NOTE

Downward Regulation of Plasma LH by LHRH Agonist, Leuprolide Acetate, Resulting in Inhibited Renal Growth and Function in the Castrated Male Rat

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Abstract. We previously reported that ovine and porcine luteinizing hormone (LH) stimulated kidney growth in castrated hypophysectomized rats. Our present study focuses on the physiological role of the renotropic activity of LH isoforms. Plasma LH levels were decreased to 10% of that of castrated control rats by injections of a slow-releasing LHRH agonist, leuprolide acetate, from microcapsules. Compared to controls, which were injected with microcapsules only, the kidney weight in leuprolide-treated castrated rats decreased 12%. Renal protein and DNA contents decreased significantly. Body, liver and spleen weights were not changed by the treatment, however. This effect on the kidney was not observed in castrated hypophysectomized rats, suggesting that leuprolide affected the kidneys indirectly, rather than directly, by suppressing LH secretion. In leuprolide-treated castrated rats, urinary fractional excretion of sodium (FENa) increased, indicating suppressed renal function at the proximal tubules. We concluded that the secretion of renotropically active LH isoforms was regulated at least partially by LHRH and played a physiological role in growth and the function of the proximal tubules.

Key words: LHRH, LH, Kidney, DNA.

(*Endocrinol Japan* 38: 693–697, 1991)

LH AND OTHER glycoprotein hormones consist of an array of isoforms with heterogeneous oligosaccharide and polypeptide structures [1]. We previously reported that some ovine and porcine LH isoforms stimulated [³H]thymidine incorporation into renal DNA in castrated hypophysectomized rats [2, 3], and increased renal weight and protein and DNA contents in castrated hypophysectomized mice [4]. To further clarify the role of LH isoforms in maintaining kidney growth, we studied the effect of the selective removal of LH isoforms on kidney growth.

We injected microcapsules containing a potent

LHRH agonist, leuprolide [D-Leu⁶-(des-Gly¹-NH₂)-LHRH ethylamide] acetate, subcutaneously into castrated rats and studied its effect on plasma LH and kidney weight. Microcapsules slowly released leuprolide for a month, causing LH secretion to be suppressed [5], which may demonstrate the effect on the kidney of selectively reducing gonadotropins. We studied changes in both renal growth and renal function. Rats were castrated to exclude the indirect effect on the kidney of leuprolide via testosterone. Castrated hypophysectomized rats were also studied to clarify whether or not leuprolide affected the kidney directly.

Received: July 10, 1991

Accepted: October 7, 1991

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Materials and Methods

J1a: Wistar male rats were purchased from Nihon Ikagaku Doubutsu Shizai Kenkyujo,

Tokyo. The rats were castrated or castrated and hypophysectomized at four weeks of age. Hypophysectomy was performed by the external auditory canal method [6]. Leuprolide acetate (0.3 mg) in microcapsules [5], kindly donated by Takeda Chemical Industries, Ltd., Osaka, were injected subcutaneously. Microcapsules without leuprolide were similarly injected into control rats. Four weeks after the first injection of microcapsules, the rats were sacrificed or given a second injection of microcapsules with or without 0.66 mg of leuprolide. The rats given second injections were sacrificed seven weeks after the first injection. In castrated hypophysectomized rats, the second dose was 0.3 mg rather than 0.66 mg because of their lower body weights. Blood, kidney, liver, and spleen were collected for further study. Kidney, liver, and spleen were lyophilized to measure their dry weights, then immersed and homogenized in distilled water to measure their protein and DNA contents as described previously [4]. The plasma LH concentration was measured by specific RIA provided by the National Hormone and Pituitary Program, NIDDK, Baltimore, MD [7] with NIDDK-rLH-RP-3 as a standard. The creatinine concentration was determined by an automated picric acid method and sodium was determined by

flame photometry. The results were reported as the mean $\pm$ SE. The analysis of variance and Duncan's new multiple range test were used for statistical analysis [8].

Results

Effects of leuprolide on castrated rats

Leuprolide decreased the plasma LH level to about 10% of that of control rats both at four and seven weeks (Fig. 1). As shown in Table 1, the body, liver, and spleen weights of leuprolide-treated rats did not differ from those of the controls. The dry kidney weight decreased to 88% of that of the controls at seven weeks (Table 1). Renal protein and DNA contents were also signi-

Fig. 1. Plasma LH concentration after treatment with leuprolide (LH-RH_A). Plasma LH concentration was significantly decreased in castrated rats by four and seven weeks' treatment. ** $p < 0.01$ vs. controls.

Fig. 2. Renal contents of protein and DNA after treatment with leuprolide (LH-RH_A). Renal protein and DNA contents were significantly decreased in castrated rats by seven weeks' treatment with leuprolide. ** $p < 0.01$ vs. controls.

