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The Ohio State University, 1988
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UMI
THE EFFECTS OF DIABETES AND DIURESIS ON
IN VIVO AND IN VITRO FUNCTION OF THE
RAT URINARY BLADDER

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Elizabeth M. Kudlacz, B.S., R.Ph.

*****

The Ohio State University
1988

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First, and foremost, I would like to express sincere gratitude to my adviser, Dr. L. Wallace, for his patience, guidance and, especially, for establishing in me the confidence to believe in myself. I would also like to thank all members of my committee; Dr. Gerald, Dr. McKay and Dr. Brueggemeier for their participation in various aspects of my graduate career as well as for their friendship. Finally, I would like to acknowledge a number of individuals without whom I may not have been able to endure the trials and tribulations of this academic program; i.e., my colleagues. Thanks to Mark, Tom, Bob, Tim, Raye Ann, Xiao, etc., for sharing the good times and bad. Best of luck to all.
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CHAPTER I

INTRODUCTION

1.0. Diabetes mellitus and its complications

1.0.1. General aspects

Diabetes mellitus is a disease characterized by abnormal fat, protein and carbohydrate metabolism which results from a deficient amount or effect of insulin (Guyton, 1986). Although the disease affects a significant proportion of the population, the etiology of the condition remains unknown. Diabetic patients are typically classified as one of two types: Type I (insulin-dependent) diabetics or Type II (insulin-independent) diabetics. As the name implies, the Type I diabetic must receive exogenous insulin to compensate for a failure of the body to manufacture sufficient quantities of the hormone. For the Type II diabetic, through dietary alterations and/or oral hypoglycemic agents, endogenous insulin acts more effectively (Stauffacher and Renold, 1971; Skillman and Tzagournis, 1986).
Insulin is a polypeptide hormone produced by beta-pancreatic islet cells. One of the major functions of insulin is to increase the rate of glucose transport through cell membranes, most importantly in adipose tissue and skeletal muscle. This movement is attributed to an insulin-induced stimulation of the facilitated diffusion of glucose (Guyton, 1986). The mechanism by which insulin modifies this process is believed to involve binding of insulin to its receptor followed by internalization of the complex and subsequent activation of glucose transport by either a direct action of the hormone or through the production of intracellular mediators (Jacobs and Cuatrecasas, 1983). In those tissues which rely upon insulin for glucose uptake (i.e. adipose tissue and skeletal muscle), absence of the hormone, such as in diabetes mellitus, is responsible for a number of metabolic perturbations. Adipose tissue responds to insulin deficiency with lipolysis and excessive free fatty acid outflow. Diminished protein synthesis and enhanced catabolism occur in skeletal muscle in response to the same stimulus (Stauffacher and Renold, 1971; Skillman and Tzagournis, 1986). Cells which do not require insulin for glucose uptake include the lens, retina, nerve, kidney, blood vessels and islet cells, and their permeability to glucose exposes them to ambient blood glucose levels (Gabbay, 1973). The majority of chronic complications of diabetes arise in these tissues, undoubtedly as a result of persistent hyperglycemia. Chronic complications of diabetes therefore include microvascular and macrovascular disease, nephropathy, eye disease and neuropathy (Meissner and Legg, 1971; Skillman and Tzagournis, 1986).
In order to approximate the human condition of diabetes mellitus, several different animal models have been developed. Genetic models include animals with spontaneously occurring diabetes (Brown, Dyck, Sima, McClearn, Powell and Porte, 1982). For example, non-obese rodents such as the Chinese hamster and BB Wistar rat, as well as obese rodents such as ob/ob and db/db mice have been used (Brown et. al. 1982). Chemically-induced diabetes is usually produced by either alloxan or streptozotocin (STZ) treatment (Grodsky, Anderson, Coleman, Craighead, Gerritsen, Hansen, Herberg, Howard, Lernmark, Matschinsky, Rayfield, Riley and Rossini, 1982). These chemicals are specifically toxic for insulin-producing cells and induce their detrimental effects almost immediately after exposure by a largely unknown mechanism (Grodsky et. al., 1982). By adjusting the dose and frequency of STZ injections, diabetes of varying severity can be induced, including insulin-dependent and insulin-independent diabetes mellitus (Brown et. al., 1982). The STZ-diabetic rats have many clinical, metabolic and electrophysiological features in common with insulin-dependent diabetic humans, which makes them an important experimental model (Brown et. al., 1982).

1.0.2. Diabetic neuropathy

General aspects. Diabetic nerve disease is perhaps the most common chronic complication of diabetes mellitus. Diabetic neuropathy is defined as the presence of clinical or subclinical evidence of peripheral nerve dysfunction which is more prevalent amongst diabetic than nondiabetic individuals and cannot be attributed to other disease processes (Pfeifer and Greene, 1985). The incidence of diabetic
neuropathy is difficult to ascertain because there is no exact definition of the syndrome. This may explain estimates of the disease which range from 0-93% (Thomas, Ward and Watkins, 1982). Other problems in diagnosis arise from the fact that diabetic neuropathies comprise a polymorphous group of disorders, some of which may be rapid in onset and reversible compared with others that may be insidious in onset and irreversible (Kozak, 1982). In general, however, the onset of diabetic neuropathy is usually clinically detectable approximately 5 years after diagnosis of the disease, and the prevalence of clinically overt neuropathy is believed to increase progressively with duration of diabetes (Pfeifer and Greene, 1985).

A superficial classification of diabetic neuropathies may be made on the basis of whether the neuropathy is subclinical or clinically overt. A patient with clinically overt neuropathy experiences symptoms in addition to signs or objective measurements of peripheral nerve impairment. By contrast, subclinical neuropathy is not accompanied by subjective symptoms and may be determined through the use of sensitive diagnostic measures such as the speed at which impulses are conducted along nerves (Pfeifer and Greene, 1985). Impairment in nerve conduction velocity has been repeatedly demonstrated in diabetic patients who did not experience neurologic symptoms. Diabetic neuropathy may affect autonomic or somatic nerves in either a diffuse or focal manner. A common method of classification is based upon the neuroanatomical site of the lesion. Focal and multifocal diabetic neuropathies are characterized by nerve impairment confined to the distribution of either one nerve (mononeuropathy) or several neurons (mononeuropathy multiplex).
or to focal lesions of the brachial or lumbosacral plexuses (plexopathy) or of the nerve roots (radiculopathy). These neuronal disorders are characterized by a sudden onset with at least partial recovery without treatment. Because of the specificity of the neurons involved, the cause of the dysfunction is believed to be vascular. That is, thrombosis in a small arteriole supplying a nerve or nerve bundle may starve the tissue, resulting in a loss of function in a specific locale.

The most common form of diabetic peripheral neuropathy is the slowly developing distal symmetrical polyneuropathy in which all somatic nerves are diffusely impaired in both the upper and lower extremities. Both motor and sensory nerves are affected, although sensory involvement usually precedes and overshadows motor involvement. The signs and symptoms which are expressed depend upon the number, type and size of damaged nerve fibers and the extent to which damage has occurred. These give rise to a number of subtypes of the disease such as mixed sensorimotor, large-fiber sensory and small-fiber sensory neuropathies. Two of the major symptoms are transient pain and paresthesias which cause varying degrees of patient discomfort. Although in most patients distal symmetrical polyneuropathy causes only mild sensory symptoms and rare motor weaknesses, mechanical and traumatic lesions which go unnoticed because of sensory denervation may pose significant health hazards, such as foot ulceration.

_Autonomic neuropathy._ Diabetic nerve dysfunction affects not only peripheral somatic motor and sensory nerves but autonomic nerves as well. The incidence of autonomic nervous system dysfunction is reported
to be 20-40% of the diabetic population (Niaken, Harati and Comstock, 1986). The neuropathy is typically subclinical and insidious in its onset, creating difficulties in diagnosis. Once the patient is to the point where he/she complains of symptoms, neurological damage has usually progressed significantly. The localization of a particular neurologic lesion in diabetic autonomic neuropathy can be difficult for a number of reasons (Pfeifer and Greene, 1985). First of all, the autonomic nervous system innervates nearly every organ in the body. It has been traditionally divided into the parasympathetic and sympathetic divisions. In general, activity in the sympathetic nervous system increases body activity whereas the parasympathetic nervous system acts inversely to decrease body activity. Confusion arises because a given response may reflect either increased parasympathetic or reduced sympathetic nervous stimulation. Also, the distinction between dysfunction in afferent (sensory) vs. efferent (motor) nerves is often difficult because stimulation of one limb of an intact reflex arc necessitates a response in the other. Methods used to determine subclinical neuropathies in peripheral somatic nerves, i.e. nerve conduction velocities, are impractical when applied to autonomic dysfunction since autonomic nerve fibers in an intact organism are not easily accessible. Thus, other methods must be utilized to analyze diabetic autonomic neuropathy. Clinical tests have been developed to assess subclinical autonomic neuropathy and have demonstrated asymptomatic defects in the cardiovascular system, iris, esophagus and urinary bladder. Even when the clinical manifestations of diabetic autonomic neuropathy are found in one organ, asymptomatic nerve damage
is believed to affect other organs. There appears also to be a high incidence of somatic nerve dysfunction in patients with autonomic neuropathy (Pfeifer, Weinberg, Cook, Reenan, Halar, Halter, LaCava and Porte, 1985).

Functional alterations attributed to diabetic dysfunction of parasympathetic and/or sympathetic autonomic nerves have been described in a number of visceral organs. Alterations in the cardiovascular system of diabetic patients have been studied extensively (Pfeifer and Greene, 1982; Niakan et al., 1986; Kozak, 1982; Thomas et al., 1982). The most common cardiovascular abnormalities include postural hypotension, resting tachycardia and painless myocardial infarction. Painless MI has been ascribed to a loss of afferent cardiac nerves (Ewing, Campbell and Clarke, 1976; Faerman, Faccio and Milei, 1977). Postural hypotension is attributed to a defect in the sympathetic reflex arc (Saito, Sato and Miura, 1982; Tohmeh, Shah and Gryer, 1979). Loss of vagal input is believed to contribute to high resting heart rate (Ewing, Campbell and Clarke, 1980; Clarke and Ewing, 1982), while cardiac adrenergic supersensitivity may contribute to arrhythmias and coronary spasms (Christensen, 1979). Experimental diabetes also produces cardiovascular abnormalities. Decreased sympathetic nervous activity has been demonstrated in the heart of diabetic rats (Yoshida, Nishioka, Nakamura and Kondo, 1985; Yoshida, Nishioka, Yoshioka, Nakano, Kondo and Terashima, 1987) and mice (Giachetti, 1978). A reduction in the number of alpha- and beta-adrenoceptors in diabetic rat hearts has been described (Williams, Schaible, Scheuer and Kennedy, 1983; Heyliger, Pierce, Singal, Beamish and Dhall, 1982; Latifpour and McNeill, 1984).
Changes in vascular responsiveness to autonomic agonists, in particular reduced adrenergic sensitivity, have been observed in diabetic rats (Carrier, Jackson and Scarborough, 1983; Longhurst and Head, 1985).

Another major site of diabetes-induced functional deficit is the gastrointestinal (GI) system. Constipation is the most frequent GI symptom encountered in the patient population, although diarrhea is not uncommon, even in the same individual (Pfeifer and Greene, 1985). Abnormalities have been found in both sympathetic and parasympathetic components of gut innervation (Hensley and Soergel, 1968); however, there is no clear association between the nervous anomalies and symptoms (Whalen, Soergel and Grenen, 1969). Furthermore, reductions in beta-adrenergic and serotonergic receptor activities were observed in the intestine of diabetic rats (Altan, Yildizoglu and Ozturk, 1987).

