating LH was held at a normal physiologic level. This was accomplished by suppressing endogenous LH secretion with testosterone- and estrogen-filled silicone elastomer (silastic) capsules and maintaining circulating LH with mini-osmotic pumps containing ovine LH.

Materials and Methods

Animals

Male Sprague-Dawley rats (220 to 270 g) were kept in a climate-controlled environment with a 14-hour light: 10-hour dark cycle and had free access to food and water.

Testicular Vein Sampling

Testicular blood samples were collected as described by Mock and Frankel (1982) with some minor modifications. At the time of sampling, rats were anesthetized with ether, and the unoperated testis was exposed through an abdominal incision. A large testicular vein beneath the testicular capsule was incised with the tip of a scalpel blade, and the venous effluent was collected in a heparinized hematocrit tube. Time between administration of anesthesia to collection of sample was approximately 2 minutes. After centrifugation, tubes were scored and broken above the line that divided the red blood cells from plasma. Using an Eppendorf pipette, 10 μL plasma was removed from the tube and subsequently diluted 1:100 with phosphate-buffered saline containing 0.1% gelatin. Diluted plasma samples were stored at −20°C until assay for androgen content.

Experiment 1

This experiment was performed to better define when the doubling in testicular vein androgen secretion in response to UC occurs and whether this phenomenon was associated with a change in circulating LH. Between 8:00 and 9:00 AM, all rats underwent sham operations (SO) or UC through a midscrotal incision while under ether anesthesia. The left testis was removed in this and subsequent studies because Frankel et al (1989a) had found that a more robust response was obtained. Groups of SO and UC rats were again anesthetized in an identical fashion at either 3, 6, 12, or 24 hours after surgery, and an abdominal incision was made to permit collection of a blood sample from a testicular vein of the exposed testis and a systemic sample from the abdominal aorta. Each animal provided only a single sample of systemic and testicular vein blood. Systemic blood samples were permitted to clot at 4°C for the collection of serum. Sera and plasma were stored at −20°C until assayed for androgen and gonadotropin.

Experiment 2

This experiment was performed to ensure that normal circulating androgen and testicular responsiveness to LH stimulation were not significantly ($P > 0.05$) altered by the hormone replacement regimen provided by the steroid capsules and osmotic pumps. The hormone replacement regimen used was similar to that described by Ewing et al (1983), with minor modifications. In brief, testosterone and estrogen (Sigma Chemical Company, St. Louis, MO) were administered by Silastic capsules of 2.5 and 0.3 cm, respectively (inner diameter, 1.98 mm; outer diameter, 3.18 mm; Dow Corning, Midland, MI; Catalog # 602-305). Capsules were placed subcutaneously while animals were anesthetized with ether. Exogenous oLH S-26 (National Institutes of Health) in 0.1 mol/L borate buffer also was administered continuously at a rate of 12 μg/day by subcutaneously implanted Alzet mini-osmotic pumps. Silastic capsules or osmotic pumps were incubated in physiologic saline for 1 to 3 hours before implantation. Control groups received empty capsules and pumps. Where indicated, a bolus of 5 μg oLH S-26/200 μL saline was given by a single intraperitoneal injection 2 hours before sampling to evaluate testicular responsiveness to LH (Moger and Armstrong, 1974).

Rats were divided into four groups: group 1 received control implants of empty capsules and pumps; group 2 received steroid-filled capsules; group 3 received LH pumps; and group 4 received both steroid capsules and LH pumps. After 5 days, animals were anesthetized with ether and a jugular blood sample was taken. Immediately after the blood sample, animals received an intraperitoneal injection of LH. Two hours after injections, rats were decapitated and the trunk blood was collected. All blood samples were permitted to clot at 4°C for the collection of serum. Sera were stored at −20°C until assayed for androgen and gonadotropin.

Experiment 3

This experiment was performed to evaluate the response to UC when circulating LH was held constant. Five days after receiving either control implants or steroid capsules and LH pumps, rats underwent SO or UC through a midscrotal incision under ether anesthesia. One and 7 days after SO or UC, rats were anesthetized with ether and samples of blood from a testicular vein and aorta were collected and processed as described for experiment 1. Because osmotic pumps only function for 1 week, the group sampled 7 days after UC had their LH pumps replaced at the time of UC.

