

Effects of ONO-2235, an Aldose Reductase Inhibitor, on Muscarinic Receptors and Contractile Response of the Urinary Bladder in Rats with Streptozotocin-Induced Diabetes

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ABSTRACT—This study was conducted to evaluate effects of the aldose reductase inhibitor ONO-2235 on the contractile response to acetylcholine of the urinary bladder dome of streptozotocin-induced diabetes mellitus (DM) rats and simultaneously observe the changes in the function and number of muscarinic receptors and the sorbitol content of the bladder. The contractile response to acetylcholine increased 51% in the DM rat bladder dome compared to the normal rats; however, this was attenuated to a 10% increase by administration of 100 mg/kg ONO-2235 for 2 weeks. Treatment with ONO-2235 significantly decreased the specific [³H]quinuclidinyl benzilate binding in DM rats. However there was no significant dose-dependency among the ONO-2235-treated groups. The sorbitol levels of the sciatic nerve and the bladder were higher in the DM rats compared to the control rats; ONO-2235 decreased the level, although it did not completely reverse them to the control level. These results suggest that an aldose reductase inhibitor attenuates the increase of the muscarinic receptor number and normalizes the enhanced contractile response to acetylcholine caused by hyperglycemia and diuresis, probably through suppression of the polyol-pathway in the DM rat bladder dome.

Keywords: Diabetes mellitus, Bladder dome (rat), Aldose reductase inhibitor, Polyol pathway, Muscarinic receptor

The function of the urinary bladder is often changed in patients with diabetes mellitus (DM) (1–6). In the early stages of DM, there is a reduction in the sensation of bladder fullness that eventually progresses to bladder atony and urinary retention (7). The motor activity of the smooth muscle, especially that of the bladder dome, is mainly regulated by the parasympathetic nervous system (8, 9). Histochemical and functional studies have shown a DM-induced alteration in cholinergic innervation as well as an altered sensitivity to cholinergic agonists (10–13). Recent in vitro studies showed that the contractile response to muscarinic agonists was increased about 50% in the bladder dome of rats with streptozotocin (STZ)-induced DM (14, 15), and this was accompanied by significant increases in the densities of muscarinic receptors in the bladder dome of rats (14, 16, 17). Interestingly, similar to the increase of the muscarinic receptors in STZ-induced DM rat bladder domes, which can cause

neurogenic bladder, the up-regulation was also observed in sucrose-fed diuretic rats (14, 16). These results suggest that the increased contractile response and the increased number of muscarinic receptors in the DM rat bladder dome might have been caused by both the physical responses to diuresis and the DM induced peripheral and/or autonomic neuropathy (3, 18, 19).

Although the exact mechanisms are unknown, the polyol pathway is clearly involved in DM neuropathy, and the rate-limiting step in the polyol pathway is catalyzed by aldose reductase (19). ONO-2235, 5-[(1*E*,2*E*)-2-methyl-3-phenylpropenylidene]-4-oxo-thioxo-3-thiazolidineacetic acid, is a potent aldose reductase inhibitor that is used to treat the disturbance of the peripheral and/or autonomic nervous system associated with DM (20). The present study was designed to simultaneously investigate the effects of ONO-2235 on the up-regulation of muscarinic receptors, the contractile responses and, for the first time, the sorbitol content in the urinary bladder of STZ-induced DM rats.

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MATERIALS AND METHODS

Animals and preparations

Male STD-Wistar rats, 13-weeks-old and weighing about 250–280 g, were used. They were obtained from the Animal Laboratory for Research of Yamanashi Medical University. Experiments were performed in accordance with the Guidelines for Animal Experiments of Yamanashi Medical University.

