AT₁ blockade during lactation as a model of chronic nephropathy: mechanisms of renal injury

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Machado FG, Poppi EP, Fanelli C, Malheiros DM, Zatz R, Fujihara CK. AT₁ blockade during lactation as a model of chronic nephropathy: mechanisms of renal injury. Am J Physiol Renal Physiol 294: F1345–F1353, 2008. First published April 9, 2008; doi:10.1152/ajprenal.00020.2008.—Suppression of the renin-angiotensin system during lactation causes irreversible renal structural changes. In this study we investigated 1) the time course and the mechanisms underlying the chronic kidney disease caused by administration of the AT₁ receptor blocker losartan during lactation, and 2) whether this untoward effect can be used to engender a new model of chronic kidney disease. Male Munich-Wistar pups were divided into two groups: C, whose mothers were untreated, and Lact, whose mothers received oral losartan (250 mg·kg⁻¹·day⁻¹) during the first 20 days after delivery. At 3 mo of life, both nephron number and the glomerular filtration rate were reduced in Lact rats, whereas glomerular pressure was elevated. Unselective proteinuria and decreased expression of the zonula occludens-1 protein were also observed, along with modest glomerulosclerosis, significant interstitial expansion and inflammation, and wide glomerular volume variation, with a stable subpopulation of exceedingly small glomeruli. In addition, the urine osmolality was persistently lower in Lact rats. At 10 mo of age, Lact rats exhibited systemic hypertension, heavy albuminuria, substantial glomerulosclerosis, severe renal interstitial expansion and inflammation, and creatinine retention. Conclusions are that 1) oral losartan during lactation can be used as a simple and easily reproducible model of chronic kidney disease in adult life, associated with low mortality and no arterial hypertension until advanced stages; and 2) the mechanisms involved in the progression of renal injury in this model include glomerular hypertension, glomerular hypertrophy, podocyte injury, and interstitial inflammation.

nephrogenesis; angiotensin II

STIMULATION of AT₁ receptors (AT₁-1R) by angiotensin II (ANG II) has long been known to exert diverse important biological effects in adult mammals. Depending on which intracellular downstream system is activated, ANG II binding to AT₁-1R can promote renal and systemic vasoconstriction, blood pressure elevation, and sodium conservation on one hand, or enhance the immune response and inflammatory events, favoring the development of chronic kidney disease, on the other (21, 28). Accordingly, suppression of the renin-angiotensin system (RAS) with angiotensin-converting enzyme (ACE) inhibitors and/or AT-1R blockers has become a mainstay of treatment of chronic progressive nephropathies.

More recently, it has been shown that, beside its hemodynamic and proinflammatory effects, ANG II and AT-1R play an important role in the final phases of nephrogenesis, participating both in the process of nephron maturation and in the formation of the ureters (11). In rodents, nephrogenesis is completed in the first 2 wk after birth (32). During this period, ACE activity, ANG II concentration, and the density of AT1-R are all increased in renal tissue (8). Accordingly, several research groups have shown that suppression of the RAS at this phase causes irreversible renal structural and functional changes in adult life, affecting the maturation of nephrons and renal vessels and leading to a substantial reduction in nephron number (8, 32, 35).

The aim of the present study was to investigate 1) the time course, the long-term outcome, and the pathogenesis of the chronic kidney disease that develops in rats given AT-1R blockers during the final phase of nephrogenesis; and 2) the possibility that such treatment be employed to construct an experimental model of chronic kidney disease that could mimic more closely than current models the development and outcome of the corresponding human disease.

