

Recent advances in understanding the biology of diabetes-associated bladder complications and novel therapy

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Accepted for publication 12 October 2004

KEYWORDS

diabetes mellitus, detrusor, smooth muscle, nerve growth factor, urothelium

INTRODUCTION

Diabetes mellitus (DM) is at epidemic proportions and becoming a major problem in the USA. According to the Centers for Disease Control and Prevention, 18 million people in the USA have DM and the prevalence of DM increased from 4.9% in 1990 to 7.3% in 2000 [1]. Urological complications have increasingly become a concern in those affected by DM (both Type I and II). More than a quarter of diabetic patients will develop costly and debilitating urological complications, e.g. incontinence, infections, loss of sensation and retention of urine. The total annual cost of diabetes in 1997 has been estimated at more than \$98 billion (<http://www.diabetes.org>).

In addition to diabetic bladder dysfunction, there is a greater incidence of asymptomatic and symptomatic bacteriuria, which can progress to kidney infection and kidney damage. This increase in infection has been attributed to numerous causes, from incomplete bladder emptying to changes in bladder wall components and immune dysfunction. A confounding factor for all basic studies on the bladder is the lack of published data on the urothelial cell, vascular, neurological and smooth muscle function, and interactions in bladder tissue from nondiabetic sources that can be used for comparison with the diabetic.

An important question is whether bladder dysfunction is secondary to an inherent neuropathology induced by diabetes, or caused by changes associated with bladder

overdistension. Many animal models have been used to elucidate this and other questions associated with diabetic cystopathy. Streptozotocin (STZ)-induced diabetic rats and sucrose-drinking rats (sucrose induces a polyuria similar to that seen in diabetic patients) have generally been used. Paro *et al.* [2] noted that alloxan-induced diabetic rats had decreased and irregular contractions, while sucrose-fed rats had normal bladder contractions. This suggests that in alloxan-induced DM, contractile dysfunction is secondary to an inherent diabetic cystopathy, while bladder hypertrophy in sucrose-fed rats is an organ adaptation to polyuria. Other differences between STZ-induced diabetes and sucrose-induced bladder distension include a decrease in noradrenaline uptake and in choline acetyltransferase activity [3], and cystometrographic and supraspinal reflex latencies between the groups [4].

Clinically, the diagnosis of diabetic cystopathy is most readily made with urodynamic testing [5,6]. The most common urodynamic findings include elevated residual urine volume, impaired bladder sensation, involuntary detrusor contractions, increased cystometric capacity and decreased bladder contractility. Cystometry may show detrusor areflexia, which is usually found in patients with an impaired sensation of bladder filling [7–9]. Detrusor overactivity is also common in patients with DM [10]. Other aspects of the severity of DM, e.g. duration, glycaemic control and microvascular complications resulting in damage to innervation of the bladder, have been suggested as possible mechanisms for incontinence [11,12].

PATHOPHYSIOLOGY

The biology of DM-associated bladder complications is multifactorial and they can

be a result of an alteration in the physiology of the detrusor smooth muscle cell, the innervation or function of the neuronal component, or urothelial dysfunction (Fig. 1). The experimental model most often used to assess bladder complications is the STZ rat model. As bladder smooth muscle contraction is mediated by acetylcholine released by the pelvic nerve acting on muscarinic receptors, a series of pharmacological studies have focused on the impact of STZ-DM on the responsiveness of bladder strips to externally applied muscarinic agonists. Neuronal dysfunction may reflect a deficiency of axonal transport of nerve growth factor (NGF) and be important in inducing diabetic neuropathy [13–15]. The urothelium undergoes changes in DM; thus, in the STZ-induced DM rat model, there are progressive increases in total bladder tissue, with hypertrophy of the bladder wall and dilatation of the bladder [16,17]. Both smooth muscle and urothelium have been shown to increase significantly with time. Thus there is strong evidence that DM adversely affects the bladder smooth muscle, nerves and the urothelium (Fig. 1).

DM AND DETRUSOR SMOOTH MUSCLE FUNCTION

DM has been shown to alter detrusor smooth muscle function in experimental animals, with the vast majority of these studies conducted on the STZ rat model. However, because there are no longitudinal studies conducted under similar experimental conditions, there is still uncertainty about the time course, magnitude and mechanism of DM-related changes in detrusor smooth muscle cell function.

