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Yoshida A[1], Ito Y[1], Hagiwara E[1], Yoshizawa T[2], Takahashi S[2], Yamada S[1]
***1.** University of Shizuoka, **2.** Nihon University*

TIME-DEPENDENT ALTERATION OF PHARMACOLOGICALLY RELEVANT RECEPTORS IN THE BLADDER OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

Hypothesis / aims of study

Diabetic cystopathy is a common complication of diabetes mellitus and clinically, up to 80% of patients with diabetes mellitus develop bladder dysfunction. It is known that the symptoms of diabetic cystopathy are characterized by the decreased bladder sensation, increased bladder capacity and impaired detrusor contractility with increased residual urine. However, these symptoms are not always observed in diabetic patients and several studies have shown that urinary frequency and urgency with detrusor hyperactivity also occur. Muscarinic and purinergic receptors in the bladder are significantly involved in the regulation of urination. Previous studies have suggested the possible involvement of these pharmacologically relevant receptors in the pathogenesis of urinary dysfunction of diabetic rats. Thus, the present study was conducted to test whether the development of diabetic state in streptozotocin (STZ)-induced diabetic rats causes the time-dependent alteration of muscarinic and purinergic receptors in the bladder.

Study design, materials and methods

Diabetes was induced in 7-week old female wistar rats by single intraperitoneal injection of STZ (50 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5). The control rats were injected same volume of buffer instead of STZ. Rats with higher levels of plasma glucose than 250 mg/dl at 24 h after STZ-treatment were used for the further study. At 1, 4, 8, 12-week after treatment of STZ, muscarinic and P2X receptors were measured by the radioligand binding assay using [^{3}H]NMS and [^{3}H]αβ-MeATP, respectively. The apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [^{3}H]NMS and [^{3}H]αβ-MeATP were estimated by nonlinear regression analysis.

Results

STZ-treated rats displayed significantly higher plasma glucose than that of age-matched control rats as shown in Table 1. In STZ-treated rats compared with age-matched control rats, the body weight was significantly decreased and bladder weight was significantly increased. After 4-, 8- and 12-week after STZ treatment, the B_{max} for specific [^{3}H]NMS binding was significantly (1.3, 1.3 and 1.2 times, respectively) increased in the diabetic rats compared with age-matched control rats, whereas there was little significant change at 1-week of STZ treatment (Table 2). Similarly, the B_{max} for specific [^{3}H]αβ-MeATP binding increased in the bladder of STZ-treated rats compared with control rats at all time points and the increases were greater by 1.6 times (1-week), 1.8 times (4-week), 2.0 times (8-week) and 1.3 times (12-week), respectively. In contrast, the K_d values for specific binding of [^{3}H]NMS and [^{3}H]αβ-MeATP in the bladder were similar between control and STZ-treated rats at all time points (Table 2).

Interpretation of results

The significant enhancement of Bmax for [^{3}H]NMS and [^{3}H] αβ-MeATP without a change in the Kd in the bladder of STZ-induced diabetic rats indicated the increased density of muscarinic and P2X receptors. The up-regulation of pharmacologically relevant receptors in the bladder may indicate the decreased efferent nervous activity arising from diabetic neuropathy.

Concluding message

The time dependent increases of density of pharmacologically relevant (muscarinic and P2X) receptors occurs in the bladder of STZ-induced diabetic rats, and these receptor alteration may be partly associated with the pathophysiology of urinary dysfunction due to the diabetes.

Table 1. General features of control and STZ-treated rats.

Time after STZ treatment	Body weight (g)	Bladder weight (mg/g B.W.)	Plasma glucose (mg/dl)
1 week:			
Control	207 ± 2	0.26 ± 0.01	156 ± 2
STZ	195 ± 2**	0.37 ± 0.02**	391 ± 23**
4 week:			
Control	253 ± 3	0.24 ± 0.01	166 ± 6
STZ	220 ± 4**	0.59 ± 0.08**	530 ± 33**
8 week:			
Control	297 ± 4	0.22 ± 0.01	150 ± 4
STZ	250 ± 4**	0.71 ± 0.06**	545 ± 23**
12 week:			
Control	309 ± 4	0.26 ± 0.01	164 ± 4
STZ	266 ± 5**	0.62 ± 0.08**	578 ± 20**

Values are expressed as mean±S.E. (n=10-39). Asterisks show a significant different from age-matched control, **P<0.001.

Table 2. K_d and B_{max} for specific binding of [^{3}H]NMS and [^{3}H]□ □-MeATP in the bladder of rats at 1, 4, 8, 12 week after STZ treatment.

Time after STZ treatment	[^{3}H]NMS		[^{3}H]□ □-MeATP	
	K_d (pM)	B_{max} (fmol/mg tissue)	K_d (nM)	B_{max} (fmol/mg tissue)

1 week:				
Control	283±16	9.7 ± 0.7	1.22±0.05	29.7 ± 2.7
STZ	301±12	11.3 ± 0.6	1.44±0.07	48.9 ± 3.7*
4 week:				
Control	293±16	9.3 ± 0.8	1.01±0.10	23.2 ± 1.6
STZ	315±23	12.4 ± 0.8*	1.26±0.14	42.0 ± 4.6*
8 week:				
Control	313±10	10.9 ± 0.4	1.14±0.13	27.2 ± 2.3
STZ	325±23	14.5 ± 1.2*	1.46±0.20	54.5 ± 5.8*
12 week:				
Control	303±18	11.8 ± 0.7	1.19±0.13	27.9 ± 3.2
STZ	306±13	14.3 ± 0.6*	1.01±0.06	37.3 ± 4.3

Values are expressed as mean±S.E. (n=4-9). Asterisks show a significant difference from age-matched control, *P<0.05.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**This study was done in accordance with recommendations of the US National Institutes of Health and the Experimental Animal Ethical Committe of the Univsesity of Shizuoka.**