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Characteristics of ureteral bolus in diabetic rats

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Abstract Changes in the dynamics of the ureteral bolus in diabetics was investigated by using videomicroscopic imaging. Diabetes mellitus (DM) was induced in 8-week-old male rats by the administration of streptozotocin. The pressure of the renal pelvis (via nephrostomy) and visualization of the dynamics of the ureteral bolus were recorded in three groups (DM, sucrose-fed, and control) 8 weeks after administration. Peristaltic velocity, frequency and bolus length were analyzed on the basis of image processing using indigo carmine dissolved in a saline solution. This was perfused via the nephrostomy. The dilated ureters were macroscopically observed in all diabetic rats. There were significant decreases in the velocity of the bolus in diabetics (4.3 ± 0.8 mm/s) compared to sucrose-diuretic (11.0 ± 3.2 mm/s) and control (8.3 ± 1.4 mm/s) rats. In addition, the length of the bolus in diabetics (9.3 ± 1.4 mm) was about twofold longer than those of control rats (4.2 ± 0.6 mm). There were also significant decreases in the frequency of renal pelvis contraction in diabetics (19.5 ± 1.9 min^{-1}) compared to sucrose-diuretic (25.3 ± 1.0 min^{-1}) and control (29.0 ± 2.3 min^{-1}) rats. These results indicate that the decrease in the velocity of the bolus and the frequency of renal pelvis contraction result in the disability of urine transport in the upper urinary tract in DM although the volume per bolus increases. Besides hyperglycemia, these changes may predispose diabetics to upper urinary tract infections such as pyelonephritis.

Keywords Ureteral peristaltic function · Diabetic rats · Imaging analysis

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Introduction

It is well established that diabetes mellitus (DM) induces dysfunction of the lower urinary tract, which occurs in 26–87% of all cases [7]. This is manifested as an inability to perceive distension of the bladder resulting in a large bladder that empties incompletely. Later stages are associated with hypocontractile bladder and urinary retention [2, 11]. In the end stage, hydronephrosis is observed due to chronic urinary retention and/or the obstruction of the ureterovesical junction due to the fibrotic changes of the bladder wall caused by chronic urinary tract infection.

Although some studies have shown that the incidence of urinary tract infections in diabetics may not be different from non-diabetics, several recent studies have documented the fact that DM is associated with a high frequency of infections [5]. Logically, bacteriuria ought to be closely related to a hypocontractile bladder and high residual volumes. Diabetic patients are at a high risk for upper urinary tract infections, with a spectrum of diseases ranging from acute pyelonephritis to renal corticomedullary abscess. The changes in the function of urine transport in the upper urinary tract seem to cause a predisposition to these complications. In this study, we assessed upper urinary tract function in streptozotocin-induced diabetic rats.

Material and methods

Treatment with streptozotocin

Diabetes was induced in 8-week-old male Sprague-Dawley rats (300–320 g) by administering an intraperitoneal injection of streptozotocin (STZ) (65 mg/kg) dissolved in a 0.1 M citrate-phosphate buffer (pH 4.2). Age-matched control and sucrose-diuretic animals received the same volume of the vehicle. All treated animals had free access to food and water (plus 5% sucrose for the sucrose-diuretic group). Daily water consumption and urine production were measured by placing the animals in a metabolic cage. Blood glucose levels were measured by a glucose-oxidase method using

Glucoscot II (Kyoto Daiichi Kagaku, Kyoto, Japan) before the investigations.

Surgical preparations and recordings

Videomicroscopic imaging of the upper urinary tract and the measurement of the renal pelvis pressure were performed under urethane anesthesia (1.2 g/kg s.c.) 8 weeks after the administration of STZ. A midline incision was made on the abdomen to expose the bladder, left ureter, and kidney. The investigations were made according to methods described in detail by Tillig and Constantinou [17]. Briefly, a silicon tube (0.30×0.64 mm) was placed in the left renal pelvis through a nephrostomy for perfusion of the upper urinary tract with indigo carmine to visualize the movement of the urine bolus and also for recording the renal pelvis pressures as shown in Fig. 1. The location of the distal end of the nephrostomy tube was carefully placed within the renal pelvis using a stereomicroscope (Olympus OME-1000). To avoid the influence of intravesical pressure, a cystostomy was placed at the apex of the bladder dome using a 24 G catheter. External pelvic filling with indigo carmine, dissolved in a physiological saline solution (10 mg/ml), was performed using an infusion pump (TOP 5200) at the constant rates of 0.1 or 1.0 ml/h. The nephrostomy tube was connected to an external pressure transducer (Gould P2310) for the measurement of intrarenal pressure, which was recorded on a polygraph (Nihon Koden WI-641G).

