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PHARMACOLOGICAL PROPERTIES, FUNCTIONAL ALTERATIONS, AND GENE EXPRESSION OF MUSCARINIC RECEPTORS IN YOUNG AND OLD TYPE 2 GK DIABETIC RAT BLADDERS

Hypothesis / aims of study
Diabetic cystopathy, a form of urinary bladder dysfunction, is a major complication of diabetes, occurring in 25 to 83% of patients with diabetes mellitus. The streptozotocin (STZ)-induced diabetic rat is the most commonly used and well-investigated experimental model for type 1 diabetes. However, only limited information is available about type 2 diabetic rat cystopathy. We investigated pharmacological properties and gene expressions of the muscarinic receptor system in young and old Goto-Kakizaki (GK) rat bladders.

Study design, materials and methods
Twelve- and 70-week-old male GK rats and age-matched male Wistar rats were used in this study. The bladder functions were estimated by voiding behavior studies, cystometric studies, and functional studies using KCl, carbachol, and various concentrations of subtype selective muscarinic antagonists, i.e., atropine, pirenzepine, methoctramine, and 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP). The participation levels of M_2 and M_3 receptor mRNAs in the bladder were investigated by real-time PCR.

Results
In the voiding behavior studies, although there were no significant differences in urine output, an age-related decrease in micturition frequency and an age-related increase in single voided volume were observed in both GK and Wistar rats. In the cystometric studies, although there were no significant differences in maximum detrusor pressure or bladder capacity, residual urine volume was significantly increased in the 70-week-old GK rats. In the functional studies, carbachol-induced contractility of the detrusor was significantly increased in GK rats of both age groups. The pA_2 values calculated for series of muscarinic antagonists were similar in all groups and were rank-ordered as atropine > 4-DAMP > methoctramine > pirenzepine. The slopes of the Schild plots for these muscarinic antagonists were similar between groups. The estimated pA_2 values indicate that the carbachol-induced contractile response is mediated through the M_3 receptor subtype in all groups. Furthermore, muscarinic M_2 and M_3 receptor mRNAs were significantly upregulated in the 70-week-old GK rat bladder.

A: twelve-week-old Wistar rats, B: twelve-week-old GK rats, C: seventy-week-old Wistar rats, and D: seventy-week-old GK rats.

Table 1. Voiding behavior studies in the experimental rats

	micturion frequency (/day)	urine production (ml/day)	single voided volume (ml)
A	12.3 ± 0.8	10.7 ± 1.1	0.87 ± 0.0
B	13.6 ± 0.9	12.8 ± 0.9	0.94 ± 0.08
C	9.1 ±1.7 *	12.8 ± 0.9	1.40 ± 0.22*
D	8.1 ± 1.2 *	13.2 ± 1.3	1.62 ± 0.32*

*)significantly different from younger group (group A VS group C, and group B VS group D).

Table 2. Cystometrogram data in the experimental rats

	Pdet (cmH_2O)	single voided volume (ml)	residual urine (ml)
A	42.4 ± 5.7	0.31 ± 0.03	0.054 ± 0.033
B	33.7 ± 2.9	0.34 ± 0.04	0.035 ± 0.010
C	35.3 ± 8.7	0.52 ± 0.13	0.044 ± 0.020
D	44.1 ± 4.3	0.51 ± 0.08	0.230 ± 0.057* **

Pdet: maximum detrusor pressure during voiding *) significantly different from age-matched Wistar group (group A VS group B, and group C VS group D). **)significantly different from younger group (group A VS group C, and group B VS group D).

Table 3. Functional studies in the experimental rats

	Emax / KCl	ED_{50} (10^{-6} M)
A	1.57 ± 0.05	1.19 ± 0.12
B	1.88 ± 0.09*	2.88 ± 0.92
C	1.39 ± 0.05**	2.01 ± 0.55
D	1.61 ± 0.05* **	2.22 ± 0.49

E_{max} and ED_{50} values are for carbachol. KCl means contractile force to 100 mM KCl. *) significantly different from age-matched Wistar group (group A VS group B, and group C VS group D). **)significantly different from younger group (group A VS group C, and group B VS group D).

Table 4. Expression of muscarinic M_2 and M_3 receptor mRNAs in the bladder dome

	M_2 /b-actin (x 10^{-3})	M_3 / b-actin (x 10^{-3})
A	2.57 ± 0.89	4.69 ± 0.88
B	2.72 ± 0.73	4.68 ± 0.17
C	1.50 ± 0.40	5.28 ± 0.11

D	4.48 ± 1.38*	8.26 ± 0.19* **

Expression of muscarinic M_2 and M_3 receptor mRNAs were normalized with that of **b**-actin mRNAs. *) significantly different from age-matched Wistar group (group A VS group B, and group C VS group D). **)significantly different from younger group (group A VS group C, and group B VS group D).

Interpretation of results: In the present study, we demonstrated the hypercontractility of detrusor smooth muscle to carbachol in GK rats at both ages compared to age-matched control rats. We also demonstrated that 70-week-old GK diabetic rats had significantly increased residual urine volume compared to both age-matched control rats and 12-week-old GK rats. Findings from the pA_2 values and slopes indicate that alteration of the contractile response via the muscarinic M_3 receptor subtype is not due to changes in muscarinic receptor affinity in diabetic rat detrusor. Rather, such changes appear to be the result of quantitative rather than qualitative changes in the muscarinic receptor system. The results (Table 4) showed that the mRNA levels of both muscarinic M_2 and M_3 receptors were increased under diabetic conditions at 70 weeks, as compared to age-matched controls. Based on our previous and present data, it appears likely that the overexpressions of muscarinic M_2 and M_3 receptor mRNAs are related to the hypercontractility of detrusor in 70-week-old diabetic rats. The diabetes-associated neuropathy may inhibit the release of acetylcholine from cholinergic nerves, in turn inducing the overexpression of muscarinic receptors in the diabetic detrusor. Such overexpression may enhance signaling downstream of these receptors and may increase detrusor contraction, according to the results of the present organ bath study.

Concluding message: Our data indicate that non-insulin-dependent diabetes induces alterations of the muscarinic receptor system that may contribute to the development of diabetic cystopathy.

References

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Tottori University Ethics Committee**