Animals were allowed free access to food and water. They were randomly divided into 6 groups: 1) control (C), 2) diabetic rats (DM), 3) DM rats administered orally with 20 mg/kg ONO-2235 once daily for 2 weeks (DM-ONO 20), 4) those administered orally with 50 mg/kg ONO-2235 once daily for 2 weeks (DM-ONO 50), 5) those administered orally with 100 mg/kg ONO-2235 once daily for 2 weeks (DM-ONO 100) and 6) non-DM rats administered orally with 100 mg/kg ONO-2235 once daily for 2 weeks (C-ONO 100). DM was induced by a single intraperitoneal injection of STZ (50 mg/kg) dissolved in 10 mM citrate buffer (pH was adjusted to 4.5 with HCl). Age-matched control rats received an injection of the same volume of citrate buffer. Three days after STZ injection, the presence of DM was confirmed by measuring the serum glucose level in peripheral blood obtained from the tail vein. Four weeks after the induction of DM, STZ-injected rats were randomly separated into 4 groups. The DM group was not treated for 2 weeks, while the others (DM-ONO 20, 50, 100) were administered with 20, 50 or 100 mg/kg of ONO-2235, respectively. Two weeks after the drug treatment, i.e., 6 weeks after the initial injection of STZ or vehicle, the rats were sacrificed by decapitation. Blood samples were taken for the serum glucose measurement. The dome of the bladder was quickly removed and divided into two sections; one portion was used for functional studies examining contractile responses, while the other was frozen in liquid nitrogen and subsequently stored at -80°C for the receptor binding assay experiment and the measurement of sorbitol. Sciatic nerves were also obtained, and the changes of the sorbitol level in the nerve tissue were measured.

Measurement of serum glucose

Serum glucose was measured by the glucose oxidation method by using a Dry-Chem 100 Analyzer (Fuji Photo Film Co., Tokyo).

Measurement of sorbitol

Small samples of the bladder domes and sciatic nerves (one sample was about 10 mg) were homogenized in distilled water, precipitated by the addition of an equal volume of ice-cold 6% perchloric acid and centrifuged at 3000 rpm for 5 min. To neutralize the supernatant, 0.5 ml

of 2 N K_2CO_3 was added. The sorbitol concentration was determined by a fluorometric enzyme assay as previously described (21).

Functional studies

The bladder dome was dissected away from the whole bladder, and longitudinal strips about 3-mm-wide and 7-mm-long were cut from the entire anterior surface. Muscle strips were mounted in 20-ml chambers containing Krebs-Henseleit solution of the following composition: 119.0 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgSO_4 , 24.9 mM NaHCO_3 , 1.2 mM KH_2PO_4 , 10.0 mM glucose and 0.057 mM ascorbic acid. The solution was gassed with 95% O_2 and 5% CO_2 at $36 \pm 1^{\circ}\text{C}$.

The isometric force of the muscle strips was recorded with a force-displacement transducer (Nihon Kohden, Tokyo) attached to a pen recorder (Nihon Kohden) and a MacLab/8 system (AD Instruments Pty Ltd., Castle Hill, Australia). Muscle strips were equilibrated for 60 to 90 min before drug administration. The resting tension was adjusted to 1.0 g throughout the experiment. After stimulation by 50 mM K Krebs-Henseleit solution (isoosmotic solution prepared by substituting KCl for NaCl in the Krebs-Henseleit solution) and the washout, cumulative dose-response curves to acetylcholine were constructed relative to the 50 mM K contraction. The concentration-response curve to acetylcholine was obtained in the presence of 1 μM physostigmine.

Preparation of membrane particulate

Frozen tissues were thawed, cut into small pieces, and homogenized in 40–50 vol. of ice-cold 50 mM Tris-HCl buffer (pH 8.0) by using a Polytron Brinkman, twice, each burst lasting 30 sec. Each burst was separated by a 30-sec interval. The homogenate was centrifuged at $49,000 \times g$ for 20 min at 4°C . The supernatant was discarded, and the resultant pellets were rehomogenized, filtered through a 106- μm nylon mesh and recentrifuged under the same conditions described above. The pellets were suspended in a 30-vol. aliquot of incubation buffer (50 mM Tris-HCl, pH 8.0) to yield a membrane particulate that contained 0.02 to 0.03 mg protein/ml.