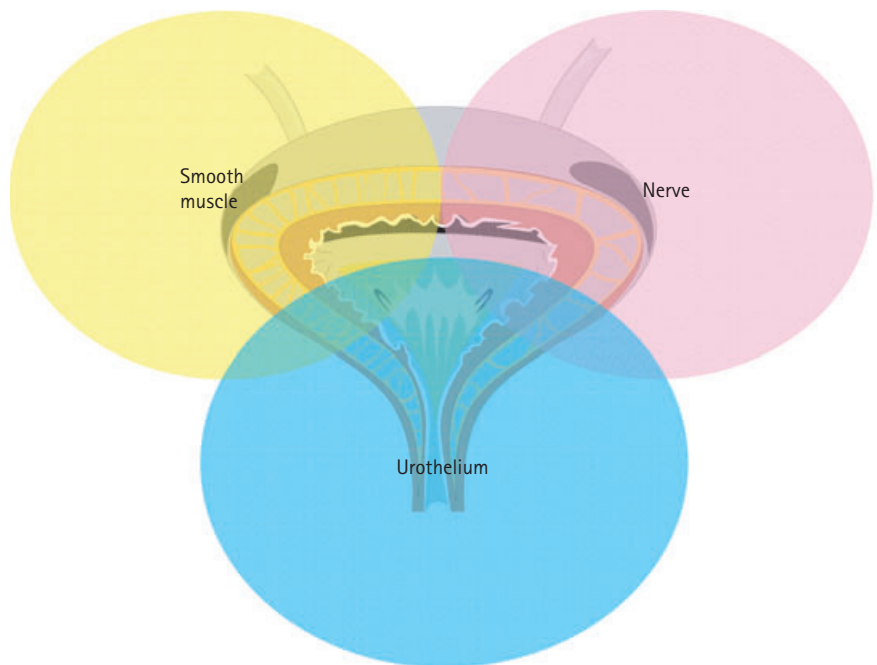
STZ-DM: Pharmacological studies on isolated bladder strips have generated much confusion. While there are generally changes in isolated detrusor smooth muscle cell strips

there is no agreement on either the phenomenon or the mechanism. For example, several studies documented an increase in responsiveness of DM bladder strips to externally applied muscarinic agonists [17,18] but others reported a decrease or no change in the muscarinic component [19]. There was an increase in muscarinic receptor density at both 2 and 8 weeks after STZ-induced DM [20]. A recent study found an increase in the β_1 -receptor-mediated relaxation response in isolated detrusor smooth muscle strips from 8–10 week STZ-DM rats [21]. Moreover, there was an increased contractile response to 5-hydroxytryptamine from 4-week STZ-DM rats.

One DM-related change that most experts agree on is an increased responsiveness of isolated rat bladder strips to electrical field stimulation (EFS) [22,23]. However, there is no consensus on the putative mechanism for this increased responsiveness to EFS. Theories include that the increased response to EFS is caused by DM-related changes in membrane lipid composition or other destabilizing membrane changes, or increased neurotransmitter release [24]. Belis *et al.* [25] suggested that the changes are related to increased calcium-channel activity, while Waring and Wendt [23] found no evidence for altered calcium regulation, and therefore suggested that the increased responsiveness may be a result of enhanced calcium sensitivity. Most recently, Bezuijen *et al.* [26] reported that decreased function was more notable in strips from diabetic rats with enlarged bladders. This does not elucidate the mechanism, but could explain some of the observed variability from previous studies. In addition, this same group recently showed that DM increases the rate of development of at least some aspects of bladder decompensation in rats with partial urethral outlet obstruction [27]. Such observations further highlight the multifactorial nature of diabetic cystopathy, and the potential array of causal mechanisms and clinical symptoms that might be apparent in an ageing population.

Hashitani and Suzuki [28] found increased depolarization of myocytes in STZ-DM rat bladder strips on applying acetylcholine, indicating enhanced muscarinic sensitivity in the diabetic bladder. They further noted decreased spontaneous electrical activity in the myocytes, presumably related to altered purinergic transmission. These observations are consistent with the effects generally

FIG. 1. Three important aspects of the diabetic cystopathy that may overlap.



associated with a decrease in neuronal transmitter release.