The diffuse effects of diabetic autonomic neuropathy are further made apparent by the fact that the disorder is also responsible for pupillary abnormalities, thermoregulatory changes, sudomotor dysfunction and disorders of the urogenital system (Pfeifer and Greene, 1982; Niakan et. al., 1986; Kozak, 1982). Neuropathic erectile impotence is perhaps the most common and often the only symptom of diabetic autonomic neuropathy (Rubin and Babbott, 1958). Changes in the VIPergic, cholinergic and adrenergic nerves in the penis of diabetic impotent males have been described, which suggests a multifaceted etiology (Lincoln, Crowe, Blacklay, Pryor, Lumley and Burnstock, 1987). Retrograde ejaculation is another problem associated with diabetic autonomic neuropathy. Damage to sympathetic innervation causes semen to be forced into the bladder.
Experimental evidence also exists for changes in the adrenergic component of the autonomic nervous system in the ventral prostate of the rat (Crowe, Milner, Lincoln and Burnstock, 1987). Diabetes-related disorders of the genital tract are often accompanied by urinary bladder dysfunction, which is probably a consequence of their common innervation. Diabetic urinary bladder dysfunction will be discussed in a later section.

Neuropathological alterations. Axonal loss and segmental demyelination are the most common pathological changes observed in diabetic neuropathy (Duchen, Anjorin and Watkins, 1980; Low, Walsh and Huang, 1975; Ward, 1981). The number of both myelinated and unmyelinated nerve fibers is reportedly diminished with a relatively greater loss of small myelinated and unmyelinated axons (Said, Slama and Selva, 1983). After only four weeks of experimental diabetes, the estimated mean cell body volume of lower motor and primary sensory nerves was significantly reduced (Sidenius, 1982). Axonal damage is believed to account for most of the clinically and electrophysiologically detectable nerve impairment (Behse, Buchtal and Carlsen, 1977). Demyelination is believed to occur subsequent to axonal loss (Sharma and Thomas, 1974; Jakobsen, 1976). Segments of myelin loss have been demonstrated between nodes in a patchy distribution which may indicate abnormal Schwann cell function (Ward, 1981). Perturbations in lipid metabolism may interfere with the maintenance of myelin, thus giving rise to demyelination (Thomas et. al., 1982). Alterations in the microvasculature of peripheral nerves have also been reported. Deposition of PAS positive material, generalized thickening and often
occlusion of the vessel lumen has been observed (Ward, 1981). Connective
tissue changes have also been described including endoneurial collagen
deposition (Behse et al., 1977) and increased perineurial laminae
(Asbury and Johnson, 1978). Progressive sclerosis of small vessels is
believed by some to be the cause of diabetic nerve damage (Fagerberg,
1979); however, the fact that some forms of diabetic neuropathy are
reversible and diffuse suggests that the vascular hypothesis of diabetic
nerve dysfunction applies only to select forms of the disease.

Studies specifically focussing on autonomic nerve abnormalities are
not as numerous as those involving other types of peripheral nerves.
Preganglionic fibers of the autonomic nervous system are myelinated,
whereas postganglionic fibers are usually unmyelinated (Bhagat, 1971).
Segmental demyelination and axonal degeneration similar to that seen in
diabetic peripheral somatic nerve have been described in vagal
preganglionic fibers innervating the heart and esophagus (Kristensson,
Nordborg, Olsson and Sourander, 1971; Smith, 1974) and in postganglionic
sympathetic fibers innervating the intestine (Low, Walsh and Huang,
1975). Giant neurons have been described in the sympathetic ganglia of
diabetic patients (Appenzeller and Richardson, 1966). Experimental
diabetes has been shown to induce changes in the morphology of
sympathetic preganglionic nerve fibers as well as diminish cell
synthesis and secretion (Kniel, Junker, Perrin, Bestetti and Rossi,
1986). Widespread degenerative changes were observed in sympathetic
ganglion cells by two weeks after STZ treatment in rats (Monckton and
Pehowich, 1980). By contrast, dystrophic axonopathy was observed in the
alimentary tract of diabetic rats but was not reproducibly demonstrated
in the autonomic innervation of other organs (Schmidt, Plurad and Modert, 1983). Hence, pathological alterations may be discerned in diabetic autonomic neuropathy, although some studies indicate that they may not always be temporally correlated with altered function.

Etiology of diabetic neuropathy. A number of theories have been proposed to explain the cause of diabetic neuropathy (Winegrad, 1987; Gabbay, 1973; Clements, 1979; Greene, Lattimer, Ulbrecht and Carroll, 1985). However, the general consensus currently is that some metabolic alteration is responsible for the dysfunction. This conclusion is based upon evidence of a correlation between the severity of diabetic neuropathy and blood glucose levels (Clements, 1979) and the fact that administration of insulin can, in some cases, ameliorate the symptoms (Pietri, Ehle and Raskin, 1980; Gregerson, 1968; Greene, DeJesus and Winegrad, 1975). High glucose concentrations produce several biochemical abnormalities in peripheral nerves which are believed to contribute to diabetic neuronal dysfunction. These include increased polyol pathway activity, decreased nerve myoinositol and nonenzymatic protein glycosylation.

Increased polyol (sorbitol) pathway activity. The polyol pathway of glucose metabolism is comprised of two enzymatic reactions that convert nonphosphorylated glucose to fructose. The polyol pathway consists of the following reactions:

\[
glucose \rightarrow \text{sorbitol} \rightarrow \text{fructose}
\]
The enzyme responsible for the metabolism of glucose to sorbitol is aldose reductase. Sorbitol dehydrogenase catalyzes the conversion of sorbitol to fructose. These enzymes are located in the cytoplasm of numerous mammalian tissues including nerve (Clements, 1979). Polyol pathway activity is regulated by intracellular glucose concentration. Thus, in tissues such as nerve, which do not require insulin for glucose transport, persistent hyperglycemia results in an intracellular elevation of polyol pathway intermediates; i.e. sorbitol and fructose (Stewart, Sherman, Kurien, Moonsammy, and Wisgerhof, 1967; Clements, 1979). An increase in the levels of these sugars in peripheral nerve axons and Schwann cells has been correlated with a reduction in motor nerve conduction velocity (Winegrad, 1987). The same effects occur when rats are administered excessive dietary galactose. Galactose is also reduced by aldose reductase to its corresponding sugar alcohol, galactitol, which is not, however, metabolized further (Stewart et. al., 1967). This is believed to explain the more rapid and greater accumulation of galactitol in nervous tissue as compared to sorbitol and fructose. Galactosemia, like diabetes, also produces a decrease in motor nerve conduction velocity (Gabbay and Snider, 1972). Intracellular sorbitol and galactitol accumulation has been associated with an increase in nerve water content attributed to endoneurial edema (Greene et. al., 1985). However, the actual contribution of osmosis to the generation of diabetic nervous dysfunction has been severely criticized (Greene et. al., 1985). While the exact mechanism by which an increase in polyol pathway activity contributes to neuronal dysfunction is uncertain, further evidence of its importance is
suggested by studies utilizing compounds which reduce pathway activity. Inhibition of aldose reductase in diabetic animals has been shown to decrease the accumulation of sorbitol in nerves (Poulsom and Heath, 1983; Peterson, Sarges, Aldinger and MacDonald, 1979) and improve motor nerve conduction velocity (Kikkawa, Hatanaka, Kobayashi, Yasuda and Shigeta, 1982; Yue, Hanwell, Satchell and Turtle, 1982; Dvornik, Simard-Duquesne, Krami, Sestanj, Gabbay, Kinoshita, Varma and Merola, 1973; Mayer and Tomlinson, 1983b,c). Administration of aldose reductase inhibitors have also been shown to lower galactitol levels by 60% with a delay in the appearance of a defect in conduction velocity (Peterson et. al., 1979; Dvornik et. al., 1973). These compounds have been tested clinically for their ability to improve conditions of both somatic and autonomic neuropathy with variable success (Jaspan, Towle, Maselli and Herold, 1986; Fagius and Jameson, 1981; Judzewitch, Jaspan, Polonsky, Weinberg, Halter, Halar, Pfeifer, Vukadinovic, Berstein, Schneider, Liang, Gabbay, Rubenstein and Porte, 1983).

Decreased nerve myoinositol (MI) content. MI is a cyclic polyalcohol which is derived from the diet and is a precursor of polyphosphoinositides. These compounds are present in all cell membranes, including excitable membranes where they may function in the regulation of ion transfer (Thomas et. al., 1982). The peripheral nerve maintains a tissue-to-plasma MI concentration gradient of 90-100 fold (Greene et. al., 1985). The establishment or maintenance of the nerve concentration gradient occurs by means of a specific, high affinity, sodium-dependent, carrier mediated MI transport system (Greene, Lewis, Lattimer and Brown, 1982). Nerve MI content is decreased in both
diabetic humans (Mayhew, Gillon and Hawthorne, 1983) and diabetic animals (Greene, DeJesus and Winegrad, 1975; Greene, Yagihashi, Lattimer and Sima, 1984; Palmano, Whiting and Hawthorne, 1983). This may be due to competition between glucose and MI for uptake via the transport system (Greene, Lewis, Lattimer and Brown, 1982). MI affects the activity of sodium-potassium ATPase by altering the cell membrane content of phospholipids (Greene, Lattimer, Ulbrecht and Carroll, 1985). This interrelation creates a self-perpetuating and self-reinforcing metabolic derangement in which decreased MI reduces sodium-potassium ATPase activity thereby further inhibiting MI uptake.

There appears to be a correlation between reduced nerve MI content, decreased sodium-potassium ATPase activity and reduction in motor nerve conduction velocity (Greene et al., 1985). Changes in these three parameters can be reversed by insulin, dietary myoinositol supplementation or, aldose reductase inhibitors (Clements, 1979; Greene and Lattimer, 1984a; Greene and Lattimer, 1984b; Mayer and Tomlinson, 1983b,c). Data from the latter group of compounds suggests that increased polyol pathway activity may contribute to altered nerve MI content. The result is the formation of an integrated metabolic hypothesis (see Figure 1).

**Nonenzymatic protein glycosylation.** Hyperglycemia promotes nonenzymatic glycosylation of proteins which may alter their function in various tissues. Peripheral nerve protein glycosylation has been demonstrated to increase in acute experimental diabetes (Vlassara, Brownlee and Cerami, 1981), although the identity of these proteins and
their functions is unknown. Brain tubulin, however, has been shown to be nonenzymatically glycosylated in vivo and in vitro, resulting in marked changes in its self-assembly and solubility characteristics (Williams, Howarth, Devenny and Bitensky, 1982). This could potentially alter axonal transport as well as physical characteristics of the axon cylinder. Impairment of axonal transport in experimental diabetes has been described by a number of researchers (Tomlinson, Willars and Cathrop-Owen, 1987; MacLean, 1987; Schmidt, Plurad, Saffitz, Grabau and Yip, 1985; Mayer and Tomlinson, 1983a; Willars, Tomlinson and Robinson, 1986). These effects may also contribute to the functional and structural changes associated with diabetic neuropathy (Figure 1).