MATERIALS AND METHODS

Experimental groups. Twenty-one adult female Munich-Wistar rats, 10–12 wk of age and weighing 160 to 200 g, obtained from a local facility at University of São Paulo, were housed in individual plastic boxes after mating, fed regular rodent chow containing 0.5 Na and 22% protein (Nuvital Labs, Curitiba, Brazil), and given tap water. Rats were maintained at 23 ± 1°C and 60 ± 5% relative air humidity under an artificial 12:12-h light-dark cycle. After delivery, six pups were kept with each dam until weaning. During the first 20 days of lactation, the dams received the AT-1R antagonist losartan (250 mg·kg⁻¹·day⁻¹) dissolved in the drinking water. In preliminary experiments, this was found to be the highest dose not causing death or severe growth stunting of the pups. After being weaned on day 25 after birth, the male offspring were housed in cages in groups of no more than four and given unrestrained access to food and water. Only male pups were utilized in this study, since a marked gender difference exists regarding the effect of ANG II receptor blockers and ACE inhibitors, females being much less susceptible (17). Thirty-three male offspring from losartan-treated dams (Lact) and 33 age-matched control rats never exposed to losartan (C) were utilized in these studies. To avoid bias, no group received more than two pups from the same litter. In this manner, groups were homogeneous regarding the origin of the animals. All experimental procedures were approved by the local Research Ethics Committee (CAPPesq, process no. 0166/07) and developed in strict conformity with our institutional guidelines and with international standards for manipulation and care of laboratory animals.

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**Functional studies.** At 3 mo of age, five C and five LLact were anesthetized with Inactin (100 mg/kg ip) and prepared for renal functional studies, as described earlier (3), to measure mean arterial pressure (MAP), glomerular filtration rate (GFR), renal plasma flow (RPF), and renal vascular resistance (RVR) using conventional clearance techniques (3). The glomerular hydraulic pressure (POc) was determined by performing direct micropuncture measurements on superficial glomeruli (3).

**Glomerular counting.** At 3 mo of age, five rats from each group were anesthetized with ketamine (50 mg/kg ip) and xylazine (10 mg/kg ip), and the kidneys were rapidly perfused retrogradely through the abdominal aorta with a 20% India ink solution as described previously (5). Thereafter, the right kidney was gently decapsulated, sliced, and kept in a 50% hydrochloric acid solution at 37°C for 1 h, then in saline solution at 4°C overnight. After the material was resuspension in 15 ml of saline, a 1-ml aliquot was examined under a stereomicroscope at ×50 magnification for glomerular counting. Counting was performed independently by two observers (F. G. Machado and C. Fanelli). The glomerular count attributed to each rat was the average of the two counts thus obtained. The procedure was repeated if a discrepancy of >10% between the two observers occurred.

**Renal morphology: short term studies.** Ten rats from each group were anesthetized with ketamine (50 mg/kg), and a 300-μl blood sample was collected from the abdominal aorta for measurement of the circulating concentrations of creatinine (SCreat), sodium (PNa), and potassium (P K). The kidneys were then retrogradely perfusion-fixed through the abdominal aorta with Dubosq-Brazil solution after a brief washout with saline to remove blood from the renal microcirculation. After being weighed, two midcoronal renal slices were postfixed in buffered 4% formaldehyde, then embedded in paraform using conventional sequential techniques, and 4-μm-thick sections were examined for assessment of glomerular size, the extent of glomerular injury, and the fractional interstitial area, as well as for immunohistochemical detection of macrophages, ANG II-positive cells, and glomerular zona occludens-1 (ZO-1) protein.

**Renal morphology and time course of renal disease: long term studies.** Thirteen rats from each group were followed from 3 to 10 mo of age with determination of tail-cuff pressure (TCP) and 24-h urine albumin excretion rate (U AlbV at 3, 6, and 10 mo of age. TCP was assessed using a commercially available (Visitech Systems, Apex, NC) automated method (13), under light restraining, and after light warming. To avoid any interference of stress, all rats were preconditioned to the procedure and were invariably calm at the time of TCP determination. In addition, TCP values were always taken as the average of at least three consecutive measurements that varied by no more than 2 mmHg, that is, after stabilization of blood pressure.

At the end of the study period, rats were anesthetized and blood and renal tissue were obtained and processed as described for the short-term experiments.