Receptor binding studies

In the saturation studies with [^3H]quinuclidinyl benzilate (QNB), aliquots of membrane preparations that contained approximately 70–80 μg of protein were incubated with increasing concentrations of [^3H]QNB (0.0125–0.5 nM) for 60 min at 23°C . The protein concentration was determined by the method of Lowry et al. (22). Using a Brandel Cell Harvester (Gaithersburg, MD, USA) at the end of the incubation period, the mixture was rapidly filtered in a vacuum through a Whatman GF/B fiber glass

filter, which was pretreated for 1 hr with 0.05% polyethylenimine. Filters were rinsed twice with 2 ml of ice-cold Tris-HCl buffer and then transferred into a scintillation cocktail (Clear-sol; Nacalai Tesque, Kyoto) and shaken for 30 min. The radioactivity of each filter was measured at an efficiency of 50% to 55%. Specific binding, defined as the difference in binding in the presence or absence of 1 μ M atropine, ranged from 71% to 92% of the total binding depending on the concentration of [3 H]QNB. In the competitive inhibition experiments, aliquots of membrane particulate were incubated with approximately 0.4 nM [3 H]QNB in the presence of different concentrations of a non-labeled compound. Inhibition studies with acetylcholine were performed in the presence of 1 μ M physostigmine.

Drugs and chemicals

[3 H]QNB (34.7 Ci/mM) was obtained from New England Nuclear (Boston, MA, USA) and stored at -20°C . Acetylcholine chloride, atropine sulfate, physostigmine sulfate, streptozotocin tetraacetate and sorbitol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). NAD and sorbitol dehydrogenase was purchased from Boehringer Mannheim Co. (Indianapolis, IN, USA). ONO-2235 was donated by Ono Pharmaceutical Co. (Osaka).

Data analyses

Data are expressed as a mean \pm standard error of the means (S.E.M.). One-way or two-way analysis of variance (ANOVA) was used to compare the results in each group. If the ANOVA value reached a significant level, comparisons were made by Bonferroni's *F*-test to localize the significant difference. Saturation data were calculated by a linear regression of bound/free versus bound fractions, according to the method of Rosenthal (23), to calculate the maximum number of binding sites (B_{max}) and the equilibrium dissociation constants (K_D). B_{max} values were normalized by the volume of protein (mg protein).

The IC_{50} (calculated concentration to produce 50% inhibition) values were determined by regression analysis of displacement curves. K_i values were calculated by the following equation: $K_i = \text{IC}_{50} / (1 + L/K_D)$, where *L* is the concentration of radioligand (24). Hill coefficients were also obtained as described by Bennet and Yamamura (25). All values in this study were determined by triplicate determinations of three to five separate experiments. Statistical analysis was done by Stat View 4.0 statistical software (Abacus Concepts, Inc., Berkeley, CA, USA) and Excel 4.0 (Microsoft Corporation, Redmond, WA, USA). A level of $P < 0.05$ was accepted as statistically significant.

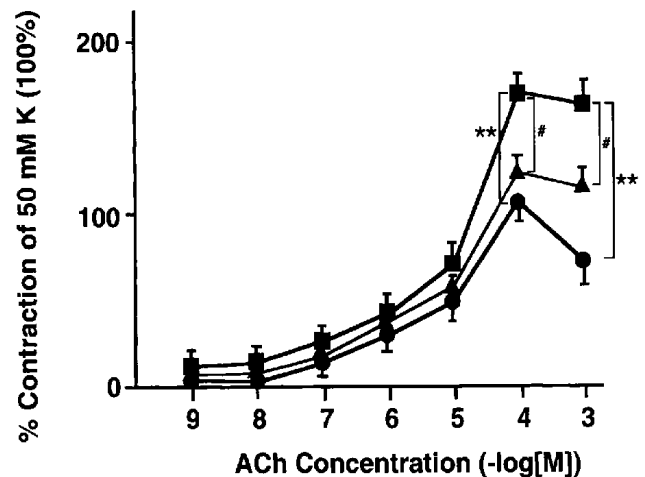


Fig. 1. Concentration-dependent contraction of the rat bladder in response to acetylcholine. The contractile response is expressed as a percentage of the contraction induced by 50 mM KCl. At concentrations over 100 mM, differences between the C group and DM group ($P < 0.01$, **) and between the DM group and DM-ONO 100 ($P < 0.05$, #) were significant. C group = control group (●), DM group = diabetes group (■) and DM-ONO 100 group = DM rats administered ONO-2235 at 100 mg/kg (▲). All data are expressed as the mean values from 3–5 individual experiments.