As shown in Fig. 1, the left pelvis and ureter were visualized using a stereomicroscope equipped with a video system (Olympus OTV-SE), and video clips of the ureteral bolus movement were recorded for subsequent image analysis. The imaging system was calibrated using a ruler put beside the ureter. After digitizing a representative part of each video clip, frames were selected and analyzed for measurement of the velocity, length, and frequency of the urine bolus using a personal computer (Macintosh Performa 6310) and software package (Adobe Video Shop and Photoshop).

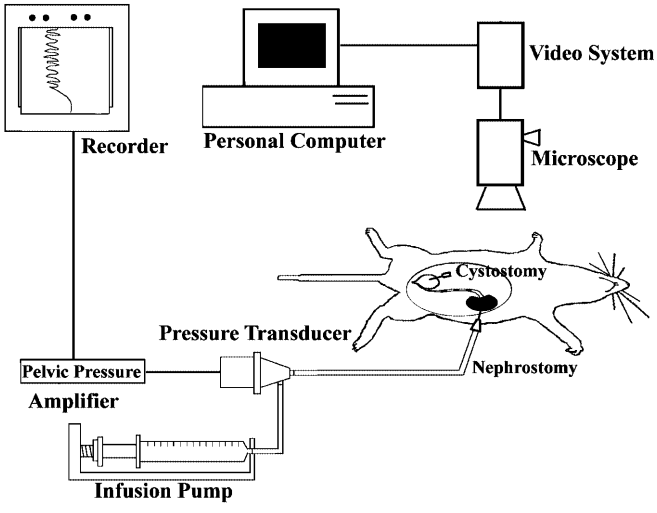

Fig. 1. Schematic diagram illustrating the experimental setup

Renal pelvic pressure and contraction frequency were continuously recorded for a period of 10 min at each filling rate, while the movement of the urine bolus was simultaneously recorded. The data for each parameter were obtained for the last 1 min of both filling rates.

Statistical analyses

All results are expressed as mean ± SE and were compared by the unpaired Student's *t*-test with $P < 0.05$ accepted as statistically significant.

Results

General observations

Diabetic rats ($n = 6$) weighed significantly less after the induction of DM than did the control ($n = 6$) or sucrose-drinking ($n = 6$) rats (Table 1). There were no differences between the weights of the control and sucrose-drinking rats. Serum-glucose concentrations were significantly higher in the STZ-diabetic rats than in the control or sucrose groups. Sucrose consumption had no significant effect on serum glucose concentrations. Diabetic and sucrose-drinking rats consumed and excreted significantly greater volumes of fluid than did controls, while there were no differences between the volumes of diabetic and sucrose-drinking rats.

Macroscopic observations of each ureter

Figures 2, 3 and 4 show the typical macroscopic features of ureters in the control, sucrose and diabetic rats, which were infused with indigo carmine dissolved in a physiological saline via nephrostomy (1 ml/h). There was less fatty tissue in the retroperitoneal space in the diabetic rats than in the other rats. In addition, the ureters of diabetic rats were dilated compared to those of the other groups. Similar features were seen in all diabetic rats.

Pelvic function and the dynamics of the ureteral bolus

Representative examples of pelvic pressure recordings obtained using this system are shown in Fig. 5. The baseline pressure of the pelvis increased with the infusion rate of 1 ml/min in each rat. The numerical values of the frequency of pelvic contraction are shown in Fig. 6. The frequency of pelvic contraction at the filling

Table 1. Effect of STZ-diabetes or sucrose-feeding on body weight and other parameters. Each value is a mean ± SE

	Control ($n = 6$)	Sucrose ($n = 6$)	Diabetic ($n = 6$)
Body weight (g)	457 ± 9	462 ± 9	187 ± 10*,#
Serum glucose (mg/dl)	106 ± 10	100 ± 7	529 ± 40*,#
Daily urine production (ml)	22 ± 2	99 ± 5*	106 ± 4*
Daily water consumption (ml)	27 ± 5	101 ± 4*	94 ± 2*