**Histomorphometric analysis.** All morphometric evaluations were performed in 4-μm-thick sections by a single observer (D. M. Avancini Costa Malheiros) blinded to the groups. For each rat, the extent of glomerular injury (GS) was estimated in sections stained by the periodic acid-Schiff (PAS) reaction by attributing to each glomerulus a score corresponding to the scleroded fraction of the tuft area and deriving a glomerulosclerosis index (GSI) for each rat as described previously (3). At least 120 glomeruli were examined per rat. The percentage of the renal cortical area occupied by interstitial tissue, used as a measure of the degree of interstitial expansion, was estimated in Masson-stained sections by a point-counting technique (12).

Mean glomerular random cross-sectional area (A G) was determined by averaging individual values for 50 consecutive glomerular tuft profiles in a section from the left kidney of each rat by counting points falling within the tuft profiles. Glomeruli were examined at ×200 under a 144-point grid, covering a 64,900-μm² area. The average glomerular tuft volume (V G) for each rat was then calculated as V G = 1.25 (A G)³/² (10).

**Immunohistochemical analysis.** Immunohistochemistry was performed on 4-μm-thick sections mounted on glass slides coated with 2% silane. Sections were deparaffinized and rehydrated by conventional techniques, then heated in citrate buffer for antigen retrieval. Incubation with the primary antibody was carried out overnight at 4°C and omitted in the negative control experiments.

For ED-1 detection, sections were preincubated with 5% normal rabbit serum to prevent non-specific binding, then incubated overnight at 4°C with a monoclonal mouse anti-ED-1 antibody (Serotec, Oxford, UK) diluted in BSA at 0.5%. After being rinsed with Tris-buffered saline, sections were incubated with a 2% solution of rabbit anti-mouse immunoglobulin (Dako, Glostrup, Denmark) in BSA, then with an alkaline phosphatase anti-alkaline phosphatase (APAAP) complex (Dako). Sections were then developed with a fast red dye solution (Sigma-Aldrich, St. Louis, MO), counterstained with Mayer’s hemaluma, and covered with a glycercin-gelatin mixture.

For ANG II cells staining positively for ANG II, an indirect streptavidin-biotin alkaline phosphatase technique was employed. Sections were preincubated with avidin and biotin solutions (Biotin Blocking System, Dako) to block non-specific binding of these compounds, and with horse serum diluted at 2% in a 2% solution of nonfat milk in Tris-buffered saline to avoid non-specific protein binding. Sections were then incubated overnight at 4°C with a polyclonal rabbit anti-human ANG II antibody (Peninsula Labs, San Carlos, CA) diluted at 0.25% in BSA. Thereafter, sections were incubated for 45 min at room temperature with biotinylated anti-rabbit IgG (Vector Labs, Burlingame, CA) diluted at 0.1% in BSA, then with a streptavidin-biotin alkaline phosphatase complex (Dako) for an additional 30 min, and finally developed, counterstained, and mounted as described above.

The ZO-1 molecule was detected using a rabbit polyclonal antibody (Zymed Laboratories, San Francisco, CA) and a commercially available kit (Novocastra Labs, UK). Sections were otherwise processed as described above.

The extent of renal infiltration by macrophages or ANG II-positive cells (cells/mm²) was evaluated in a blinded manner at ×200 magnification. For each section, 25 microscopic fields (corresponding to a total area of 1.6 mm²) were examined. To estimate the intensity of the glomerular expression of ZO-1, the fraction of the tuft area staining positively for this molecule was determined by an adapted point-counting technique (12) in 50 consecutive glomerular profiles, examined at ×200 under a 144-point grid.

**Analytical techniques.** U AlbV was determined by radial immunodiffusion (18). S creat, P Na, P K, and urine osmolality were assessed by conventional laboratory techniques. Fractionation of urinary proteins was performed by SDS-PAGE, utilizing an adaptation of the discontinuous system described by Laemmli (15). A selectivity index (SI) was calculated as the ratio of the 180- to the 68-kDa bands.

