Differential Regulation of Bladder β-Adrenergic and Muscarinic Cholinergic Receptors in Experimental Diabetes

JAMSHID LATIFPOUR, TADASHI NISHIMOTO, MOAZEZ J. MARIAN, MASAKI YOSHIDA, AND ROBERT M. WEISS

To determine the contribution of diuresis-induced bladder hypertrophy, which accompanies the diabetic state, on the biochemical and functional alterations observed in the diabetic bladder, we compared three experimental groups: 8-wk streptozocin (STZ)-induced diabetic rats, 8-wk sucrose-fed diuretic rats, and age-matched controls. Diabetic and sucrose-fed rats had higher water intake, higher urine output, and larger bladders than controls. Diabetic rats had lower serum insulin levels, lower body weights, and higher serum glucose levels than either control or sucrose-fed animals. Receptor binding studies with [^{3}H]quinuclidinyl benzilate in bladder dome demonstrated an upregulation of muscarinic receptors in diabetic and sucrose-fed rats compared with controls. Parallel binding studies with [^{3}H]dihydroalprenolol and [^{125}I]iodopindolol showed an upregulation of β-adrenergic receptors in diabetic but not in sucrose-fed bladder domes. Carbachol induced larger contractile responses in diabetic and sucrose-fed than in control bladder dome muscle strips. Isoproterenol relaxed KCl-contracted detrusor strips from both diabetic and sucrose-fed rats to a greater degree and with a higher affinity than detrusor strips from controls. Our data show that overdistension and increased workload per se contributed to the upregulation of muscarinic but not to the upregulation of β-adrenergic receptors in STZ-induced diabetes. Furthermore, the magnitude of carbachol-induced contractions correlated with muscarinic receptor upregulation, whereas the magnitude of isoproterenol-induced relaxation did not correlate with changes in the density of the β-adrenergic receptors. Thus, it appears that different regulatory mechanisms are involved in diabetes-induced alterations in muscarinic and β-adrenergic receptors in bladder dome. *Diabetes* 40:1150–56, 1991

From the Section of Urology, Yale University School of Medicine, New Haven, Connecticut.

Address correspondence and reprint requests to Jamshid Latifpour, PhD, Section of Urology, Yale University School of Medicine, PO Box 3333, New Haven, CT 06510.

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Vesical dysfunction is a common complication of diabetes mellitus and is manifested by impaired bladder sensation, detrusor hypotonicity, bladder distension, and incomplete bladder emptying, which at times may lead to urinary retention (1–4). These abnormalities have been attributed in part to peripheral autonomic neuropathy (5,6).

Early studies suggested that bladder dysfunction is a late manifestation of poorly controlled diabetes (7,8). However, more recent clinical studies have demonstrated not only a high incidence (83%) of neurogenic bladder dysfunction in diabetic subjects who have signs of peripheral neuropathy (9,10) but also a significant incidence of bladder disorders in diabetic patients without other manifestations of peripheral neuropathy (5,11). The induction of diabetes by compounds such as streptozocin (STZ) and alloxan, which selectively cause pancreatic islet necrosis, has lead to the development of valuable animal models for studying abnormalities that occur in insulin-dependent diabetes (12–15). Although data derived from studies with these diabetic models have shown significant changes in autonomic innervation and in the contractile response of the bladder to various stimuli, there has been no consensus, because muscarinic agonists and/or electrical field stimulation have been reported to increase (12), decrease (16), or to have no effect (17,18) on contractile responses of bladder smooth muscle.

We reported that bladder domes of STZ-induced diabetic (STZ-D) animals were hypertrophied and contained an increased amount of muscarinic cholinergic receptors. Furthermore, muscle strips from the diabetic bladder dome produced a larger contractile response to muscarinic agonists (19). Certain characteristics of the diabetic bladder are similar to those observed with bladder outflow obstruction and/or denervation. Similarities include bladder distension,