Poladia and Bauer [29] studied the changes in nitric oxide synthase (NOS) and reactive nitrogen species formation during DM-related bladder remodelling, using the STZ-DM rat model. They found early, time-dependent and cell-specific changes in the three isoforms of NOS, and region-specific increases in protein nitration. Endothelial NOS was significantly up-regulated in the lamina propria, neuronal NOS in the urothelium, lamina propria and in the smooth muscle layer, whereas inducible NOS was up-regulated only in the urothelium. They suggested that changes in NO production and impaired NO control are early events in diabetic cystopathy, and that mechanisms leading to increased oxidative stress and proteasomal activation may be key participants leading to organ dysfunction.

BB/W rat: There are only a few published studies with the BB/W rat diabetic model [14,22]. As with the STZ-rat model, the diabetic BB/W rat has the expected *in vivo* phenotypic characteristics, e.g. decreased overall body weight, and corresponding increases in voiding volumes and voiding frequency. From a mechanistic standpoint, Longhurst [30] reported an apparent absence

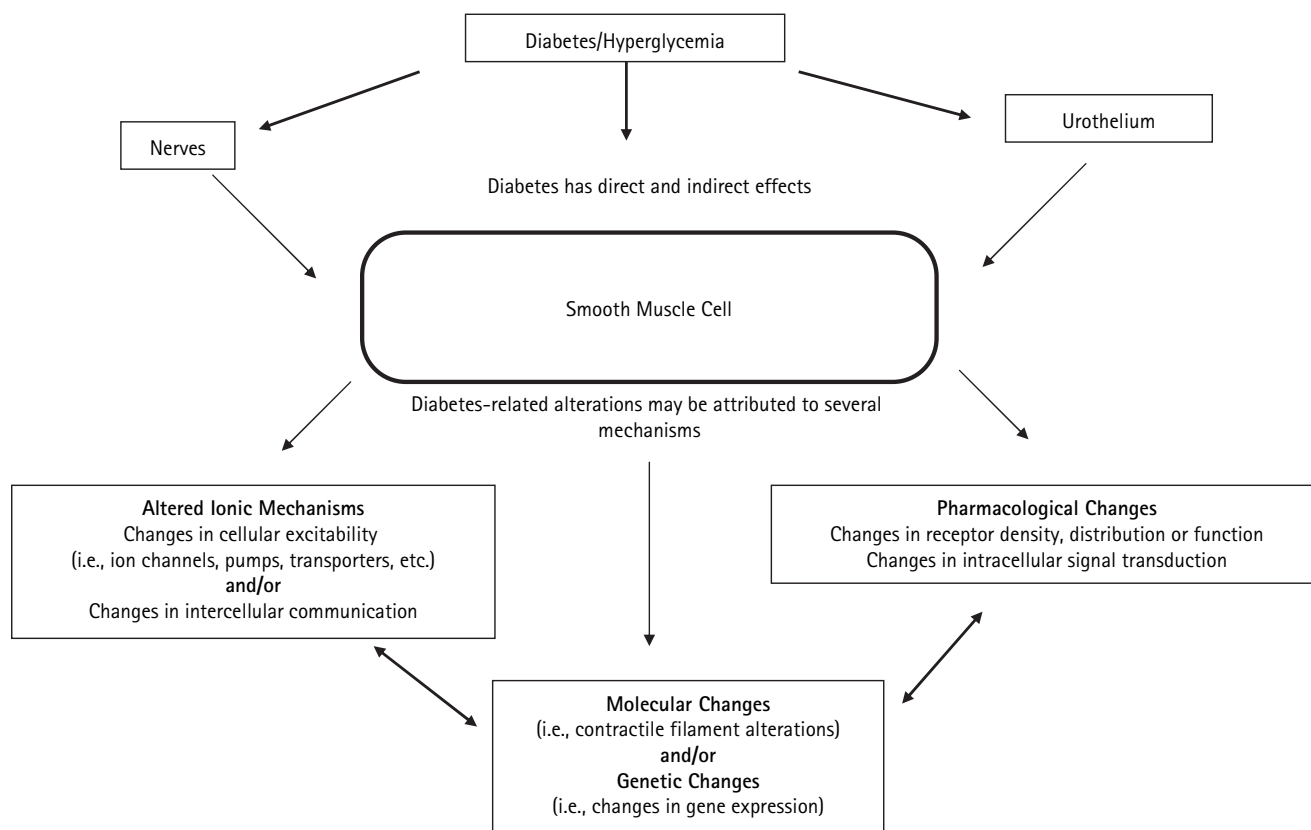
of detectable effects of 6 months of DM in the BB/W rat on the pharmacology of isolated detrusor (i.e. bladder body) strip contractions. However, there were modest but statistically significant decreases in the sensitivity and magnitude of carbachol and ATP-induced contractions of detrusor strips when the data were normalized for tissue weight.