**Statistical analysis.** Statistical differences were assessed by twoway ANOVA, with treatment and time as intervening factors and with pairwise posttest comparisons according to the Bonferroni method (34). Student’s unpaired t-test was used in the functional studies and in nephron number analysis, which involved only simple comparisons between LLact and C rats. Since S creat, U AlbV, GSI, and the density of interstitial ANG II-positive cells exhibited non-Gaussian distributions, the corresponding data were subjected to log transformation before statistical analysis. To assess deviations from Gaussian distribution of A G, the Kolmogorov-Smirnov test was performed using GraphPad Prism version 4.00 for Windows [GraphPad Software, San Diego CA (www.graphpad.com)]. Results are expressed as means ± SE.

**RESULTS**

**Functional studies.** Functional parameters measured in rats at 3 mo of age are shown in Table 1. No difference in body weight was observed between groups. In agreement with ear-
lier reports (35), the number of nephrons was reduced by nearly 33% in L<sub>Lact</sub> compared with C rats (P < 0.05). MAP was similar between L<sub>Lact</sub> and C. However, GFR and RPF were reduced by nearly one-third in L<sub>Lact</sub> rats, whereas RVR was increased by 46%. Although L<sub>Lact</sub> rats were normotensive, P<sub>GC</sub> was 10 mmHg above this in C group (P < 0.05).

**Long-term studies.** Renal and functional parameters obtained at 3 and 10 mo of age are represented in Table 2. Body weight was similar between C and L<sub>Lact</sub> rats at 3 mo and also at 10 mo of age. Left kidney weight did not differ between groups but increased with time in C rats. No differences between groups or an effect of time were observed regarding P<sub>Na</sub> or P<sub>K</sub>. S<sub>creat</sub> was similar between 3 mo of age but rose by 46% at 10 mo. Although L<sub>Lact</sub> rats were normotensive, P<sub>GC</sub> was reduced by nearly one-third in L<sub>Lact</sub> rats, whereas RVR was increased by 46%. Although L<sub>Lact</sub> rats were normotensive, P<sub>GC</sub> was 10 mmHg above this in C group (P < 0.05).

The time course of TCP is described in Fig. 1A. As in the functional studies, L<sub>Lact</sub> rats were normotensive at 3 mo of age. TCP remained at normal levels at 6 mo of age. However, TCP rose by ~40 mmHg in L<sub>Lact</sub> rats at 10 mo of age, while little variation was observed in controls (P < 0.05 L<sub>Lact</sub> vs. C). Figure 1B depicts the time course of the urine albumin excretion rate. Albuminuria was always higher in L<sub>Lact</sub> rats and exhibited a clearly progressive nature, reaching values ninefold higher than C at 10 mo of age.

Data on the integrity of the glomerular barrier are given in Fig. 2. The SI was markedly increased in L<sub>Lact</sub> rats at 3 mo of age, denoting loss of the ability of the glomerular barrier to retain high-molecular-weight proteins. This difference persisted at 10 mo of age, although the difference between values for 3 and 10 mo of age in L<sub>Lact</sub> rats did not reach statistical significance. The quantitative immunohistochemical expression of the ZO-1 molecule was lower than C in L<sub>Lact</sub> rats at 3 mo. This difference persisted at 10 mo, with a further decrease of this parameter in L<sub>Lact</sub> rats while the C value remained stable.

As described previously (7), L<sub>Lact</sub> rats exhibited variable degrees of papillary atrophy under macroscopic examination. The analysis of renal structural injury and inflammation is detailed in Figs. 3 and 4. Glomerular segmental sclerotic lesions, which were infrequent in L<sub>Lact</sub> rats at 3 mo of age, were abundant at 10 mo of age (Figs. 3A and 4A). Expansion and inflammation of the interstitial tissue were already observed at 3 mo in L<sub>Lact</sub> rats and were drastically increased at 10 mo of age (Figs. 3B and 4B). Infiltration of the renal interstitium by macrophages (Figs. 3C and 4C) and by cells staining positively for ANG II (Figs. 3D and 4D) followed a similar pattern, with intensity roughly proportional to that of interstitial expansion.

