The Renotropic Effect of Ovine Luteinizing Hormone on Subtotally Nephrectomized Rats

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Abstract. Some of luteinizing hormone (LH) isoforms can stimulate renal growth. The objective of this study is to determine whether the administration of LH modifies subtotal nephrectomy-induced chronic renal failure. Castrated 3/4-nephrectomized male rats were divided into four groups of seven each and fed a low-protein (6%) diet. Ovine LH with renotropic activity (40 µg/day) or vehicle only (control) was given for three weeks or six weeks. Compared with controls, remnant kidney weights (% body weight) in LH-treated rats had increased significantly at three weeks (0.385 ± 0.019 vs 0.443 ± 0.052, P<0.02), but not at six weeks (0.281 ± 0.004 vs 0.272 ± 0.013). 24h creatinine clearance (ml/day/100 g body weight) increased significantly both by three weeks (242 ± 58 vs 301 ± 36, P<0.05), and six weeks (323 ± 55 vs 395 ± 10, P<0.01). Urinary thromboxane B2 excretion increased in LH-treated rats, suggesting that hemodynamic changes may play a role in increasing creatinine clearance. Our results suggest that renotropically active olH stimulated the glomerular function in castrated rats with reduced renal mass. Further study may clarify its clinical usefulness.

Key words: LH, Kidney, Chronic renal failure.
Materials and Methods

Extraction and purification of LH with renotropic activity

Lyophilized ovine pituitary glands were obtained from Waitaki International Bioscience Ltd. (Christchurch, New Zealand). Renotropically active LH preparation was purified by a method described previously [1]. Bioactivity was confirmed by bioassay based on the ability to stimulate $^3$H-thymidine incorporation into the renal DNA of the castrated-hypophysectomized rat. Significant activity was demonstrated at a dose of more than 4 µg [1, 9].

Experimental procedures

Twenty-eight male Sprague-Dawley rats weighing 170–220 g were obtained from Charles River Japan Co. (Atsugi, Japan) and divided into four groups of seven rats each. There was no significant difference among the four groups in body weight, mean arterial blood pressure, urinary protein excretion, serum creatinine or 24 h creatinine clearance (Table 1). They were anesthetized with ether for surgical manipulation. We used the castrated rats to study the direct effect of LH, not the indirect effect mediated by LH-stimulated testicular androgen. One week after castration, the lower half of the left kidney was infarcted by ligation of the renal parenchyma through a lumbar incision. Thereafter, the rats were fed a low-protein (6%) diet (Nihon Clea Co., Ltd., Tokyo, Japan). One week later, the right kidney was removed. The ratio of kidney weight per body weight was the same in all four groups. We then started administering a renotropically active LH preparation. Forty µg of ovine LH was resolved in 0.15 ml of 0.05 M sodium borate, pH 8.6, with 0.1% (w/v) bovine serum albumin (Bovine F-V, Nakarai Chemicals Ltd., Kyoto, Japan), and was given subcutaneously every day for three weeks (Group 2) or six weeks (Group 4). Control rats were given vehicle only for three weeks (Group 1) or six weeks (Group 3). 24 h-urine was collected in a metabolic cage. The blood pressure was measured by means of a tail cuff without anesthesia. At the end of the three-week or six-week experiments, blood was collected by puncturing the aorta, and the remnant left kidney was excised. Serum and urine creatinine were analyzed with a Beckman Creatinine Analyzer 2 (Beckman, Palo Alto, CA, U.S.). Urine protein was measured by refractometry [10], with bovine serum albumin as a standard. Urine thromboxane

