Temporal diabetes- and diuresis-induced remodeling of the urinary bladder in the rat

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Liu, Guiming, and Firouz Daneshgari. Temporal diabetes- and diuresis-induced remodeling of the urinary bladder in the rat. Am J Physiol Regul Integr Comp Physiol 291: R837–R843, 2006. First published March 2, 2006; doi:10.1152/ajpregu.00917.2005.—The natural history of diabetes mellitus-induced remodeling of the urinary bladder is poorly understood. In this study, we examined temporal remodeling of the bladder in diabetic and diuretic rats. Male Sprague–Dawley rats were divided into three groups: streptozotocin-induced diabetic, 5% sucrose-induced diuretic, and age-matched control. Micromorphologic and morphometric characteristics were evaluated using metabolic cages and light-microscopic examination of the bladder 4 days and 1, 2, 3, and 9 wk after induction. Digital image analysis was used to quantify equatorial cross-sectional areas of bladder tissue and lumen, as well as relative content of the three primary tissue components: smooth muscle, urothelium, and collagen. Diabetes and diuresis caused significant increases in fluid intake, urine output, and bladder weight. In both groups, progressive increases were observed in lumen area from 4 days to 3 wk after induction and in wall area from 2 to 3 wk after induction. Wall thickness decreased within the first 2 wk in the diabetic and diuretic rats but returned to control at 3 and 9 wk. As a percentage of total cross-sectional area, smooth muscle area increased, urothelium area was unchanged, and collagen area decreased in diabetic and diuretic rats after 2–3 wk compared with control rats. In conclusion, diabetes and diuresis induced similar bladder remodeling. Diabetes-induced diuresis caused adaptive physical changes in rat bladder by 4 days after induction; remodeling was observed by 2–3 wk after induction and remained stable from 3 to 9 wk.

However, the full spectrum of morphological changes in the urinary bladder of diabetic rats and the role of diuresis remain to be determined.

Diuresis, induced by feeding 5% sucrose, instead of water, to animals, causes significant increases in bladder weight but does not affect body weight or serum glucose concentration (20). Experimentally induced diuresis in rats and rabbits causes bladder hypertrophy and increased contractility, capacity, and compliance, similar to changes observed in diabetic rats (3, 33). We and others have shown that diuresis and STZ-induced diabetes cause some of the same, as well as some different, changes in neurological regulation of bladder contraction (17, 19). The similarities between the findings in diabetic and diuretic rats suggest that bladder hypertrophy in diabetic animals may be a physical adaptation to increased urine production and that changes in the physical properties of the bladder may be a significant factor in development of vesicle dysfunction in diabetes. Therefore, the use of a diuretic group is crucial in distinguishing morphological changes produced by diabetes from those possibly induced by the effect of increased urine output alone. The extent to which diuresis alters the detailed morphology in diabetic animals is not known.

A more detailed characterization of organ remodeling in heart diseases has been studied. Cardiac hypertrophy occurs in response to long-term increases in hemodynamic load, but the pattern of hypertrophy differs, depending on the pathological conditions (6, 26). In pressure-overload hypertrophy (e.g., hypertension), an early stage of concentric hypertrophy, with an increase in left ventricular mass and no change in ventricular volume, is followed by late development of ventricular dilation. Volume-overload hypertrophy (e.g., aortic regurgitation) is characterized mainly by eccentric ventricular hypertrophy. Conceptually, hypertrophy should provide functional benefits by increasing the numbers of sarcomeres per myocyte and/or by decreasing wall stress. Quite surprisingly then, cardiac hypertrophy developing in these pathological conditions often progresses from a compensated stage, where cardiac function is well maintained, to a decompensated stage with clinical signs of heart failure. Heart failure is almost always accompanied by some degree of hypertrophy. So hypertrophy itself may be a therapeutic target in heart failure (6, 26). In comparison, time-dependent remodeling in the diabetic bladder has not been well documented.

The bladder is composed of urothelium, smooth muscle, and connective tissue, mainly including collagen, and is capable of responding to mechanical stresses by increasing in mass through numerous cellular and structural changes. If the ratio
of muscle to collagen is changed, bladder wall hypertrophy will result in an alteration in compliance. Therefore, it is important to understand which tissue compartments (urothelium, detrusor muscle, or connective tissue) undergo hypertrophy and the stage of the disease at which hypertrophy occurs.