bladder hypertrophy, detrusor instability, increased residual urine, and lower bladder collagen concentrations (20–25). Furthermore, alterations in the passive and active length-tension relationships of the diabetic bladder wall (26) have been shown to be similar to those observed in the rat bladder subjected to intravesical outflow obstruction (21) and denervation (27). Significant bladder hypertrophy, distension, and increase in muscle mass also have been reported in nondiabetic rats when urine output is increased by supplementing their drinking water with 5% sucrose (28–31). In addition, studies with sugar-induced diuretic rats demonstrated a cystometric deficit similar to that observed in the STZ-D rat bladder (29). These findings indicate that changes in the physical properties of the bladder may be a significant factor in the development of vesical dysfunction in insulin-dependent diabetes. To test this hypothesis and to further investigate the mechanisms by which bladder dysfunction develops in diabetes mellitus, we compared the biochemical and functional characteristics of muscarinic cholinergic and β-adrenergic receptors, two of the major physiological regulatory systems of the bladder, in bladder domes of diabetic rats, sucrose-fed diuretic rats, and age-matched controls.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats (55–56 days old, 230–280 g body wt) obtained from Charles River (Wilmington, MA) were randomly divided into three groups: control, diabetic, and sucrose-fed rats. Diabetes was induced by injection of 65 mg/kg i.v. STZ that was prepared in 0.1 M citrate buffer, pH 4.5. Control and sucrose-fed groups were injected intravenously with similar volumes of citrate buffer. The drinking water for the sucrose-fed rats contained 5% sucrose. Animals were housed in groups of two in suspended cages and were allowed free access to normal food and drinking water. Twenty-four and 48 h after injection of STZ, the induction of diabetes was confirmed by measuring urinary glucose with Labastic (Ames, Elkhart, IN). Animals were kept under identical conditions, and water intake and urine output were measured at specified time intervals. Care was taken to supply sufficient drinking water, especially for the diabetic and sucrose-fed groups. Eight weeks after the injections, rats were fasted overnight and killed by decapitation, and blood samples were collected in chilled test tubes for measurement of serum glucose and insulin levels. The whole bladder was removed and transferred into cold saline.

Serum insulin levels were determined with a radioimmunoassay kit (Ventrex, Portland, ME), and serum glucose concentrations were measured with the enzymatic (hexokinase) method (Glucose H.K., Sigma, St. Louis, MO).

Bladder domes were separated at the level of the ureteral orifices and then were either frozen in liquid N_2 and stored in −80°C for receptor binding assays or were cut into strips for functional (mechanical) studies.

For radioligand binding assays, membrane particulate preparations and saturation studies were performed as reported previously (19). Frozen tissue was thawed, cut into small pieces, homogenized in 40–50 vol of ice-cold 50 mM Tris-HCl (pH 8 at 25°C) with a Brinkman Polytron set at speed 6 for two 20-s periods, and centrifuged at 49,000 × *g* for 20 min at 4°C. The supernatant was discarded, and the pellet was homogenized in the cold Tris-HCl buffer, filtered through a nylon mesh (105 μm for [^{3}H]quinuclidinyl benzilate [QNB] and [^{3}H]dihydroalprenolol [DHA] binding and 250 μm for [^{125}I]iodopindolol [PIN] binding studies) and centrifuged under the same conditions. The final pellet was suspended in 50 mM Tris-HCl buffer, pH 8 for [^{3}H]QNB and [^{3}H]DHA binding, and in 50 mM Tris-HCl buffer, pH 8, containing 2.5 mM $MgCl_2$ and 0.5 mM EDTA for [^{125}I]PIN binding experiments. Tissue concentrations were adjusted as needed.

In saturation experiments, aliquots of membrane particulate fractions were incubated with 0.025–0.75 nM [^{3}H]QNB, 0.04–1 nM [^{3}H]DHA, or 0.004–0.125 nM [^{125}I]PIN at 23°C. Protein concentrations and incubation times for [^{3}H]QNB, [^{3}H]DHA, and [^{125}I]PIN experiments were 0.025 mg/ml for 60 min, 0.35 mg/ml for 30 min, and 0.25 mg/ml for 45 min, respectively. Nonspecific binding of muscarinic and β-adrenergic antagonists were determined with parallel sets of tubes incubated with 1 μM atropine and 1 μM (±)propranolol, respectively. After the incubation periods, the mixtures were filtered on Whatman GF/B filter papers (Clifton, NJ) with a 24-sample manifold (Brandel Cell Harvester, Gaithersburg, MD). Before filtration, the filter papers were placed in 0.05% polyethylenimine solution for 30 min to reduce the extent of nonspecific binding to filter paper. Filters were washed with 8–10 ml ice-cold Tris-HCl buffer, and the radioactivity retained on the filters was determined with a Packard Tri-Carb 1600 CA liquid-scintillation analyzer at an efficiency of 50–55% for [^{3}H]QNB and [^{3}H]DHA or with a Packard Multi-prias1 Analyzer at an efficiency of 70–75% for [^{125}I]PIN. Crude membrane particulates from two control, one diabetic, or one sucrose-fed bladder dome were used for a single binding experiment with [^{3}H]QNB or [^{125}I]PIN. For studies with [^{3}H]DHA, four to five control and two to three diabetic or sucrose-fed bladder domes were used for a single saturation experiment. Protein concentrations were determined by the method of Lowry et al. (32) with bovine serum albumin as the standard.