*$P < 0.05$ compared with control value
#$P < 0.05$ compared with sucrose value

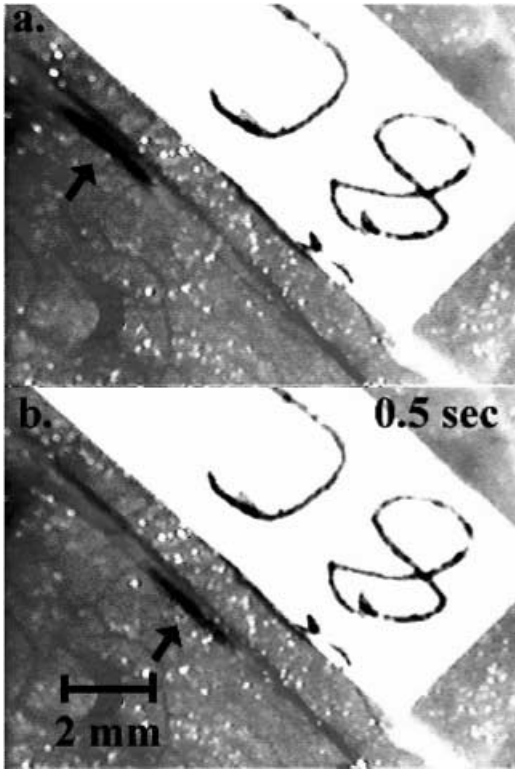

Fig. 2. The video frames of consecutive urine boli traversing the ureter in a control rat. The *arrow* indicates the bolus. The *lower frame* is recorded 0.5 s later. The *horizontal bar* shows the length. (Magnification: ×20)

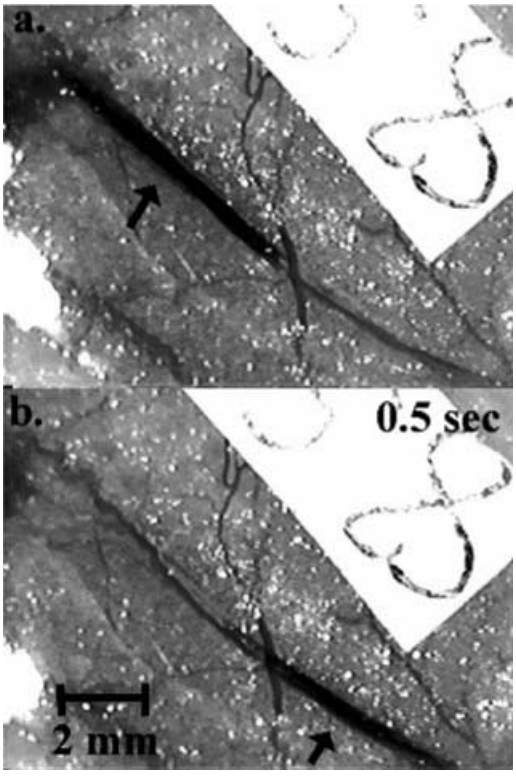

Fig. 3. The video frames of consecutive urine boli traversing the ureter in sucrose-fed rat. The *arrow* indicates the bolus. The *lower frame* is recorded 0.5 s later. The *horizontal bar* shows the length. (Magnification: ×20)

rate of 1 ml/h decreased in the diabetic group compared with the other two groups.

A comparative illustration demonstrating a sequence of images using this system is shown in Figs. 2, 3 and 4. These figures show that the length of the ureteral bolus in both sucrose-drinking and diabetic rats seems to be longer than that in the control. In addition, the velocity of the ureteral bolus in diabetic rats is slower than in the control. The numerical values of these parameters are shown in Fig. 7. The frequency of a ureteral bolus at the filling rate of 1 ml/h decreased in the diabetic group compared with the other two groups. The bolus velocity in diabetics significantly decreased compared to that in the other groups. On the other hand, the statistical results of bolus length are complicated, while polyuria induced by diabetes or sucrose-drinking seems to increase the bolus length. This parameter in sucrose-drinking rats significantly increased compared to that in the control at the filling rate of 0.1 ml/h but did not in the diabetic rats. On the contrary, at the filling rate of 1 ml/h, the length of the bolus in diabetic rats was significantly elongated. This was not the case in sucrose-drinking rats.

Discussion

Our study is the first report concerning the visual characteristics of the ureteral bolus in diabetic rats. It demonstrates that the frequency of renal pelvis contraction decreases and that the velocity of the bolus also decreases in diabetic rats. The urine transport disability induced by the impairment of pelvic contraction frequency did not seem to be compensated by the increases in the volume of a bolus.