Table 1. Effects of LH-RH agonist on body, kidney, liver, and spleen weights

Treatment period		Body weight (g)		kidney weight (g)	liver weight (g)	spleen weight (g)
		initial	final			
Experiments on castrated rats						
Controls	4 weeks (6)	86.2±1.1	234.9±4.2	0.363±0.006	3.208±0.117	0.171±0.006
LH-RH _A	4 weeks (6)	86.4±1.6	234.0±4.2	0.376±0.010	3.098±0.066	0.170±0.004
Controls	7 weeks (6)	88.7±1.1	309.0±9.0	0.468±0.018	3.754±0.157	0.197±0.008
LH-RH _A	7 weeks (5)	86.2±2.5	308.6±7.6	0.414±0.005 ^{a)}	3.649±0.113	0.174±0.012
Experiments on castrated hypophysectomized rats						
Controls	4 weeks (11)	108.3±1.3	114.5±2.9	0.162±0.004	1.395±0.072	N.D.
LH-RH _A	4 weeks (10)	108.1±1.1	107.7±3.2	0.153±0.004	1.255±0.044	N.D.
Controls	7 weeks (7)	95.9±2.0	121.7±3.3	0.162±0.007	1.361±0.007	0.066±0.004
LH-RH _A	7 weeks (6)	96.2±1.6	115.0±4.4	0.161±0.006	1.176±0.063 ^{b)}	0.059±0.003

Controls indicate rats treated with microcapsules without leuprolide. LHRH_A indicates rats treated with leuprolide in microcapsules. The number in parenthesis indicates the animal number used. The dose injected is indicated in "Materials and Methods". Means±SEM are shown. ^{a)}, $p<0.05$; ^{b)}, $p<0.01$ vs Controls. N.D. means not determined.

Fig. 3. Renal function after treatment with leuprolide (LH-RH_A). Creatinine clearance (Ccr) and fractional excretion of sodium (FENA) were determined. * $p<0.05$ vs. controls.

ificantly decreased in rats treated for seven weeks (Fig. 2). Creatinine clearance tended to be lower in leuprolide-treated rats. FENA tended to be higher in leuprolide-treated rats with a significant difference at four weeks of treatment (Fig. 3).

Effects of leuprolide on castrated hypophysectomized rats

Hypophysectomy reduced body and organ weights. Treatment with leuprolide did not affect body, kidney or spleen weights, but reduced liver weight further at seven weeks (Table 1).

Discussion

A significant relationship has been reported between testicular function and renal growth [9]. Premature male rats undergo renal growth with increased renal DNA contents (renal hyperplasia). After puberty, however, the increased plasma level of testicular androgen inhibits renal DNA synthesis and stimulates renal protein synthesis (renal hypertrophy). When mature male rats were castrated, renal DNA synthesis was again initiated [9]. Because our present work was to study the physiological role of renotropically active LH isoforms characterized as mitogenic for the proximal tubules, we used castrated rats in this experiment, thereby excluding the influence of testicular androgen. In castrated rats treated with leuprolide, plasma LH levels decreased significant-

ly to 10% of those of castrated control rats but were still slightly higher than those of noncastrated male rats (0.1–0.2 ng/ml) [7]. Although the aim of our study, i.e., the removal of plasma LH, was partially achieved, the outcome may not represent the maximum effect. When leuprolide-treated and untreated castrated rats were compared, the only significant effect observed was in kidney weight, but not in body, liver, and spleen weights. Leuprolide did not decrease kidney weight in castrated hypophysectomized rats, suggesting that this kidney-specific effect was not directly from leuprolide but was the result of decreased plasma levels of gonadotropins, LH, and FSH. Because FSH had no renotropic effect [10, 11], the decrease in kidney weight may be attributable to reduced LH. It was also evident that the secretion of renotropically active LH isoforms was regulated at least partially by LHRH.

The decrease in kidney weight due to leuprolide treatment was relatively minor, only by 12% from that of the controls. The effect was much weaker than that induced by hypophysectomy, perhaps for the following reasons: First, LH isoforms stimulated proliferation in only limited cells, proximal tubular cells, among the heterogeneous renal cells [12]. Second, plasma LH levels were not suppressed completely, so the effect may not be the maximum possible. Third, other pituitary factors may contribute to maintain kidney growth directly or indirectly. For example, although GH,

IGF-I, thyroid hormone and testosterone did not stimulate renal DNA synthesis [13, 11], these hormones exhibit anabolic effects on the kidney [14–16] and maintain kidney growth.

Liver weight was decreased after seven weeks of treatment with leuprolide in castrated hypophysectomized rats. This finding is inconsistent with our previous one that, in castrated hypophysectomized mice, five daily injections of renotropically active ovine LH isoforms reduced liver weights [4]. Such a difference remains to be further studied.

Renal function also appeared to be inhibited in castrated rats treated with leuprolide. Sodium reabsorption at the proximal tubules, FENa, was reduced significantly at four weeks, so it seems likely that renotropically active LH isoforms stimulated both renal growth and the function of proximal tubules. A more detailed study may clarify the role of LH isoforms with regard to the functions of the proximal tubules.

Acknowledgments

We thank Ms. Jun Setoguchi for editing this manuscript. This study was supported in part by the Foundation for Growth Science in Japan and by a Grant-in-Aid from the Japanese Ministry of Education, Science, and Culture for Encouragement of Young Scientists (03771838).

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