Radioimmunoassays

An established radioimmunoassay (RIA) was used to measure androgen levels. The cross-reactivities for dihydrotestosterone, 5α-androstane-3α, 17β-diol, and 5α-androstane-3β, 17β-diol, were 98%, 58%, and 24%, respectively (Jansz and Pomerantz, 1985). Testicular vein plasma and systemic serum samples were extracted with ether before assay. The sensitivity of this assay was 3 pg androgen/tube and both the intra-assay and interassay coefficients of variation were below 5%.

To measure immunoactive LH, modifications were made to the LH RIA described by Matteri et al (1987). This assay uses a monoclonal antibody (MAb $518B_7$) generated against the β-subunit of bovine LH as the primary antibody. NIH rLH RP-2 was used as the reference preparation. The following changes were made to the assay: The primary antibody dilution was 200 pg/100 μL assay buffer containing 1.5% normal mouse serum. The secondary antibody (goat anti-mouse serum; Antibodies Inc, Davis, CA) was diluted 1:300 in 0.05 mol/L sodium phosphate buffer containing 5% polyethylene glycol (7000 to 9000 MW). Sensitivity of the LH RIA averaged 10 pg/tube, and both the intra-assay and interassay coefficients of variations were below 5%.

Serum FSH was determined by RIA using a kit provided by the National Hormone and Pituitary Program. NIH rFSH RP-2 was used as the reference preparation. Sensitivity of the assay was 300 pg/tube, and the intra-assay coefficient of variation was below 5%. Testosterone, immunoactive LH, and FSH concentrations were calculated using logit transformation and unweighted least squares regression analysis.

Bioassay

To measure circulating bioactive LH from intact male rats, modifications of the mouse interstitial cell test (MICT) described by Ellinwood and Resko (1980) were made. Sensitivity of this assay has been improved to approximately 3 pg LH-RP2/tube with the inclusion of 3-isobutyl-1-methyl-xanthine (0.1 mmol/L) and forskolin (1.5 μmol/L) in the incubation medium (Debertin and Pomerantz, 1992). In brief, testes from young mice (Swiss Webster CD-1 strain, 4 to 8 weeks of age) that had been asphyxiated with carbon dioxide were decapsulated and minced with fine scissors. Testicular tissue was added to 25 mL of incubation medium (medium 199 with Hank's salts and L-glutamine containing 25 mmol/L Hepes, 0.35 mg/mL $NaHCO_3$, and 0.2% bovine serum albumin at pH 7.35) and was gently stirred with a magnetic stirring bar for 10 minutes. Remaining clumps of tissue were dispersed by repeatedly drawing the mixture into a fire-polished Pasteur pipette. The suspension then was filtered through a nylon mesh (100 μm) into a 50-mL centrifuge tube. The filtrate, in the capped vertical tube, was preincubated for 1 hour in room air at 34°C in a shaking water bath at 60 cycles/min. After preincubation, cells were collected by centrifugation at 400g for 15 minutes at 4°C. Supernatant was discarded and the tissue pellet was resuspended in fresh incubation medium (3.75 mL/testis) containing 60.8 μg/mL of 3-isobutyl-1-methyl-xanthine, (Sigma), which resulted in a concentration of 0.1 mmol/L in the final incubation volume. Cell viability was routinely greater than 80%, as assessed by staining with 0.1% trypan blue. Testicular tissue from two mice yielded sufficient cells for a 200-tube assay.

Standards and samples (10 μl) were added to 12 × 75-mm glass tubes containing 50 μL incubation medium, which contained 12.3 μg/mL forskolin (Calbiochem, San Diego, CA), thus achieving a concentration of 1.5 μmol/L in the final incubation volume of 205 μL. NIH rLH RP-2 dissolved in incubation medium was used as the reference preparation. Serum volume was adjusted to 10 μL in all assay tubes using serum from hypophysectomized rats. At this time, incubation volume was adjusted to 130 μL using fresh incubation medium in all assay tubes. Finally, while gently stirring the cell suspension with a magnetic stirring bar, 75 μL cell suspension was added to each assay tube so that the total incubation volume equalled 205 μL. Assay tubes were incubated for 4 hours at 34°C in a shaking water bath at 60 cycles/min in room air. After incubation, assay tubes were centrifuged for 10 minutes at 2000g. Supernatant was collected and stored at −20°C until the time of androgen assay. Androgen production for the standard curve was plotted against the log of LH concentrations. Bioactive LH concentrations were calculated using unweighted least squares regression analysis. Both the intra-assay and interassay coefficients of variation were below 10%.