1.1 Bladder Structure and its Relation to Function

The urinary bladder is a hollow, muscular organ comprised of two principle regions whose function is to store and expel urine: the bladder body and bladder base (Gosling, 1979; Tanagho, 1984a; Hald and Bradley, 1982) (Figure 2). The bladder body consists of a transitional epithelium, beneath which is an extensive mucosal layer formed primarily of connective and elastic tissues. The detrusor muscle lies external to the submucosa and consists of three ill-defined layers; outer and inner longitudinal layers and an intermediate circular layer. The result is the formation of a complex meshwork of smooth muscle. Individual smooth muscle cells and smooth muscle bundles are enclosed in collagen. Collagen fibrils in smooth muscle are characterized by their uniformity and narrow diameter whereas elastin, which is also present, may occur as fibers running at right angles to the long axis of smooth muscles. The
adaptation of the detrusor muscle to increases in distension, such as occurs in urine storage, has been correlated with elastic properties of smooth muscle and collagen (Bradley, 1986; Apter, Mason and Lang, 1972; Zinner, Ritter, Sterling and Donker, 1977). Contractility of bladder smooth muscle depends upon the initial resting length of muscle fibers and the state of their elasticity (Carpenter, 1968). Therefore, changes in the composition of the bladder result in altered organ function. For example, the progressive deposition of collagen in response to overdistension leads to reduced elasticity of the rabbit bladder and increased micturition (Lloyd-Davies, Clark, Prout, Shuttleworth and Tighe, 1970). The reduction in the ability of fibrotic cat bladders to relax is attributed to a decreased elasticity which is also attributed to an increased collagen component (Zinner et. al., 1977).

Cystometry is a method of measuring the storage capacity of the urinary bladder, which is primarily a function of the detrusor (Tanagho, 1984b). A cystometrogram is a graphical representation of the change in intravesicular bladder pressure in response to infused gas or fluid (see Figure 3). In the human with a normal bladder, organ capacity is about 400-500 ml, and the resting bladder filling pressure is less than 10-15 cm H$_2$O. The patient first experiences a desire to void at 100-200 ml of infused fluid. Intravesicular pressure remains fairly constant at approximately 8-10 cm H$_2$O as fluid is continuously infused. Pressure does not rise until 350-450 ml of fluid is introduced, at which point a sensation of fullness (capacity) is reached and a slight rise in pressure observed. If filling is continued beyond this point, patient distress occurs and the intravesicular pressure rapidly increases. The
result is an involuntary expulsion of bladder contents around the infusion catheter.

The cystometrogram appears to be a reflection of both bladder composition and innervation (Zinner et al., 1977). The importance of bladder innervation in the modulation of bladder tonus and the micturition reflex was examined by Tang and Ruch (1955). The nervous system was found to influence the micturition reflex but not control of tonus. Stretch or distension was the only factor found to influence bladder tonus. The portion of the cystometrogram characterized by an absence of intraluminal pressure change in spite of increasing fluid volume (termed plateau pressure) may be a result of two factors. First of all, smooth muscle stretches to accommodate increased fluid. Also, elastin fibers undergo a relatively large increase in length in response to a small tension. Because collagen fibers are coiled initially, the bladder wall must be extended considerably before these fibers straighten and contribute to tension. Once they reach their working length, they resist further stretching. Hence, the rapidly rising portion of the curve reflects the tension contribution from collagen. The obvious safeguard to overdistension of the bladder is the micturition reflex (Alexander, 1971).
Figure 1. Integrated metabolic hypothesis of diabetic neuronal dysfunction. The diagram illustrates hypothesized relationships between altered polyol pathway activity, nerve myoinositol content, and nonenzymatic protein glycosylation in the production of structural and functional changes in the diabetic nerve. Adapted from Sima, Brismar and Yagehashi, 1987.
Figure 2. General structure of the urinary bladder and its innervation. The schematic diagram illustrates major regions of the urinary bladder and the location of parasympathetic and sympathetic innervation. Adapted from Griffin, 1983.
Figure 3. Representative cystometrograms from normal and diabetic patients. Panel A illustrates a cystometrograph obtained from the bladder of a normal individual. Panel B illustrates the most common cystometrograph observed in diabetic patients with autonomic neuropathy affecting the bladder. The organs from these individuals are characterized by sensory loss and large capacity. Panel C illustrates the cystometrograph of a diabetic individual whose bladder exhibits hyperreactivity, an effect which occurs to a lesser degree in the patient population. Adapted from Kozak, 1982.
The second major portion of the urinary bladder is the bladder base (Khanna, Barbieri, Altamura and McMichael, 1981; Tanagho, 1984a). The bladder base is composed of the trigone muscle and the bladder neck. The trigone muscle consists of small, smooth muscle fibers in a rather dense collagen matrix which is embryologically distinct from the rest of the bladder. During bladder filling, while the bladder body (detrusor) and trigonal detrusor muscle (deep base) relax, the superficial trigone contracts to keep the base flat and fixed. During micturition, the contraction of the trigonal detrusor will cause the base to assume a conical shape for efficient urine expulsion. The bladder neck consists of the region beginning at the level of the ureters and extending rostrally to include the outlet and proximal portion of the urethra. The urethra consists also of smooth muscle in a matrix of collagen and elastin. There are three urethral muscular components; 1. longitudinal layer which is an extension of the detrusor, 2. another longitudinal layer which is an extension of the superficial trigone, and 3. a circular smooth muscle layer unrelated to the circular muscle of the detrusor. The urethral structure contributes to its primary function of maintenance of urinary continence.

1.2 Innervation of the Urinary Bladder

The function of the urinary bladder in both the storage and expulsion of urine depends upon the integration of the central and peripheral nervous systems.
1.2.1 Central control of urinary bladder function

A simple reflex arc exists between the bladder and the sacral spinal cord (Griffin, 1983). Bladder filling stimulates stretch receptors in the detrusor muscle which results in muscle contraction mediated by efferent nerves. Higher centers, including the cerebral cortex, modulate the reflex activity. In the adult human, voluntary control of detrusor contractions promotes continence. Voluntary control of micturition in animals has not been ascertained; therefore, it is at times difficult to extrapolate micturition data from the conscious animal to the human condition (Maggi and Melli, 1986).

The central nervous system contribution to the control of urinary bladder function has been organized into four loops (Bradley, 1986). Loops I and II control detrusor innervation whereas loops III and IV modulate innervation of periurethral and pelvic striated muscle. Loop I consists of axons connecting cortical nuclei in the frontal lobe and thalamus with the brain stem detrusor motor nucleus. The net effect of the loop is an inhibitory one on the brain stem detrusor nucleus. The brain stem detrusor nucleus, or pontine-mesencephalic region, located between the pons and midbrain, contains essential detrusor motor structures as determined by lesioning experiments (Bradley and Conway, 1966; Barrington, 1925; Sillen, Rubenson and Hjalmas, 1981). Hyperreactivity in rat bladders is observed after peripheral administration of L-DOPA, which is believed to be the result of stimulation of this locale (Sillen, Rubenson and Hjalmas, 1979; Sillen, Rubenson and Hjalmas, 1982). Bladder hyperreactivity also results from
damage to Loop I structures. Decreased cortical inhibition can occur as a result of cerebrovascular accidents, Parkinson's disease, Alzheimer's dementia, multiple sclerosis and neoplasms (see Williams and Pannill, 1982).

Loop II is comprised of sensory axons arising from the detrusor via the pelvic nerve to the sacral spinal cord where they ascend, without synapsing, to the brain stem detrusor nucleus. This pathway amplifies detrusor reflex contractions which travel to the brain stem detrusor nucleus. Distension of the cat urinary bladder has been shown to produce unit discharge in these nuclei (Bradley and Conway, 1966). Impulses which originate in the brain stem detrusor nucleus descend to the conus medullaris where motor neurons in this nucleus send axons through the second, third and fourth sacral roots to synapse in the pelvic ganglia. From the pelvic ganglia, postganglionic parasympathetic axons arise which initiate detrusor contractions. Loop II is thus the primary reflex arc of detrusor innervation. Dysfunction in loop II may occur when excitability of afferent pathways occurs, such as that due to bladder infection, inflammation, etc., which promotes detrusor instability. By contrast, impaired sensory input due to factors such as diabetes mellitus or tabes dorsalis, may result in atonic bladder.

The function of loop III is to produce passive relaxation of the pelvic floor musculature during bladder filling. Loop IV allows voluntary control over pelvic floor muscles and the external sphincter. Together, these four loops communicate bladder volume status to the brain. Detrusor contractions occur at a threshold urine volume
(amplified by loop II) unless inhibited by higher cortical centers (loop I). These contractions produce voiding when the act is desired, and are aided by contractions of abdominal and pelvic floor musculature along with relaxation of the bladder outlet and urethral sphincter (loops III and IV).

1.2.2 Peripheral innervation of the urinary bladder

Peripheral autonomic nerves which innervate the smooth muscle of the urinary bladder include the visceral efferent (motor) nerves and visceral afferent (sensory) nerves. The integrity of both components is required for micturition to occur since one aspect of a reflex arc depends upon the other.

1.2.2.1 Visceral efferent innervation

The autonomic efferent innervation of the bladder may be divided into parasympathetic, sympathetic and non-adrenergic-non-cholinergic input. As in most other organs, the parasympathetic and sympathetic nervous systems act in opposition to one another. Sympathetic nervous stimulation facilitates urine storage whereas parasympathetic nervous stimulation promotes urine expulsion.

Sympathetic innervation. Sympathetic preganglionic nerve fibers of the lower urinary tract originate in the intermediolateral nuclei of the lower thoracic and upper lumbar segments of the spinal cord (T10-L2) (Tanagho, 1984b; Andersson and Sjogren, 1982; Taira, 1972) (see Figure 2). The majority of neurons synapse in the inferior mesenteric and hypogastric plexuses from which postganglionic sympathetic fibers run in
the hypogastric nerve to the pelvic plexus. Response of the bladder to hypogastric nerve stimulation depends upon the species and individual animal (Taira, 1972). Hypogastric nerve stimulation of dog, cat and rabbit bladders in vivo promotes bladder relaxation after an initial contraction (Langley and Anderson, 1895). In the rat bladder, postganglionic sympathetic denervation caused no detectable change in adrenergic or AChE-positive nerves in the bladder, while parasympathetic decentralization or denervation produced a total disappearance of adrenergic fibers. Electrical stimulation of the hypogastric or pelvic nerves distal to the pelvic ganglia elicited contraction of the rat detrusor muscle. Therefore, in the rat urinary bladder, postganglionic adrenergic fibers appear to be supplied primarily by the pelvic nerves and only to a lesser extent via hypogastric nerves (Alm and Elmer, 1974).

The smooth muscle of the bladder body has a sparse supply of adrenergic nerves. In this region, adrenergic nerves are found only between groups of muscle fibers or between muscle bundles except around blood vessels (Taira, 1972). By contrast, in the bladder base, especially in the area of the trigone muscle, adrenergic innervation is much richer. Besides quantitative differences, there also appear to be qualitative differences between the adrenergic neurons innervating the bladder body and base regions. After reserpine and 6-hydroxydopamine pretreatment, no adrenergic nerve fluorescence was observed in the apical and body portion of the rat urinary bladder (Alm and Elmer, 1975). However, the adrenergic nerve fluorescence was unchanged in the trigone region. The data are consistent with other observations which
suggest that neurons of the peripheral adrenergic nervous system can be divided into two groups based upon functional and anatomical differences. Short adrenergic nerves, which innervate the vas deferens and seminal vesicles, have long preganglionic and short postganglionic fibers (Sjostrand, 1965). The transmitter fluorescence of these nerves is unaffected (or to a much slower extent) by small doses of reserpine and 6-hydroxydopamine (Owman and Sjoberg, 1967; Malmfors and Sachs, 1986; Sjostrand and Swedin, 1968). By contrast, long adrenergic nerves possess short preganglionic and long postganglionic fibers. Furthermore, transmitter fluorescence in these nerves is diminished by reserpine and 6-hydroxydopamine. Hence, the bladder body appears to be innervated by long adrenergic nerves whereas the bladder base is innervated by short adrenergic nerves.

