#79 Pathophysiological investigation of underactive bladder in chronic diabetic rat model induced by streptozotocin

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Hypothesis

Underactive bladder (UAB) has been described as a symptom complex suggestive of detrusor underactivity (DU) that is usually characterized by prolonged urination time with or without a sensation of incomplete bladder emptying, usually with hesitancy, reduced sensation on filling, and slow stream [1].
Diabetes mellitus (DM) is one of the causes of the UAB, and streptozotocin (STZ)-induced DM rats have been widely used as an animal model of UAB [2].
Diabetic bladders may undergo a transition from a compensated to a decompensated state [3], but this time point has not been fully investigated.
The aims of this study are to assess the time-dependent in vitro bladder functional changes and to characterize lower urinary tract dysfunction in the STZ-induced chronic diabetic rats.

Materials & Methods

Male Wistar rats (9-weeks old) were received intraperitoneal injection of 60 mg/kg of STZ.

•*In vitro* muscle strip experiments (at 4, 8, 12, 16 weeks)
- ✓ full-thickness of longitudinal strips (bladder body)
- ✓ contractile responses to
  - ➢ high potassium chloride (high K^{+}: KCl 62 mM)
  - ➢ carbachol (CCh: 10^{-3}-10^{-8} M)
  - ➢ electrical field stimulation (EFS; 2-64 Hz, 50 V, 0.8 msec pulse duration, 5 s train duration, 2 min interval)

•*In vivo* cystometry (CMG; at 16 weeks)
- ✓ under conscious and restraint condition
- ✓ single CMG measurements (at 2 days after a catheter-implantation)
- ✓ saline-instillation at a rate of 6 mL/hour
- ✓ Three micturition cycles were averaged.

•*In vivo* simultaneous recordings of bladder pressure under an isovolumetric condition and urethral perfusion pressure (UPP) (at 16 weeks) [4-6] (Figure 1)
- ✓ under urethane-anesthetized condition (1.0 mg/kg subcutaneously)
- ✓ ligation of the ureters and bladder neck
- ✓ A bladder catheter and a double lumen urethral catheter were separately inserted.

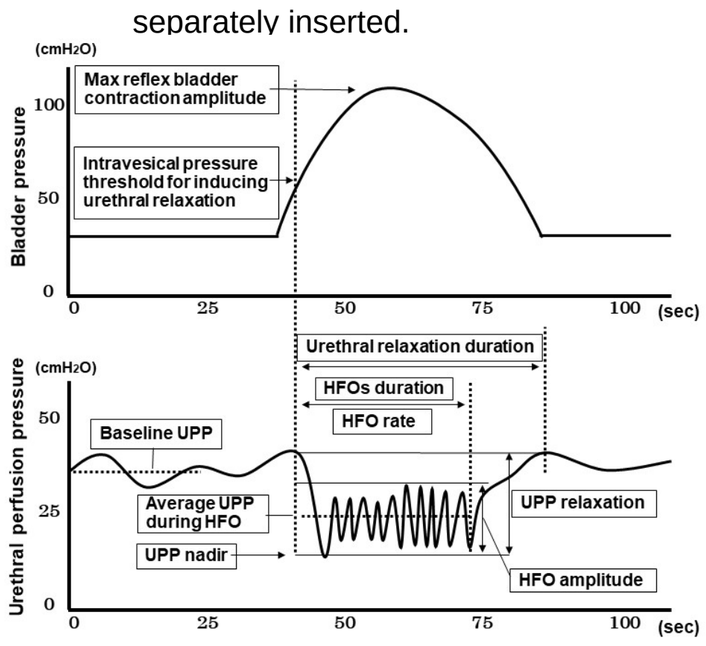

Figure 1. Schematic diagram showing parameters of bladder pressure under an isovolumetric condition and urethral perfusion pressure.

HFO: high-frequency oscillation

Results

Diabetic rats

- higher serum glucose level and bladder weight, and lower body weight at all time points (4, 8, 12, and 16 weeks)
- contractile responses to
  - ✓ high K^{+} : no change
  - ✓ CCh: higher at all time points (Figure 2A)
  - ✓ EFS: tended to be higher at 4, 8, 12 weeks, reversed at 16 weeks (Figure 2B)
- CMG measurements (Table 1 and Figure 3)
  - ✓ higher voided volume, residual volume, bladder capacity, maximal voiding pressure, and the amplitude and frequency of NVCs
- simultaneous recordings of bladder pressure under isovolumetric conditions and UPP (Table 2 and Figure 4)
  - ✓ higher UPP nadir and average UPP during HFO

Discussion

- *In vitro* muscle strip experiments suggested
  - ✓ hypersensitivity of the muscarinic receptors in the detrusor smooth muscle occurs at from early (4 weeks) to late (16 weeks) phases of STZ-induced diabetic bladder
  - ✓ efferent nerve impairment of the bladder occurs at a late phase (16 weeks) induced by STZ
- *In vivo* CMG measurements suggested
  - ✓ diabetic bladder dysfunction as a model of UAB (with or without detrusor overactivity) occurs at least more than 16 weeks after induction of DM by STZ
  - ✓ bladder contractile dysfunction is relatively limited, but rather urethral relaxation during voiding could be impaired at a late phase of DM induced by STZ
- *In vivo* simultaneous recordings of bladder pressure and UPP suggested
  - ✓ urethral relaxation during voiding is impaired at a late phase of STZ-induced DM

Conclusions

The present results suggest that diabetic bladder dysfunction as a model of UAB occurs at least more than 16 weeks after DM-induction by STZ-injection.