Functional (mechanical) studies were performed as follows. Longitudinal strips from the entire anterior part of the bladder dome (1.5-mm wide and 5- to 9-mm long) were mounted in a 3.5-ml chamber containing Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.52 mM $CaCl_2$, 1.2 mM $MgSO_4$, 24.88 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 5.55 mM glucose, and 2 mM sodium pyruvate [pH 7.4]) at 37°C and were gassed with 95:5 O_2/CO_2. Force was monitored with an isometric Statham UC-2 transducer, which was connected to a Grass model 7A physiograph. In a series of preliminary experiments, muscle strips were stimulated with 80 mM KCl to determine the length of optimal force development. Before constructing concentration-response curves, muscle strips were stretched to the length of optimal force development and then equilibrated for 90 min. During this period, the buffer was replaced every 15 min. In examining the contractile response to the muscarinic agonist carbachol, cumulative concentration-response curves were constructed in a stepwise manner after the response to the previous concentration had reached a plateau. Contractile response to carbachol was determined by subtracting the baseline force and the magnitude of the spontaneous contractions from the peak response. Alternatively, the muscle strip was

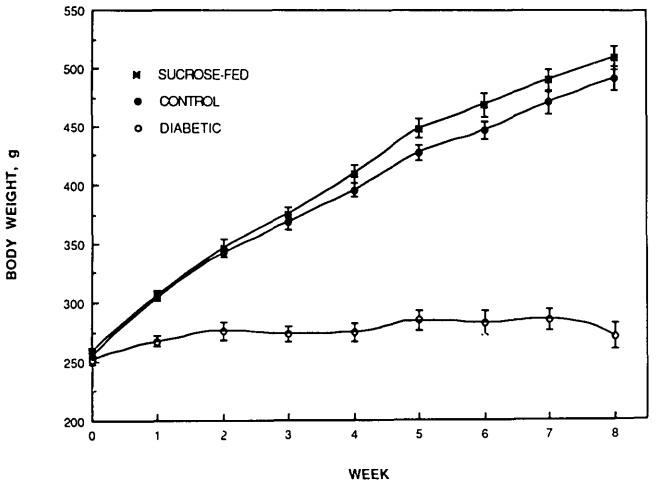

FIG. 1. Body weight gain curves of experimental animals. At wk 0, diabetes was induced by injection of 65 mg/kg i.v. streptozocin. Sucrose-fed rats received 5% sucrose in their drinking water. Animals were killed after 8 wk. Data points are shown as means ± SE of 20–30 determinations/group.

contracted with 80 mM KCl, and after tonic force had reached a stable plateau, cumulative concentration-response curves to the β-agonist isoproterenol were constructed. The response to isoproterenol was studied in the presence of 1 μM phentolamine. In studying the effect of (−)isoproterenol on KCl-contracted muscles, the 100% level of relaxation was determined from the force recorded in calcium-free Krebs-Henseleit solution containing 1 mM EGTA.

[^{3}H]DHA (99.9 Ci/mmol), [^{3}H]QNB (34.7 Ci/mmol), and [^{125}I]PIN (2200 Ci/mmol) were obtained from Du Pont-NEN (Boston, MA) and stored at −20°C. Atropine, (±)propranolol, (−)isoproterenol, phentolamine, and carbachol (carbamylcholine) were purchased from Sigma. Other chemicals and materials were of analytical grades and were obtained from commercial sources.

Saturation data were analyzed according to Rosenthal (33) with linear regression of bound/free versus bound to calculate the maximum number of binding sites (B_{max}) and equilibrium dissociation constant (K_d).

Contractile data were calculated in terms of grams of active force per cross-sectional area in square milimeters. The cross-sectional area was calculated according to the equation cross-sectional area = weight/(length × 1.05), where 1.05 is the assumed density of the muscle. E_{max} was obtained from the maximum contractile force or from the maximum relaxation response developed, and ED_{50} was calculated from a semilogarithmic plot of the percentage of maximum response versus drug concentration. K_d and ED_{50} values were calculated as geometric means, whereas B_{max} and E_{max} values were calculated as arithmetic means (34,35). Statistical analyses between groups and between concentration-response curves were performed with analysis of variance and the multiple-comparison Fisher's test.

RESULTS

The induction of glucosuria was demonstrated in STZ-injected rats by the presence of significant amounts of glucose (56–112 mM) in the urine as early as 24 h after the injection of STZ. Diabetic rats showed increases in their water intake and urine output and developed severe diarrhea. Although sucrose-fed rats did not develop glucosuria, their daily water intake and urine output were similar to that of diabetic rats and were significantly higher than that of controls. During the 8-wk study period, body weight gain curves showed similar patterns for control and sucrose-fed animals. Diabetic rats did not show any significant weight gain during the 8 wk after administration of STZ (Fig. 1). At the time of decapitation, diabetic animals were significantly smaller, had higher serum glucose levels, and had lower serum insulin levels than age-matched sucrose-fed and control rats. Furthermore, diabetic and sucrose-fed rats had markedly larger bladder domes than control animals (Table 1). Although bladder dome protein concentrations were not different among the three groups, protein contents were significantly higher in bladder domes from diabetic and sucrose-fed rats than in tissues from control rats because of their larger size (data not shown). These features of diabetic and sucrose-fed rats were similar to those reported by other investigators (29,31).

