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Pituitary Response to LHRH During Chronic Renal Insufficiency in the Male Rat

STANLEY J. NAZIAN, JOHN R. DIETZ, AND MELISA D. ROWLAND

Chronic renal insufficiency was induced in young adult male rats by a 2-step 5/6 nephrectomy. Four weeks after surgery the rats were anesthetized with ketamine and injected with saline or 1, 5, 10, 50, or 100 ng of LHRH/100 g BW and blood samples were collected at various times and assayed for LH. A second group of rats was injected with saline or 5, 10, 50, 100, or 200 ng of LHRH/100 g BW and serum samples assayed for FSH. A third experiment tested for the presence of a self-priming effect of LHRH on LH secretion. Rats were primed with three iv injections of 10 ng of LHRH/100 g BW or saline at 30-min intervals and challenged with 50 ng of LHRH/100 g BW 30 min after the last priming injection. Ten min after the challenge injection, the LH increment was determined. The data indicate that male rats suffering from chronic renal insufficiency have an impaired LH response to low doses of LHRH, esentially normal FSH responses to most doses of LHRH examined, and lack a self-priming effect of LHRH on LH secretion. These results suggest that part of the reproductive abnormalities found in chronic renal failure may be due to alterations in pituitary LH responses.

From the Department of Physiology and Biophysics, College of Medicine, University of South Florida, Tampa, Florida.

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Chronic kidney failure in the adult human male is accompanied by a number of endocrine and reproductive abnormalities (Emmanouel et al, 1980; Handelsman, 1985). Among these differences are reduced libido, impotence, decreased testicular size, severely impaired spermatogenesis and gynecomastia. In general, these defects are not alleviated by renal dialysis but are rapidly reversed by successful renal transplantation.

Studies performed with a rat model of renal insufficiency have suggested concomitant defects in both the hypothalamic/pituitary unit and the testis (Briefel et al, 1982; Blackman et al, 1982; da Costa e Silva et al, 1984; Handelsman et al, 1985a, b;

Reprint requests: Stanley J. Nazian, Department of Physiology & Biophysics, College of Medicine, Box 8, University of South Florida, Tampa, FL 33612.

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Tsitouras et al, 1985; Nazian and Dietz, 1987). Serum levels of testosterone (T) are depressed in male rats suffering from chronic renal insufficiency (Blackman et al, 1982; da Costa e Silva, 1984; Handelsman et al, 1985a, b; Nazian and Dietz, 1987), and testicular responses to human chorionic gonadotropin (hCG) are reduced (Handelsman et al, 1985a; Tsitouras et al, 1985). Although not all reports agree (da Costa e Silva, 1984), most studies (Blackman et al, 1982; Handelsman et al, 1985a, b; Nazian and Dietz, 1987) indicate that serum concentrations of LH are also depressed by chronic renal insufficiency, while FSH concentrations have been reported to be both reduced (Blackman et al, 1982; Handelsman et al, 1985a) and increased (Nazian and Dietz, 1987) 4 weeks after the induction of renal insufficiency in the male rat.

In vivo studies of the hypothalamic control of the anterior pituitary of rats suffering from chronic renal insufficiency appear to be limited to one report (Handelsman et al, 1985b). These workers reported "normal" LH and FSH responses to LHRH in rats suffering from chronic uremia. Since the LH response to naloxone administration was absent in such animals, they attributed their reduced serum LH and FSH concentrations to an alteration in the inhibitory tone of the opioid system. However, their LHRH studies utilized a single extremely large (2-μg/rat) dose of LHRH that was not adjusted for body weight. Since chronic renal insufficiency results in a highly significant weight reduction (Handelsman et al, 1985a; Nazian and Dietz, 1987), they could easily have missed more subtle shifts in pituitary responsiveness to LHRH (Handelsman et al, 1985b).

The studies reported here were designed to examine *in vivo* the effect of a range of more physiologic doses of LHRH on LH and FSH secretion in the male rat suffering from chronic renal insufficiency. Chronically uremic male rats were also examined for the presence of a self-priming effect of LHRH on LH secretion (Nazian, 1986).