Statistical Analysis

Bartlett's test was used for detection of heterogeneity of variance within experiments. In cases where heterogeneity was found, logarithmic transformations of the data were used before analysis. One-way, two-by-two, or two-by-four analyses of variance were used for comparisons within experiments. Statistical analyses for multiple comparisons were performed by the Tukey Kramer method, and Student's *t* test was used for individual comparisons (Sokal and Rohlf, 1981).

Results

Experiment 1

In this experiment, we determined when the compensatory androgen secretion first occurred during the 24 hours after UC, and whether immunoactive or bioactive serum LH changes at this time. Figure 1A shows serum androgen concentrations 3, 6, 12, and 24 hours after UC. Two-by-four analysis of variance detected a significant interaction between the effect of surgery and time on the concentration of

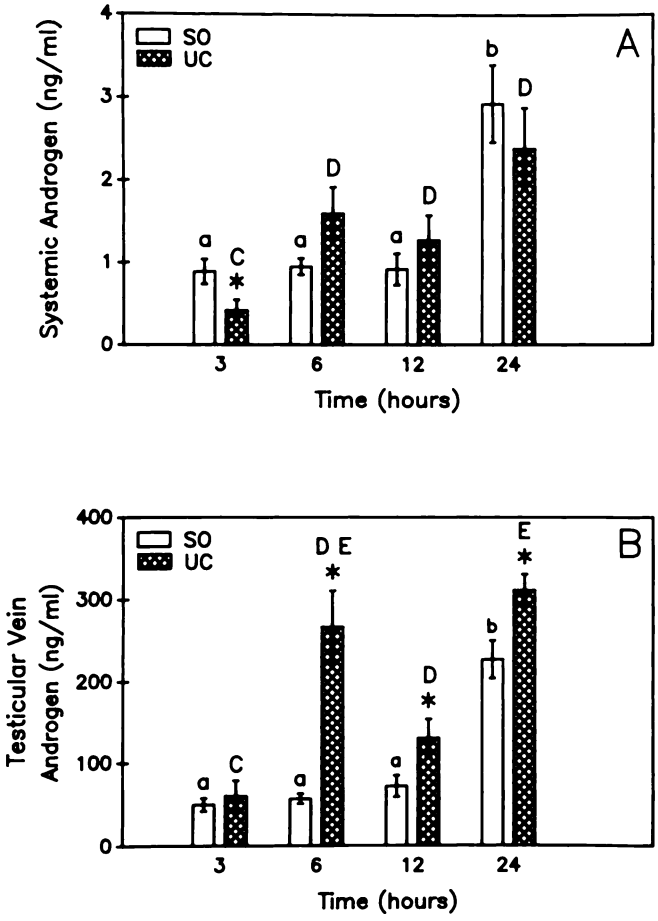

FIG. 1. Concentrations of androgen in systemic serum (A), and testicular vein plasma (B) at 3, 6, 12, and 24 hours after sham operation (SO) or unilateral castration (UC). In this and following graphs, each bar represents the mean ± SEM (n = 6–13). An * indicates significant differences between SO and UC groups ($P < 0.05$). Different lower case letters indicate significant differences in androgen concentrations at different times in SO rat ($P < 0.05$). Upper case letters are used for significant differences between groups of UC rats ($P < 0.05$).

circulating androgen ($P < 0.001$). This interaction is attributed to the concentration of androgen in the serum of SO rats being significantly elevated ($P < 0.05$) at 24 hours compared with the previous sampling times. Three hours after surgery, but not at other sampling times, serum concentrations of androgen were lower in UC rats ($P < 0.05$) compared with SO rats.

A significant interaction between the effects of time and surgery was observed for testicular vein androgen concentrations (Fig 1B, $P < 0.001$). This interaction was attributed to the SO rats having unchanged concentrations of androgen in plasma from the testicular veins until 24 hours after surgery, whereas the UC groups showed an increase ($P < 0.05$) in the concentration of androgen between 3 and 6 hours after surgery, which was maintained for the duration of the experiment. Compensatory androgen secretion, observed as significant increases in concentrations of androgen in the testicular vein of UC rats, was not present at 3 hours, but occurred 6, 12, and 24 hours after UC ($P < 0.05$).