A statistical analysis of the glomerular dimensions is shown in Fig. 5. In rats at 3 mo of age, glomerular areas followed a pattern not significantly different from a Gaussian distribution in normal controls (Fig. 5, A and B), the calculated glomerular volume averaging 0.90 ± 0.03 × 10<sup>6</sup> μm<sup>3</sup>. In L<sub>Lact</sub> rats, a large number of unusually small glomerular profiles were observed. The appearance of these glomeruli was inconspicuous (Fig. 5A), whereas in several of them the hilum was clearly visible (Fig. 5A, inset), ruling out the possibility that these profiles represented solely glomeruli sectioned close to a pole. The presence of these small glomeruli skewed the frequency distribution of glomerular areas to the left, promoting a significant deviation from normality. Accordingly, the percentage of profiles with areas <3,000 μm<sup>2</sup> was 18 ± 3% in L<sub>Lact</sub> compared with only 6 ± 1% in C rats (P < 0.05). The mean glomerular volume in L<sub>Lact</sub> rats at 3 mo of age was 1.18 ± 0.06 × 10<sup>6</sup> μm<sup>3</sup> (P < 0.05 vs. control). When the profiles with areas under 3,000 μm<sup>2</sup> were excluded from the calculation, the average glomerular volume for L<sub>Lact</sub> rats was even higher (1.50 ± 0.08 × 10<sup>6</sup> μm<sup>3</sup>), further underlining the occurrence of glomerular compensatory growth in L<sub>Lact</sub> rats. In agreement with this concept, the fraction of glomerular profiles with areas exceed-

### Table 1. Renal function study at 3 mo of age

<table>
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<tr>
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<th>C (n = 5)</th>
<th>L&lt;sub&gt;Lact&lt;/sub&gt; (n = 5)</th>
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<tr>
<td>BW, g</td>
<td>323 ± 18</td>
<td>285 ± 14</td>
</tr>
<tr>
<td>NN, ×10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>27 ± 1</td>
<td>18 ± 1*</td>
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<tr>
<td>MAP, mmHg</td>
<td>108 ± 2</td>
<td>104 ± 1</td>
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<tr>
<td>GFR, ml/min</td>
<td>1.42 ± 0.07</td>
<td>0.94 ± 0.06*</td>
</tr>
<tr>
<td>RPF, ml/min</td>
<td>4.13 ± 0.24</td>
<td>2.77 ± 0.27*</td>
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<tr>
<td>RVR, mmHg·ml·min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>14.3 ± 0.7</td>
<td>20.9 ± 2.3*</td>
</tr>
<tr>
<td>P&lt;sub&gt;vac&lt;/sub&gt;, mmHg</td>
<td>53 ± 1</td>
<td>63 ± 2*</td>
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</table>

Values are means ± SE. n, No. of rats; BW, body weight; NN, nephron number (per 1 kidney); MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; RVR, renal vascular resistance; P<sub>GC</sub>, glomerular hydraulic pressure. *P < 0.05 vs. C.

