Streptozocin-induced Diabetes Affects Rat Urinary Bladder Response to Autonomic Agents

MALAK G. KOLTA, LANE J. WALLACE, AND MICHAEL C. GERALD

SUMMARY

The response of the urinary bladder body and base to autonomic agents was studied in streptozocin (STZ)-diabetic rats. The bladder body region from 6-wk diabetic rats showed no changes in response to acetylcholine, phenylephrine, or isoproterenol. In contrast, the bladder base region showed a 39% increase in contractile response to acetylcholine and a 37% increased response to phenylephrine. In tissues from 47-wk diabetic animals, the bladder body showed a 51% increased contractile response to acetylcholine and a 37% increased relaxation response to isoproterenol. The bladder base showed a 66% increased contraction to acetylcholine. Thus, in the bladder base, enhanced responses to acetylcholine are detected soon after induction of diabetes and continue to increase as the diabetic state progresses. Moreover, in the same bladder region, an increase in responsiveness to alpha-adrenergic stimuli occurs. In the bladder body, enhanced responses to cholinergic and to beta-adrenergic stimuli occur, but are only observed in a more chronic diabetic state. The data suggest that an effect associated with autonomic diabetic neuropathy of the urinary bladder is an increased postsynaptic responsiveness to cholinergic stimuli in both regions.

DIABETES 1985; 34:917–21.

Neuropathies of the autonomic nervous system as a complication of diabetes mellitus have been well recognized since the early studies of Jordan and Crabtree[1] and Rundles.[2] Such abnormalities cause, among a wide range of symptoms, disturbances of vascular reflexes and cardiac rhythm, visual blurring, abdominal cramps, pain, sexual impotency, and dysfunction of the urinary bladder.[3] The earliest abnormality in the urinary system of a diabetic subject appears to be a loss of bladder sensation and poor control of the tone of the bladder base (neck).[4] This, however, occurs without impairment of efferent motor function, resulting in a hypotonic, large-capacity bladder that, nevertheless, empties completely.[2,5] As the diabetic condition becomes more severe and chronic, a complete bladder paralysis develops, resulting in urine retention and overflow incontinence. This situation sometimes leads to infection resulting from bacterial contamination of the residual urine.[6]

The lower urinary tract is innervated and functionally regulated by both divisions of the autonomic nervous system.[7–9] Activation of the parasympathetic motor fibers to the bladder[10] causes an intense stimulation of the muscarinic receptors in the bladder body, resulting in a strong and efficient bladder contraction causing emptying of the bladder. By contrast, during the urine storage phase, sympathetic nerve activity acting on beta-adrenergic receptors relaxes the bladder body while simultaneously acting on alpha-adrenergic receptors to prevent urine passage through the bladder base.[8] We hypothesized that one aspect of urinary bladder dysfunction in chronic diabetes might be an altered response of the organ to autonomic stimuli. This paper reports the effects of muscarinic, alpha-adrenergic, and beta-adrenergic agonists in the body and the base of urinary bladders from diabetic rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats were 14 wk of age and weighed 250–300 g at the beginning of the experiments. Animals were housed in groups of four per cage at a controlled temperature of 23 ± 1°C with a lighting schedule of 12 h of light and 12 h of dark (light on at 0600 h). Purina lab chow and water were provided ad libitum. After a period of not less than 14 days after arrival in our laboratory, diabetes was induced by a single injection of streptozocin (STZ, 40 mg/kg, i.v.). The success of the treatment was determined by measuring blood glucose 5 days later. STZ-treated animals with blood glucose levels of <300 mg/dl were eliminated from the ex-

From the Division of Pharmacology, College of Pharmacy, Ohio State University, 500 W 12th Avenue, Columbus, Ohio 43210.
Address reprint requests to L. J. Wallace at the above address.
Received for publication 23 July 1984 and in revised form 24 December 1984.