Materials and Methods

General

Young adult male rats (300 to 350 g body weight (BW)) of a Sprague-Dawley-derived strain were purchased from the Holtzman Co. (Madison, WI). They were housed three to five per cage in hanging wire mesh cages under controlled lighting conditions (14L:10D) and temperature (24 C). Food and water were available *ad libitum*. One to 2 weeks after receipt, these animals were subjected to a 5/6 nephrectomy or sham surgery as described previously (Nazian and Dietz, 1987). Four to 5 weeks following nephrectomy, the animals were anesthetized with ketamine HCl, weighed and the right jugular vein was exposed.

Experimental

Three separate experiments were performed. In the first, a saline control, 1, 5, 10, 50, or 100 ng LHRH/100 g BW were injected iv. LHRH concentrations in this and all other experiments reported here were adjusted so that approximately 0.2 ml of fluid was injected with each dose. Blood samples were obtained by heart puncture just prior to the injection (1.0 ml) and at 10, 20, 30, 45, and 60 min after the injection (0.5 ml). Duplicate 150-μl aliquots of serum were obtained from the time 0 sample, diluted with 350 μl of assay buffer and frozen (−22 C) until assayed for LH by radioimmunoassay (RIA). Duplicate 80-μl aliquots diluted with 420 μl of buffer were used at the other time points. Serum urea concentrations in the time 0 (or in a few cases the 10-min) blood sample was analyzed. In the second experiment, saline, 5, 10, 50, 100, or 200 ng LHRH/100 g BW were injected iv. Blood samples were collected, as above, just prior to and 15, 30, 60, and 90 min after the injection. Serum was obtained as above and assayed for urea and FSH. Duplicate 80-μl aliquots diluted with 420 μl of buffer were used for the FSH assay. In the third experiment, animals were examined for a self-priming effect of LHRH on LH secretion (Nazian, 1986). Rats were primed with three injections of saline or 10 ng LHRH/100 g BW at 0.5-h intervals via the right jugular. Thirty min after the third priming injection, a 1.0-ml blood sample was obtained by heart puncture and all animals received a 50-ng LHRH/100 g BW challenge injection via the left jugular. A final blood sample was obtained 10 min after the challenge injection. Duplicate 100-μl aliquots from the pre-challenge sample were diluted with 400 μl of buffer. Duplicate 30-μl aliquots from the post-challenge samples were diluted with 470 μl of buffer. All aliquots were frozen until assayed for LH by RIA. Urea concentrations in the pre-challenge sample were also determined.

Assays and Statistics

Serum LH and FSH concentrations were determined using the RIA kits provided by the National Hormone and Pituitary Agency as described previously (Nazian, 1988). Standards used were NIH rat LH RP-2 and NIH rat FSH RP-1, respectively. All samples from a given experiment were run in the same assay. Serum from rats suffering from chronic renal insufficiency does not have any nonspecific effects in these assays (Handelsman et al, 1985b). Serum concentrations of urea nitrogen were determined by a standard spectrophotometric method using a kit obtained from Sigma (St. Louis, MO). Each group initially contained five to seven rats. Due to attrition resulting from the multiple heart punctures, some of the later (45-, 60-, or 90-min) time points contain samples from only four animals. For the first two experiments, analysis of variance was performed using the General Linear Model Procedure of the Statistical Analysis System (SAS Institute, Cary, NC) as implemented for a personal computer. When controlling for time revealed significant differences between nephrectomized and sham-operated