Given that motility disorders are an important component of diabetic cystopathy, it will be critical to more precisely determine the nature, time course, magnitude and mechanism for these changes (Fig. 2). Elucidating the contribution of detrusor myocytes to diabetic bladder disease will be important to the improved understanding, diagnosis and treatment of diabetic cystopathy. To do so will require multidisciplinary longitudinal studies in both man and experimental animals, in which the extent of DM is well characterized, and the effects of DM on bladder function *in vivo* documented.

NEURONAL DYSFUNCTION IN DM

Although the pathogenesis of diabetic neuropathy is not fully clarified, it is generally accepted that the cause of diabetic neuropathy is multifocal. Some of the

FIG. 2. Effects of DM on detrusor smooth muscle function.



proposals for pathogenesis include altered metabolism of glucose, ischaemia, superoxide-induced free-radical formation and impaired axonal transport [31]. It is also known that the neuropathies of DM caused by the metabolic derangement of the Schwann cell result in segmental demyelination and impairment of nerve conduction. This gradual process of segmental demyelination has been confirmed histologically in the bladder and is consistent with the observed impairment of nerve conduction of the visceral afferent fibres within the bladder wall. Van Poppel *et al.* [32] reported that there was less acetylcholinesterase activity in bladder biopsy specimens from patients with severe insulin-dependent DM than in normal controls.

The deficiency of axonal transport of NGF may be important in inducing DM neuropathy, which contributes to DM cystopathy [2,13]. Sasaki *et al.* [33] recently reported, using STZ-DM rats, the relation between bladder function and NGF levels in the bladder and lumbosacral dorsal root ganglia (DRG), which contain afferent neurones innervating the bladder, and the feasibility of NGF gene

therapy for treating DM cystopathy [34] (Fig. 3).

Using STZ-DM rats (65 mg/kg, intraperitoneal) the effects of DM and gene therapy, using replication-defective herpes simplex virus (HSV) vectors encoding the NGF gene (HSV-NGF) injected into the bladder wall, were assessed on A δ afferent fibre-dependent conscious voiding and C-fibre-mediated bladder nociceptive responses. This was done using metabolic cage/awake cystometry and cystometry with intravesical instillation of 0.25% acetic acid under urethane anaesthesia, respectively. In addition, NGF levels in the bladder and L6-S1 DRG were measured by ELISA methods 3, 6, 9 and 12 weeks after STZ injection, and 4 weeks after the HSV-NGF treatment [33].

In DM rats, NGF levels in the bladder and L6-S1 DRG significantly decreased 12 weeks after STZ injection. In cystometry and metabolic-cage studies, bladder capacity and postvoid residual volume were significantly increased 12 weeks after STZ injection (Fig. 3). Bladder nociceptive responses, assessed by a

reduction of intercontraction intervals after acetic acid instillation, were significantly decreased in a time-dependent manner during the 12 weeks after STZ injection.

Rat injected with HSV-NGF into the bladder wall 8 weeks after STZ injection had a significant increase in NGF levels in the bladder and L6 DRG 4 weeks after HSV-NGF treatment (i.e. 12 weeks after STZ injection). DM rats injected with HSV-NGF also had a significantly smaller bladder capacity and postvoid residual volume than DM rats injected with HSV encoding the LacZ gene (Fig. 3). However, HSV-NGF treated rats showed no significant bladder nociceptive responses after intravesical acetic acid infusion [34,35].

These results indicate that the reduced production of NGF in the bladder and/or impaired transport of NGF to L6-S1 DRG may be an important mechanism inducing DM cystopathy, which is attributable to defects in both A δ -fibre and C-fibre bladder afferent pathways. NGF gene therapy using replication-defective HSV vectors, which

restores decreased NGF expression in the bladder afferent pathways, could be effective for treating DM cystopathy [13,35] (Fig. 4).