TABLE 1
Comparison of short and long duration of diabetes

	6-wk experiment		47-wk experiment	
	Control	Diabetic	Control	Diabetic
Body wt (g)				
Initial	262 ± 2	275 ± 8	281 ± 7	273 ± 4
Final	459 ± 11	240 ± 23*	568 ± 25	263 ± 28*
Plasma glucose (mg/dl)	119 ± 4	468 ± 23*	118 ± 7	405 ± 18*
Weight of bladder body (mg)	169 ± 13	272 ± 26*	143 ± 9	269 ± 24*
Urine volume (ml)	0.14 ± 0.03	1.0 ± 0.13*	0.24 ± 0.03	0.95 ± 0.08*

Animals were made diabetic by a single injection of STZ (40 mg/kg, i.v.) and maintained for the indicated durations before analysis. Data shown are means ± SEM of data obtained from five rats.
*Significantly different from corresponding control, $P < 0.01$.

periments. Control animals received an equivalent value of the STZ vehicle, were housed under identical conditions as the diabetic animals, and served as a baseline for any age-related changes occurring in the absence of the diabetic state.

At the end of either 6 wk or 47 wk of diabetes, animals were killed by decapitation. The abdomen was opened, and the amount of urine retained in the bladder was determined after collection by inserting a catheter through either ureter. For experiments using an intact, whole-bladder preparation,[11] the organ was dissected free and suspended with 1.5-g resting tension in a 10-ml organ bath containing a physiologic saline solution of the following composition (mM): NaCl, 120; KCl, 5.4; $CaCl_2$, 1.2; $NaHCO_3$, 15.5; NaH_2PO_4, 1.2; glucose, 11.5; and 0.02 μg/ml physostigmine (eserine) sulfate. In other experiments studying bladder regions, the bladder body was dissected with a lateral cut at the entry of the ureters. The bladder base was excised with a second cut made at the level of the pelvis. The bladder body and base were suspended with 1.5- and 0.3-g resting tension, respectively, in organ baths as described above. Baths were maintained at 37°C and were aerated with 95% O_2 plus 5% CO_2. Tissues were washed every 10 min after mounting and allowed to equilibrate for a minimum of 45 min before the addition of drugs. For each tissue, the responses to acetylcholine, phenylephrine, and isoproterenol were determined sequentially. Tissues were pretreated with the beta- and alpha-adrenergic receptor antagonists propranolol or phentolamine (1 μM), respectively, 2 min before the cumulative addition of phenylephrine or isoproterenol, respectively. After the completion of concentration-response curves for all three autonomic agents, tissues were allowed to relax for about 90 min, with the bath fluid replaced every 10 min. At the end of each experiment, 0.3 M KCl was added as a nonspecific agent to induce maximum contraction of the tissue. Effects of the drugs were normalized to the KCl-induced maximum responses. Tissues from diabetic and control animals were always run in parallel experiments. Results were plotted, and the maximum response (E_{max}) and concentration of agonist producing a response equal to half of the maximum (ED_{50}) were determined from the graphs. Means for E_{max} and ED_{50} were calculated from results obtained from five separate bladders. Differences between means of treated and control experiments were analyzed using the Student *t*-test.

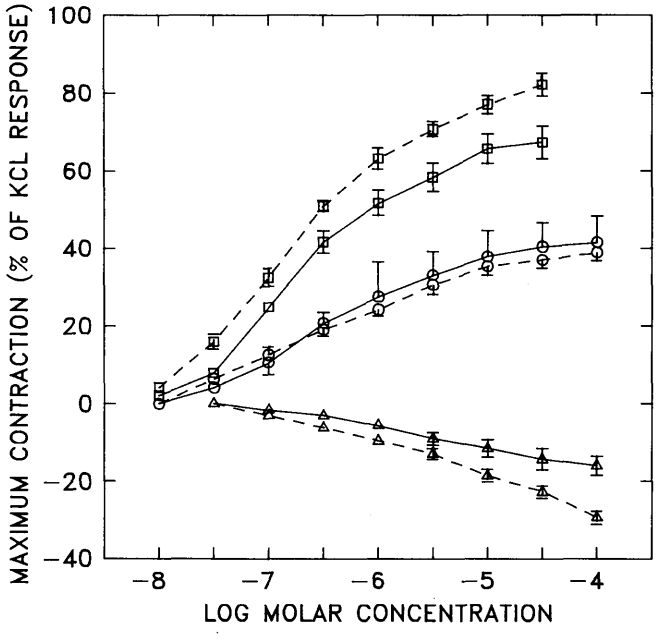

FIGURE 1. Effect of autonomic agonists on isolated, intact bladder. Isolated, intact bladders from control and 6-wk diabetic rats were examined for response to autonomic agonists as described in the text. Data are expressed as means ± SEM from five bladders. Dashed lines show data from diabetic animals, solid lines data from controls. Autonomic agents used were acetylcholine (□), phenylephrine (○), and isoproterenol (△).