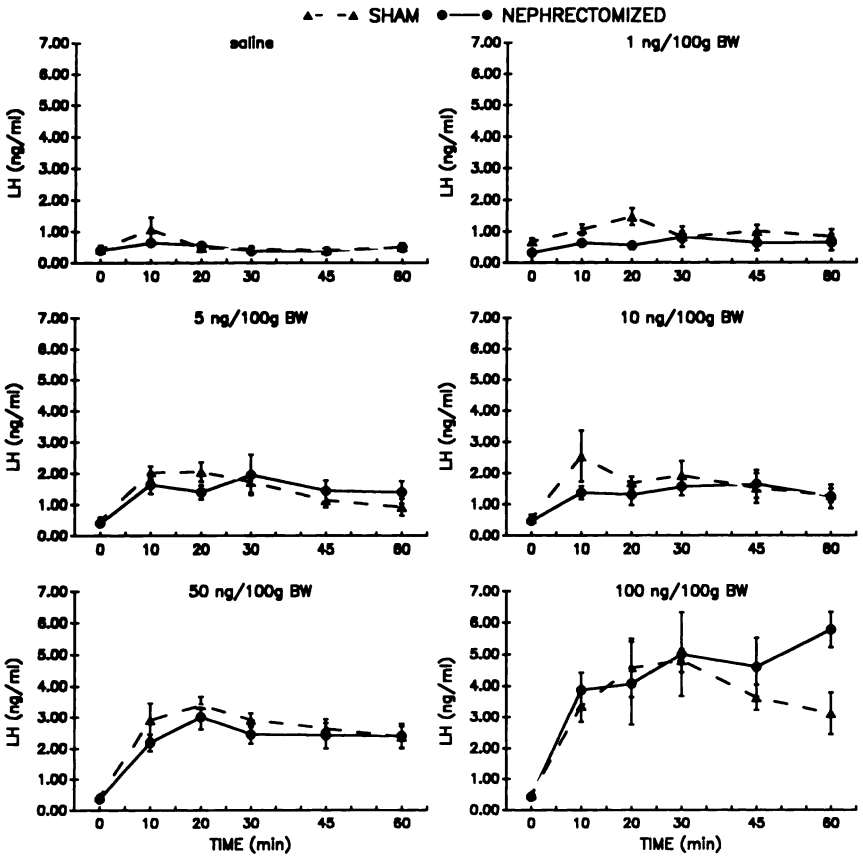

Fig. 1. Serum LH concentrations (mean ± SEM) in response to the indicated dose of LHRH injected immediately after the time 0 blood sample was collected. Male rats were subjected to 5/6 nephrectomy (closed circles) or sham surgery (closed triangles) 4 weeks prior to testing.

rats, we analyzed the data further by multiple comparisons of the mean. When appropriate, data were analyzed and significance determined using Student's t-test for unpaired data. Some of the data were first expressed as the increment of hormone concentration in response to LHRH injection (Nazian and Mahesh, 1979).

Results

Experiment 1: LH, Figure 1

Four weeks following surgery animals that had undergone a 5/6 nephrectomy had significantly ($P < 0.001$) lower body weights and significantly ($P < 0.001$) higher serum concentrations of urea nitrogen than did sham-operated controls. Mean body weight (± SEM) was 493 ± 7 g for sham-operated controls and 393 ± 8 g for 5/6 nephrectomized animals. Serum urea nitrogen was 17.3 ± 0.5 mg/dl in control rats and 97.3 ± 4.7 mg/dl in animals suffering from chronic renal insufficiency. When the data for the time 0 blood samples were pooled, serum concentrations of LH were significantly ($P < 0.05$) reduced in 5/6 nephrectomized rats compared with the controls. Sham-operated rats had serum LH concentrations of 0.51 ± 0.04 ng/ml, while 5/6 nephrectomized rats had LH levels of 0.40 ± 0.03 ng/ml.

After an iv injection of 1 ng LHRH/100 g BW, serum LH was lower in 5/6 nephrectomized rats compared with controls at 10 and 20 min postinjection ($P < 0.05$). The increment in LH (concentration minus time 0 concentration) was also significantly ($P < 0.05$) depressed at 20 min postinjection (sham: 0.80 ± 0.23; nephrectomized: 0.19 ± 0.09 ng/ml, mean ± SEM). In response to an iv injection of 100 ng LHRH/100 g BW, the pattern of serum LH concentration was essentially similar in nephrectomized animals and sham-operated controls through 45 min. Sixty min postinjection, LH concentrations in 5/6 nephrectomized rats were higher than in sham-operated controls. The response of serum LH to injections of saline, 5, 10, or 50 ng LHRH/100 g BW were essentially similar regardless of renal status. The analysis of variance indicated a significant effect of nephrectomy ($P < 0.001$) for the 1-ng/100 g BW