In addition, the present results indicate urethral relaxation failure rather than bladder dysfunction during voiding is possibly prominent in the chronic diabetic animal model induced by STZ.

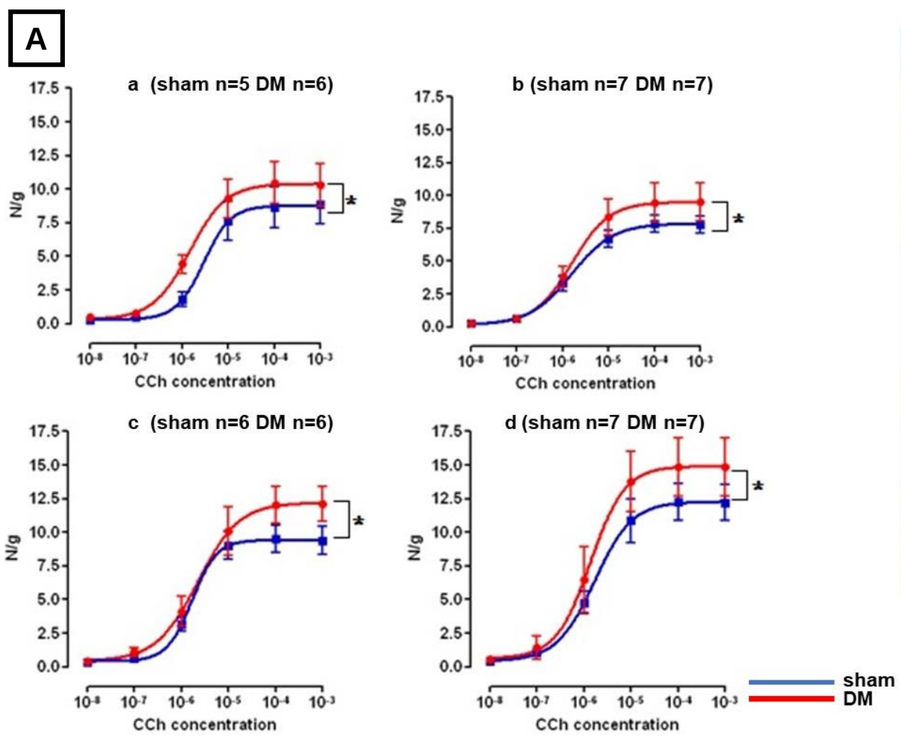

Figure 2. Contractile responses to CCh (A: upper) and to EFS (B: lower).
a: at 4 weeks, b: at 8 weeks, c: at 12 weeks, d: at 16 weeks
Values are expressed as mean ± SEM.
*P<0.05: sham vs. DM rats (Mann-Whitney U test)

Parameter	Sham (n=8)	DM (n=9)	P value
Voilded volume(ml)	0.42 ± 0.13	1.10 ± 0.55	0.01
Residual volume(ml)	0.11 ± 0.04	0.31 ± 0.32	0.03
Bladder capacity(ml)	0.53 ± 0.13	1.41 ± 0.53	0.01
Voiding efficiency (%)	79 ± 9.3	77 ± 19	0.88
Threshold pressure for inducing micturition (cmH_2O)	9.2 ± 4.6	7.5 ± 2.1	0.12
Maximal voiding pressure (cmH_2O)	35 ± 11	50 ± 8.1	0.03
Basal Pressure (cmH_2O)	5.08 ± 3.65	2.95 ± 1.81	0.14
Amplitude of non-voiding contractions (cmH_2O)	3.09 ± 0.43	4.97 ± 0.94	0.01
Frequency of non-voiding contractions (times / min)	0.84 ± 0.55	1.53 ± 0.68	0.04

Table 1. CMG parameters in sham and diabetic rats at 16 weeks after induction of DM. Values are expressed as mean ± SEM. P values are expressed by analysis of Mann-Whitney U test.