### Table 2. Renal functional and systemic parameters at 3 and 10 mo of age

<table>
<thead>
<tr>
<th></th>
<th>3 mo</th>
<th>10 mo</th>
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<tbody>
<tr>
<td></td>
<td>C (n = 10)</td>
<td>L&lt;sub&gt;Lact&lt;/sub&gt; (n = 10)</td>
</tr>
<tr>
<td>BW, g</td>
<td>278 ± 9</td>
<td>264 ± 10</td>
</tr>
<tr>
<td>LKW, g</td>
<td>1.49 ± 0.06</td>
<td>1.67 ± 0.05</td>
</tr>
<tr>
<td>P&lt;sub&gt;Na&lt;/sub&gt;, mmol/l</td>
<td>139 ± 2</td>
<td>141 ± 2</td>
</tr>
<tr>
<td>P&lt;sub&gt;K&lt;/sub&gt;, mmol/l</td>
<td>3.7 ± 0.1</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>S&lt;sub&gt;creat&lt;/sub&gt;, mg/dl</td>
<td>0.64 ± 0.11</td>
<td>0.70 ± 0.09</td>
</tr>
<tr>
<td>V&lt;sub&gt;U&lt;/sub&gt;, ml/24 h</td>
<td>23 ± 2.2</td>
<td>31 ± 1.5</td>
</tr>
<tr>
<td>U&lt;sub&gt;osm&lt;/sub&gt;, mosmol/kgH&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>1,210 ± 116</td>
<td>921 ± 45*</td>
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</tbody>
</table>

Values are means ± SE. n, No. of rats; LKW, left kidney weight; P<sub>Na</sub>, plasma sodium concentration; P<sub>K</sub>, plasma potassium concentration; S<sub>creat</sub>, serum creatinine concentration; V<sub>U</sub>, daily urinary volume; U<sub>osm</sub>, urine osmolality. *P < 0.05 vs. C. †P < 0.05 vs. respective 3-mo value.
Inhibiting AT-1R signaling during lactation resulted in a 30% reduction in the number of nephrons, confirming previous reports (29, 32, 35), although some discordant findings have been described (19), and corroborating the concept that the presence of ANG II is essential for adequate nephron maturation (8, 32). The molecular mediators and the cellular signaling pathways that may be responsible for this salutary action of ANG II on nephrogenesis are presently unclear, although evidence has been reported that activation of insulin-like growth factor (IGF) and of its receptor may play a crucial role (24).

Confirming previous reports (2, 7, 9, 32, 35), LLact rats developed severe and progressive renal functional and structural changes, that were nevertheless associated with zero mortality, making it possible to follow these rats until very advanced stages. This behavior was remarkably homogeneous: at 3 mo of age, all rats in the LLact group were diseased, and low coefficients of variation were observed for parameters such as albuminuria and TCP. It must be emphasized that in the present study the teratogenic effect of losartan was obtained by offering the drug to the dams in the drinking water, taking advantage of the fact that losartan easily crosses the blood-milk barrier (31). It was thus possible to obviate the need for individual intraperitoneal injections, utilized in many previous studies.

At 3 mo of age, GFR was diminished by 30% in LLact rats compared with C rats. This finding is in agreement with most previous reports (7, 8, 29, 35), despite a few discordant observations (17). Since the reduction in GFR and in RPF was proportional to the reduction in the number of nephrons, it is tempting to conclude that no compensatory hyperperfusion or hyperfiltration, such as observed in rats with uninephrectomy or 5/6 renal ablation (3, 33), occurs in this model. However, it must be noted that a substantial fraction of glomeruli in LLact rats was found to be hypotrophic, a proportion likely underestimated since, unlike normal glomeruli, hypotrophic glomeruli are easily overlooked if sectioned close to one of the poles. Since these hypotrophic glomeruli, which probably result from nephron malformation, are expected to contribute little to the overall GFR and may even have lost their connection with the respective tubules (4), some compensatory hyperfiltration must have occurred in the remaining nephrons. On the other hand, the finding that a substantial number of glomeruli in LLact rats...
exhibited mean cross-sectional areas in excess of 15,000 \( \mu \text{m}^2 \) (the frequency of such glomeruli was negligible in controls) indicates that compensatory glomerular hypertrophy also occurred in these animals.