RESULTS

Characteristics of the animals and tissues used in the experiments are shown in Table 1. The marked loss of body weight, increase in plasma glucose, increase in weight of the bladder body, and increased volume of retained urine in the diabetic animals are evident.

Whole, intact bladders from 6-wk-old diabetic rats and their corresponding controls were tested for response to acetylcholine, phenylephrine, and isoproterenol (Figure 1). While the maximum contractile responses of the whole bladders to the nonspecific agent KCl were equivalent (9.3 ± 0.9 and 10.5 ± 0.9 mm) for the control and diabetic rats, respectively, the maximum response to acetylcholine-induced contractions was 22% greater ($P < 0.05$) and the relaxation response to isoproterenol was 1.75-fold greater ($P < 0.01$) in bladders obtained from diabetic rats than in those from control animals. With intact bladders, no significant differences between diabetic and control states were observed in responses to phenylephrine.

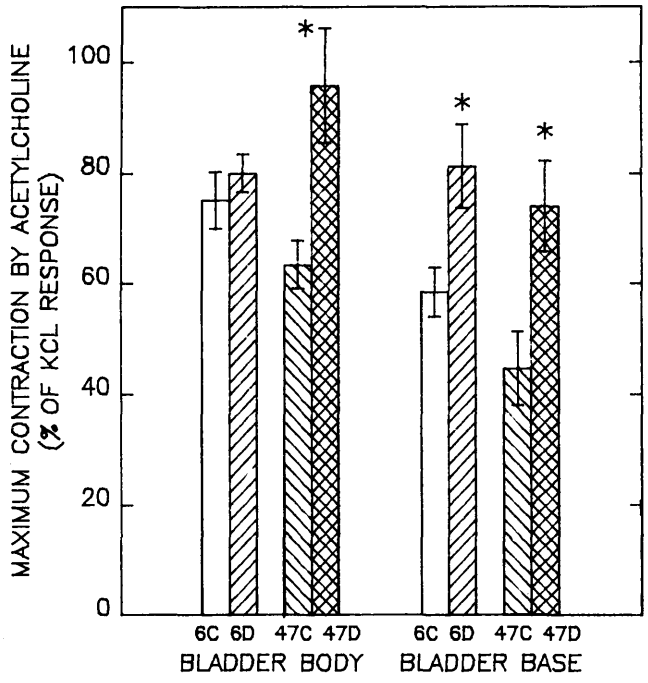

FIGURE 2. Effect of acetylcholine on the maximum contractile response of the bladder body and base from diabetic rats. E_{max} was determined from concentration-response curves as described in the text. Conditions were: 6C, 6-wk controls; 6D, 6-wk diabetic animals; 47C, 47-wk controls; and 47D, 47-wk diabetic animals. Data are means ± SEM from five bladders. *Indicates significantly different from age-matched control, $P < 0.025$.

Since the bladder body and base differ in morphology, embryologic origin, autonomic receptor distribution, and function, the effects of the diabetic state on bladder response to autonomic agents were more thoroughly investigated by analyzing the two major bladder regions at both 6 and 47 wk postinduction of diabetes. Diabetes had no significant effect on the magnitude of KCl-induced contractions in either bladder region. The ranges of the mean KCl responses for the bladder body were 10.1–12.8 and 6.7–7.8 and for the neck were 12.0–12.3 and 9.0–9.9 mm for 6- and 47-wk groups, respectively.

Six weeks of diabetes was associated with a 39% increase in the maximum contraction induced by acetylcholine in the bladder base but no significant change in the bladder body (Figure 2). By 47 wk of diabetes, the maximum contraction was 51% and 66% increased over control values in the body and base regions, respectively (Figure 2).

With phenylephrine, the bladder base showed a 37% increase in maximum response 6 wk postinduction of diabetes (Figure 3). The response of tissues from control animals to this alpha-adrenergic agonist increased (65%) in the bladder base and decreased (69%) in the bladder body during the 47-wk experiment. There were no differences in response to phenylephrine when comparing long-term diabetic animals and their age-matched controls (Figure 3).