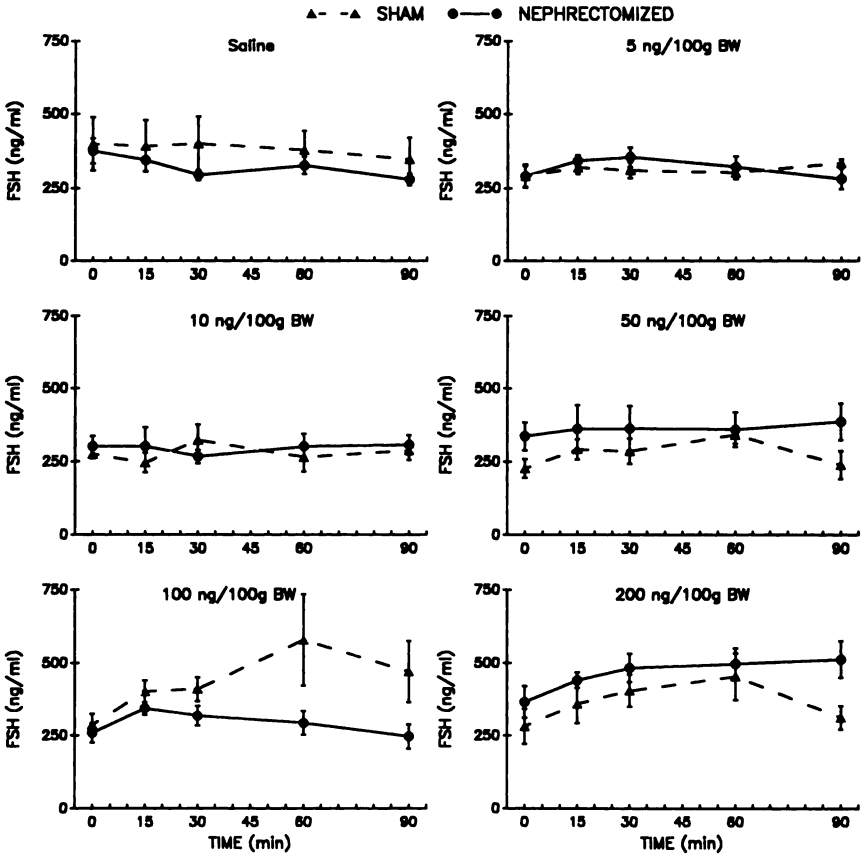

Fig. 2. Serum FSH concentrations (mean ± SEM) in response to the indicated dose of LHRH injected immediately after the time 0 blood sample was collected. Male rats were subjected to 5/6 nephrectomy (closed circles) or sham surgery (closed triangles) 4 weeks prior to testing.

dose only. Other doses showed no statistically significant effect of kidney status.

Experiment 2: FSH, Figure 2

Body weights in 5/6 nephrectomized animals were significantly ($P < 0.001$, 397 ± 8 g and 492 ± 7 g, respectively) lower compared with sham-operated controls and serum urea nitrogen values were significantly ($P < 0.001$) higher (96.3 ± 4.5 mg/dl and 20.7 ± 0.5 mg/dl, respectively). When the data from the time 0 samples were pooled, there were no significant differences in FSH concentrations between rats suffering from chronic renal insufficiency (294 ± 22 ng/ml) and control animals (316 ± 17 ng/ml). The response of serum FSH to saline, 5, 10, or 50 ng LHRH/100 g BW was essentially similar regardless of renal status. Analysis of variance indicated that the response of FSH to 100 ng LHRH/100 g BW was affected by 5/6 nephrectomy ($P < 0.01$). There appeared to be a decreased response in the nephrectomized rats 60 and 90 min after injection of LHRH. Analysis of variance also suggested that the FSH response to 200 ng LHRH/100 g BW was increased by nephrectomy ($P < 0.01$). Serum FSH concentrations in 5/6 nephrectomized animals were higher compared with the sham-operated controls 90 min after LHRH injection.