Table 1. General features of experimental rats

	C (n=14)	DM (n=12)	DM-ONO 20 (n=15)	DM-ONO 50 (n=14)	DM-ONO 100 (n=13)	C-ONO 100 (n=6)
Body weight (g)						
13 weeks	274 \pm 4	256 \pm 5**	272 \pm 2	280 \pm 2	269 \pm 7	269 \pm 7
19 weeks (Final)	355 \pm 6	255 \pm 6**	246 \pm 16**	211 \pm 4**	216 \pm 9**	341 \pm 8
Bladder dome weight (mg)	55 \pm 3	159 \pm 19**	130 \pm 8**	130 \pm 3**	135 \pm 6**	58 \pm 4
Serum glucose (mg/dl)	101 \pm 6	363 \pm 24**	377 \pm 33**	352 \pm 25**	364 \pm 22**	108 \pm 8

Data are means \pm S.E.M. of 6–15 animals per group. **: Significantly different from the normal control (C) group ($P < 0.01$). DM-ONO 20, DM-ONO 50, DM-ONO 100 = diabetic rats administered with 20, 50 and 100 mg/kg ONO-2235, respectively; C-ONO 100 = non-diabetic rat administered with 100 mg/kg ONO-2235.

RESULTS

Serum glucose concentrations and physiological characteristics

The serum glucose level ranged from 200 to 400 mg/dl 3 days after the administration of STZ as shown in Table 1, along with the body and bladder dome weight data. At the time of sacrifice, the body weights of the DM and DM-ONO groups were significantly lower than those of the C and C-ONO 100 groups. The bladder dome of the DM and the DM-ONO groups weighed more than those of the C and C-ONO 100 groups. The serum glucose level was higher in the DM and DM-ONO groups compared to those in the C and C-ONO 100 groups, showing that the treatment with ONO-2235 had no effect on the blood glucose level.

Functional studies

Acetylcholine (10^{-9} to 10^{-3} M) induced stronger contractions in the muscle strips obtained from bladder domes of the DM groups than those of the C group. The DM-ONO groups exhibited a weaker contractile response compared to the DM group (Fig. 1). When the contractile responses were normalized as a percentage of the response to 50 mM K Krebs-Henseleit solution, the acetylcholine responses were increased by 51%, 25%, 17% and 10% in the DM, DM-ONO 20, DM-ONO 50 and DM-ONO 100 group, respectively, compared to those of the C group. There was no significant difference in the acetylcholine response between the C and C-ONO 100 groups. While the attenuation of the enhanced

acetylcholine responses in the DM-ONO groups tended to depend on ONO-2235 doses, the difference was not statistically significant. Not only the maximum responses, but also the sensitivity to acetylcholine, as evaluated by the EC_{50} value, of the DM group (9.3×10^{-6} M) were significantly changed from those of the C group (3.7×10^{-6} M), but there were no significant differences in the EC_{50} values among the DM-ONO groups (4.0 to 5.1×10^{-6} M) and the DM groups. The results were the same when the absolute contractile responses expressed per square meters of cut bladder dome to acetylcholine and 50 mM KCl responses were compared as in Table 2.

Receptor binding studies

The presence of a single class of specific, non-interacting binding sites for muscarinic agonist was identified in saturation binding studies using [3 H]QNB. As shown in Table 3 and Fig. 2, the B_{max} value in the DM group is significantly greater than that of the C group (DM, 245 ± 34 ; C, 105 ± 8 fmol/mg protein, $P < 0.01$). In the DM-ONO groups, the B_{max} values had returned to nearly normal levels, but still exceeded those of the C group (DM-ONO 20, 178 ± 14 ; DM-ONO 50, 176 ± 14 ; DM-ONO 100, 166 ± 23 fmol/mg protein, $P < 0.05$). No significant difference was observed between the B_{max} value of the C and the C-ONO 100 groups. K_D values were almost the same throughout all the groups. Competitive inhibition experiments showed no differences in the K_i values and Hill coefficients (n_H) for acetylcholine among the groups (Table 4).

Table 2. Contractions of ACh and KCl in rat bladder dome

	C (n=5)	DM (n=4)	DM-ONO 20 (n=4)	DM-ONO 50 (n=4)	DM-ONO 100 (n=6)	C-ONO 100 (n=4)
Contraction to 50 mM KCl (g/mm ²)	0.161 ± 0.01	0.182 ± 0.019	0.171 ± 0.013	0.179 ± 0.009	0.187 ± 0.022	0.17 ± 0.016
Maximum contraction to ACh (g/mm ²)	0.163 ± 0.009	0.311 ± 0.027**	0.224 ± 0.032* [#]	0.191 ± 0.014* [#]	0.209 ± 0.022* [#]	0.169 ± 0.002 [#]

Data are means ± S.E.M. of 4–6 determinations in each group. * and [#]: significantly different from the normal C and DM group, respectively ($P < 0.05$), **: significantly different from the C group ($P < 0.01$).