The present study was designed to examine time-dependent (from 4 days to 9 wk) remodeling of the bladder during diabetes and to define the extent to which these changes can be attributed to diuresis.

**MATERIALS AND METHODS**

**Experimental animals.** Male Sprague-Dawley rats were matched by date of birth (270–310 g, 10 wk-old; Harlan) and housed in a facility with a 12:12-h light-dark cycle, with food and water provided ad libitum. The animals were randomly allocated to three groups: diabetic (n = 30), diabetic (n = 30), and age-matched control (n = 20). Diabetes was induced in the rats by intraperitoneal injection of STZ (65 mg/kg dissolved in 0.1 M citrate buffer, pH 4.5), and diuresis was induced by addition of 5% sucrose to the drinking water. Blood samples were taken 72 h after administration of STZ and at the time of death to confirm diabetes (blood glucose >350 mg/dl). Blood glucose levels were measured with the ACCU-CHEK Advantage blood glucose-monitoring system (Roche Diagnostics, Indianapolis, IN). At 4 days and 1, 2, 3, and 9 wk after injection, characteristics of micturition were evaluated using metabolic cages; then the animals were killed by a single injection of pentobarbital sodium (200 mg/kg ip). The urinary bladder was removed at the level of the bladder neck/proximal urethra caudally for morphological study. Morphometric characteristics were evaluated by gross examination of the bladder (hematoxylin-eosin and Masson’s trichrome staining). All experimental protocols and procedures were approved by the Cleveland Clinic Foundation Institutional Animal Care and Use Committee.

**Drinking and micturition.** Drinking and characteristics of micturition were measured for all rats. Rats were placed in individual metabolic cages (Nalgene, Nalge), and the previous food, water, and light-dark conditions were maintained for ≥24 h. After this familiarization period, a known volume of water or 5% sucrose was placed in the drinking bottles. Clean plastic beakers were used to collect urine. At the end of 24 h, the volume of liquid remaining in the drinking bottles was measured. The volume consumed was calculated, and the voided volume was measured for each treatment group.

**Bladder fixation and staining.** For characterization of the morphological changes of the bladder in diabetic and diuretic rats, the bladders were equilibrated for 20 min at 37°C in Krebs buffer aerated with 95% O2-5% CO2 to maintain pH 7.4. The composition of the Krebs solution was as follows (in mM): 133 NaCl, 4.7 KCl, 2.5 CaCl2, 7.0). After fixation, the tissues were dehydrated and embedded in the equatorial midline and fixed in 10% neutral buffered formalin (pH 4.5). Masson’s trichrome-stained slides were used to determine the three components (urothelium, collagen and smooth muscle) of bladder tissues. The software can distinguish regions stained with different colors and accurately measure the areas. This color segmentation method was employed to determine the percentage of the tissue area that was stained “pink” (urothelium), “blue” (collagen), and “red” (smooth muscle). In all cases, the images were processed by the same investigators, who were unaware of treatment group assignments.

**RESULTS**

**General characteristics.** General physical characteristics of the animals are shown in Table 1. The initial mean body weight was similar for all three groups, but the diabetic group weighed significantly less than the diuretic and control groups at 4 days and 1, 2, 3, and 9 wk after induction (P < 0.05). There were no significant differences in body weight between the diuretic and the control animals at any time (P > 0.05). Blood glucose concentrations were significantly higher in the diabetic than in the control or diuretic group by 4 days after STZ injection and were maintained throughout the study (P < 0.01). Mean blood glucose levels were almost four to five times higher in the diabetic than in the age-matched control and diuretic rats. There were no significant differences in blood glucose levels between the control and the diuretic animals (P > 0.05). Bladder weight increased markedly in the diabetic and diuretic rats compared with the control group and appeared to increase faster (4 days) in the diabetic rats, but there were no significant differences in bladder weights between the diabetic and the diuretic animals at any time. Bladder weight of the diabetic and diuretic rats reached a plateau (nearly double that of control rats) at 3 wk. The ratio of bladder weight to body weight was significantly higher in the diabetic group than in the age-matched control and diuretic rats.