Table 1. Systemic and whole-kidney parameters before (0 week) and after (3 or 6 weeks) treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample (40 µg/day)</th>
<th>Period (weeks)</th>
<th>BW (g)</th>
<th>MABP (mmHg)</th>
<th>UV (ml/day)</th>
<th>Ht (%)</th>
<th>sCr (mg/dl)</th>
<th>Ccr (ml/day)/100 g BW</th>
<th>Urine protein (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>0</td>
<td>208.4</td>
<td>120.0</td>
<td>12.2</td>
<td>44.9</td>
<td>0.41</td>
<td>918</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>± 14.8 ± 12.0</td>
<td>± 2.3</td>
<td>± 1.8</td>
<td>± 0.03</td>
<td>± 154</td>
<td>± 1.6</td>
<td>± 50.6</td>
<td>242</td>
<td>± 1.9</td>
</tr>
<tr>
<td>3</td>
<td>LH</td>
<td>0</td>
<td>211.0</td>
<td>124.0</td>
<td>14.3</td>
<td>45.0</td>
<td>0.40</td>
<td>930</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>± 6.5 ± 7.0</td>
<td>± 3.7</td>
<td>± 1.6</td>
<td>± 0.05</td>
<td>± 233</td>
<td>± 1.6</td>
<td>± 20.5</td>
<td>301</td>
<td>± 2.0</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>0</td>
<td>189.9</td>
<td>116.0</td>
<td>13.7</td>
<td>45.0</td>
<td>0.43</td>
<td>918</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>± 11.3 ± 8.0</td>
<td>± 3.4</td>
<td>± 1.7</td>
<td>± 0.09</td>
<td>± 131</td>
<td>± 1.8</td>
<td>± 23.6</td>
<td>323</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>LH</td>
<td>0</td>
<td>243.1</td>
<td>142.1</td>
<td>20.4</td>
<td>39.8</td>
<td>1.16</td>
<td>323</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>± 23.6 ± 9.2</td>
<td>± 9.2</td>
<td>± 9.8</td>
<td>± 0.15</td>
<td>± 165</td>
<td>± 1.5</td>
<td>± 14.7 ± 13.2</td>
<td>41.4</td>
<td>± 1.0 ± 0.1</td>
</tr>
</tbody>
</table>

BW, body weight; MABP, mean arterial blood pressure; UV, urine volume; Ht, hematocrit; sCr, serum creatinine; Ccr, 24 h creatinine clearance. Values are the mean±SD. Seven rats were used in each group. *P<0.02; **P<0.05; ***P<0.01 were observed between each pair of groups.
(Tx) B₂ was measured by a method described elsewhere [11] with a commercial radioimmunoassay kit (Daiichi RI, Tokyo, Japan).

Microscopic study

The excised kidney was weighed and incised longitudinally. Half of the kidney was immersed in 10% buffered formalin. Microscopic study was performed blindly on sections stained with periodic acid-Schiff. A semiquantitative score, the sclerosis index, was used to evaluate the degree of glomerular sclerosis based on the method of Raij et al. [12]. Briefly, at least 20 glomeruli were examined in each specimen to grade the severity of sclerosis from 0 to 4+ by the percentage of glomerular involvement. Grade 1+ represented an involvement of 25% of the glomeruli, while grade 4+ indicated 100% involvement. A sclerosis index was then calculated by multiplying the degree of damage (0 to 4+) by the percent of glomeruli with the same degree of damage.

Quantification of glomeruli

At least 20 glomeruli per section were studied to measure the area of Bowman’s capsule and that of the glomerular capillary tuft (Image Analyzer SP-500, Olympus, Tokyo, Japan). The mean volume of Bowman’s capsule or the capillary tuft was calculated by the Weibel method [13]. This method requires the determination of the mean glomerular random cross-sectional area, i.e., A(G), by counting points hitting randomly selected glomerular profiles and counting these profiles. The mean glomerular volume, V(G), is obtained by:

\[ V(G) = \frac{\beta}{\kappa} (A(G))^{3/2}, \]

where \( \kappa = 1.1 \) (distribution coefficient), \( \beta = 1.38 \) (coefficient considering spherical shape), A(G): mean cross-sectional area of Bowman’s capsule or the capillary tuft.

Statistical analysis

The analysis of variance and Duncan’s new multiple-range test were used for all statistical analyses. All data was presented as the mean ± SD.

Results

Systemic and whole kidney parameters after subtotal nephrectomy in castrated rats

Subtotal nephrectomy increased blood pressure and serum creatinine and decreased hematocrit, indicating the establishment of chronic renal failure (Table 1). However, there was no significant difference between control and LH-treated groups in these parameters either at three or six weeks. The remnant kidney was significantly heavier in LH-treated rats (Group 2) than in control ones (Group 1) at three weeks. At six weeks, remnant kidney weights decreased in both groups, and their difference disappeared (Fig. 1). Ccr decreased after subtotal nephrectomy (Table 1 and Fig. 2), then increased at six weeks. Either at three or six weeks, Ccr in LH-treated rats was significantly higher than that in controls. The urinary excretion of Tx B₂ was higher in LH-treated rats than controls both at three and six weeks, and was higher at three weeks than at six weeks (Fig. 3).
Fig. 2. 24-h creatinine clearance per 100 g body weight after three or six weeks' administration of LH. *: P<0.001, Group 1 vs. Group 3; **: P<0.02, Group 2 vs. Group 4.