Receptor binding experiments showed the following results. Saturation experiments with [^{3}H]QNB demonstrated greater amounts of muscarinic cholinergic receptors (B_{max})

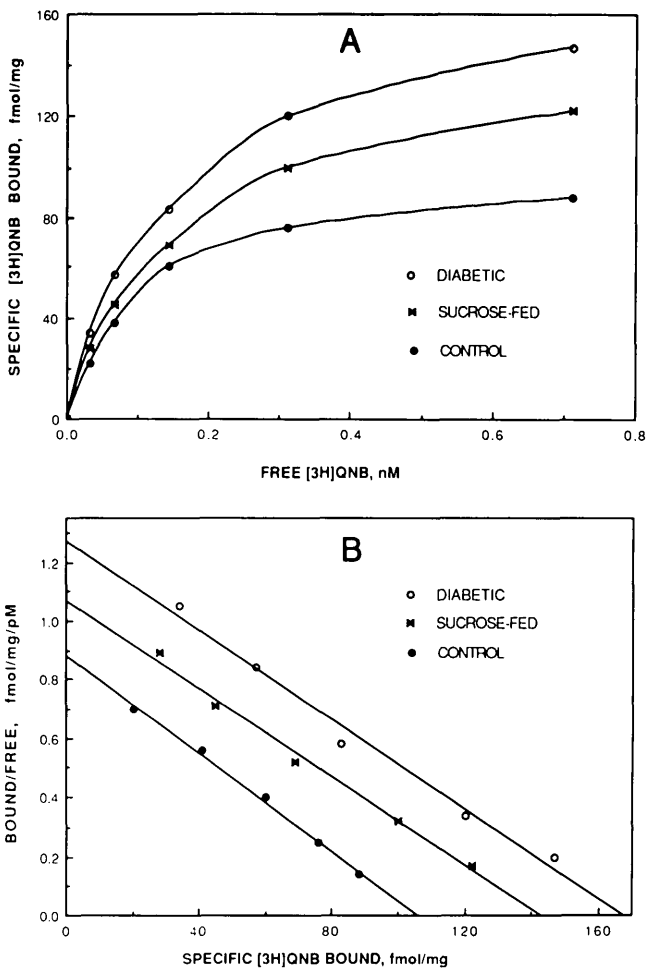

FIG. 2. Saturation curves (*A*) and Rosenthal plot (*B*) of [^{3}H]quinuclidinyl benzilate (QNB) binding to rat bladder dome. Aliquots of membrane particulates were incubated in triplicates with 0.025–0.75 nM of [^{3}H]QNB for 60 min at 23°C. Specific binding was defined in presence and absence of 1 μM atropine.

TABLE 1
General features of experimental animals

	Control	Diabetic	Sucrose fed
Initial body weight (g)	254 ± 4	251 ± 3	258 ± 4
Final body weight (g)	491 ± 10	270 ± 11*	502 ± 7
Bladder dome weight (mg)	103 ± 3	220 ± 12†‡	173 ± 11†
Serum glucose (mM)	7.3 ± 0.2	33.3 ± 1.1*	7.6 ± 0.2
Serum insulin (pM)	137.9 ± 10.8	20.1 ± 1.4*	170.9 ± 13.6

Values are means ± SE for between 20 and 30 determinations/group. Diabetic animals received injection of 65 mg/kg i.v. streptozocin, and sucrose-fed rats received 5% sucrose in their drinking water. Rats were killed after 8 wk.
*$P < 0.001$ vs. control and sucrose fed.
†$P < 0.001$ vs. control.
‡$P < 0.001$ vs. sucrose fed.

in bladder membrane particulates from both diabetic and sucrose-fed rats than from controls. The increase was significantly higher in the diabetic than in the sucrose-fed group (Fig. 2). The differences were significant whether data were normalized on the basis of protein content, tissue wet weight, or whole bladder dome. K_ds for [^{3}H]QNB, however, were similar in all three groups (Table 2).