**Fluid consumption and excretion.** In general, 24-h fluid consumption and urine output peaked in the diabetic rats, which consumed and excreted 7- and 17-fold more volume, respectively, and the diuretic rats, which consumed and excreted 4.2- and 7.7-fold more volume, respectively, than the control rats at 4 days after induction (Fig. 1, A and B). Fluid consumption and urine output peaked in the diabetic rats 1 wk after induction at 6.1- and 13.4-fold, respectively, greater than in the control rats, whereas the peak occurred at 2 wk in the diuretic rats, at 7.2- and 14.5-fold, respectively, greater than in the control rats. The amounts of fluid consumed and excreted by the diabetic and diuretic rats declined slightly during the next 1–2 wk but remained stable from 3 to 9 wk.

**Morphometric analysis.** Histological examination by light microscopy showed bladder hypertrophy and lumen dilation in the diabetic and diuretic animals relative to the control animals (Fig. 2, A–C). Automated digital imaging was used to quantify
the cross-sectional area and composition of bladder tissue at various times (Fig. 2, D–H).

The total cross-sectional area of the bladder lumen (at the equatorial midline) increased significantly as early as 4 days after induction in the diabetic and diuretic rats relative to the control rats ($P < 0.01$) and then gradually increased until 3 wk, with no further increase at 9 wk (Fig. 3A). The lumen areas reached ~3.4 and 3.9 times greater in the diabetic and diuretic rats, respectively, than in the control rats at 9 wk. The total cross-sectional area of the bladder wall (at the equatorial midline) of the diabetic and diuretic rats increased by 2 wk after induction and continued to increase through 3 and 9 wk, reaching levels that were ~1.5 times those of the control rats (Fig. 3B). The wall thickness in the diabetic and diuretic rats decreased within the first 2 wk but gradually returned to control values at 3 and 9 wk (Fig. 3C). There were no significant differences in lumen area, wall area, or wall thickness between the diabetic and diuretic rats at any time ($P > 0.05$). These results show that lumen enlargement and hypertrophy are two obvious responses to polyuria in the early stage of diabetes.

The three different components of the bladder wall changed in a time-dependent manner in the diabetic and diuretic rats (Fig. 4). The absolute cross-sectional area of the urothelium increased gradually in the diabetic and diuretic rats and was significantly greater than in the control rats at 3 and 9 wk after induction ($P < 0.05$; Fig. 4A). However, when expressed as percentage of the total tissue area, the urothelium area was not significantly greater during progression of diabetes and diuresis ($P > 0.05$). The collagen was mainly localized in the lamina propria and within and between the muscle bundles (Fig. 2G). The actual collagen cross-sectional area did not change significantly during the progression of diabetes or diuresis but decreased in the diabetic and diuretic groups as a percentage of the total tissue area at 3–9 wk ($P < 0.05$). The smooth muscle area of the bladder wall increased significantly, as did smooth muscle area as a percentage of total tissue area ($P \leq 0.05$), at

### Table 1. Body weight, bladder weight, and blood glucose levels of diabetic, diuretic, and age-matched control rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Body Wt, g</th>
<th>Blood Glucose, mg/dl</th>
<th>Bladder Wt, mg</th>
<th>Bladder wt/Body Wt, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 days</td>
<td>Control</td>
<td>280.00±1.37</td>
<td>319.50±2.22</td>
<td>125.25±3.49</td>
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<td></td>
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<td>284.33±2.76</td>
<td>278.33±4.23†</td>
<td>587.33±2.40†</td>
</tr>
<tr>
<td></td>
<td>Diuretic</td>
<td>275.33±2.23</td>
<td>321.33±3.62</td>
<td>133.17±1.58</td>
</tr>
<tr>
<td>1 wk</td>
<td>Control</td>
<td>295.75±2.04</td>
<td>342.50±2.55</td>
<td>117.50±4.18</td>
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<tr>
<td></td>
<td>Diabetic</td>
<td>297.83±2.52</td>
<td>269.83±3.82†</td>
<td>564.50±10.19†</td>
</tr>
<tr>
<td></td>
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<td>302.17±4.92</td>
<td>340.67±3.74</td>
<td>131.83±2.39</td>
</tr>
<tr>
<td>2 wk</td>
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<td>295.50±6.37</td>
<td>351.25±6.31</td>
<td>127.25±2.20</td>
</tr>
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<td></td>
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<td>586.50±2.93†</td>
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<td>293.25±2.34</td>
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<td>251.83±9.92†</td>
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<tr>
<td>9 wk</td>
<td>Control</td>
<td>293.50±6.13</td>
<td>454.75±14.31</td>
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</tr>
<tr>
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<td>Diuretic</td>
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<td>455.50±8.16</td>
<td>133.33±2.40</td>
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</table>

Values are means ± SE means of 4–6 rats. *Significantly different from corresponding control ($P < 0.05$). †Significantly different from corresponding control and diuretic ($P < 0.05$).
2 wk in the diabetic and diuretic animals compared with the control animals. There were no significant differences between the diabetic and diuretic rats in areas of any of the three tissue components at any time ($P > 0.05$). These results show that the significantly increased bladder wall areas in the diabetic and diuretic rats are due primarily to increased smooth muscle and urothelium.