Although hydronephroureter caused by urinary retention is often seen in diabetic patients, there are few morphological studies on the upper urinary tract in diabetes [9]. Deil et al. have reported moderate hydronephrosis and the hypertrophy of the renal cortex in the kidney of a diabetic hamster [4]. In more severe cases of hydronephrosis, the cortex becomes extremely thin because of the pressure from the expanding renal pelvis. As a result of the filling of the pelvis, and of the contraction, the pelvic pressure rises so that urine flows into the ureter. Thus, the enlarged capacity of the pelvis seems to result in the increase of the bolus volume observed in diabetic rats. Thulesen et al. have also reported that the histological examination of macroscopically enlarged ureters from diabetic rats revealed tubes with an open circular lumen [16]. In contrast, polyuria produced by sucrose-water was shown to induce growth of the bladder and the adjacent distal part of the ureter. These results concur with the macroscopic observations in the present study. It is reasonable to assume that the open circular lumen of the ureter cannot produce the proper pressure for contraction waves to move a bolus because

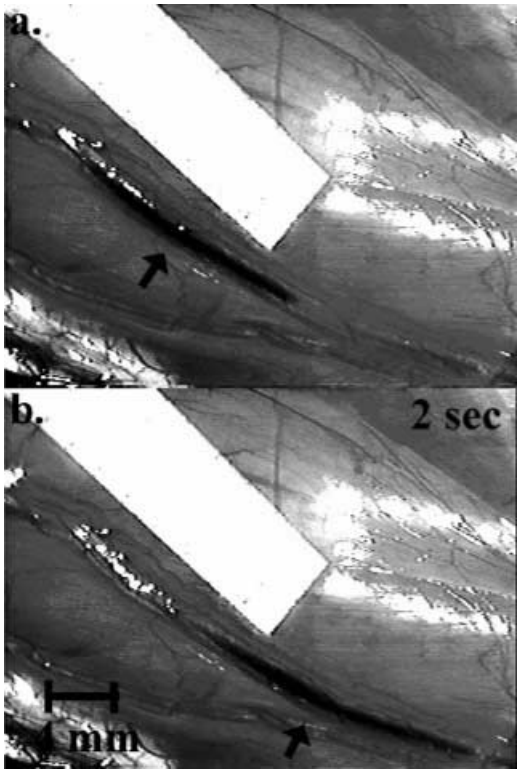

Fig. 4. The video frames of consecutive urine boli traversing the ureter in diabetic rat. The *arrow* indicates the bolus. The *lower frame* is recorded 2 s later. The *horizontal bar* shows the length. (Magnification: ×12)

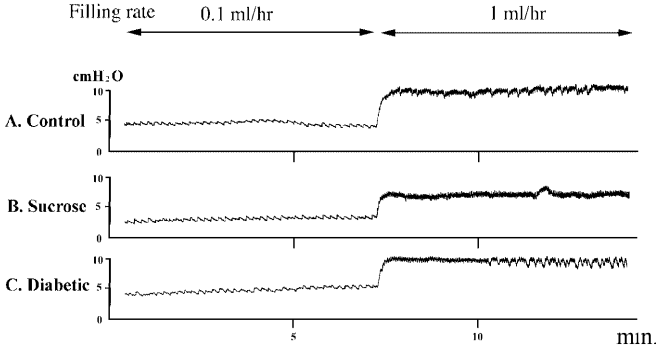

Fig. 5. Typical records of pelvic pressure in control, sucrose-fed, and diabetic rat

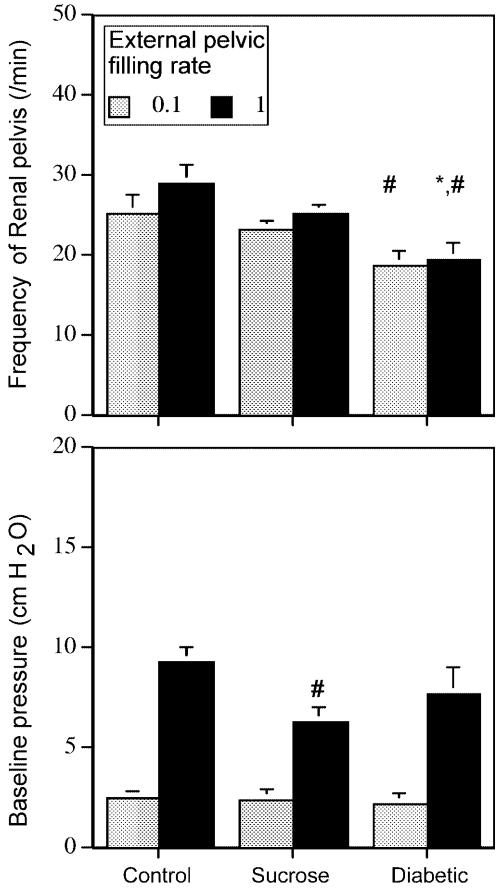

Fig. 6. The frequency of pelvic contraction and renal pelvic baseline pressures at each filling rate in control, sucrose-fed, and diabetic rats. *: $P<0.05$ compared with control value. $^{\#}P<0.05$ compared with sucrose value

the passive pressure produced by the elasticity of the ureter wall decreases [8]. Thus, these changes resulted in a decrease in the velocity of a moving bolus in hydroureter observed in diabetics. These results and our results indicate that the hydronephroureters seen in diabetics are due to the lack of urine expulsion from the pelvis.