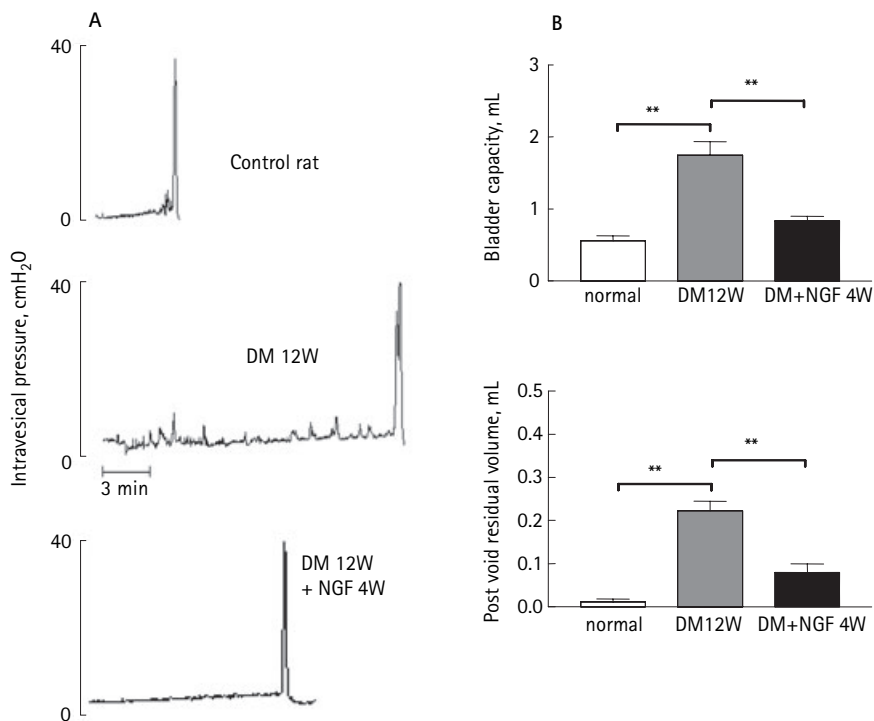
UROTHELIAL DYSFUNCTION IN DM

The location of the urothelium suggests that it is important for regulating permeability, transport and endocytosis. However, it has become increasingly clear that the urothelium is not only a passive barrier against urea and ion diffusion, but that it can also function as a sensor, controlling bladder function and dysfunction. The urothelium may have receptors and ion channels similar to those in bladder nerves, and injury or inflammation may alter the response of both urothelial cells and sensory afferents to nociceptive and other stimuli. Many mediators, e.g. ATP, NO and prostanoids, can be released from the urothelial cells [36,37]. Vanilloid receptors are expressed on urothelial cells [38], and it has been shown that ATP can potentiate the response to vanilloids by lowering the threshold for, e.g. protons and capsaicin [39]. This means that the large amounts of ATP released from damaged/sensitized cells in response to injury/inflammation may influence afferent nerves and contribute to the variety of abnormalities in DM-induced bladder dysfunction.

In the STZ-DM rat model there are progressive increases in total bladder tissue with hypertrophy of the bladder wall and dilatation of the bladder [15,16]. Both smooth muscle and urothelium (percentage of total tissue) increase significantly in a time-dependent manner. Pinna *et al.* [15] found that the epithelium from STZ-DM rat bladders was at least twice as thick and heavy as that from controls. In isolated urothelial layer preparations from bladders of STZ-DM rats, the absolute amount of endogenous prostaglandins E₂ and F_{2α} was higher than in corresponding preparations from control animals, but when prostaglandin F_{2α} production was expressed as a fraction of tissue weight, it was reduced in the diabetic epithelium.

ATP and bradykinin significantly increased the endogenous release of both prostaglandins from the urothelium when compared with the release under basal conditions. This increase was time-dependent and was higher in diabetic than in control tissues. Bradykinin-induced release of prostaglandin E₂ has also been reported in primary cultures of human

FIG. 3. Cystometric analyses in an awake condition, to evaluate the efficacy of HSV vector-mediated NGF delivery to the bladder in diabetic rats. (A) Representative traces of cystometrograms in a normal rat (upper trace), an untreated diabetic rat (12 weeks after inducing DM, middle trace) and a 12-week diabetic rat injected with HSV expressing NGF gene 8 weeks after inducing DM (lower trace). (B) The mean bladder capacity inducing voiding (upper graph) and postvoid residual volume (lower graph) (seven normal rats, six untreated diabetic rats and eight diabetic rats injected with HSV-NGF). **P < 0.01.