While there were no significant changes after 6 wk of diabetes, the relaxation induced by isoproterenol was 37% greater in the bladder body of 47-wk diabetic animals than in that of their age-matched controls (Figure 4). During the 47-wk experiment, however, bladder regions from control animals increased in the relaxation responses to the beta-adrenergic agonist by 182% and by 79% in bladder body and base, respectively (Figure 4).

DISCUSSION

Experimental diabetes induced by either alloxan or STZ is often used as an animal model of diabetes in humans.[12] In our experiments, the 6 wk of STZ-induced diabetes caused a 60% increase of the bladder body weight and more than a sevenfold increase in urine retained in the bladder (Table 1). Distension of the urinary bladder is one of the first abnormalities appearing in this organ in diabetic humans.[13–15] Initially, the enlarged bladder retains the capacity for efficiently expelling urine, but as the disease progresses this capacity is lost, and urine retention occurs.[13,15–18] The distension observed in our experiments could result either from a decreased to-void sensation coupled with an increased capacity of the bladder or from an inability to expel urine. Determination of which mechanism applies to the rat bladder involves in vivo experiments beyond the scope of the present study. However, the available data do suggest that the urinary bladder from diabetic rats might be a suitable model for studying mechanisms that contribute to urinary problems in diabetic humans.

The maximum contraction elicited by KCl was not affected by induction of diabetes, even though the bladder weight was greater in the treated animals. This suggests that the increased bladder weight results from increases in components other than functional smooth muscle. The greatly distended diabetic bladder relaxed when urine was removed so that, in the tissue bath, whole bladders and bladder re-

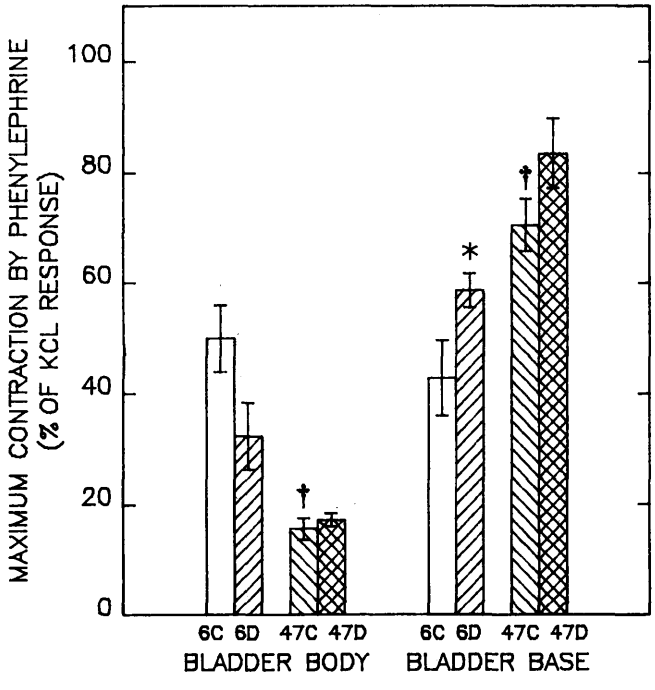

FIGURE 3. Effect of phenylephrine on the maximum contractile response of the bladder body and base from diabetic rats. E_{max} was determined from concentration-response curves as described in the text. Conditions were: 6C, 6-wk controls; 6D, 6-wk diabetic animals; 47C, 47-wk controls; and 47D, 47-wk diabetic animals. Data are means ± SEM from five bladders. *Indicates significantly different from age-matched control, $P < 0.025$. †Indicates significantly different from 6-wk control, $P < 0.025$.

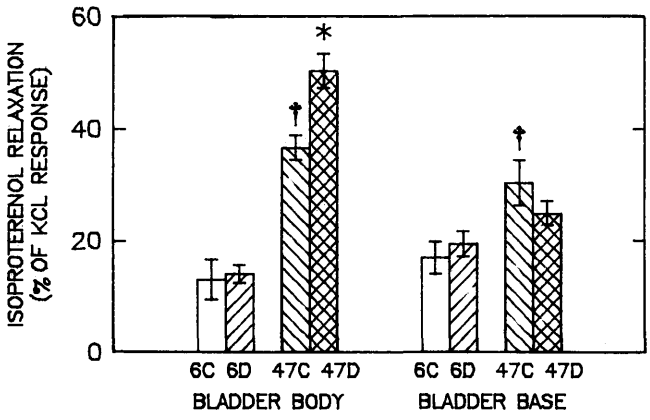

FIGURE 4. Effect of isoproterenol on the maximum relaxation of the bladder body and base from diabetic rats. E_{max} was determined from concentration-response curves as described in the text. Conditions were: 6C, 6-wk controls; 6D, 6-wk diabetic animals; 47C, 47-wk controls; and 47D, 47-wk diabetic animals. Data are means ± SEM from five bladders. *Indicates significantly different from age-matched control, $P < 0.025$. †Indicates significantly different from 6-wk control, $P < 0.025$.