Table 3. Saturation of [3 H]QNB binding in rat bladder dome

	C (n=6)	DM (n=5)	DM-ONO 20 (n=5)	DM-ONO 50 (n=5)	DM-ONO 100 (n=6)	C-ONO 100 (n=3)
B_{max} (fmol/mg protein)	105 ± 8	245 ± 34**	166 ± 23* [#]	176 ± 14* [#]	178 ± 14* [#]	104 ± 8
K_D (pM)	102 ± 2	97 ± 4	100 ± 3	100 ± 2	91 ± 4	103 ± 6

Data are means ± S.E.M. of 3–6 determinations in each group. Data are analyzed by the linear regression of bound versus bound/free. B_{max} = maximum number of binding sites, K_D = equilibrium dissociation constant. * and [#]: significantly different from the C and DM group, respectively ($P < 0.05$), **: significantly different from the C group ($P < 0.01$).

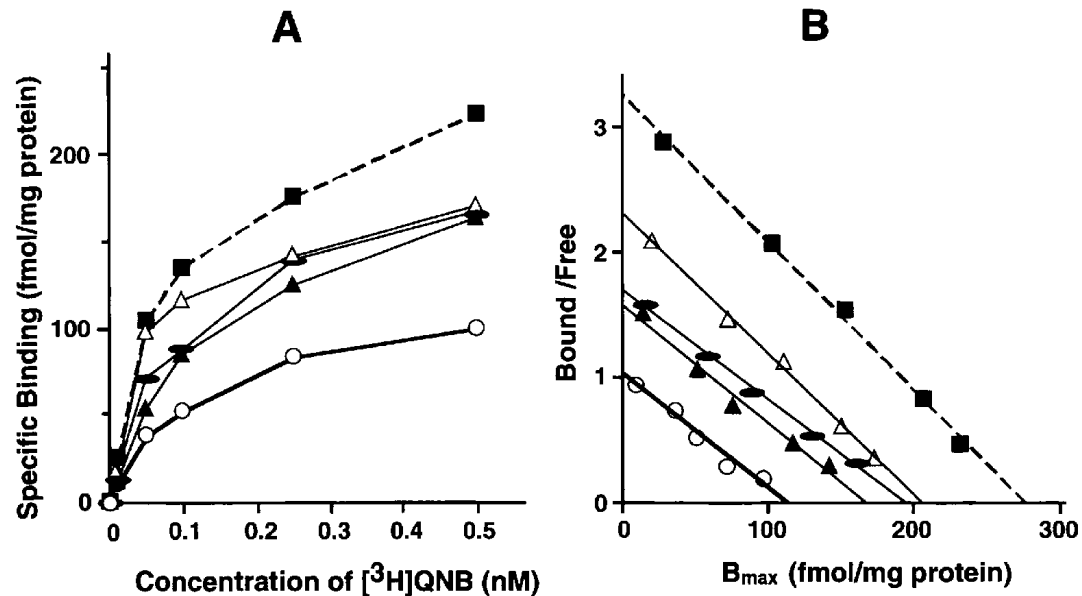


Fig. 2. Saturation binding by [³H]QNB in rat bladder dome. Data are expressed as the mean values from 3–5 experiments. Panel A is the saturation curve, and panel B represents the same data plotted according to Rosenthal (23). Nonspecific binding was obtained in the presence of 1 mM atropine. ○, ■, △, ● and ▲ indicate the control group, diabetes group, groups of DM rats administered ONO-2235 at 20, 50 and 100 mg/kg, respectively.

Table 4. Inhibition of [³H]QNB binding to rat bladder by acetylcholine

	C (n=4)	DM (n=3)	DM-ONO 20 (n=3)	DM-ONO 50 (n=3)	DM-ONO 100 (n=4)	C-ONO 100 (n=3)
K _i (nM)	509.0 ± 8	484 ± 18	499 ± 14	489 ± 19	457 ± 18	511 ± 10
n _H	0.9 ± 0.03	0.97 ± 0.23	0.95 ± 0.01	0.96 ± 0.01	0.95 ± 0.03	0.92 ± 0.15

Data are means ± S.E.M. of separate 3–4 experiments. K_i = inhibition constant. n_H = Hill coefficient. No significant differences were observed among all groups.