Figure 2A and B illustrate the circulating concentration of immunoactive and bioactive LH of the animals in Figure 1. Two-by-four analysis of variance detected a significant interaction of the effects of surgery and time for both immunoactive and bioactive LH ($P < 0.05$ and $P < 0.001$, respectively). This interaction was due to unaltered concentrations of immunoactive and bioactive LH in SO animals during the course of the experiment, but altered concentrations of LH in the UC groups. Six hours after UC, the concentration of both immunoactive and bioactive LH in serum was significantly higher ($P < 0.05$) than that in SO

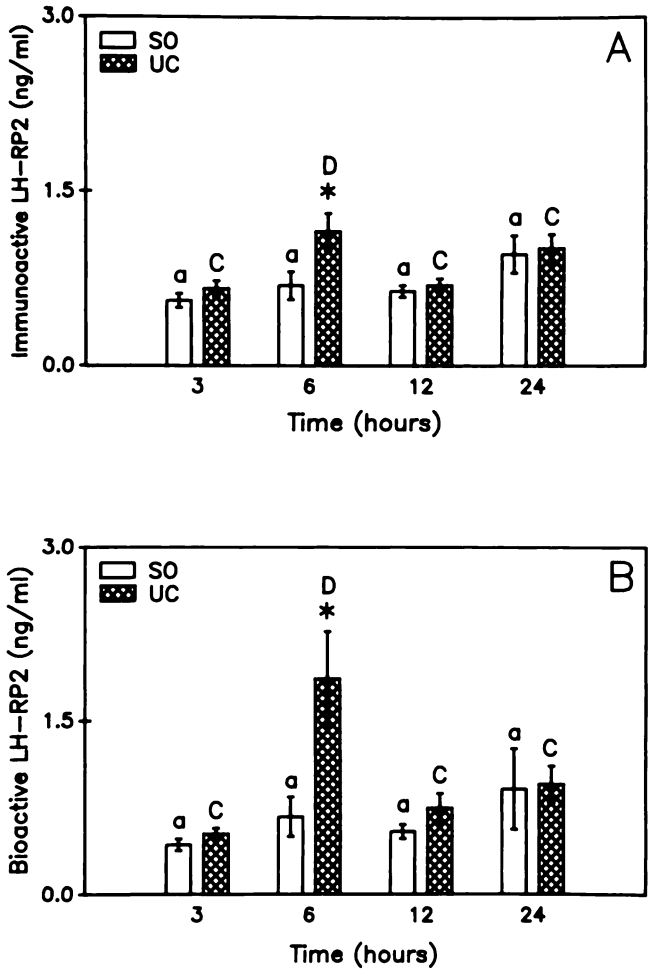

FIG. 2. Concentrations of immunoactive luteinizing hormone (LH) (A), and bioactive LH (B) in systemic serum at 3, 6, 12, and 24 hours after sham operation (SO) or unilateral castration (UC) (n = 6–13). An * indicates significant differences between SO and UC groups ($P < 0.05$). Different lower case letters indicate significant differences in LH concentrations at different times in SO rats ($P < 0.05$). Upper case letters are used for significant differences between groups of UC rats ($P < 0.05$).

rats. No such differences were encountered at the other times of sampling.

Experiment 2

Figure 3 depicts serum androgen concentrations before and after an acute stimulation with LH in rats receiving different combinations of capsules supplying steroids or pumps supplying LH at a constant rate. Basal concentration of androgen in serum was similar in all treatment groups, indicating that normal circulating androgen was unaltered by these treatments. Serum LH was 1.44 ± 0.32 ng/mL in rats with control implants, and was below the level of sensitivity of the RIA in rats bearing the steroid capsules (data not shown), suggesting that the testosterone/estrogen treatment was effective in suppressing endogenous LH secretion.

Serum androgen concentration after *in vivo* LH stimulation was used as an index of testicular responsiveness to LH. Two hours after receiving a bolus of 5 μg of LH, serum androgen was significantly elevated in all treatment groups. The rats bearing the steroid capsules, however, had a significantly lower response ($P < 0.05$) compared with the other groups, suggesting a loss of testicular responsiveness to LH. The rats receiving LH pumps, or the combination of steroid capsules and LH pumps, had a response to the bolus of LH similar to that of the rats bearing control implants. The concentration of FSH in the same basal sera from Figure 3 was determined; the group bearing control implants had 8.4 ± 1.2 ng/mL, and this was unaltered by any of the steroid/LH treatments (data not shown).

Experiment 3

In this experiment, we evaluated the response to UC when LH was held constant by the steroid capsule/LH pump regimen described previously. Figure 4A and B depict circulating androgen concentrations 1 and 7 days after SO or UC. Serum androgen was similar between UC and SO rats in the rats bearing control and steroid/LH implants at both times after surgery. Figure 5A shows the concentration of androgen in the testicular vein in control and implanted groups 24 hours after UC. A significant interaction ($P < 0.05$) between the effects of surgery and hormone treatment was observed. This interaction was due to an approximate doubling in the concentration of androgen in the testicular vein ($P < 0.05$) after UC in animals whose LH was permitted to change, whereas this doubling did not occur when LH was prevented from changing in the rats receiving the steroid/LH implants. Similar results also were observed 7 days after surgery in control and hormone-implanted rats (Fig 5B). The doubling of the concentration of androgen in the testicular vein observed 1 and 7 days after UC in the sham-implanted rats occurred without an apparent change in circulating concentration of immunoactive LH (Fig 6).