There are several mechanisms by which the sympathetic nervous system promotes urine continence and prevents bladder activity during the collecting phase. The first mechanism is through direct activation of detrusor muscle beta-adrenoceptors. Beta-adrenoreceptors have been identified primarily in the bladder body by receptor binding (Levin and Wein, 1979) and correlated with tissue relaxation by in vitro and in vivo methods (Levin and Wein, 1979; Edvarsen and Setekleiv, 1968; Ganguly and Vedasiromoni, 1976). The receptor has been demonstrated to be of the beta-2 subtype in rats (Maggi and Meli, 1982) and beta-2 in cats (Nergardh, 1977) but neither beta-1 nor beta-2 in humans (Nergardh, Boreus and Naglo, 1977). In vivo analysis of the effects of beta-adrenoreceptor stimulation suggests that the major role for sympathetic nervous innervation in this region is to prevent bladder activity during
the collecting phase. Administration of beta-adrenoceptor agonists suppressed spontaneous bladder contractions in anesthetized rats whereas administration of beta-adrenoceptor antagonists increased spontaneous motility (Maggi and Meli, 1982). In the in vivo dog bladder, isoproterenol reduced the maximum voiding pressure and increased bladder capacity (LaGrange, 1971). Propranolol had a rising effect on intravesical pressure in cat cystometric readings, which may be attributed to a blockade of beta-adrenergic inhibitory receptors. However, unchanged micturition contraction after the administration of the drug suggest that adrenergic inhibitory receptors do not operate during the expulsion of urine (Edvardsen, 1968). These data provide evidence for the activity of beta-adrenoceptor mediated effects during bladder filling.

Although the major adrenoceptor type in the bladder body is the beta-adrenoceptor which mediates tissue relaxation, a sparse supply of alpha-adrenoceptors has also been demonstrated in this region. In contrast to the beta-adrenoceptor mediated response, alpha-adrenoceptor activation of this region results in smooth muscle contraction. Alpha-adrenoceptors are believed to be responsible for the initial detrusor contraction observed upon hypogastric nerve stimulation in vivo (Edvardsen, 1968). Detrusor muscle strips respond to norepinephrine or epinephrine with relaxation which can be converted to contraction by propranolol (Edwardsen and Setekleiv, 1968). Dog detrusor muscle strips have a variable response to norepinephrine which depends upon strip length (Benson, Raezer, Wein and Corriere, 1975). When strips are near resting length, norepinephrine causes relaxation but when strips are
stretched, norepinephrine causes an increase in tension. The receptor specificity of the in vivo and in vitro effects is suggested by alpha-adrenoceptor antagonism. In normal detrusors, alpha-adrenoceptor agonists have minimal effect on contractility (Andersson and Sjogren, 1982). The number of alpha-adrenoceptors in the bladder body can be altered. In patients with autonomous (decentralized) bladders produced by parasympathetic lesions, increases in catecholamine fluorescence and sensitivity to alpha-adrenoceptor agonists have been described in vivo and in vitro (Sudin, Dahlstrom, Norlen and Svedmyr, 1977).

In addition to the direct relaxant effect sympathetic nerve stimulation has on detrusor smooth muscle beta receptors, there is a second site at which adrenergic innervation can promote urine storage. This occurs indirectly through the modification of parasympathetic nervous influences on the detrusor muscle. Parasympathetic nerve stimulation causes detrusor contraction, thereby opposing sympathetic effects and promoting urine expulsion. Evidence for this modulatory effect of sympathetic nerves is derived from several sources. Anatomically, adrenergic nerve terminals are in close apposition to nonadrenergic, presumably parasympathetic, neurons in the vesical ganglia of the cat (Hamberger and Norberg, 1965). Neurologically, the primary effects of electrical stimulation of the hypogastric nerve is alpha-adrenoceptor mediated inhibition of vesical ganglionic transmission (DeGroat and Saum, 1971; DeGroat and Saum, 1972). The function of adrenergic modulation in the vesical ganglia appears to be negative feedback control of bladder activity. That is, activation of vesical afferents in the pelvic nerve by bladder distension or
electrical stimulation causes reflex firing in the hypogastric nerves and inhibition of ganglionic transmission. These effects may reflect presynaptic depression of the transmitter release or a hyperpolarizing action on postganglionic elements.

The third major method by which the sympathetic nervous system modulates urine continence involves the bladder neck region. Stimulation of the hypogastric nerve results in contraction of the smooth muscle of the bladder outlet (Taira, 1972). In contrast to the bladder body, the primary adrenergic receptor population in the bladder base consists of alpha-adrenoceptors (Levin and Wein, 1979) located in the trigonal and urethral smooth muscles (Taira, 1972). Stimulation of the bladder outlet by alpha-adrenergic agonists results in increased resistance to urine flow because of smooth muscle contraction (Nergardh, 1974; Kleeman, 1970) which can be abolished by alpha-adrenergic antagonists such as phentolamine (Tulloch, 1975). Clinically, alpha-adrenergic agonists are used to increase sphincter resistance in patients with stress incontinence (Williams and Pennill, 1982). Failure of the bladder to empty may be the result of increased outlet resistance which is generally treated by the administration of alpha-adrenergic antagonists (Wein, 1984). In general, clinical observations suggest that although the bladder possesses sympathetic receptors, sympathomimetic drugs have little effect on bladder function. Their most useful clinical applications are to increase or decrease bladder outlet resistance (Finkbeiner, Bissada and Welch, 1978).
Parasympathetic innervation. The preganglionic parasympathetic nerve fibers which innervate the bladder originate in the intermediolateral nuclei of the sacral cord segments S2-S4 and run in the pelvic nerves to the pelvic plexus at the bladder base where they form synapses with postganglionic neurons (Tanagho, 1984b; Andersson and Sjogren, 1982, Taira, 1972) (see Figure 2). Parasympathetic nerve fibers are related to smooth muscle cells in such intimacy that every smooth muscle has an associated axon terminal (Taira, 1972). The function of the parasympathetic nervous system is to promote urine expulsion. Stimulation of the pelvic nerve causes detrusor muscle contraction although this effect is only partially blocked by atropine (Vanov, 1965; Carpenter, 1981; Sjogren, Andersson, Husted, Mattiasson and Moller-Madsen, 1982). The presence of muscarinic cholinergic receptors mediating the contractile response to released acetylcholine has been established through receptor binding studies using radioligands such as $^3$H-quinuclidinyl benzilate (QNB) (Levin, Shofer and Wein, 1979). The greatest density of muscarinic receptor binding is found in the detrusor muscle and lesser amounts in the bladder neck. Acetylcholinesterase positive fibers follow the same pattern of distribution (Kluck, 1980). All orientations of muscle in the bladder body appear to be equally sensitive to acetylcholine. By contrast, longitudinal but not circular muscle strips derived from the bladder neck respond with contraction to cholinergic muscarinic stimulation (Khanna et al, 1981). Although the importance of cholinergic stimulation of the bladder body in the expulsion phase of micturition is certain, it is questionable whether muscarinic receptors in the bladder neck are significantly involved in
normal reflex reduction of urethral resistance (Nergardh and Boreus, 1973).

**Non-adrenergic-non-cholinergic innervation.** The concept of norepinephrine and acetylcholine as the only neuromodulators of autonomic nervous function is becoming obsolete. A variety of endogenous substances have been found to elicit effects in the bladder smooth muscle. Some of these may be released in conjunction with the classical neurotransmitters ACh and NE in a process referred to as co-transmission (Campbell, 1987; Bartfal, 1985). This particular aspect has been extensively studied in particular to ascertain the identity of the neurotransmitter(s) involved in the atropine-insensitive portion of pelvic nerve stimulation. As was alluded to previously, stimulation of the pelvic nerve produces contraction only partially inhibited by atropine. Hence, some other non-cholinergic substance is believed to be released concurrently with acetylcholine from parasympathetic nerves innervating this region.

**Purinergic neurotransmission.** Purine nucleosides and nucleotides are ubiquitous throughout all cells where they participate in a number of cellular metabolic processes as well as cell replication. Adenosine and adenosine-5'-triphosphate (ATP) have also been shown to produce marked pharmacological actions and have been designated as neurotransmitters based upon classical criteria (Williams, 1987). Specific receptors for adenosine and ATP have been termed $A_1$ and $A_2$, respectively. ATP causes contraction of bladder strips from a variety of species (Levin, Jacoby and Wein, 1981; Andersson, Husted and Sjogren, 1980). In the rabbit
urinary bladder, the distribution of ATP receptors is equivalent to that of cholinergic receptors (Levin, et. al., 1979). This was determined from observations of a greater contractile response to ATP in bladder body strips compared to bladder base strips. ATP is believed by some investigators to be the "missing" excitatory neurotransmitter released by pelvic nerve stimulation. Burnstock, Cocks, Kasakov and Wong (1978) showed that ATP was released upon electrical stimulation in the isolated guinea-pig urinary bladder. Downie and Dean (1977) came to the same conclusion using the rabbit urinary bladder. Intra-arterial injection of ATP in proximity to the dog bladder produced a response corresponding to about 50% of that produced by nerve stimulation (Creed and Tulloch, 1978). However, the importance of purinergic transmission in the bladder has yet to be resolved.

**Prostaglandins.** Prostaglandins are endogenously generated fatty acid derivatives that function in a number of physiological processes and are produced by many tissues. Prostaglandins of the E and F series have been shown to cause contraction of bladder tissues of several species (Ueda, Yoshida, Yano, Mutoh, Ikegami and Sakanashi, 1985; Ambache and Zar, 1970; Hills, 1976). At least in human tissues, bladder dome smooth muscle appears to be more sensitive to prostaglandins than trigone smooth muscles (Ueda et. al. 1985). The contractile response has been attributed to activation of prostaglandin receptors (Khanna, Barbieri and McMichael, 1978).

Not only is the bladder responsive to prostaglandins but it also has the ability to synthesize them. Production of prostanoids of the E and F
series, as well as prostacyclin (PGI2), by the bladder has been demonstrated (Mikhailidis, Jeremy and Dandona, 1987). The quantity of prostanoids produced in the bladder body was found to be greater than that by the base, although their relative proportions were reportedly similar (Leslie, Pavlakis, Wheeler, Siroky and Krane, 1984). The production of prostanoids has been localized to the smooth muscle layer and the bladder epithelium of rat and rabbit (Brown, Zenser and Davis, 1980, Jeremy, Mikhailidis and Dandona, 1984). Prostanoid synthesis is stimulated by factors such as bladder distension, increased urine osmolarity and receptor stimulation (Jeremy et. al., 1984). The activation of several receptor types in the bladder has been correlated with prostaglandin production. In vitro prostanoid synthesis by the rat urinary bladder is stimulated by activation of postganglionic muscarinic receptors (Jeremy, Mikhailidis and Dandona, 1986). Stimulation of ATP-activated purinoceptors may also result in release of prostanoids (Andersson et. al., 1980). Finally, one study suggests that the contractile effect of substance P on the bladder may be partially the result of prostanoid release (Johns, 1981).