Parallel saturation experiments with [^{3}H]DHA demonstrated a higher density of β-adrenergic receptors per milligram of protein in diabetic than in sucrose-fed or control bladder membrane particulates (Fig. 3). To ensure the accuracy of the saturation experiments with [^{3}H]DHA, in parallel experiments, we checked the binding of [^{3}H]DHA to inert materials such as glass-fiber filters (data not shown). Bladder domes from sucrose-fed and control rats showed similar B_{max} values for [^{3}H]DHA binding sites per milligram of protein. The differences in the receptor densities between these three groups held when the B_{max} was calculated on the basis of tissue wet weight. However, when the total β-receptors in the bladder dome were calculated, both sucrose-fed and diabetic groups had more β-receptors than controls (Table 2). This was not unexpected, because the control bladder domes were significantly smaller than either sucrose-fed or diabetic bladder domes (Table 1). Because this is the first report, to our knowledge, of labeling rat bladder β-adrenergic receptors with [^{3}H]DHA, we also used another radiolabeled β-receptor antagonist, [^{125}I]PIN, which has a significantly higher specific activity than does [^{3}H]DHA. The higher specific activity of [^{125}I]PIN permitted us to use a smaller amount of tissue for a single binding experiment. The results of saturation experiments with [^{125}I]PIN are shown in Fig. 4 and Table 2. The B_{max} values obtained with [^{125}I]PIN are similar to those obtained with [^{3}H]DHA, confirming the higher densities of β-receptors in diabetic than in sucrose-fed and control bladder domes and indicating the labeling of the same sets of β-receptors with these two radiolabeled β-antagonists. As with [^{3}H]DHA, K_d values for [^{125}I]PIN were similar in all three groups (Table 2).

Regarding the functional experiments, the mean resting stress at the length of optimal force development for these three groups of muscles was similar and ranged from 1.8 to 2.1 g/mm^2. The muscarinic agonist carbachol induced significantly greater maximum contractile responses in diabetic and sucrose-fed than in control bladder domes (Fig. 5). Although carbachol induced larger contractile responses in diabetic than in sucrose-fed bladder domes, the difference

TABLE 2
Binding characteristics of muscarinic and β-adrenergic antagonists to rat bladder dome

	Control	Diabetic	Sucrose fed
[^{3}H]quinuclidinyl benzilate			
B_{max}			
fmol/mg of protein	89 ± 15	195 ± 16*†	140 ± 8‡
fmol/g of tissue	1878 ± 233	3708 ± 443§	3129 ± 271‡
Total receptors in dome (fmol)	195 ± 25	773 ± 123*	502 ± 67‡
K_d (pM)	83 ± 9	85 ± 10	100 ± 12
[^{3}H]dihydroalprenolol			
B_{max}			
fmol/mg of protein	9.3 ± 0.98	14.3 ± 0.9*‖	9.2 ± 0.9
fmol/g of tissue	223 ± 20	291 ± 24†‡	207 ± 21
Total receptors in dome (fmol)	22.4 ± 2.1	53.8 ± 5.3*¶	34.8 ± 3.1‡
K_d (pM)	205 ± 36	140 ± 19	174 ± 20
[^{125}I]iodopindolol			
B_{max}			
fmol/mg of protein	6.1 ± 0.8	11.1 ± 0.6*¶	7.4 ± 0.5
fmol/g of tissue	206 ± 33	327 ± 19†§	251 ± 23
Total receptors in dome (fmol)	23.4 ± 4.3	74.2 ± 7.2*¶	48.6 ± 3.1§
K_d (pM)	42 ± 2	37 ± 1	36 ± 3

Values are means ± SE of 6–9 separate experiments. Diabetic animals received injection of 65 mg/kg i.v. streptozocin, and sucrose-fed rats received 5% sucrose in their drinking water. Rats were killed after 8 wk.
*$P < 0.001$, ‡$P < 0.05$, §$P < 0.01$, vs. control.
†$P < 0.05$, ‖$P < 0.001$, ¶$P < 0.01$, vs. sucrose fed.