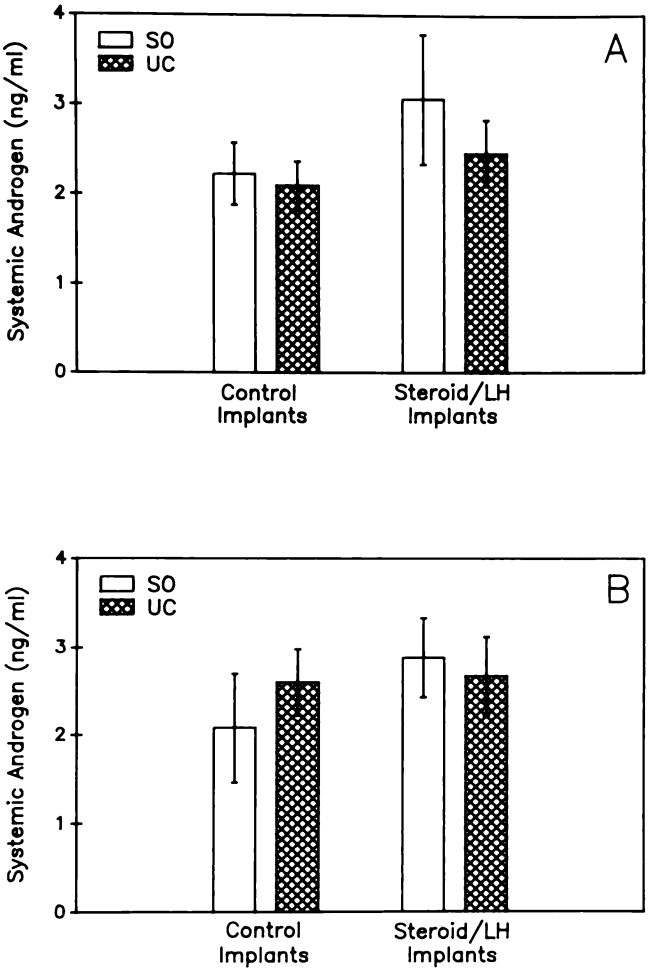

FIG. 4. Concentrations of androgen in systemic serum 1 day (A) and 7 days (B) after sham operation (SO) or unilateral castration (UC) in rats receiving control implants or testosterone–estrogen capsules and LH pumps (n = 5). No significant differences were noted.

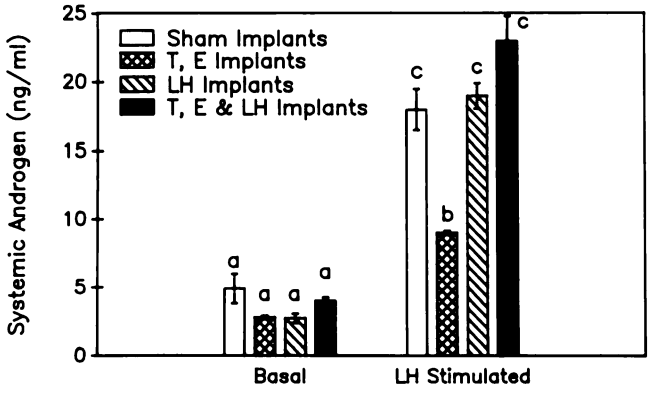

FIG. 3. Serum androgen before (basal) and 2 hours after (LH stimulated) intraperitoneal injection with 5 μg oLH in rats receiving subcutaneous empty implants as control; or testosterone–estrogen-filled capsules, and/or osmotic mini-pumps infusing LH at a constant rate of 12 μg/day for 5 days (n = 6). Different letters indicate significant differences ($P < 0.05$).