gions from diabetic and control animals were of approximately the same length. The increased weight of the diabetic bladder appears to result from an increased thickness of bladder walls rather than from increased length or width of the organ.

Since experiments involving the chronic, uncontrolled diabetic state were carried out for nearly 11 mo, it was conceivable that changes in bladder function resulting from the normal aging process could have occurred during this time. Therefore, control animals were maintained in parallel for this time period under identical environmental conditions as the diabetic animals. When comparing animals for the 47-wk experiment to those for the 6-wk experiment, a 65% increase in maximum response to phenylephrine was observed in the bladder base and a 69% decrease in this parameter in the bladder body (Table 2). Age-related increases (2–3-fold) were observed in the maximum responses to isoproterenol in both regions. The large age-related changes in the adrenergic agonist effects on bladder regions suggest that sympathetic control of this region might be minimal before maturity and may increase markedly as animals advance in age.

The changes observed in tissues from diabetic animals occurred mainly in the response to acetylcholine (Table 2). Relative to the age-matched controls, the maximum contractile response increased by 39% in the bladder base at 6 wk after diabetes onset. The responsiveness was further increased to 51% and 66% greater than control in the bladder body and neck, respectively, after 11 mo of diabetes. The increased responsiveness to acetylcholine is consistent with the observation that the diabetic bladder still responds to the muscarinic agonist bethanechol.[13] In contrast with the acetylcholine results, the adrenergic agonists produced only regionally specific changes in either short-term or chronic diabetes relative to their age-matched controls. These results compare well with the recent finding of Lincoln et al.,[19] which shows a trend toward increased response to acetylcholine in the bladder body after 8 wk of STZ-induced diabetes; longer durations of the diabetic state were not examined in this study. Thus, diabetes appears to have a much greater effect on postsynaptic actions of the parasympathetic nervous system than on the sympathetic nervous system, and the changes induced by diabetes occur earlier in the bladder base than in the bladder body.

If the hypothesis proposed by Khanna et al.[20] that muscarinic-stimulated contraction of either the bladder body or base facilitates the expulsion of urine is valid, then the increases in contractile response observed in the present study should result in the more efficient elimination of urine in the diabetic animals. While this prediction conflicts with the observed retention of urine, it also assumes the existence of a normally functioning presynaptic parasympathetic nervous system. A loss of function of the parasympathetic efferents to the bladder might result in urine retention. The typical postsynaptic (i.e., receptor) response to loss of nerve input is an increased ability to respond to the remaining activity. Our observations of augmented contractile responses would be compatible with the development of an increased postsynaptic response that compensates for diminished presynaptic activity. However, the experiments reported in this study were not designed to investigate the functioning of presynaptic nerves. Additional complications in relating the present observations to in vivo bladder function are presented when one considers that other regulators, such as the noncholinergic nonadrenergic contractile stimuli, may also be affected by diabetes as well as by spinal and supraspinal influences.

TABLE 2
Changes in the response of the bladder body and base of diabetic rats to autonomic agents

Region	Autonomic agent	6-wk experiment		47-wk experiment	
		Control	Diabetic	Control	Diabetic
Bladder body	Acetylcholine	100	106	84	127‡
	Phenylephrine	100	65	31†	34
	Isoproterenol	100	108	282†	387‡
Bladder base	Acetylcholine	100	139*	76	126‡
	Phenylephrine	100	137*	165†	195
	Isoproterenol	100	115	179†	147

E_{max} of concentration-response curves was determined for control and diabetic rats of a 6-wk and a 47-wk experiment. The response of the control for the 6-wk experiment was assigned a value of 100, and all other results were normalized to this response.
Statistics were performed using means of the original untransformed data from five animals. *6-wk diabetic are significantly different from 6-wk control, †47-wk control are significantly different from 6-wk control, and ‡47-wk diabetic are significantly different from 47-wk control. Level of significance was set at $P < 0.025$.