The function of prostaglandins in the urinary bladder remains speculative. Although prostaglandins are released during stimulation of excitatory nerves (Johns and Paton, 1977), the slowness of the bladder response to these agents makes it unlikely that they are directly involved in bladder evacuation. Alternative hypotheses suggest a role for the prostanoids in the maintenance of tone or spontaneous activity in the bladder muscle or as modulators of transmission (Andersson and Sjogren, 1982). In vivo studies suggest that prostanoids may enhance
myogenic contractile activity, which might lower the micturition threshold by increasing afferent transmission from the detrusor muscle to supraspinal centers (Maggi, Santicioli, Furio and Meli, 1985; Maggi, Evangelista, Grimaldi, Santicioli, Giolitti and Meli, 1984).

5-Hydroxytryptamine (serotonin). Serotonin is a biologically active amine which, like the prostaglandins, is an autocoid, or local hormone. In certain adrenergic peripheral nerves, such as those innervating the pineal gland, serotonin may be co-localized with norepinephrine (Jaim-Etcheverry and Zieher, 1974). Serotonin promotes rat bladder contraction in vitro and in vivo (Vanov, 1965). The in vivo effect appears to be biphasic. The initial transient contraction which is observed is believed to be due to stimulation of the autonomic ganglia. The more prolonged contraction which follows is due to a direct effect on bladder smooth muscle itself. The physiological relevance of these effects, however, is questionable.

Neuropeptides. A variety of neuropeptides have been shown to influence the micturition reflex when administered centrally or peripherally (Maggi and Meli, 1986). The discussion which follows will be restricted to the peripheral effects of a few of these compounds. Neuropeptides which have been demonstrated to exhibit significant effects in isolated bladder tissues include; angiotensins, tachykinins, bradykinins and bombesin-like peptides (Erspamer, Ronzoni and Erspamer, 1981; Erspamer, Negri and Piccinelli, 1973). Peptidergic nerves are believed to affect bladder motility in several ways. First of all, peptidergic nerves may release peptides in response to nervous
stimulation. This could result in 1) an action on the effector itself, 2) modulation of the release of other neurotransmitters from nerve fibers, and 3) modulation of the response of effector cells to other neurotransmitters. The latter two functions suggest that peptides may play a role in cotransmission (Campbell, 1987; Bartfai, 1985). Cotransmission is the control of a single target cell by two or more substances released from one neuron in response to the same neuronal event. Some examples of putative transmitters coexisting in one nerve include; acetylcholine and vasoactive intestinal peptide, serotonin and substance P and norepinephrine and neuropeptide Y. Differential regulation of release of coexisting neurotransmitters may occur when coexisting neurotransmitters are stored in separate populations of vesicles.

Substance P is an undecapeptide first detected in extracts of equine brain and intestinal tissues (von Euler and Gaddum, 1931). Its earliest discernable pharmacological effects were the production of hypotension and gut contraction. Later studies demonstrated nervous tissue, e.g. intestinal intrinsic nerve plexuses, to be a rich source of substance P activity. The peptide was also shown to be preferentially concentrated in dorsal or sensory roots of spinal nerves, which led researchers to suggest that the peptide might be a sensory neurotransmitter (Lembeck, 1953).

Substance P has been localized to nerve fibers distinct from adrenergic and cholinergic nerves in the genitourinary tract (Alm, Alumets, Brodin, Hakansson, Nilsson, Sjoberg and Sundler, 1978). In the
rat bladder, substance P fibers were found primarily in the submucosal layers of the bladder wall with few fibers scattered in the smooth muscle (Mattiasson, Ekblad, Sundler and Uvelius, 1985). Of 29 peptides tested in the human bladder, the tachykinins were found to be second in contractile potency after angiotensin (Erspamer et al., 1981). In rat, guinea pig and hamster bladders, substance P has been shown to be 4-8 times more potent than acetylcholine in producing contraction (Erspamer et al., 1973). The contractile response is not affected by tetrodotoxin or physostigmine, which suggests that substance P is acting directly on the smooth muscle (Sjogren, Andersson and Husted, 1982). This is supported by data which demonstrate the blockade of substance P effects by its antagonist (Maggi, Santicioli, and Meli, 1985). The release of prostaglandins has been proposed by some investigators (Erspamer et al. 1973) to be partially responsible for substance P induced contractility, although this has been refuted by others (Sjogren et al., 1982). In vivo, substance P elicits a tetrodotoxin resistant tonic contraction followed by a series of rhythmic, tetrodotoxin sensitive, phasic contractions (Maggi, Santicioli and Meli, 1984). These data suggest that a direct effect of substance P is followed by stimulation of reflex efferent nervous activity. Although the neuropeptide produces marked contraction in vivo and in vitro, there does not appear to be any evidence to support the role of substance P as the atropine-resistant component of nerve mediated contractile response in the bladder (Sharkey, Williams, Schultzberg and Dockray, 1983, Sjogren et al., 1982). The major function of substance P in the urinary bladder is as a sensory neurotransmitter which will be discussed
in further detail in a later section. Other postulated roles for substance P in the urinary bladder include the potentiation of efferent neurotransmission and the induction of changes in vascular tone and permeability (Maggi and Meli, 1986).

Another peptide whose role in urinary bladder function has been explored more recently is VIP, a 29 amino-acid peptide which relaxes many smooth muscles, especially in sphincteric areas (Cooper, Bloom and Roth, 1982). In the rat urinary bladder, there are a moderate number of VIP-immunofluorescent fibers located in smooth muscle and blood vessels (Mattiaison et al., 1985). However, the highest concentrations of VIP have been found around the ureteric orifices, in the trigone and urethra (Maggi and Meli, 1986). VIP may affect the micturition reflex at spinal or supraspinal sites as well as acting on the bladder musculature itself (Maggi and Meli, 1986). Although VIP is one of the most abundant neuronal peptides, it causes only weak contraction and small potentiation of nerve-induced contractions in the guinea pig bladder strips (Johns, 1979) and is inactive in the human bladder strips (Ersparmer, Ronzon and Ersparmer, 1981). However, in the cat (Larsen, Ottesen, Fahrenkurg and Fahrenkrug, 1981), rabbit (Levin and Wein, 1981), pig and human (Klarskov, Gerstenberg and Hald, 1984), VIP caused a reduction in spontaneous contractions and tone. Some clinical studies have described a reduction in VIP levels in patients suffering from idiopathic detrusor instability (Gu, Restorick, Blank, Polak, Bloom and Mundy, 1983), although other studies do not support the role of VIP in bladder disorders (Klarskov, Fahrenkrug, Holm-Betzen, Norgaard, Ottesen, Walter and Hald, 1984). With so much conflicting data, the role of VIP
as a bladder neurotransmitter remains speculative.

**Vesical afferent innervation.** Hypogastric and pelvic nerves contain not only motor but also sensory nerves (Kuru, 1965). Afferent axons accompanying sympathetic nerves are thought to be responsible for the conscious visceral sensation of bladder fullness. Afferent axons in the parasympathetic nerves are believed to convey to the spinal cord unconscious information which is important in producing detrusor contractions in response to bladder fullness.

Afferent information initiating reflex micturition is generated in the bladder wall by the activation of mechanoreceptors (Iggo, 1955). Sensory endings can be found in every layer of the vesical wall (Kuru, 1965). Two major types of sensory receptors have been localized in the bladder; encapsulated and non-encapsulated. When the intravesicular pressure rose as the cat bladder was filled with fluid, increased impulses were recorded from single afferent fibers in the the cat pelvic plexus (Iggo, 1955). Since micturition is transiently inhibited after spinal cord transection, it follows that afferent information from bladder mechanoreceptors is transferred to supraspinal centers, including the pontine-mesencephalic center, allowing activation of normal bladder voiding (Kuru, 1965).

The role of substance P as the bladder sensory nerve neurotransmitter is well established (see Maggi and Meli, 1986). A great deal of evidence is derived from experimental studies involving the specific sensory neurotoxin, capsaicin, 8-methyl-N-vanillyl-6-nonenamide. Capsaicin, the pungent ingredient of hot
peppers, was first shown to produce sensory nerve dysfunction when a specific and long-lasting insensitivity to chemical irritants occurred after topical cutaneous administration of the substance (see Buck and Burks, 1983). Systemic administration of capsaicin demonstrated similar effects that were of long duration. Capsaicin was subsequently shown to deplete substance P from the dorsal horn of the rat spinal cord (Jessell, Iversen and Cuello, 1978). Later studies have demonstrated depletion of the peptide in other primary afferent neurons and the tissues innervated by them (see Buck and Burks, 1983). Capsaicin treatment of adult rats depletes substance P levels in bladder afferent nerves (Maggi, Santicioli, Geppetti, Furlo, Frilli, Conte, Fanciullacci, Giuliani and Meli, 1987), while neonatal administration of the neurotoxin destroys the sensory fibers (Sharkey et al., 1983). Part of the effects of capsaicin are believed to be due to excitation of substance P containing neurons and the subsequent depletion of the transmitter from them. In vitro and in vivo experiments have shown that the administration of capsaicin initially produces bladder contraction which is due to the release of substance P (Maggi et al., 1984; Maggi et al., 1985). The effects of capsaicin administration on bladder function are striking. Urine retention and a marked increase in the micturition threshold, which could not be attributed to changes in motor function, were produced in capsaicin-treated neonatal rats (Sharkey et al., 1983). Pretreatment of adult rats with capsaicin increases the volume and pressure threshold for micturition (Santicioli, Maggi and Meli, 1985) but has only modest effects on the ability of conscious rats to excrete a water load (Holzer-Petsche and Lembeck, 1984). These data
support the view that capsaicin-sensitive bladder afferents are primarily involved in regulation of reflex micturition (Maggi and Meli, 1986).

1.3. **Urinary Bladder Dysfunction in Diabetes Mellitus**

1.3.1. **Clinical studies**

Diabetic urinary bladder dysfunction has been attributed to autonomic neuropathy. The incidence of bladder dysfunction in the diabetic population has been reported to be 26-87% (Niakan, et al., 1986). An increase in duration of the disease is associated with increased incidence of bladder disorders, although other researchers suggest that the severity of diabetes is a more important indicator (Buck, McRae and Chisholm, 1974). A significant correlation has been found between incidence of bladder dysfunction and other diabetic complications such as somatic neuropathy, retinopathy and impotence (Ellenberg, 1980; Bartley, Brolin, Fagerberg and Wilhemsen, 1966). Like other autonomic neuropathies, bladder dysfunction is usually insidious in its onset and often remains undetected until the appearance of secondary effects such as urinary tract infections. There appears to be at least two stages of diabetic bladder dysfunction (Ellenberg, 1980). The initial stage is characterized by infrequent voiding, which is believed to be the result of sensory nerve impairment. Therefore, when patients in this stage do micturate, they completely expel large volumes of urine at each voiding. In the second, more advanced stage of diabetic bladder dysfunction, the bladder has progressed to the point of decompensation. A symptom characteristic of the stage is the inability to void completely, which
is attributed to parasympathetic motor nerve dysfunction. The presence of an abnormal residual urine and stasis results in complications such as urinary tract infections. Morphological data demonstrating pathological changes in bladder motor nerves supports the theory of diabetes-induced decompensation. The detrusor muscle is innervated by non-myelinated nerve fibers which are the earliest and most severely damaged in diabetes (Bartley et al., 1966). Other studies have attributed diabetic vesical dysfunction to segmental demyelination in the peripheral nerves supplying the detrusor muscle (Anderson and Bradley, 1976). At least one histological study has been performed on bladder tissue of diabetic patients in which the most important observation was a reduction or absence of cholinesterase activity at the muscular and nerve axon levels (Faerman, Glocer, Celener, Jadxinsky, Fox, Maler and Alvarez, 1973).