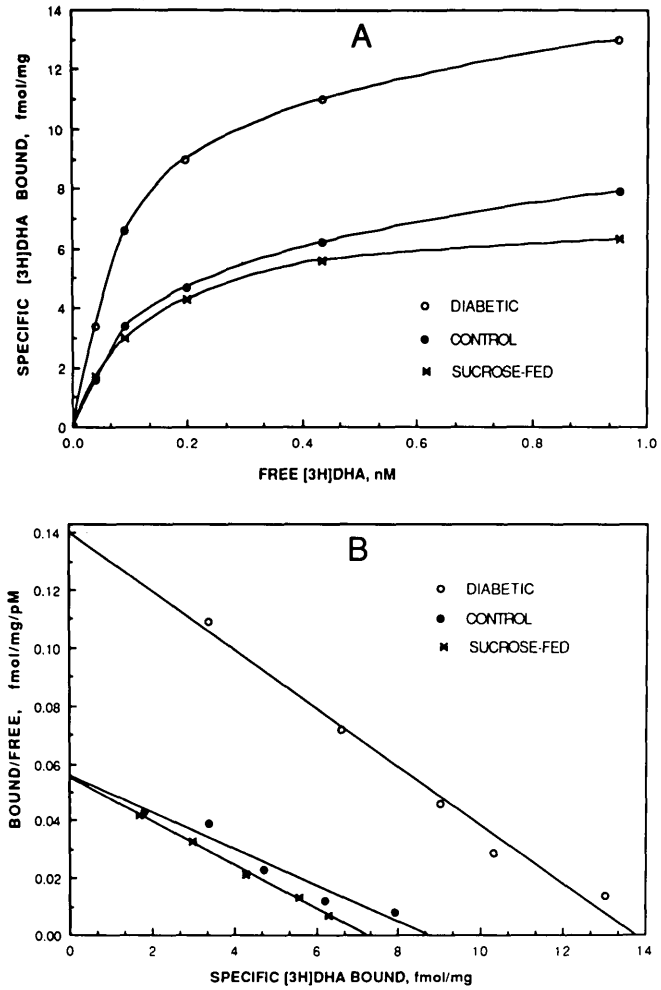

FIG. 3. Saturation curves (*A*) and Rosenthal plot (*B*) of [^{3}H]dihydroalprenolol (DHA) binding to rat bladder dome. Aliquots of membrane particulates were incubated in triplicates with 0.04–1 nM of [^{3}H]DHA for 30 min at 23°C. Specific binding was defined in presence and absence of 1 μM (±)propranolol.

was not statistically significant (Table 3). The ED_{50} values for carbachol were similar in all three groups (Table 3).

The effect of isoproterenol on KCl-contracted muscles is shown in Fig. 6. Isoproterenol induced a greater percentile relaxation of KCl-induced contractions in diabetic and sucrose-fed muscles than in control bladder muscle strips. Furthermore, the relaxation response to isoproterenol was greater in bladder muscle strips from diabetic than from sucrose-fed rats. The ED_{50} values for isoproterenol were significantly smaller in diabetic and sucrose-fed tissues than in control muscle strips (Table 3).

DISCUSSION

In this study, biochemical and functional techniques were used to study the mechanisms involved in the development of vesical dysfunction in diabetes mellitus. Because diabetes-induced diuresis has been implicated as a major factor in the development of bladder dysfunction, we compared bladder domes from STZ-D and sucrose-fed diuretic rats with those from age-matched controls. Our data demonstrated that STZ-D and sucrose-induced diuresis caused an upregulation of bladder dome muscarinic receptors, which was associated with a larger contractile response to the muscarinic agonist carbachol. Although β-adrenergic receptor density was upregulated in the diabetic but not in the sucrose-fed rat bladder domes, the β-agonist isoproterenol relaxed KCl-contracted detrusor strips from both diabetic and sucrose-fed rats to a greater degree and with a higher affinity than detrusor strips from control animals.

Our data showing an upregulation of the muscarinic receptor density, an increase in muscarinic agonist-induced contractile responsiveness, and similar affinity constants suggest that, in diabetes, there is a quantitative rather than qualitative change in the bladder dome muscarinic receptor system. Further support for this contention is provided by the similar affinity constants of various muscarinic agonists and antagonists as determined by biochemical (receptor binding) and functional (contractile responses) studies for both diabetic and age-matched control bladder domes (19). The finding that the density of muscarinic receptors increases in bladder domes of both diabetic and sucrose-fed rats is suggestive of diuresis- and/or hypertrophy-regulated changes in this receptor system. However, the possibility that parasympathetic neuropathy also plays a role in the diabetic pathological process may not be ruled out because

