**226**

Miyamae K[1], Yoshida M[1], Otani M[1], Iwashita H[1], Masunaga K[1], Ueda S[1]
1. Department of Urology, Graduate School of Medical Sciences, Kumamoto University

# PHARMACOLOGICAL AND HISTOLOGICAL CHARACTERISTICS OF BLADDER SMOOTH MUSCLE OF TYPE 2 DIABETES RAT

## Hypothesis / aims of study

Lower urinary tract dysfunction is one of the well-known complications in diabetes mellitus. Voiding symptoms or post-void residual are observed in patients with diabetes mellitus. In many reports, the experimental animals had characteristics of type 1 diabetes mellitus, which were induced by administration of streptozotocin (STZ) or alloxan. However, the largest population of diabetic patients has type 2 diabetes mellitus. In this study, to evaluate the histological change in type 2 diabetes rat, we performed hematoxylin and eosin (H-E) and S-100 immunohistochemical staining in GK (type 2 diabetes mellitus model) and Wistar rat (control) bladder. Furthermore, to evaluate the pharmacological characteristics of type 2 diabetes rat bladder, we performed the functional experiments of bladder strips using muscle bath and microdialysis technique.

## Study design, materials and methods

8 and 32 weeks-old female GK rats and age-matched female Wistar rats. The bladder of both groups was removed, weighed and fixed by immediate immersion in 10% formalin and 0.1 mol/l, phosphate buffered, 4% formaldehyde, pH 7.4, for a minimum of 6 and a maximum of 12 h. Fixed specimens were dehydrated in graded ethanol and embedded in paraffin. Five microns sections were cut, mounted onto pre-coated slides and stained for hematoxylin and eosin by standard methods. 4% formaldehyde fixed specimens were immunohistochemically stained for polyclonal S-100 antibodies. In staining procedure, sections were pretreated with 3% $H_2O_2$ for 15 min in order to remove endogenous peroxidase activity of the tissue, and immersed in a solution (consisting of 1% bovine serum albumin, 5% NaCl, 1% gelatin, 0.15% glycine and 1% Tween20/ per 100 ml of 20 mmol/l TRIS-HCl) for 15 min to block non-specific background. Between the various steps 5-min rinses were made with 50 mmol/l Tris-buffer solution to remove excess antibodies. The treated sections were exposed to the polyclonal antibody S-100 at room temperature for 60 min. They were then exposed to the secondary antibody for primary antibody for 30 min. The reaction site was stained by diaminobenzidine tetrachlorohydrate and counter stained by hematoxylin for 1 min. The sections were dehydrated through graded alcohols, cleared in xylene and mounted. All sections were examined using a microscope. A quantitative evaluation of nerve density in the muscular and stromal layer was done by counting the number of stained fibers or bundles per high power field on a constant magnification (x 200). A nerve density score was calculated as previously described [1, 2]. After counting 5 HPF the mean nerve density score can be calculated. In the functional experiment, each strip (2 x 5 mm) was suspended in a 20 ml organ bath filled with modified Krebs-Henseleit solution. The solution was maintained at 37°C and continuously gassed with 5% $CO_2$ and 95% $O_2$. Each muscle preparation was connected to an isometric force displacement transducer and isometric forces recorded and monitored on an ink-writing recorder, and we measured the contractile responses induced by KCl, Carbachol (CCh), ATP and EFS using muscle bath technique, and measured the amount of acetylcholine (ACh) release from bladder strips by High Performance Liquid Chromatography with Electro Chemical Detector.

## Results

In the histological study, H-E staining demonstrated no apparent changes in the cell size and smooth muscles organization of bladder in 8 and 32 weeks-old rats of both groups. However, the number of nerve fibers or bundles of 32 weeks-old GK rats was significantly decreased as compared with age-matched control rats. In the functional experiment using isolated bladder smooth muscle strips, in 8 weeks-old rats, there were not significant differences in the contractile responses induced by CCh, ATP and EFS, and the amount of ACh release from bladder between groups. In 32 weeks-old rats, the contractile responses induced by CCh, ATP were significantly higher than that of age-matched control rats, on contrast, the contractile response induced by EFS and the amount of ACh release from bladder of GK rats were significantly lower than that of age-matched control rats.

## Interpretation of results

The present results demonstrated the following findings. In the early phase of type 2 diabetes mellitus, there may not be significant changes in autonomic nervous system, peripheral nerves in bladder, and pharmacological characteristics of bladder smooth muscles.

The up-regulation of post muscarinic receptor mediated-response and increase in ATP-induced bladder contractile responses were observed in the late phase of diabetes mellitus.

In the late phase of diabetes mellitus, the decreased number of nerve fibers or bundles in bladder may cause the decrease in the amount of ACh release, which contribute to the voiding dysfunction.

## Concluding message

In the late phase of diabetes mellitus, the decreased number of nerve fibers or bundles in bladder may cause the decrease in the amount of neurotransmitters release, which contribute to the voiding dysfunction.

## References

[1] Urol Int 1988; 43: 145-148.
[2] Eur J Histochem 1995; 39: 127-132.