The typically asymptomatic nature of diabetic bladder dysfunction necessitates the use of a series of clinical tests to assess organ status. One of the most routinely used of these methods is cystometry (Tanagho, 1984b). As explained previously, cystometry involves the distention or stretching of the detrusor muscle with air or fluid with concurrent recording of intravesical pressure. It thus provides a measure of pressure in the bladder as it fills. The most important clinical observation that can be made during a cystometrographic procedure is whether the patient develops a detrusor reflex and can consciously suppress it. Upon infusion of fluid into the bladder of a normal patient, there is a slow rise in intravesical pressure up to 10 to 20 cm H$_2$O. As the infusion continues, there is an increasing desire
to void (Kozak, 1982) (see Figure 3). This continues up to a volume of 500 ml or so at which point there is a maximum urge to void and a voiding contraction may be induced. Based upon cystometrographic studies, two profiles of diabetic bladder dysfunction have been established (Kozak, 1982; Bradley, 1980). The most frequently observed cystometrographic profile demonstrates a bladder of large volume, marked loss of sensation and a minimal rise of pressure of 5-10 cm, even up to a volume of 1000 ml (see Figure 4). This profile occurs predominantly in insulin-dependent patients with disease of long duration (Bradley, 1980). Impairment of the bladder muscle sensory reflex is known to affect the generation of a detrusor reflex. A second cystometrographic profile observed, although less frequently is that of the patient with an uninhibited hypotonic bladder (Kozak, 1982; Bradley, 1980). This patient's cystometrogram is characterized by sudden, involuntary, high increases in pressure, usually in the early stages of filling at 100-200 ml, which are associated with a strong urge to void (see Figure 3). This behavior often results in an episode of urinary incontinence. Detrusor hyperreflexia is believed to be the result of interruption of cortical or spinal regulatory tracts (Bradley, 1980). Besides cystometry, detrusor dysfunction has been assessed by roentgenological investigations in the form of micturition urethrocystography (Bartley et. al., 1966). In a study of 75 diabetic patients, more than half of the cases had an abnormally large bladder, and an abnormal organ shape (atony) was observed in 40% of the cases. Hence, these studies indicate that detrusor dysfunction is common in diabetic bladder disease.
Measurement of urinary flow rate is another common test used to evaluate bladder function. The act of voiding occurs because of a synchronized interaction between the bladder body and the base. Therefore, an abnormal flow rate may reflect dysfunction of either component. Urine flow is simply measured by having a patient void into a flowmeter (Tanagho, 1984b). Two types of urine flow patterns have been observed in diabetic patients (Bradley, 1980). In some patients, low peak flow and prolonged duration of flow associated with increased residual volume occurs. In another group of diabetics, urine flow is characterized by a straining pattern of voiding marked by short, interrupted spurts of urine. However, these studies do not indicate whether the bladder body or base is affected by diabetes. Bladder body dysfunction can be assessed by the methods described above. The function of the bladder base can be evaluated either by recording the electromyographic activity of the voluntary component of the sphincteric mechanism or by recording the activity of both smooth and voluntary components by measuring the intravesical pressure in the sphincter (Tanagho, 1984b). In a subgroup of diabetic patients with detrusor hyperreflexia, abnormal sphincter patterns were also observed (Bradley, 1980). Disorders of the internal and external sphincter have also been diagnosed by roentgenological investigations (Bartley et al., 1966). Approximately 23% of patients presented with disorders of the internal sphincter, while 30% displayed dysfunction of the external sphincter. Hence, clinical studies demonstrate that the function of both the bladder body and bladder base are affected by diabetes.
1.3.2. Experimental studies

The most striking finding upon dissection of a diabetic animal is the presence of a grossly enlarged bladder, which may be observed as early as two weeks after induction of the disease (Lincoln, Crockett, Haven and Burnstock, 1984). The increased organ weight in 8-week STZ rats was accompanied by significant increases in bladder diameter, muscle wall thickness and muscle wall area (Lincoln, Haven, Sawyer and Burnstock, 1984). The increased muscle wall area was attributed to increased cell size rather than to hyperplasia. These data are supported by studies in 5 week alloxan treated rats in which an increase in the mean cross-sectional area of the detrusor smooth muscle cells was observed using electron microscopy (Uvelius, 1986). In the same study, total bladder collagen content was found to increase although collagen concentration decreased due to the hypertrophy of smooth muscle cells. Changes in the structural composition of the bladder may result in altered smooth muscle contractile properties. Using diabetic detrusor strips, Longhurst and Belis (1986) demonstrated a reduction in maximum contractile response to KCl compared to control. However, Kolta, Wallace and Gerald (1985) and Uvelius (1986) did not demonstrate any diabetes-induced changes in the contractile responses of detrusor strips to KCl.

Diabetes-related changes in the autonomic innervation of the rat urinary bladder have been reported by a number of researchers. Total diabetic bladder levels of choline acetyltransferase and acetylcholinesterase were significantly increased after 2 weeks of STZ-
induced diabetes with greater changes by 8 weeks, although the concentration of these enzymes was reduced because of an increased organ weight (Lincoln et al., 1984). However, no significant increase in responsiveness of bladder strips to cholinergic stimulation was observed. In other studies, cholinergic stimulation of diabetic rat bladder body strips has resulted in a decrease in maximum contractile force (Longhurst and Belis, 1986) or an increased contractile response (Kolta et al., 1985). Nerve-stimulation of these preparations has also yielded conflicting results (Uvelius, 1986; Longhurst and Belis, 1986; Lincoln et al., 1984). In genetic animal models of diabetes, such as the diabetic Chinese hamster, abnormalities in pelvic visceral nerves have also been reported (Dail et al., 1977).

The effects of diabetes on the responsiveness of the bladder to adrenergic stimulation have not been studied as extensively as the cholinergic aspect. Detrusor strips from diabetic rats demonstrated increased (Kolta et al., 1985) or no change (Uvelius, 1986) in phenylephrine responsiveness. In addition to alpha-adrenoceptor mediated contraction, beta-adrenoceptor mediated bladder relaxation has been studied in diabetic rat bladders as well. No changes were reported in the responsiveness of diabetic bladder strips to isoproterenol (Kolta et al., 1985; Lincoln et al., 1984); however, when an intact organ was used, diabetic bladders exhibited an increased response (Kolta et al., 1985). An examination of neurochemical parameters revealed no change in total norepinephrine content after 2 weeks of STZ-induced diabetes, although a threefold increase in total dopamine content was observed (Lincoln et al., 1984). Following 8 weeks of diabetes, norepinephrine
and dopamine levels per bladder were not different from control levels.

The effects of diabetes on the responsiveness of the bladder base to autonomic agents has also been studied. Longurst and Belis (1986) reported decreased sensitivity of STZ-induced diabetic rat bladder base to nerve stimulation but not to norepinephrine, acetylcholine, ATP or KCl. However, only norepinephrine exhibited a change in the maximal responsiveness. By contrast, Kolta et. al. (1985) reported enhanced responsiveness in bladder base region to cholinergic and adrenergic agonists in the 6 and 47 week STZ-diabetic rat.

Changes in a few of the less well characterized putative bladder transmitters have been demonstrated in diabetic bladders. Organs derived from untreated, non-ketonuric diabetic rats of 62 days duration produced more total PGI₂ as well as more PGI₂ per unit weight (Jeremy, Mikhailidis, Thompson and Dandona, 1986). VIP content in the urinary bladder of diabetic Chinese hamsters was reportedly increased on the basis of pmol/organ (Diani, Peterson, Sawada, Wyse, Blanks, Gerritsen, Terenghi, Varndell, Polak, Blank, and Bloom, 1985). Qualitative immunochemistry suggested that the increased VIP was due to expanded distribution of the peptide. However, there is the possibility that one or more of these non-adrenergic, non-cholinergic transmitters may contribute to or compensate for diabetic bladder dysfunction.

In summary, urinary bladder dysfunction is affected by both organ structure and nervous innervation. Bladder elasticity can affect urine storage as well as expulsion. In addition to physical aspects, organ accommodation is also affected by bladder innervation. Sympathetic
nervous input facilitates urine storage whereas expulsion is mediated by parasympathetic nervous input, presumably in conjunction with non-adrenergic-non-cholinergic neurotransmission. Motor nerve stimulation, and hence, bladder contraction, is dependent upon distension-induced activation of sensory receptors in the bladder wall. Urinary bladder dysfunction associated with diabetes mellitus is believed to result from biochemical abnormalities in autonomic nerves. Sensory and motor nerve dysfunction are believed to be responsible for the atonicity and large capacity characteristic of the diabetic bladder. However, considerable discrepancies occur amongst laboratories as to the etiology of the condition in experimental diabetes mellitus.
Although urinary bladder dysfunction occurs at a relatively high frequency in diabetic patients, studies thus far utilizing animal models of the disease have failed to examine the disorder in a comprehensive manner. Despite the fact that the initial stage of diabetic bladder dysfunction has been attributed to sensory deficit, there has been no analysis of afferent nervous function in experimental diabetes. Assessment of efferent nervous function has primarily been restricted to cholinergic and some adrenergic responses of bladder strips from diabetic (of varied duration of disease) and control animals. Interpretation is difficult because of the marked inter-laboratory variability in the data. Furthermore, in vivo analysis of bladder function in diabetic animals has not been reported. Finally, while searching for functional changes believed to be the result of autonomic neuropathy, the effect(s) of increased diuresis which occurs in diabetes has been overlooked.
The purpose of these studies was to provide a more complete assessment of urinary bladder dysfunction in the short-term diabetic rat. This was accomplished through the use of in vivo techniques to allow for examination of all neurological aspects involved in the micturition response, including the sensory component. In vitro organ techniques were utilized to determine if structural alterations occur in the bladder of diabetic and diuresed rats. In vitro studies were also performed to examine functional organ responsiveness to classical as well as non-classical neurotransmitters. And for each of these techniques, results from diabetic animals were compared with animals which had undergone parallel diuresis. This was performed in order to eliminate the possibility of a non-specific, diuretic effect from diabetic bladder dysfunction, thereby allowing the determination of true autonomic neuropathic involvement.
CHAPTER III

SPECIFIC AIMS

1. To determine whether changes occur in urinary bladder function of the 4 week STZ-diabetic rat.

2. To determine if changes in diabetic bladder function occur because of an effect of diuresis on structural parameters.

3. To determine if changes in diabetic bladder function have a neurological basis.

4. To determine the identity of the neurological alterations and to determine whether they result from diabetes induced metabolic factors or diuresis.