Fig. 3. Urinary thromboxane B2 excretion after three and six weeks' administration of LH.

Discussion

To avoid the possibility that normal or high-protein diets would worsen chronic renal failure enough to offset the renotropic effect of LH, we fed these rats a low-protein (6%) diet. It has been reported that a low-protein diet protects against glomerular sclerosis [14] and reduced total kidney size [15]. Our results agreed, i.e., a low-protein diet decreased the weight of the remnant kidney while C\textsubscript{cr} increased. In terms of total kidney weight, the renotropically active LH could stimulate renal growth at three weeks, but not at six weeks. This failure at six weeks may be explained as follows. First, LH may be able to exhibit its renotropic effect on total kidney growth under good nutritional conditions, but not under a chronic low-protein diet. Second, serum LH levels are increased in castrated rats and also in chronic renal failure [16]. Endogeneous LH with or without renotropic activity may influence or mask the effect of exogeneously administered LH. Finally, it should be emphasized that our study was done with a fixed dose (40 \mu g). Although a single injection with this dose was enough to stimulate renal DNA synthesis in castrated hypophysectomized rats [1], daily injections with a higher dose may be necessary to increase the kidney weight of castrated 3/4 nephrectomized rats in six weeks. However, an additional experiment with a higher dose was not done mainly because of a shortage of purified renotropically active LH.

Although LH failed to have any growth-promoting activity at six weeks, it could increase the C\textsubscript{cr}, through experimental periods up to six weeks. LH increased total kidney weight at least at three weeks without a significant change in glomerular size (volume of Bowman's capsule). Because LH stimulates tubular cell proliferation without glomerular cell proliferation [3], this finding appears reasonable. In the remnant kidney model, the single nephron glomerular filtration rate and glomerular capillary pressure are increased, resulting in glomerular damage, hypertension, proteinuria, and azotemia with hyalinization and obsolescence of glomeruli [12, 17, 18]. Thus, total kidney growth and glomerular growth are regulated independently [19, 20], and the increase in C\textsubscript{cr} without total kidney growth was not an unusual finding.

Because creatinine can be secreted in the pro-

Microscopic study

Although the volume of Bowman's capsule at six weeks (0.91 ± 0.43 × 10^6 \mu m^3) was significantly smaller than that at three weeks (1.24 ± 1.12 × 10^6 \mu m^3, P<0.03) in LH-treated rats, no significant change was observed between control and LH-treated groups (data not shown). No significant change in the sclerosis index was observed following treatment with LH (data not shown). Different degrees of interstitial changes, e.g., tubular dilatation and atrophy, and interstitial cell infiltration, were also observed in all groups.
ximal tubules [21]. LH-stimulated tubular growth may increase the tubular secretion of creatinine and, as a result, increase the GFR. However, at six weeks, LH failed to increase kidney size but succeeded in increasing C\textsubscript{cr}. This finding may support the concept that LH actually increases GFR directly or indirectly. Progression of kidney disease of rats with subtotal renal ablation was at least partially dependent on increased Tx A\textsubscript{2} levels in the glomerulus [20]. Urinary Tx B\textsubscript{2} is a stable metabolite of Tx A\textsubscript{2}, and was reportedly derived from platelets, mesangial cells, and arterioles in the glomerulus [20, 22, 23]. Urinary Tx B\textsubscript{2} excretion was lower at six weeks than at three weeks, explaining, at least partially, why C\textsubscript{cr} was higher at six weeks than at three weeks. Three and six weeks' administration of renotropically active LH increased urinary Tx B\textsubscript{2} excretion compared to controls. This may suggest that vasoconstrictor thromboxanes and possibly vasodilator prostaglandins may be involved to modulate glomerular hemodynamics, although our study did not clarify the mechanism for elevation of Tx A\textsubscript{2} synthesis.

The effect of LH on the glomerular function appeared to be independent of that on tubular growth. To the best of our knowledge, no evidence has ever been presented to demonstrate that Tx A\textsubscript{2} is involved in proximal tubular proliferation.

Insulin-like growth factor I (IGF-I) has also been reported to stimulate GFR [24]. Epidermal growth factor (EGF) has also been reported to improve function in postischemic acute renal failure [25]. It may be interesting to compare the effects of LH and these growth factors.

In summary, our study suggests that renotropically active LH stimulates kidney growth and improves kidney function in castrated 3/4 nephrectomized rats.

Acknowledgments

We thank Ms. Jun Hara for editing this manuscript. This study was supported in part by the Foundation for Growth Science in Japan.

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