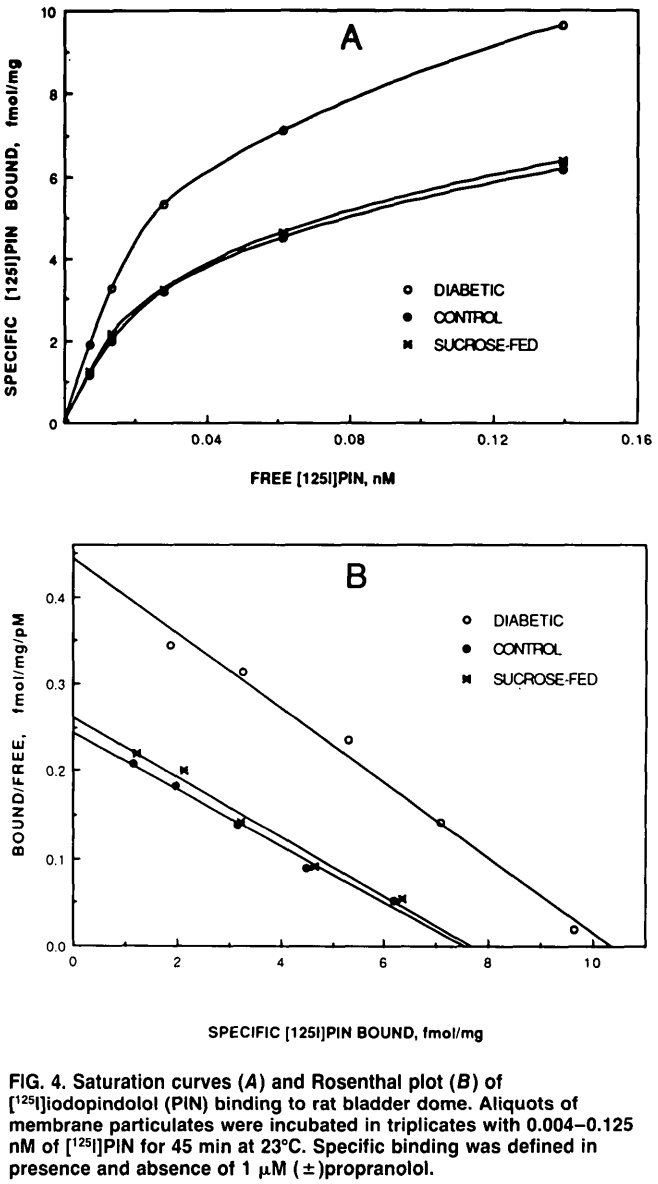

FIG. 4. Saturation curves (*A*) and Rosenthal plot (*B*) of [^{125}I]iodopindolol (PIN) binding to rat bladder dome. Aliquots of membrane particulates were incubated in triplicates with 0.004–0.125 nM of [^{125}I]PIN for 45 min at 23°C. Specific binding was defined in presence and absence of 1 μM (±)propranolol.

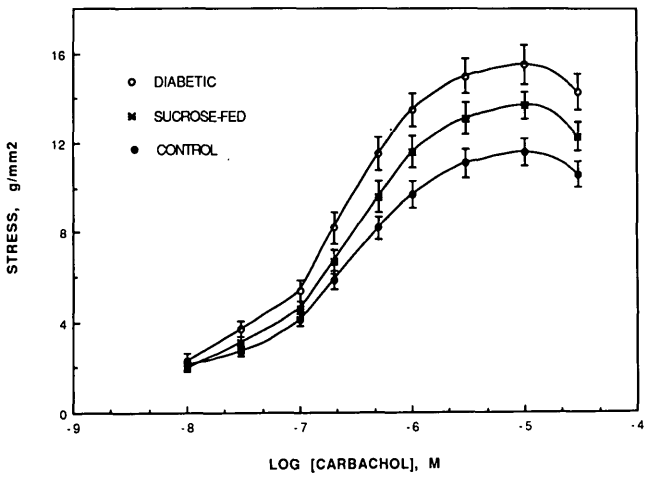

FIG. 5. Concentration-response curves of rat bladder dome muscle strips to carbachol. Muscle strip was stretched to length of optimum force development and equilibrated for 90 min before cumulative administration of agonist. Data points are shown as means ± SE of 18 independent experiments.

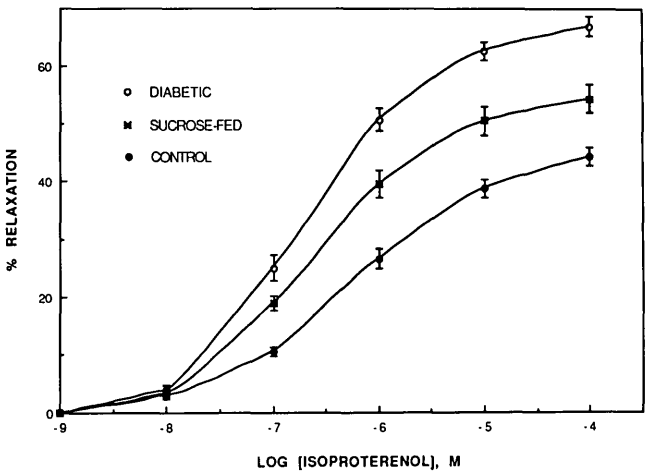

FIG. 6. Concentration-response curves of rat bladder dome muscle strips to isoproterenol. Muscle strip was stretched to length of optimum force development and equilibrated for 90 min, then it was contracted with 80 mM KCl. When tonic force had reached plateau, cumulative concentrations of agonist were added. Data points are means ± SE of 8 independent experiments.

a similar muscarinic receptor upregulation has been reported in the parasympathetic denervation-induced hypertrophied bladder (36).