urothelial cells [40]. Pinna *et al.* [15] showed that ATP evoked a phasic and tonic contraction in bladder strips from nondiabetic rats; in preparations from DM, but not from normal animals, the tonic contraction was abolished by removing the urothelium. Bradykinin evoked a long-lasting tonic contraction that was reduced significantly by removing the urothelium only in DM rat bladders. Part of the effects of both ATP and bradykinin on DM bladders thus seemed to depend on the generation and release of prostaglandins from the urothelium. This implies that both ATP (P2X) and bradykinin receptors might be present in the urothelium, and that these receptors may be important in, e.g. prostaglandin generation and release. In turn, prostaglandins may sensitize sensory nerves and increase the sensitivity of bladder smooth muscle to contractile stimuli, which may contribute to some of the bladder abnormalities, e.g. detrusor overactivity, observed in DM.

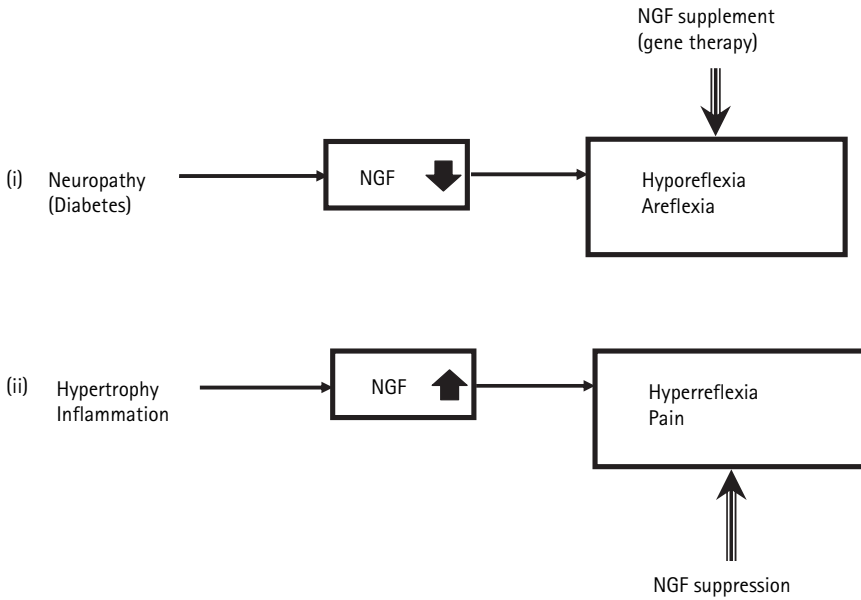
The urothelium may also be important in DM-related UTI. It was reported that women with

DM have bacteriuria more often than women without. Geerlings *et al.* [41] showed that Type 1 fimbriated *Escherichia coli* adhered twice as well to diabetic as to control epithelial cells. The receptors for these Type 1 fimbriae are glycoproteins (uroplakins), and it was proposed that diabetic uroepithelial cells have a different glycosylation of the receptor on their cells, resulting in higher adherence.

CONCLUSIONS

Although urological complications and major health problems in men and women with DM are common, data to define the expected prevalence, incidence and risk factors, and interventions to reduce the risk of developing these complications, are limited. New research initiatives are needed to further understand the basic disease mechanisms, to develop safe and effective prevention and treatment of the urological complications of DM. A better understanding of the biology of how DM affects the muscle, nerve and urothelium of the urinary bladder could lead to improved

FIG. 4. The relationship between bladder function and NGF: (i) In conditions of peripheral neuropathy such as DM, reduced NGF production in the bladder or deficiency in NGF transport to the bladder afferent pathway is an important factor in the pathogenesis of diabetic cystopathy that induces bladder hyporeflexia and decreased sensation. NGF supplement therapy may be useful to restore bladder function in these conditions. (ii) In conditions of bladder hypertrophy induced by BOO, spinal cord injury or bladder inflammation, there is increased NGF that can induce detrusor overactivity and bladder pain. Reducing NGF expression may be effective in normalizing bladder function in these conditions.



care of the diabetic patient with lower urinary tract dysfunction.

CONFLICT OF INTEREST

None declared. Sources of Funding: NIH HD397658, DK55045, NIH DK57267, DK68557, NIH DK55076, DK60037 and DK60204 and Swedish Research Council, grant no. 6837.

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Abbreviations: DM, diabetes mellitus; STZ, streptozotocin; NGF, nerve growth factor; EFS, electrical field stimulation; NOS, nitric oxide synthase; DRG, dorsal root ganglia; HSV, herpes simplex virus.