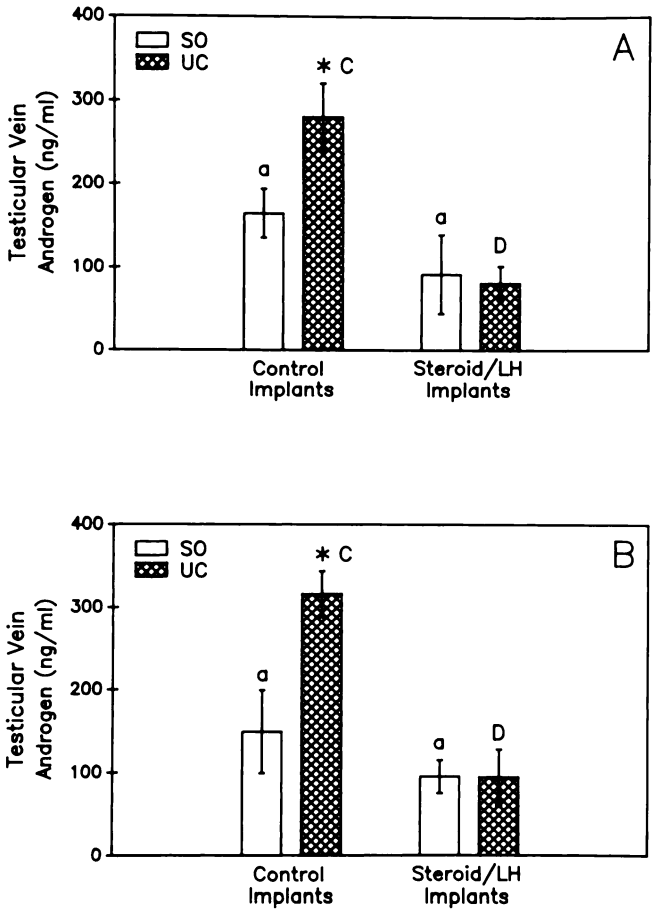

FIG. 5. Concentrations of androgen in testicular vein plasma 1 day (A) and 7 days (B) after sham operation (SO) or unilateral castration (UC) in rats receiving control implants or testosterone–estrogen capsules and LH pumps (n = 5). An * indicates significant differences between SO and UC groups ($P < 0.05$). Different lower case letters indicate significant differences in androgen concentrations between hormone treatments in SO rats ($P < 0.05$). Upper case letters are used for significant differences between groups of UC rats ($P < 0.05$).

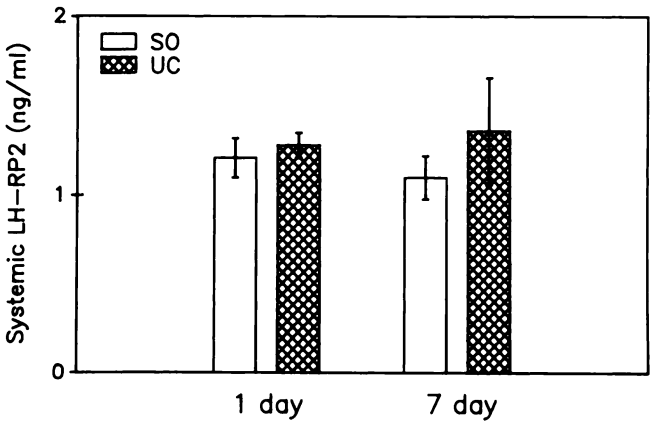

FIG. 6. Concentrations of immunoactive luteinizing hormone (LH) in systemic serum 1 day and 7 days after sham operation (SO) or unilateral castration (UC) in rats receiving control implants (n = 5). No significant differences were noted.

Figures 7A and 7B show serum FSH concentrations in rats bearing sham and hormone-filled implants 1 and 7 days after SO or UC. A significant effect of hormone treatment ($P < 0.005$) on serum FSH concentrations was observed 1 day after SO or UC (Fig 7A), where a significant decrease in serum FSH was observed in SO ($P < 0.05$) and UC ($P < 0.01$) rats bearing the hormone implants. Similar FSH concentrations were observed in SO and UC rats bearing either the empty or hormone-filled implants. A significant effect of surgery ($P < 0.001$) and hormone treatment ($P < 0.001$) was observed 7 days after SO or UC (Fig 7B). Serum FSH was significantly decreased in SO ($P < 0.05$) and UC ($P < 0.01$) hormone-treated rats, whereas FSH was significantly increased after UC in both the sham-implanted rats ($P < 0.05$) and hormone-implanted rats ($P < 0.01$).