Sorbitol concentration

Significant increases in the sorbitol concentration in the bladder domes and sciatic nerves were observed in the DM group relative to the C and the C-ONO 100 groups (Table 5). No significant difference was observed be-

tween the C and the C-ONO 100 groups. ONO-2235 decreased the sorbitol concentration but the levels in the DM-ONO group were still significantly higher than those of the C group. Though there was a dose-dependent decrease of the sorbitol concentration in the sciatic nerve, it was not seen in the sorbitol concentration of the bladder dome.

Table 5. Sorbitol in sciatic nerve and bladder dome

Group	Sorbitol (nmol/g wet weight)	
	Sciatic nerve	Bladder dome
C (n=4)	100 ± 28	75 ± 8
DM (n=3)	1079 ± 246*	985 ± 40*
DM-ONO 20 (n=3)	783 ± 44*	532 ± 80*. [#]
DM-ONO 50 (n=4)	448 ± 45*. [#]	567 ± 49*. [#]
DM-ONO 100 (n=4)	338 ± 28*. [#]	576 ± 53*. [#]
C-ONO-100 (n=3)	99 ± 26 [#]	81 ± 9 [#]

Data are means ± S.E.M. of 3–4 determinations in each group. * and [#]: significantly different from the C and DM group, respectively.

DISCUSSION

Effects of aldose reductase inhibitors on the muscarinic responses of DM rats, especially on those of the urinary bladder, have not been reported in the context of the polyol pathway, although there have been reports on the changes in the function of muscarinic receptors induced by drug treatments (14, 16, 18, 26) and on the changes in the polyol pathway activities responsible for the DM neuropathy (27–30). Our data showed an increase of muscarinic receptor function in DM rats, and this response

was attenuated by the treatment with a potent aldose reductase inhibitor, ONO-2235.

Rats with STZ-induced DM are routinely used for studying the complications of DM (29–32); and in our present study, changes produced by 4 weeks of the STZ-induced DM and changes produced by 2 weeks of aldose reductase inhibition were observed. The reduction of the body weight in our DM rats is consistent with the previously reported ones (11–13, 31, 32). However the body weight, bladder weight and the blood glucose level were not significantly different among the DM and DM-ONO groups, indicating that ONO-2235 did not alter the DM state and had no effect on the rats in a diuretic state for the last 2 weeks. Our result is consistent with reports that show increases in the contractile responses of the DM bladder muscle to acetylcholine and electrical stimulation (10, 11, 13). However there is a report that the maximal contractile responses of the DM bladder to acetylcholine (12) and electrical stimulation (30) are reduced. The difference might have been produced by the differences in the duration of DM (2 to 8 weeks in Ref. 13 and 3 months in Ref. 30), age (55–56 days in Ref. 13 and not specified in Ref. 30) or the rats (Sprague-Dowley rats in Ref. 13 and BB/Wor rats in Ref. 30).

ONO-2235 lessened the enhanced contractile responses to cholinergic stimulation of the diabetic rat bladder domes, though without any good dose-response relationships. This may be due to the choice of the doses, since former studies on ONO-2235 used up to 50 mg/kg in the rats (19, 20), while we used higher doses up to 100 mg/kg that might have produced almost the maximal response. Although the blood glucose level and the weight of the bladder did not decrease, the receptor number and the sorbitol content increased by diabetes were decreased. Because the sorbitol content in the peripheral and/or central nervous system increases by the activation of the polyol pathway (33–35) and the rate limiting enzyme in this pathway is aldose reductase (19), our results with ONO-2235 showed that the sorbitol content in the bladder dome decreased concomitantly with the decrease in cholinergic receptors. So although the mechanism for the increase of the cholinergic up-regulation in the DM bladder is not known, there may exist a close link between the polyol pathway and the elevated bladder contractions to cholinergic stimulation.