Experiment 3: Self-Priming, Figure 3

Removal of 5/6 of the kidney mass resulted in a significant ($P < 0.001$) decrease in body weight and a significant ($P < 0.001$) increase in the concentration of urea nitrogen in the peripheral serum. Mean body weight was 461 ± 7 g for the sham-operated controls and 385 ± 12 g for the 5/6 nephrectomized rats. The serum urea nitrogen concentration in the controls was 19.7 ± 1.1 mg/dl and 88.4 ± 7.5 mg/dl in rats suffering from chronic renal insufficiency. In both groups, three priming injections of 10 ng LHRH/100 g BW resulted in significant ($P < 0.001$) increases in LH secretion 30 min after the last priming injection (0.30 ± 0.03 vs. 4.76 ± 0.93 ng/ml and 0.35 ± 0.03 vs. 6.67 ± 1.07 ng/ml, control and 5/6 nephrectomized rats, respectively). There was a significant self-priming effect in sham-operated control rats. Animals that

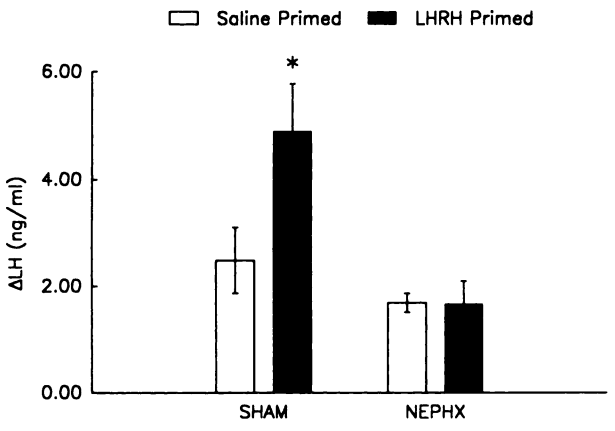

Fig. 3. The increment in serum LH (mean ± SEM) in response to the LHRH challenge injection in rats pretreated with saline (open bars) or LHRH (solid bars). SHAM: sham-operated male rats; NEPHX: 5/6 nephrectomized male rats. * $P < 0.05$.

were pretreated with LHRH released significantly ($P < 0.05$) more LH in response to the LHRH challenge than did animals pretreated with saline. Rats suffering from experimentally induced chronic renal insufficiency did not show this effect. The response of serum LH to the challenge injection was similar regardless of the type of priming.

Discussion

Previous workers (Handelsman, et al, 1985b) have reported "normal pituitary responses to LHRH stimulation *in vivo*" in rats made chronically uremic by a 5/6 nephrectomy. The data reported here indicate that with respect to LH secretion the response of the anterior pituitary to low doses of LHRH is impaired in male rats suffering from chronic renal insufficiency. The LH response to an injection of 1 ng LHRH/100 g BW is reduced in such animals. Assuming normal values for blood volume and hematocrit in the rat, this dose of LHRH results in a serum concentration of approximately 300 pg/ml. Since the volume of distribution of LHRH is similar in control rats and animals suffering from chronic renal insufficiency (Handelsman et al, 1985b), it is probably a valid approximation. This value approaches the 100 to 200 pg/ml concentration reported for LHRH in the portal plasma of anesthetized male rats (Valenca et al, 1987) and is certainly a more physiologic dose than the 2 μg/rat used previously (Handelsman et al, 1985b). Our results suggest that at least part of the reproductive dysfunction of the hypothalamic/pituitary unit seen in chronic renal insufficiency may be due to a reduced pituitary sensitivity to physiologic amounts of LHRH.