The intimate mechanisms underlying the compensatory increase in single-nephron GFR in \( L_{\text{Lact}} \) rats are not entirely clear. Part of the compensatory response likely involved an elevation of \( P_{\text{GC}} \), at least at 3 mo of age (unfortunately, the severe deformation of the renal surface due to interstitial expansion/inflammation precluded the determination of \( P_{\text{GC}} \) by micropuncture at 10 mo of age). Since systemic hypertension was absent in these rats at the time the functional studies were performed, glomerular hypertension, at least at this phase, must have resulted from afferent arteriolar dilatation and/or efferent arteriolar vasoconstriction. It is unclear whether this arteriolar dysfunction is merely part of a compensatory response or whether arteriolar malfunctioning in adult life constitutes another ill effect of losartan administration during nephrogenesis. It should be noted that the number of litters (21) and the number of functional observations (5/group) were both relatively small and, although differences between groups were almost invariably statistically significant, the present functional measurements inevitably carry some degree of uncertainty. Thus the results of the present functional studies should be interpreted with caution, and definitive conclusions in this regard will have to await confirmation by future studies.

\( L_{\text{Lact}} \) rats developed several aspects of chronic glomerular structural and functional injury. Albuminuria was already evident at 3 mo of age and exhibited a clearly progressive nature, reaching levels one order of magnitude higher than in C rats at 10 mo of age. Albuminuria was associated with limitation of the size-selective properties of the glomerular walls, as assessed by the increase in a selectivity index, already observed at 3 mo of age. However, since the selectivity index was not further increased at 10 mo of age, the aggravation of albuminuria observed at this time must have resulted from factors in addition to a size defect, such as rarefaction of the negative surface charge of the glomerular walls. The reduced expression of the ZO-1 molecule suggests that podocyte injury may explain, at least in part, the development of albuminuria in \( L_{\text{Lact}} \) rats. Since podocytes are responsible for the synthesis of part of the negatively charged molecules that contribute to limit the passage of polyanions through the glomerular walls, podocyte damage can explain the development of proteinuria by a
combined defect in the size- and charge-selective properties of
the glomerular wall.

Although albuminuria was already present at 3 mo of age, glomerular injury at this time was only mild, although significant, in LLact rats compared with C rats. Glomerulosclerosis was much more pronounced at 10 mo of age, the GSI reaching levels over 50-fold higher, whereas serum creatinine was significantly elevated, compared with age-matched C rats. Although the exact mechanisms leading to the development of glomerular injury in this model are not entirely clear, an obvious possibility is that the maladaptive glomerular hypertension and hypertrophy represented important pathogenic factors. Glomerular hypertension and/or hypertrophy have been postulated to initiate and maintain progressive glomerular injury in several experimental models (22, 36) by promoting mechanical stretch to the glomerular walls according to Laplace’s law (26) and, as a consequence, podocyte injury with formation of sinchniae with Bowman’s capsule (20, 30), mesangial expansion (25), and infiltration by inflammatory cells (27). This process tends to acquire a progressive nature as a growing number of glomeruli drop out due to severe sclerosis, and an additional burden is imposed on the remaining nephrons. Absence of early renal hemodynamic changes may be one of the reasons female rats fail to develop progressive nephropathy after neonatal exposure to ANG II receptor blockers or angiotensin-converting enzyme inhibitors (17).

A progressive increase in the fractional cortical interstitial area was, along with glomerular injury, a prominent feature of the nephropathy that developed in the LLact group. Interstitial expansion was associated with clear signs of chronic inflammation, such as interstitial fibrosis and infiltration by mononuclear cells, a large fraction of which were macrophages. Curiously, many of these inflammatory cells stained positively for ANG II, suggesting that, although its presence during nephrogenesis is essential for adequate nephron maturation, ANG II exerts in this model a similar proinflammatory role as in other experimental models of progressive nephropathy (6). The pathogenesis of interstitial expansion/inflammation in this model is unclear. Interstitial injury could be a direct consequence of glomerular injury due to propagation of tuft inflammation through sinchniae with Bowman’s capsule and direct passage of circulating inflammatory mediators to the periglomerular interstitium (14). In addition, the exaggerated filtration of proteins due to impairment of the glomerular barrier might induce the synthesis of cytokines, chemokines, and growth factors by proximal tubular cells in association with augmented protein endocytosis (1). However, interstitial expansion and inflammation were already evident in LLact rats at 3 mo of age, when albuminuria and glomerular injury were still mild. Therefore, the development of marked interstitial injury in LLact rats cannot have resulted exclusively from glomerular injury, and must have been influenced by other factors. One speculative explanation could be that the tubular cells associated with hypotrophic glomeruli might, as postulated for other experimental models, undergo epithelial-mesenchymal transforma-