In summary, rats made diabetic with STZ appear to be a good model for examining peripheral mechanisms of urinary bladder dysfunction associated with diabetes. We can conclude from our data that an effect of the disease on autonomic control of urinary bladder appears to be a marked increase in contractile response to acetylcholine in both bladder regions. By contrast, the response to alpha- or beta-agonists is regionally specific.

ACKNOWLEDGMENT

This work was supported in part by USPHS grant no. AG04622.

REFERENCES

[1] Jordan, W. R., and Crabtree, H. H.: Paralysis of bladder in diabetic patients. Arch. Intern. Med. 1935; 55:17–25.

[2] Rundles, R. W.: Diabetic neuropathy—general review with report of 125 cases. Medicine 1945; 24:111–60.

[3] Brown, M. R., Dyck, P. J., McClean, G. E., Sima, A. A. F., Powell, H. C., and Porte, D.: Central and peripheral nervous system complications. Diabetes 1982; 31:65–70.

[4] Giachetti, A.: Diabetic neuropathies: pathogenetic mechanisms and therapeutic perspectives. Pharmacol. Res. Commun. 1981; 13:101–109.

[5] Ellenberg, M.: Diabetic neurogenic vesical dysfunction. Arch. Intern. Med. 1966; 117:348–54.

[6] Buck, A. C., McRae, D. U., Reed, P. I., and Chisholm, G. D.: The diabetic bladder. *Proc. R. Soc. Med.* 1974; 67:81–83.

[7] Crane, D. B.: The urinary bladder; micturition. *In* Urology in Practice. Devine, C. J., and Stecker, J. F., Eds. Boston, Little and Brown, 1978:55–66.

[8] Bissada, N. K., and Finkbeiner, A. E.: Neuropharmacology of the lower urinary tract. *In* Pharmacology of the Genitourinary Tract and Male Reproductive System. Finkbeiner, A. E., Barbour, G. L., and Bissada, N. K., Eds. New York, Appleton-Century-Crofts, 1982:199–216.

[9] Elmer, M.: Cholinergic mechanisms in the rat bladder body muscle. Acta Pharmacol. Toxicol. 1978; 43:63–68.

[10] Bradley, W. E., Timm, G. W., and Scott, F. B.: Cystometry. II. Central nervous system organization of detrusor reflex. Urology 1975; 5:578–80.

[11] Hukovic, S., Rand, M. J., and Vanov, S.: Observations on an isolated, innervated preparation of rat urinary bladder. Br. J. Pharmacol. 1965; 24:178–88.

[12] Mordes, J. P., and Rossini, A. A.: Animal models of diabetes. Am. J. Med. 1981; 70:353–60.

[13] Schiff, H. I.: The neurogenic bladder in diabetes. NY State J. Med. 1982; 82:922–26.

[14] Buck, A. C., Reed, P. I., Siddiq, Y. K., Chisholm, G. D., and Fraser, T. R.: Bladder dysfunction and neuropathy in diabetes. Diabetologia 1976; 12:251–58.

[15] Clarke, B. F., Ewing, D. J., and Campbell, I. W.: Diabetic autonomic neuropathy. Diabetologia 1979; 17:195–212.

[16] Ellenberg, M.: Development of urinary bladder dysfunction in diabetes mellitus. Ann. Intern. Med. 1980; 92:321–23.

[17] Faerman, I., Maler, M., Jadzinsky, M., Alvarez, E., Fox, D., Zilbervar, G. J., Cibeira, J. B., and Colinas, R.: Asymptomatic neurogenic bladder in juvenile diabetics. Diabetologia 1971; 7:168–72.

[18] Kahan, M., Goldberg, P. D., and Mandel, E. E.: Neurogenic vesical dysfunction and diabetes mellitus. NY State J. Med. 1970; 2:2448–55.

[19] Lincoln, J., Crockett, M., Haven, A. J., and Burnstock, G.: Rat bladder in the early stages of streptozotocin-induced diabetes: adrenergic and cholinergic innervation. Diabetologia 1984; 26:81–87.

[20] Khanna, O. P., Barbieri, E. J., Altamura, M., and McMichael, R.: Vesicourethral smooth muscle: function and relation to structure. Urology 1981; 18:211–18.