While ureteral peristalsis is believed to be mainly caused by smooth muscle itself, neurogenic stimulation should also be taken into account [18]. Fichtner et al. have shown that oxybutynin, an anticholinergic, antispasmodic agent, lowers elevated renal pressure in the rat [6]. On the other hand, there are few studies involving a pharmacological analysis of smooth muscle of the ureter in a diabetic model. Nakamura et al. reported that 8 weeks of STZ-induced diabetes increased ureteral weights as well as the density of endothelin receptors [13]. However, they did not provide a clinical interpretation of these pharmacological results. On the contrary, there are many papers about the functional changes of the smooth muscle of the bladder. In vitro muscle bath studies detect abnormalities in detrusor function such as reduced responses to contractile agonists and antagonists or electrical stimulation [15, 10]. Thus, it is possible that diabetes reduces the contractility of the smooth muscle of the ureter similarly to that of the detrusor muscle.

Previous studies have demonstrated that the initial response of a ureter to urine flow increase was an increase in peristaltic frequency [12, 1]. Constantinou et al. reported that a further increase of mean urine flow resulted from an increase in bolus volume after the maximum peristaltic frequency was achieved [3]. Saeki et al. also reported that the ureteral peristaltic rate of dogs rose and then the bolus volume increased with a consequent increase of urine flow in acute diuresis

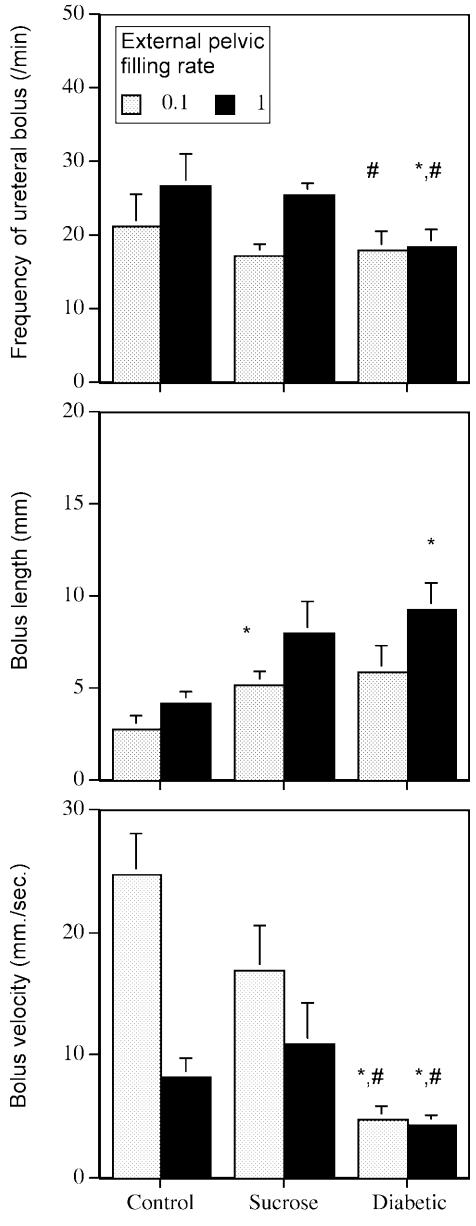

Fig. 7. The frequency of ureteral bolus, bolus length and bolus velocity at each filling rate in control, sucrose-fed, and diabetic rats. $^{*}P < 0.05$ compared with control value. $^{\#}P < 0.05$ compared with sucrose value

induced by furosemide [14]. In contrast, during a course of gradually increasing urine secretion, the ureter showed varying responses with respect to the peristaltic rate, but changes in the bolus volume consistently made up for the rate alterations to maintain urine transport ability during an increase in urine flow. They concluded that the actual mechanism of urine transport is in no way simple and that the peristaltic rate and bolus volume is ingeniously modulated. Their conclusion supports our results, which indicate that polyuria induced by diabetes or sucrose-drinking seem to make a bolus longer but that the statistical results of bolus length did not show significant differences at each filling rate as compared with the control.

Our results indicate that diabetes reduces both the frequency of pelvic contraction and the bolus velocity. We conclude that diabetes in rats reduces urine transport ability in the upper urinary tract and speculate that this changed condition may predispose diabetics to upper urinary tract infections such as pyelonephritis in addition to hyperglycemia.

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