In the saturation binding studies with [3 H]QNB, we observed a significant increase in the total number of muscarinic receptors (B_{\max}) in the bladder domes of DM rats. The increase in the muscarinic receptor number has already been demonstrated to be associated with an increase in the contractile response to muscarinic cholinergic agonists (13, 14, 16). In our present study, the B_{\max} values of the bladder dome of the DM-ONO groups

decreased significantly compared with that of the DM group, but the values of the DM-ONO groups were still higher than those of the C group, and there was no dose-dependency in the ONO-2235 effects. It is noteworthy that the extent of the ONO-2235-induced decrease of the enhanced contractile responses and that of the B_{\max} was almost the same. However, the K_D values in binding assay were barely changed regardless of the decrease of EC_{50} values in the contraction study. The reason for the unchanged K_D is not known from this study; a similar result has been reported when insulin was used to treat the up-regulation of the bladder dome of rats (26). These results indicate that there is a close relationship between the function and the number of the receptors; however, as the EC_{50} decreased without K_D changes, there may exist spare receptors for the muscarinic cholinergic agonist in the bladder dome because the sensitivity of a tissue to a particular agonist such as acetylcholine may depend not only on the affinity of the receptor for binding an agonist but also on the total concentration of receptors according to the spare receptor concept.

The fact that the administration of ONO-2235 did not completely eliminate the enhanced contractile force to normal control values may indicate: 1) that the aldose reductase inhibition was not complete even using higher doses of ONO-2235 compared to other reports (19, 20) or 2) that not the DM, but the diuresis itself induced the increased contractile response to cholinergic stimulation. This was derived from the observation that the altered contractile responses observed in the DM rat bladder were also observed in non-diabetic polyuric rats (15, 30, 36–38).

As for the first possibility, ONO-2235 has been reported to be a potent aldose reductase inhibitor, but an *in vitro* study showed that even using the highest concentration, the enzyme activity was not completely inhibited (19). This is consistent with our observation that the sorbitol content of the bladder and sciatic nerve still stayed at a fairly high level compared to that of the non-DM rats.

As for the second possibility that the enhanced cholinergic response is explained partly by the DM induced increase in the polyol pathway, resulting in receptor up-regulation, and partly by the functional response to diuresis, there are reports that non-diabetic sucrose-fed and thus polyuric rats showed significant enlargement and distention of their urinary bladder and increase in the receptor number (15, 30, 36–38). Fukumoto et al. postulated that this up-regulation might be due to an alteration of the coupling between muscarinic receptors and G proteins in rat bladder dome (16); our study does not give any new insight about this mechanism. Naturally, insulin is effective in correcting the up-regulation of muscarinic

receptors in the rat bladder domes, simultaneously preventing bladder hypertrophy (26). This may be due to the persistence of hypertrophy of the bladder resulting from the diuresis and/or the unrecoverable autonomic neuropathy. It is possible that in our study, the incomplete return of the up-regulation in the muscarinic receptor and contractile responses in the DM-ONO groups may be due in part to the diuresis as well as the hypertrophy of the bladder. The decrease in the weight of the bladder dome in DM-ONO groups is small and shows the persistence of hypertrophy of the bladder dome, while the ACh responses to unit area of the bladder dome dramatically decreased. Since the change in the sorbitol content of the non-diabetic sucrose fed rats was not known, the incomplete suppression of the receptor number and contractile function of our DM-ONO groups could not differentiate the existence of diuresis- and hypertrophy-induced changes from the sorbitol-related diabetes-induced changes. Since the total sorbitol of the bladder dome, which consists of mainly smooth muscles, was increased about tenfold, but the extent of the increase of sorbitol content only of the nervous tissues of the bladder, which causes the neuropathy, is difficult to measure, the quantitative analysis of the present results concerning the role of either the diuresis and bladder hypertrophy or the increased cholinergic receptors in the enhanced cholinergic response of the diabetic bladders seems to be difficult.

Although our study only demonstrated changes in the DM-induced bladder dome response to cholinergic stimulation, the simultaneous observations of the changes in blood glucose level, weight of the bladder, receptor number and sorbitol content did not provide a definite answer about the mechanisms of the changes. However, the clinical implication of our observation is, like other reports on the favorable effect of aldose reductase inhibitors on the DM neuropathy (20, 39), that the aldose reductase inhibitor treatment may be a favorable treatment for diabetes-induced urinary bladder dysfunction.

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