5. To compare bladder function in short term (4 week) STZ-diabetic rats with clinical diabetic bladder dysfunction.
CHAPTER IV
EFFECTS OF DIURESIS AND DIABETES ON THE RAT URINARY BLADDER:
STRUCTURE AND FUNCTION

4.0. Introduction

Polydypsia and polyuria are two of the cardinal signs of diabetes mellitus. Therefore, the urinary bladder of diabetic individuals must process greater volumes of urine than that of normoglycemic persons. The bladder of any individual can accommodate relatively large volumes of fluid through its passive elastic behavior. The elasticity of the bladder is attributed to a composite of tissue elastin and collagen which can be altered by overdistension beyond physiological limits (Alexander, 1971; Ronchi, Pricolo, Divieti, Palmi, Brigatti and Clement, 1982). The resultant change in elasticity is important because contractility of bladder smooth muscle is governed not only by the initial resting length of the muscle fibers but also by the state of their elasticity (Carpenter, 1968). Furthermore, the sensor of bladder fullness reportedly responds to changes in bladder wall tension which is derived from elastic distension due to filling (Alexander, 1971). Hence, alterations in elastic properties of the bladder, which may occur as a result of excessive distension, could result in changes in organ function.
The diabetic urinary bladder has been described as enlarged, hypotonic and insensitive. These characteristics have been attributed to initial sensory nerve damage followed by motor nerve dysfunction. However, as suggested above, diabetic patients also void large volumes of urine regardless of an underlying neuropathy simply because of the osmosis which results from the hyperglycemic state. Because factors such as bladder overdistension can lead to elasticity changes, which, in turn, promote functional alterations, we are faced with the prospect of two possible causes of diabetic bladder dysfunction; i.e. neurological and physical. The importance of ascertaining whether or not physical organ changes contribute to the bladder disease cannot be underestimated since both the deficient sensation and contractile ability of the organ, which have been described in diabetic patients, can be attributed to elastic rather than neuropathic changes.

In an attempt to examine the contribution of diuresis induced changes in elasticity to diabetic bladder dysfunction, the following model systems were employed. The streptozotocin (STZ) treated rat was utilized as the model for diabetes mellitus. The dose of STZ was chosen to be 65 mg/kg since it produces marked, although incomplete, insulin depletion which is not associated with ketonuria (Junod, Lambert, Stauffacher and Renold, 1969). The effects of diabetes on urinary bladder function were assessed four weeks after induction of the disease. At this time, diabetic rats already demonstrate alterations in bladder function as well as signs of autonomic neuropathy in other organs. In an attempt to reproduce the autonomic neuropathy believed to be responsible for diabetic bladder dysfunction, the galactosemic rat
was used as a model system. Galactosemia has been associated primarily with changes in motor nerve conduction velocity, and little research has been done on the usefulness of this system in the study of organ dysfunction attributable to autonomic neuropathy. The benefit of the galactosemic model, in terms of enhanced speed and degree of nervous dysfunction, is the result of marked accumulation of galactitol in nerves. The final treatment group utilized was chosen to represent a control for the increased diuresis which accompanies the diabetic condition. Rats which are administered modest quantities of sucrose in their drinking water will ingest and excrete large volumes of fluid (Carpenter, 1983). There is no evidence for nerve damage due to administration of this sugar, at these doses, for this particular length of time. However, it should be mentioned that chronic ingestion of a diet containing 68% sucrose is associated with nerve edema and increased endoneurial fluid pressure (see Brown et al., 1982).

4.1. Materials and Methods

Male Sprague Dawley rats (Harlan), 220-250 g, were utilized in all experiments. Animals were housed in groups of 4 per cage at a controlled temperature of 23° C on a 12 hour light/dark cycle. Purina lab chow and water were supplied ad libitum where applicable. Four experimental conditions were compared: control, diabetic, galactose fed, and sucrose fed. Rats were made diabetic by a single injection of streptozocin (U.S. Biochemical) (65 mg/kg i.p.) after an overnight fast. Approximately 5 days post-injection, a plasma sample was obtained by tail vein puncture, and the glucose level was estimated from the
peroxide produced by glucose oxidase (Sigma) (Dahlqvist, 1968). Animals with plasma glucose levels exceeding 300 mg/dl were assumed to be diabetic, and only these rats were used for further experimentation. Galactose treated rats received the sugar (U.S. Biochemical) as 50% of their diet, the sugar being mixed in appropriate proportions with pulverized Purina rat chow and supplied ad libitum. Plasma galactose levels were estimated from the peroxide produced by galactose oxidase (Sigma) (Dahlqvist, 1968). Reversibility studies were performed by removing galactose from the chow for 4 weeks. Sucrose treated rats were fed 5% sucrose (E M Science) in their drinking water and received unmodified rat chow and water ad libitum. The period of treatment for all experiments was 4-5 weeks.

Urine output over 24 hours was measured for each of the treatment groups. One rat was placed per metabolism cage with food and water ad libitum and, where applicable, galactose or sucrose supplementation. No animal was tested more than once.

Cystometrography measures pressure changes which occur in the urinary bladder in response to increasing volumes of intravesicular fluid. In vitro cystometrography, adapted from the techniques of Levin and Wein (1982), was performed to evaluate compliance changes. Rats were anesthetized with urethane (1.2 g/kg, s.c.), and a midline incision was made to expose the urinary bladder. The ureters were ligated and sectioned below the ligatures. The bladder base was then severed at the level of the pelvic bone. After emptying the bladder completely, the urethra was secured to a cannula connected to a pressure transducer and
infusion pump. The organ was maintained at 37° C in an aerated (95% O₂/5% CO₂) tissue bath containing Tyrode's buffer. After a 30 minute equilibration period, warmed Tyrode's buffer was infused into the organ at a rate of 0.05 ml/min, while the pressure was continuously monitored via a Grass polygraph. Plateau pressure and capacity derived from each cystometrogram are defined in Figure 4. Cystometrography was repeated at least three times for each bladder, and the results averaged. Variation amongst the replicates was less than 5%. Pressure-volume data were converted to strain-volume data by the formula (Ronchi et al., 1982)

\[
S = \frac{p \cdot (\sqrt[3]{V_f} + \sqrt[3]{V_f} + V_b)}{4 \cdot (\sqrt[3]{V_f} + V_b - \sqrt[3]{V_f})}
\]

where \( S \) is strain, \( P \) is pressure, \( V_f \) is the volume of buffer infused into the bladder, and \( V_b \) is the volume occupied by the bladder wall (estimated from the bladder weight).

Bladders used in in vitro cystometrography were weighed and frozen for later biochemical analysis. Protein was measured by the method of Lowry (Lowry, Rosebrough, Farr and Randall, 1951). DNA was measured by the indole method (Hubbard, Matthew and Dubowik, 1974).

The following statistical tests were performed. When multiple comparisons were made amongst treatment groups, Tukey's multiple range test was performed on ranked data. When multiple treatment groups were compared to the control group only, Dunnett's test was employed. The Student's t test was used in all other circumstances. For all tests, the level of significance was set at \( p<0.05 \).
Figure 4. Schematic representation of an in vitro cystometrogram. The figure defines the following parameters: plateau pressure, twice the plateau pressure, and fluid capacity.
4.2. Results

4.2.1. General observations

After a period of 4 weeks, control, diabetic, galactose treated and sucrose treated rats were compared. Diabetic rats were characterized by a significant loss of weight as compared to control animals and elevated plasma glucose levels of 592 ± 16 mg/dl (n=8) as compared with control levels (130 ± 2 mg/dl, n=8). Plasma glucose levels were not affected by sucrose (130 ± 5 mg/dl, n=10) or galactose feeding (127 ± 7 mg/dl, n=9). The traditional symptoms of polyuria, polydipsia and polyphagia were also evident in the diabetic animals. The presence of autonomic neuropathy was suggested by intestinal distention and diarrhea; poor peripheral blood supply (making bleeding from the tail vein difficult) and incidence of abnormal penile erection. By contrast, galactose fed animals did not exhibit any of these signs, although accumulation of the sugar (or its alcohol) was suggested by the development of cataracts and elevated plasma galactose levels (156 ± 19 mg/dl, n=8) as compared with control rats (36 ± 2 mg/dl, n=6). No remarkable differences were noted in the sucrose fed animals. There was no significant difference between the body weights of sugar fed animals and controls.

The average 24 hour urine output for sugar fed and diabetic rats was approximately 10-fold greater than for control animals (Table 1). All treatments were significantly different from control but not from one another.
Table 1
24-Hour urine output by treated and control rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>URINE OUTPUT (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.2 ± 0.9</td>
</tr>
<tr>
<td>Diabetic</td>
<td>115.6 ± 12.3*</td>
</tr>
<tr>
<td>Galactose</td>
<td>97.5 ± 28.4*</td>
</tr>
<tr>
<td>Sucrose</td>
<td>93.1 ± 9.4*</td>
</tr>
</tbody>
</table>

Urine was collected from 4-6 rats per group for 24 hours after 4 weeks of treatment. Values represent mean ± S.E.M. * indicates urine output significantly greater than control (p<0.05).
4.2.2. Biochemical studies

The wet weights of bladders derived from sugar fed and diabetic animals were significantly greater than those of control rats (Table 2). The increase in organ weight was associated with a significant increase in protein and DNA content per bladder for all three treatments. When protein and DNA were normalized to the weight of the organ, there were no significant differences between the groups.

4.2.3. In vitro cystometrography

Representative cystometrograms from treated and control bladders are shown in Figure 5. The plateau pressure and fluid capacity of bladders from treated rats were significantly different from control but not from one another (Table 3). During the course of in vitro cystometrograms derived from galactose fed and diabetic animals, large, transient increases in pressure were noted in 60% of the bladders (see Figure 3). Such peaks were limited to one per cystometrogram and were randomly observed in three consecutive cystometrograms made for a single bladder. Addition of 1 μM hexamethonium to at least one preparation did not appear to diminish the peak intensity. When the pressure values derived from the cystometrograms of control and treated bladders were converted to strain, the strain measurement, which progressively increases as a function of volume, showed a much slower rate of increase in the treated groups (Figure 6).
Table 2
Biochemical analyses of treated and control bladders

<table>
<thead>
<tr>
<th></th>
<th>RAT WT. (g)</th>
<th>BLADDER WT. (mg)</th>
<th>PROTEIN PER BLADDER (mg)</th>
<th>DNA PER BLADDER (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>388 ± 14</td>
<td>117 ± 3</td>
<td>15.4 ± 0.4</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Diabetic</td>
<td>254 ± 13*</td>
<td>155 ± 13*</td>
<td>18.8 ± 0.8*</td>
<td>0.35 ± 0.04*</td>
</tr>
<tr>
<td>Galactose</td>
<td>336 ± 17</td>
<td>182 ± 11*</td>
<td>22.3 ± 0.6*</td>
<td>0.34 ± 0.03*</td>
</tr>
<tr>
<td>Sucrose</td>
<td>377 ± 17</td>
<td>171 ± 6*</td>
<td>20.6 ± 0.8*</td>
<td>0.34 ± 0.03*</td>
</tr>
</tbody>
</table>

Rats were treated for 4 weeks with sucrose (n=11), galactose (n=10) or were made diabetic (n=11). Values represent mean ± S.E.M. * indicates significant difference from control (n=14) (p<0.05). No significant differences were present when protein and DNA were normalized to the weight of the bladder.
Figure 5. Representative in vitro cystometrographic tracings from control and treated rat bladders. Shown are typical tracings from control (A), diabetic (B) and sucrose fed (C) bladders. Cystometrograms from galactose fed rat bladders were nearly identical to those from diabetic organs.
In vitro cystometrographic parameters from treated and control rat bladders

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>PLATEAU PRESSURE (cm Hg)</th>
<th>CAPACITY (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0.76 ± 0.09</td>
<td>2.11 ± 0.12</td>
</tr>
<tr>
<td>Diabetic</td>
<td>6</td>
<td>0.34 ± 0.04*</td>
<td>3.40 ± 0.47*</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4</td>
<td>0.42 ± 0.05*</td>
<td>3.24 ± 0.11*</td>
</tr>
<tr>
<td>Galactose</td>
<td>6</td>
<td>0.32 ± 0.02*</td>
<td>4.28 ± 0.53*</td>
</tr>
</tbody>
</table>

In vitro cystometry was performed on bladders obtained from diabetic rats or from rats fed sugars for 4-5 weeks. Values represent mean ± S.E.M. * indicates significant difference from control (p<0.05).
Figure 6. Strain vs. volume plot for treated and control organs. Circles represent values from control bladders (n=7), and squares represent data from diabetic bladders (n=3). Data from galactose and sucrose treated rat bladders were nearly identical to those from diabetic rats.
Studies were designed to determine whether the effects of galactose on the bladder were reversible. After maintaining rats on a galactose diet for four weeks, animals were returned to a normal diet for an additional 4 weeks. The weights of these bladders and their plateau pressures returned to values not significantly different from control. Transient contractions were not apparent in any of these preparations. Although the fluid capacity of the bladder was reduced by almost one-half of the original galactose treated value, it remained significantly greater than control.