Additional *in vivo* evidence for a defect in pituitary function during chronic renal insufficiency comes from the results of the self-priming experiment. The normal adult male rat pituitary releases more LH in response to an LHRH challenge if the animal is pretreated with LHRH than if pretreated with saline (Nazian and Mahesh, 1979; Nazian, 1986). This effect is clearly absent in rats made chronically uremic by 5/6 nephrectomy (Fig. 3). The self-priming effect appears to require adult, or at least pubertal, T levels for its maintenance (Nazian, 1986). Therefore, the lack of such an effect in the 5/6 nephrectomized rat may be due, at least in part, to the reduced concentration of T seen in this model of renal failure (Handelsman et al, 1985a, b; Nazian and Dietz, 1987).

Although the pooled time 0 concentrations of LH are significantly suppressed in the 5/6 nephrectomized rats, the "response" to saline does not appear to maintain this difference, probably because of the long term effects of the ketamine anesthesia. Although acute exposure to ketamine does not alter LH concentrations (Nazian, 1988), it is known that longer exposure can result in decreases in serum LH levels (Nazian, 1983). Such an explanation can probably also account for the failure of the pre-challenge LH concentrations to differ in the self-priming experiment. Such shifts in basal LH concentrations probably are not important in the dynamic responses investigated in this study.

Other reports have suggested that chronic renal insufficiency in the adult male rat leads to a decreased serum concentration of FSH (Blackman et al, 1982; Handelsman et al, 1985a). Previous work from this laboratory, however, indicated that there was no change in serum FSH through the first 2 weeks following the induction of chronic renal insufficiency and an increase in serum FSH at 4 weeks (Nazian and Dietz, 1987). The data reported here suggest that 5/6 nephrectomy had no effect on baseline FSH secretion. However, these rats were anesthetized with ketamine. Recent experiments in this laboratory (Nazian, 1988) have suggested that ketamine is capable of reducing serum FSH concentrations in intact immature male rats, but not in adult animals. It was hypothesized previously (Handelsman et al, 1985b) that chronic uremia results in a regression of the hypothalamic/pituitary unit to a prepubertal state. If this is true, then the failure

to find any differences in basal serum FSH concentrations could be the result of a subtle interaction of an immature hypothalamus, low serum T and the anesthetic. The response of serum FSH concentrations to the lower doses of LHRH was similar in both control and 5/6 nephrectomized rats, suggesting that the alteration in pituitary response induced by chronic renal insufficiency is at least somewhat specific for LH.

The FSH response to the highest doses of LHRH used in these experiments was prolonged in the 5/6 nephrectomized rats compared with the sham-operated controls. The metabolic clearance rate of LHRH, as well as both LH and FSH, is reduced by 5/6 nephrectomy (Handelsman et al, 1985b). Thus, especially in the case of larger doses, more LHRH is available for a longer time and the LH and FSH released remains in the circulation longer. The FSH response to the 100-ng dose of LHRH was reduced in nephrectomized compared with sham-operated animals. This may suggest that a subtle difference in the FSH response to larger doses of LHRH exists in nephrectomized rats. If such an effect exists, it is masked by the prolonged metabolic clearance rates for LHRH and FSH described above.

It is conceivable that hyperprolactinemia may have played a role in some of our results. Male rats (Blackman et al, 1982; Handelsman et al, 1985a) and humans (Handelsman, 1985) suffering from chronic renal insufficiency have increased serum concentrations of prolactin. Elevated levels of prolactin are known to interfere with basal and LHRH-stimulated LH release as well as the ability of naloxone to induce LH release (Bartke et al, 1978; Hodson et al, 1980; Sweeney et al, 1985). These responses are similar to those seen in the male rat suffering from chronic renal insufficiency (Handelsman et al, 1985b). The effect of hyperprolactinemia on the self-priming effect has not been investigated in male rats.

In summary, the data reported here suggest that chronic renal insufficiency in male rats induced by 5/6 nephrectomy results in a decrease in the pituitary LH response to low, physiologic levels of LHRH, the elimination of the self-priming effect of LHRH on LH secretion, and little apparent change in the pituitary FSH response to LHRH.

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