**DISCUSSION**

Diabetic patients often demonstrate a variety of symptoms, including bladder hyperreflexia and areflexia and indeterminate or normal bladder function (13, 31). The heterogeneity of the clinical symptoms of DBD may result from a variety of factors, including the duration or type of diabetes and other associated risk factors, such as sex, parity, and level of hyperglycemia. DBD is associated with distinct morphological changes; however, the details and pathophysiological mechanisms of such changes are not well understood.

Diuresis alone can induce many of the effects associated with diabetes, including bladder hypertrophy, increased contractility, and increased capacity (3, 33). Therefore, to identify the disease-specific impact of diabetes on remodeling of the bladder, it is essential to consider the role of diuresis in the remodeling process.

The increase in bladder weight is one of the most noticeable responses of the bladder to diabetes and diuresis. Our findings of increased bladder weight are consistent with reports from other investigators who examined rat bladders at 2–16 wk after STZ treatment (18, 35). In the present study, we demonstrated a significant increase in bladder weight as early as 4 days after induction of diabetes and 1 wk after induction of diuresis in rats, and the bladder weight continued to increase up to 3 wk in both groups. The growth process subsided after the first 3 wk of diabetes or diuresis, probably because of sufficient adaptation of the bladder to the increased urine volume.

The increases in fluid intake and urinary output in the STZ-diabetic rats are also consistent with other functional studies in diabetic rats that have shown increased urine output, micturition volume, urinary capacity, and bladder compliance (32). In our study, increases in fluid intake and urinary output...
occurred slightly sooner in the diabetic than in the diuretic rats. Such different micturition patterns may contribute to the faster increase in bladder weight in the diabetic than in the diuretic rats. The exact stimulus that initiates the increase in bladder weight in response to polyuria is not known, but it is plausible that it is related to alterations in bladder volume, rate of filling, and rate of stretch of the bladder. Whatever the reasons, changes in cell shape can stimulate DNA synthesis, which in turn results in increased protein synthesis, causing increased cell mass and hyperplasia (5).

In the present study, dilation of the bladder lumen was observed in diabetic and diuretic rats within 4 days after induction; it continued to increase over the first 3 wk and was followed by an increase in bladder wall area. Consequently, dilation of the lumen was accompanied in both groups by an initial decrease in bladder wall thickness that gradually recovered to control levels over the first 3 wk after induction.

The mechanisms of the increased lumen area induced by polyuria are not very clear. The bladder rapidly adapts to increased urine production by increasing micturition frequency and volume (21). It was reported that an increase in water intake can increase bladder capacity within minutes (1). Such a resetting of the threshold volume for initiating a micturition reflex may involve nervous mechanisms. A possible explanation would be a functionally disturbed sensory input from the bladder.

The micturition reflex is activated primarily by filling the bladder to a threshold volume at a physiological rate (natural filling). Afferent receptors and nerve endings are “calibrated” for natural filling, which is slow, intermittent, and variable, normally averaging \( \sim 1 \text{ ml-kg}^{-1}\text{-h}^{-1} \) in humans (16). Many studies have demonstrated that the high rate of bladder filling during cystometry can affect cystometric features, particularly increases in intravesicular pressure and threshold volume (capacity) (11, 14–16, 29). High, nonphysiological filling rates may result in mechanical trauma to the afferent pathway, so that the frequency of action potentials, which results in the urge to void, will occur at a larger-than-normal volume. In addition, rapid stretch may cause temporary functional disturbances in nerve endings, detrusor smooth muscle cells, and cell junctions, leading to increased capacity. In the present study, within the first 4 days in the diabetic and diuretic rats, urine excretion increased from 8 to 136 and 61.25 ml/24 h, respectively. The increased rate of urine formation might induce an increase in bladder capacity (3, 22) and, at the same time, stimulate tissue hypertrophy and hyperplasia (30).