Discussion

We have attempted to better understand the compensatory increase in androgen secretion that occurs in response to the removal of a single testis. We found an increase in immunoreactive and bioactive LH between 3 and 6 hours after UC, and this was accompanied by compensatory androgen secretion from the remaining testis. Suppression of this LH change abolished the compensatory response. The results confirm previous studies demonstrating that the compensatory androgen response is established 24 hours after UC (Mock and Frankel, 1982; Frankel et al, 1984; Moger and Anakwe, 1986; Frankel et al, 1989a, b). Frankel and Wright (1982) observed a recovery of androgen in serum 8 hours after UC to concentrations seen in control rats, and this compensation was maintained at 24 hours. The data we have provided demonstrate a similar trend, in that the concentration of androgen in serum had recovered 6 hours after UC to concentrations seen in the SO rats, and this recovery was maintained for at least 24 hours. The mechanism of the recovery of androgen in the UC rats is evident in the data on concentration of androgen in the testicular vein, where, at each time point, the recovery of systemic androgen was associated with an approximate doubling the concentration of androgen in the testicular vein. Unique to this study was the observation that the remaining testis increases its output of androgen between 3 and 6 hours in response to UC. Mock and Frankel (1982) measured the concentration of testosterone in the testicular vein at 4 and 24 hours after UC, but only observed the increase in testicular output at 24 hours. Together, these data suggest that the compensatory secretion of androgen in response to UC occurs between 4 and 6 hours after surgery.

Accompanying the increased concentration of androgen

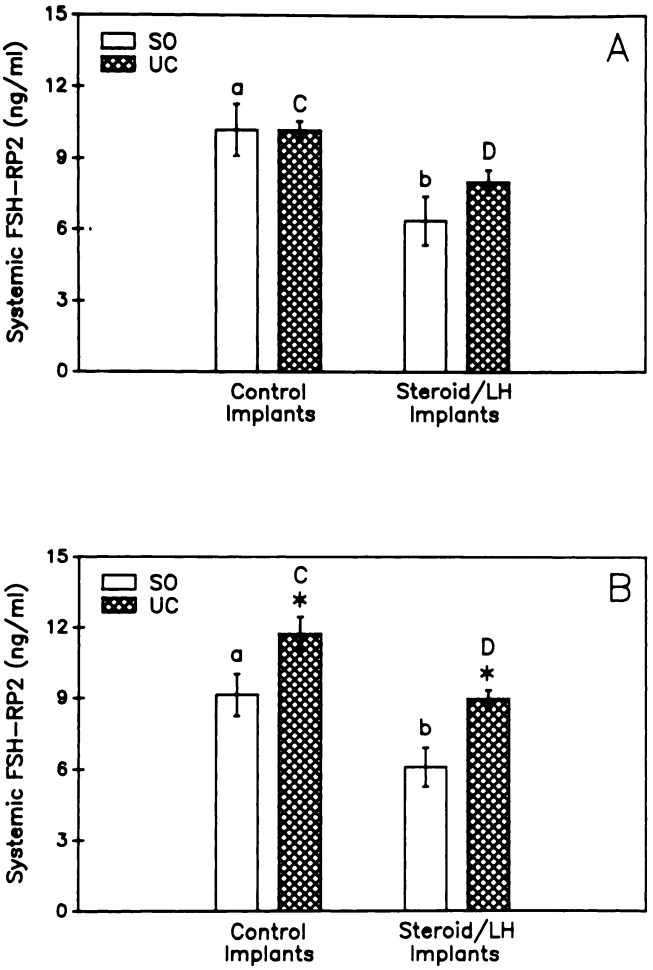

FIG. 7. Concentrations of follicle stimulating hormone (FSH) in systemic serum 1 day (A) and 7 days (B) after sham operation (SO) or unilateral castration (UC) in rats receiving control implants or testosterone–estrogen capsules and LH pumps (n = 5). An * indicates significant differences between SO and UC groups ($P < 0.05$). Different lower case letters indicate significant differences in FSH concentrations between hormone treatments in SO rats ($P < 0.05$). Upper case letters are used for significant differences between groups of UC rats ($P < 0.05$).

in the testicular vein 6 hours after UC was a significant increase in circulating immunoreactive LH. The elevated serum immunoactive LH may have provided the stimulus for the increased testicular steroid output observed at this time. In contrast, the compensatory response in androgen secretion 12 and 24 hours after UC was not accompanied by an increase in circulating immunoactive LH. Others also have been unable to associate the recovery of testosterone in serum 24 hours after UC with elevated concentrations of LH (Davidson et al, 1976; Frankel and Wright, 1982; Mock and Frankel, 1982). We also used a bioassay for LH to address the possibility that the lack of increase in immunoactive LH between 6 and 24 hours after UC did not reflect the biologically active stimulus to the Leydig cell. Bioactive LH was only significantly elevated at 6 hours; thus, a mechanism for the increased testicular output seen at times later than 6 hours remains unknown. Having seen an increase in bioactive and immunoactive LH only at 6 hours, we hypothesized that this change was necessary for the short-term (6 hours) and longer-term compensatory androgen response. If this proposal is valid, then the prevention of this change in LH should also prevent not only the short-term compensatory response, but also the long-term response seen 7 days after UC. This was assessed by ensuring that circulating LH was held constant using the described hormone regimen and then examining whether the compensatory androgen response persisted after UC.