In a rat whole-bladder preparation, nerve stimulation-induced contractions resulted in lower intravesical pressures in the diabetic and sucrose-fed bladders than in control bladders (28). Because the contractile responses induced by stimulation at 1 Hz were equally enhanced in all three groups by the ganglionic blocking agent tetra-ammonium chloride, it was proposed that prejuctional and/or contractile element activity of both the diabetic and sucrose-fed rat bladders was affected. Santicioli et al. (29), however, reported that, although there was a decrease in the contractile response of STZ-D bladder muscle strips to nerve stimulation, there was a slight increase in the contractile response of bladder muscle strips from sucrose-fed rats to nerve stimulation. They thus suggested that there was an ongoing diabetic bladder neuropathy in the STZ-D animals.

The results of saturation experiments with [^{3}H]DHA and [^{125}I]PIN demonstrated a different pattern of alterations in the properties of β-adrenergic receptors compared with muscarinic receptors. β-Adrenergic receptors were upregulated in the diabetic but not in the sucrose-fed bladder domes. Thus, it appears that, in diabetes, β-receptor density is not regulated by hypertrophy but possibly is influenced by other metabolic disorders such as hypoinsulinemia, hypothyroidism, or insufficient levels of testosterone. These diabetes-induced hormonal derangements have been shown to alter the densities of autonomic receptors in various peripheral tissues (13,14,37). Another possibility is that changes in the β-adrenergic receptors occur as a result of exposure to STZ itself. However, this would appear to be unlikely because insulin administration has been shown to prevent diabetes-induced alterations in β-adrenergic and muscarinic cholinergic receptors in heart, prostate, and bladder dome of STZ-D rats (38–41).

Despite the fact that β-adrenergic receptors were upregulated in the diabetic but not in the sucrose-fed rat bladder domes, the β-adrenergic agonist isoproterenol relaxed KCl-contracted detrusor strips from both diabetic and sucrose-fed rats to a greater degree and with a higher affinity than detrusor strips from control animals. The differences between the functional and receptor binding data may be due to alterations in various receptor-effector components. Furthermore, because K^+-induced contractions in smooth muscle are mediated through voltage-dependent Ca^{2+} channels (42), the activation of ionic channels, i.e., Ca^{2+} channels, by KCl might have modified the interaction kinetics of isoproterenol with its selective site of action, i.e., the β-receptor.

The similarities in the physical properties of bladder domes of diabetic and sucrose-fed animals (29–31) implies that bladder hypertrophy is mainly a diuresis-induced phenom-

TABLE 3
Effects of muscarinic and β-adrenergic agonists on rat bladder dome muscle strips

	Control	Diabetic	Sucrose fed
Carbachol (n = 18)			
E_{max} (g/mm^2)	9.6 ± 0.6	13.1 ± 2.5*	11.8 ± 0.7†
ED_{50} (nM)	330 ± 26	289 ± 33	315 ± 32
Isoproterenol (n = 8)			
E_{max} (% maximum relaxation)	44.2 ± 1.6	66.9 ± 1.7‡§	54.4 ± 6.4*
ED_{50} (nM)	533 ± 53	250 ± 53‡	269 ± 25‡

Values are means ± SE for n experiments. E_{max}, maximum contraction. Diabetic animals received injection of 65 mg/kg i.v. streptozocin, and sucrose-fed rats received 5% sucrose in their drinking water. Rats were killed after 8 wk.
*P < 0.01, †P < 0.05, ‡P < 0.001, vs. control.
§P < 0.01 vs. sucrose fed.

enon. The fact that the enlargement of the bladder domes in diabetic and sucrose-fed rats is accompanied by an increase in the protein content indicates that the weight gain of the bladder dome is not due to water accumulation but is rather a hypertrophic process. However, although sucrose-fed and diabetic groups were shown to have similar water intakes and urine outputs, the diabetic bladder domes were heavier than the diuretic bladder domes; thus, diuresis may not have been the sole cause of bladder hypertrophy. Furthermore, muscarinic receptor densities seem to be regulated by the extent of bladder dome hypertrophy, because the B_{max} values for [^{3}H]QNB binding sites are higher in diabetic than in sucrose-fed bladder domes. Sucrose-fed rats lacked glucosuria, had normal serum glucose and insulin levels, and had similar body weight growth curves as controls. These data suggest an absence of gross metabolic alterations in sucrose-fed animals. Alterations in urine output, water intake, and bladder distension observed in sucrose-fed rats are similar to the early manifestations of diabetes mellitus in humans (3,6).

In conclusion, this study demonstrated that, in rat bladder domes, both STZ-induced diabetes and sucrose-induced diuresis caused upregulation of muscarinic receptors and increased contractile responsiveness to a muscarinic agonist. Furthermore, although diabetes but not sucrose-induced diuresis caused an upregulation in β-adrenergic receptors, the relaxant effects of isoproterenol were increased in bladder strips from both diabetic and sucrose-fed rats. These data suggest that, in diabetic rat bladder dome, muscarinic and β-adrenergic receptors are under different regulatory mechanisms.

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