Fig. 4. Bar graph representation of the intensity of glomerulosclerosis (A), renal cortical interstitial expansion/inflammation (B), and renal cortical infiltration by macrophages (C) and cells staining positively for ANG II (D) in control (open bars, n = 10 at 3 mo and n = 13 at 10 mo of age) and LLact (filled bars, n = 10 at 3 mo and n = 13 at 10 mo of age). *P < 0.05 vs. respective control value; †P < 0.05 10-mo vs. respective 3-mo value.
Fig. 5: A: representative micrographs of renal tissue showing the variability of glomerular areas in C and L_{Lact} at 3 and 10 mo of age. A population of exceedingly small glomeruli is seen in L_{Lact}. That these are indeed small glomeruli and not normal glomeruli sectioned close to a pole is shown in the inset, in which the hilum of one of these glomeruli can be easily seen. B: frequency distribution of glomerular areas in C (top) and L_{Lact} (bottom) at 3 mo (left) and 10 mo (right). Distribution was strongly non-Gaussian in L_{Lact}, showing a subpopulation of small glomeruli at both 3 (n = 10/group) and 10 (n = 13/group) mo of age. C: bar graph showing the proportion of small, medium-size, and large glomerular areas in C and L_{Lact}. Both glomerular tuft profiles with small areas (indicating hypotrophy) and with large areas (indicating compensatory hypertrophy) were much more numerous in L_{Lact} than in C (P < 0.05).
osmolality was reduced to correspondingly increased. This abnormality was aggravated at 10 mo of age, when urinalysis showed doubling, and urine osmolality was reduced to <50% compared with age-matched C rats. These findings, which corroborate previous reports (9), suggest that the concentrating ability of Lact rats is impaired in disproportion to renal structural injury. The reasons for this abnormality are unclear. One possible explanation is that the delicate operation of the countercurrent mechanism is exquisitely dependent on the complex anatomic arrangement of tubular and vascular structures at the renal medulla and might be disrupted by papillary atrophy or even by mild expansion of the renal interstitium (9). Renal concentrating ability could be further compromised by depletion of AQP2, which has been described in rats treated with an ACE inhibitor during lactation (9). Depletion of the Na-K-2Cl cotransporter at the thick ascending limb of Henle has also been described in these rats (16) and might lead to a salt-losing state, with polyuria and diminished concentrating ability.

It is noteworthy that, even in the presence of a chronic, progressive nephropathy, with substantial reduction of the nephron number and of the GFR, systemic hypertension did not develop in Lact rats until after 6 mo of life, when renal injury had already attained an advanced stage. The reasons for this unexpected finding are not immediately apparent. However, it must be noted that, as discussed earlier, tubular function may be impaired in these rats, which may be prone to salt loss (7). Such abnormality would compensate for the inevitable trend toward salt retention imposed by the development of chronic kidney injury and prevent blood pressure elevation until nephron loss and interstitial inflammation were severely aggravated by progression of the disease.

In view of the characteristics and the time course of the ensuing nephropathy, administration of losartan during lactation constitutes a suitable model of chronic progressive nephropathy, is easily reproducible, and requires no surgery or special preparation. An additional advantage of this model is its low mortality, likely associated with the absence of hypertension during its initial stages, allowing the progression of the nephropathy at a relatively slow rate and, consequently, the development of progressive renal insufficiency and of the adaptations and maladaptations that characterize human chronic nephropathies.

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REFERENCES