Although bladder hypertrophy and, specifically, growth of detrusor smooth muscle in response to increased bladder work and distension have been reported (18, 35), the relative amounts and rates of growth of the different bladder tissue components are not well known. The present study showed that the three major components of the bladder wall (urothelium, connective tissue, and smooth muscle) did not change significantly in the control rats but did change in the diabetic and diuretic rats during the investigated period. The absolute values of smooth muscle and urothelium areas increased between 1 and 3 wk after induction of diabetes or diuresis, and the collagen area did not change significantly throughout the study period. Because the smooth muscle and urothelium areas comprise high and very low percentages, respectively, of the total tissue area, the changes in area expressed as percentage of total tissue area were an increase in smooth muscle, no change in urothelium, and a decrease in collagen. Our results from diabetic and control rats differ slightly from results reported previously (27), which showed similar changes, but with a later onset (5 wk after induction with STZ). The reason may be our use of younger rats, which may have been capable of generating a more vigorous growth response to polyuria. The present study showed that the primary source of the increased bladder weight was the smooth muscle, which progressively increased...
to comprise \( \sim 64\% \) of the bladder wall in the diabetic and diuretic rats compared with 51% in the control rats over the 9 wk of the study. A previous study demonstrated that diabetes-induced diuresis stimulates DNA synthesis and cell proliferation initially and mainly in the urothelium but is followed by transient proliferation of the smooth muscle and connective tissue compartments (4). Hypertrophy of the smooth muscle and urothelium may be involved in development of altered detrusor pressure as part of a compensatory and/or pathophysiological response to the underlying disease process. The resulting hypertrophy may enable the bladder to adapt to the polyuria associated with diabetes.

The mechanisms involved in triggering tissue hypertrophy and hyperplasia are not fully understood. It has been reported that a high filling rate is a primary factor in the induction of hyperplasia of the bladder urothelium, connective tissue, and smooth muscle (30). Acute overdistension induced a fivefold increase in \(^{3}\text{H}\)thymidine incorporation in the body of the bladder and a threefold increase at the base of the bladder. Autoradiography of overdistended bladders showed significant and substantial labeling that was confined to the urothelial basal cells (34). The proliferating urothelium might produce some factors that can modulate the proliferation of detrusor muscle cells (24, 25).

Collagen is the major constituent of the extracellular matrix in bladder. Bladder collagen has been suggested to influence the passive property of the bladder wall. Bladder compliance correlates with changes in relative amounts of collagen (2). An increase in detrusor muscle and a relative decrease in collagen density in diabetic and diuretic bladders resulted in more compliance of the bladder, i.e., a two- to threefold increase in volume without an increase in internal pressure. In addition, collagen fibrils in smooth muscle probably also play an important role in intercellular transmission of active force. A change in collagen concentration might thus also affect the contractile properties of the smooth muscle.

It is well known that an important characteristic of an overloaded heart is progressive ventricular remodeling, which appears to be a key contributor to morbidity and mortality of congestive heart failure (10, 28). Angiotensin-converting enzyme inhibitor and \( \beta \)-blocker therapy were proven to be remarkably effective in improving left ventricular remodeling.
(10) and reducing morbid events (28). Diabetic bladder remodeling might lead to some local neurogenic and myogenic changes that result in altered bladder function. Obviously, control of the blood glucose level is the best strategy in the treatment of diabetes. However, it is often difficult to maintain blood glucose at a level that can completely prevent diabetic cystopathy. Diabetic cystopathy has been reported in 25% of patients treated with oral hypoglycemic agents (8). Therefore, it is necessary to use the processes leading to bladder dysfunction as a framework for design of novel therapeutic targets. Preventing or slowing the progressive remodeling of the bladder might be an effective strategy for treatment of DBD. The approaches may include timed voiding, intermittent catheterization, and even pharmacological inhibitors to attenuate remodeling of the bladder.

In conclusion, STZ-induced diabetes and 5% sucrose-induced diuresis resulted in rapid, marked remodeling of the bladder wall, which included hypertrophy, lumen dilation, and reorganization of the relative structural relations among the three major tissue components. Morphology changed significantly within the first 3 wk after induction. Time-dependent increases in smooth muscle and in total, but not relative, amounts of urothelium and reductions in collagen density were observed in the diabetes and diuresis models. These data therefore suggest that diabetes-associated polyuria leads to remodeling of the bladder within the 9 wk of the study.

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