Parameter	Sham (n=6)	DM (n=6)	P value
Baseline UPP (cmH2O)	29.4 ± 8.37	40.4 ± 9.39	0.23
UPP relaxation (cmH2O)	26.0 ± 7.76	30.2 ± 10.6	0.30
UPP nadir (cmH2O)	7.93 ± 2.66	13.1 ± 2.55	0.03
Urethral relaxation duration (s)	55.0 ± 12.2	44.8 ± 14.6	0.17
Average UPP during HFO (cmH2O)	11.2 ± 2.37	20.2 ± 2.72	0.01
HFO amplitude (cmH2O)	3.24 ± 0.99	2.72 ± 0.63	0.30
HFO duration (s)	15.9 ± 5.35	17.3 ± 5.67	0.69
HFO rate (Hz)	3.17 ± 0.77	2.86 ± 0.62	0.81
Intravesical pressure threshold for inducing urethral relaxation (cmH2O)	21.2 ± 4.52	19.3 ± 6.72	0.47
Max reflex bladder contraction amplitude (cmH2O)	60.7 ± 11.5	69.9 ± 20.6	0.63

Table 2. UPP parameters in sham and diabetic rats at 16 weeks after induction of DM. Values are expressed as mean ± SEM. P values are expressed by analysis of Mann-Whitney U test.

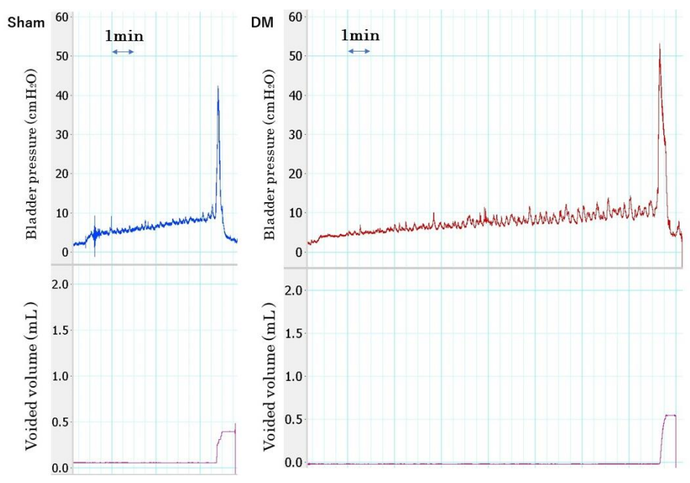

Figure 3. Representative traces of single CMG recording in sham and diabetic rats at 16 weeks after induction of DM by STZ.

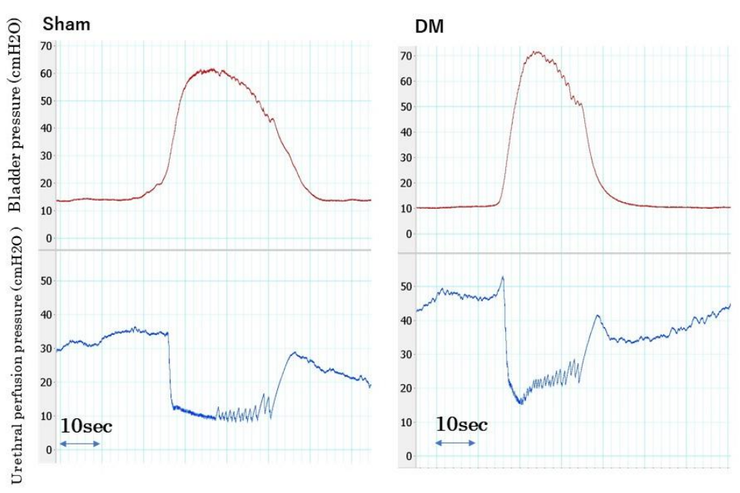

Figure 4. Representative traces of simultaneous recordings of bladder pressure under an isovolumetric condition and UPP in sham and diabetic rats at 16 weeks after induction of DM by STZ.

References

1. Chapple, C. R.; Osman, N. I.; Birder, L.; van Koeveringe, G. A.; Oelke, M.; Nitti, V. W.; Drake, M. J.; Yamaguchi, O.; Abrams, P.; Smith, P. P., The underactive bladder: a new clinical concept? European urology 2015, 68 (3), 351-3.
2. Daneshgari, F.; Leiter, E. H.; Liu, G.; Reeder, J., Animal models of diabetic uropathy. The Journal of urology 2009, 182 (6 Suppl), S8-13.
3. Daneshgari, F.; Liu, G.; Imrey, P. B., Time dependent changes in diabetic cystopathy in rats include compensated and decompensated bladder function. The Journal of urology 2006, 176 (1), 380-6.
4. Jung, S. Y.; Fraser, M. O.; Ozawa, H.; Yokoyama, O.; Yoshiyama, M.; De Groat, W. C.; Chancellor, M. B., Urethral afferent nerve activity affects the micturition reflex; implication for the relationship between stress incontinence and detrusor instability. The Journal of urology 1999, 162 (1), 204-12.
5. Torimoto, K.; Fraser, M. O.; Hirao, Y.; De Groat, W. C.; Chancellor, M. B.; Yoshimura, N., Urethral dysfunction in diabetic rats. The Journal of urology 2004, 171 (5), 1959-64.
6. Torimoto, K.; Hirao, Y.; Matsuyoshi, H.; de Groat, W. C.; Chancellor, M. B.; Yoshimura, N., alpha1-Adrenergic mechanism in diabetic urethral dysfunction in rats. The Journal of urology 2005, 173 (3), 1027-32.