The nondetectable serum immunoactive LH in rats bearing only the steroid capsules confirms previous studies showing that this treatment was effective in suppressing endogenous LH secretion (Ewing et al, 1977; Robaire et al, 1979). The administration of a continuous supply of exogenous LH maintained the testicular responsiveness to an acute challenge with LH. The inability of the rats bearing steroid capsules to respond as well as the other groups (experiment 2) to an LH challenge can be attributed to the lack of circulating LH. Luteinizing hormone has been demonstrated to maintain testicular responsiveness to acute stimulation through its trophic effects on Leydig cells. When LH is removed from circulation for a period, the steroidogenic capacity of the testes diminishes (Zirkin et al, 1982; Ewing et al, 1983; Wing et al, 1984, 1985; Keeney et al, 1988). The loss of function has been attributed to the reduction in the volume of Leydig cells and smooth endoplasmic reticulum in Leydig cells (Zirkin et al, 1982; Ewing et al, 1983; Wing et al, 1984, 1985; Keeney et al, 1988). If LH is replaced, then the volume of the cytoplasm and smooth endoplasmic reticulum is restored and the testes regain their steroidogenic capacity (Zirkin et al, 1982; Ewing et al, 1983; Wing et al, 1985). Thus, this hormone treatment effectively suppressed endogenous LH secretion while maintaining constant delivery of LH by the osmotic pumps, and maintained basal serum androgen and testicular responsiveness to LH.

Compensatory secretion of androgen occurred in the UC rats bearing empty implants (experiment 3). Again, this occurred without an observable change in circulating LH at the times sampled. In contrast, compensation did not occur in UC rats bearing the hormone implants. Frankel et al (1984) also noted that compensatory androgen secretion did not occur in response to UC when LH was removed by hypophysectomy. Such an approach does not eliminate the possibility that the compensatory response in androgen secretion requires a basal, but unchanging, level of LH secretion. The model we employed offered the advantage of maintaining LH constant as well as maintaining other pitu-

itary factors that may influence testicular function. For example, FSH was increased 7 days after UC in both the sham- and hormone-implanted rats. This increase in FSH confirms previous studies that demonstrated that FSH did not increase until after the compensatory androgen response occurred (Frankel and Wright, 1982; Gomes and Jain, 1976; Mizunuma et al, 1983). Thus, in the face of unchanging circulating LH, the stimulus of removing a single testis is insufficient to elicit increased androgen secretion. This observation may suggest that the increase in LH seen 6 hours after UC is a necessary component for establishing the compensatory androgen response at this and later times. An alternative explanation is that the implantation of the capsules containing testosterone and estrogen inhibited an as yet unidentified signal that elicits the compensatory response in androgen secretion, and that the status of LH secretion is not relevant. Additional studies are required to address this possibility.

Why androgen secretion can remain elevated 1 and 7 days after UC without an apparent increase in circulating LH remains unclear. A possible mechanism for increased testicular vein androgen is an increased testicular responsiveness to LH. In vitro studies have shown, however, that isolated Leydig cells or testicular tissue from UC and control rats secrete similar amounts of androgen when stimulated with LH human chorionic gonadotropin (Gerendai et al, 1984; Frankel et al, 1989b). Our own data also suggest that increased responsiveness to LH is not an important mechanism of compensation after UC. If this had occurred, then testicular vein androgen should have increased after UC when the rats had LH delivered at a constant rate.

We have demonstrated that the remaining testis in the adult rat increases its output of androgen as early as 6 hours after UC. This increased androgen output is associated with elevated serum LH, suggesting that LH may be the possible stimulus for the compensatory androgen secretion seen at this time. We also have demonstrated that the compensatory increase in testicular output of androgen noted 1 or 7 days after UC does not occur when LH is maintained constant by the combination of steroid capsules and osmotic pumps containing LH. This may indicate that the observed change in LH 6 hours after UC is an important component in the short- and long-term compensatory response of androgen secretion to UC.

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