The effects of acute bladder distension on the bladder capacity of a control rat were examined. The initial bladder capacity of the organ was 0.90 ml as determined by cystometrography. The organ was filled with a volume of fluid approximately 80% greater than its original capacity, then the fluid expelled and another cystometrograph generated. The bladder capacity from the second cystometrograph was increased by approximately 29%. The bladder was again filled well beyond its capacity and the same procedure repeated. Again, the bladder capacity was increased, this time by 35% of its original value. The third time the process was repeated, the bladder capacity had further increased by 46% of its original value. The plateau pressure, however, was not altered during any of these cystometrographs.
4.3. Discussion

Polyuria is a frequently noted symptom of diabetes mellitus (Thomas et al., 1982; Kozak, 1982; Hosking, Bennett and Hampton, 1978; Niakan et al., 1986). In order to promote enhanced diuresis, the sucrose fed rat was utilized as a control for non-neurogenic bladder changes that may occur in the diabetic and galactose fed rats. The weights of the bladders from all of the treatment groups were 30-50% greater than control. This suggests that diuresis alone may alter the physical properties of the organ, a finding that has been described by others (Carpenter, 1983; Lincoln et al., 1984). The increase in organ weight was accompanied by an increase in protein and DNA, which suggests that the weight gain is not simply due to water accumulation (Table 2). A similar increase in protein and DNA has been reported in the muscularis and mucosa of rat urinary bladders distended by parasympathetic denervation (Ekstrom, Henningsson, Henningsson and Malmberg, 1984). In our experiments, since microscopic analyses were not performed, the possibility of polyploid nuclei cannot be ruled out to explain the increase in DNA content. Lincoln et al. (1984) suggested that the increase in muscle wall area observed in diabetic bladders is more likely due to an increase in cell size than to hyperplasia. In addition to changes in smooth muscle size, an increase in total bladder collagen was observed in diabetic rats (Uvelius, 1986). Hence, a number of structural changes occur in the bladders of diabetic, as well as sucrose and galactose fed rats.

In order to assess whether or not changes occur in the compliance
properties of the diabetic urinary bladder, in vitro cystometrography was performed. Cystometrography is a method by which pressure changes in the urinary bladder are measured in response to increasing volumes of infused fluid. Because the organ is separated from extravesicular innervation in this preparation, the procedure only measures the stretch properties of the bladder wall. However, the presence of the transient increases in pressure observed in the diabetic and galactose fed rat bladders suggests, perhaps, activation of some intravesicular innervation. Evidence of spontaneous contractions was also reported by Moss, Lincoln and Burnstock (1987) in a similar preparation. The absence of an effect of hexamethonium on the contractions and a failure of nicotine (in other experiments) to induce stimulation of the isolated organ suggests a lack of ganglia in this preparation as has been reported in bladder strips (Taira, 1972). Perhaps the contractions are the result of the action of local modulators, such as prostaglandins, which are released from the tissue by distension (Jeremy et al., 1984) and elicit bladder contraction (Ueda, et al., 1985; Ambache and Zar, 1970; Hills, 1976). However, bladder innervation is generally not believed to play a significant role in the appearance of the cystometrogram (Tang and Ruch, 1955). Rather, stretch or distension was found to be the only factor modulating bladder tonus. In fact, the cystometrograms from these in vitro experiments are similar to those observed in vivo (see Chapter V).

In all three treatment groups associated with marked diuresis (Table 1), reduced plateau pressures and increased fluid capacities were observed (Table 3). These data reinforce the finding that elevated
diuresis alone may be sufficient to promote alterations in physical parameters of the urinary bladder; i.e., increased bladder size accompanied by an increase in the compliance of the organ. At this point, it is important to remember that we have defined capacity in a different manner than applies to the clinical situation. That is, clinically, capacity is the volume at which the patient has a strong desire to void. For our purposes, capacity represents an arbitrary value created to set an upper limit for the bladder filling volume since a micturition reflex does not occur in vitro which is the in vivo safeguard against overdistension. The importance of a conservative maximum value is apparent in the experiment which examined the effects of acute distension on the capacity of a control bladder. Obviously, some structural damage occurred during the course of repeated over-filling which caused the bladder capacity to be nearly doubled. This phenomenon might also add insight into the diuresis induced changes in bladder compliance. If acute distension of the bladder can produce compliance changes, it is likely that chronic distension can do the same. One major difference, however, is the fact that the plateau pressure is diminished in treated bladders but not in those which were acutely distended. The flat curve and large capacity associated with sugar-treated and diabetic rat bladders suggests that an increase in elasticity of these organs has occurred (Zinner et al., 1977). Initially, this seems contradictory, because total bladder collagen content, but not concentration, has been found to increase in diabetic rats (Uvelius, 1986). As a connective tissue, collagen is limited in its elasticity. However, changes in elastin and smooth muscle may
compensate for the increases in collagen. The alterations in elasticity which occur in response to diuresis are apparently reversible. Removal of the galactose fed animals from their treatment for a period of four weeks reduced the initial cystometrographic parameters to approximately those of control values. While the bladder weights and plateau pressures of galactose-removed rats were not significantly different from control, the fluid capacity remained elevated. The reason for the persistent increase in bladder capacity is unknown, but perhaps involves structural changes such as were observed in the acute overdistension experiment.

The elasticity of the bladder affects the contractile and sensory functions of the organ (Carpenter, 1968; Motzkin, 1968). The altered compliance properties in treated bladders are proposed to affect the micturition reflex in vivo as follows. Sensory nerve endings are principally located in the collagen compartment of the detrusor muscle (Hald and Bradley, 1982). Deformation of sensory receptors produces activation of afferent fibers which elicits a micturition response due to reflex stimulation of parasympathetic efferent fibers. Sensory receptors were originally believed to respond to changes in bladder wall tension rather than pressure, which is commonly measured by cystometry (Ruch and Patton, 1974). Tension is related to pressure by the Law of Laplace which states that tension = pressure x radius / 2. Hence, the wall tension required to withstand a given fluid pressure is proportional to the vessel radius (Nave and Nave, 1980). However, the problem with using tension to describe the force generated in the bladder walls is that it does not account for changes in the
thickness of the organ. The value of strain was determined to be a more physiologically relevant parameter since it does take into account the changes in bladder wall thickness during distension (Ronchi et al., 1982). Support for the contention that sensory receptors might be sensitive to strain rather than tension comes from observations of increasing strain during the plateau phase of the cystometrogram (during which time the pressure remains constant) and the fact that reflex bladder contractions appear to occur at a relatively constant value of strain (Ronchi et al., 1982). In these experiments, measurements of strain show that at all fluid volumes, the resultant strain is less in bladders of diabetic rats than in control animals (Figure 6). The same effect is seen for sucrose and galactose fed rat bladders. The functional consequence is that an increase in volume of fluid is required to elicit the sensation of fullness and hence micturition. However, this response would be expected to occur in all of the treated animals, not just the diabetic rats. Based upon these observations alone, we might propose that the physical alterations which occur in the bladder wall of the diabetic animal result from the increased urine output which accompanies the disease. Furthermore, these changes may be responsible for some of the symptoms associated with diabetic bladder dysfunction: reduced bladder sensation and hence urine retention and organ distension. The in vitro cystometrograms from diuretic models or diabetic rats are similar to those generated in diabetic patients. Both display a low plateau pressure with an increase in capacity and, in the diabetic patient, these factors are accompanied by a lack of filling sensation (Ellenberg, 1980) (see Figures 3 and 5).
CHAPTER V

EFFECTS OF DIURESIS AND DIABETES ON THE RAT URINARY BLADDER:
IN VIVO FUNCTION

5.0. Micturition Profiles: Unligated Urethra

5.0.1. Introduction

Although several studies have examined the problem of diabetic urinary bladder dysfunction using in vitro methodologies (Moss et al., 1987; Carpenter, 1983; Kolta et al., 1985; Uvelius, 1986; Lincoln, et al., 1984; Longhurst and Belis, 1986), none have utilized a whole animal preparation. The process of micturition is a complex interplay, even at the peripheral level, between sympathetic, parasympathetic and sensory nerves which cannot be addressed in an isolated system. When the bladder stores urine, direct sympathetic stimulation of bladder body beta-adrenoceptors is believed to promote tissue relaxation whereas stimulation of bladder base alpha-adrenoceptors causes contraction and, hence, urine continence. Furthermore, alpha-adrenoceptor activation in the parasympathetic ganglia exerts an inhibitory effect on parasympathetic ganglionic transmission which also promotes organ quiescence. As bladder content increases, producing greater strain in the bladder wall, sensory nerve impulses also increase until a threshold of excitation is reached.
During the expulsion phase which ensues, parasympathetic stimulation causes contraction of the detrusor muscle by muscarinic receptor activation. A decrease in outlet resistance occurs because of both the change in bladder shape during contraction and the parasympathetically mediated contraction of the longitudinal layer of urethral smooth muscle. Furthermore, the effects of parasympathetic stimulation on urine expulsion are facilitated by the cessation of the tonic influences of the sympathetic nervous system.

In vivo analysis of bladder function in the diabetic rat would provide important information about the entire micturition reflex, rather than the simple assessment of effector-receptor function obtained from in vitro studies. A major advantage for using the in vivo system is that it allows examination of the sensory component of the reflex arc. It is the afferent neurons which are believed to be initially affected in diabetic bladder dysfunction thereby leading to the clinical signs of hypotonia and organ enlargement (Ellenberg, 1980). In the previous chapter of this dissertation, it was postulated that changes in the physical properties of the diuresed rat bladder might be responsible for the proposed sensory deficit in diabetic bladders. A comparison between sensory competence in diabetic rat bladders vs. sucrose- and galactose fed rat bladders should reveal whether or not physical changes in organ structure modulate bladder sensory perception. Since galactosemia also produces enhanced diuresis, it is important also, through the use of a sucrose fed model for diuresis, to distinguish between neuropathic and diuretic induced alterations in bladder function. Rats fed a 5% sucrose solution have been utilized by other
researchers as a control for diuresis induced by diabetes mellitus and diabetes insipidus (Carpenter, 1983). A positive control for sensory nerve dysfunction is also utilized for comparative purposes. Systemic capsaicin administration to adult rats has been shown to increase the micturition threshold for up to one month after treatment (Santicioli, Maggi and Meli, 1985). This effect has been correlated with a reduction in substance P levels in sensory neurons innervating the organ (Maggi et al., 1987).

Several investigators have utilized in vivo techniques to analyze bladder function in urethane-anesthetized rats by measuring pressure changes inside the bladder in response to drug administration (Maggi & Meli, 1982; Postius and Szelenyi, 1983). Urethane is the anesthetic of choice for these experiments because it; 1) has little effect on neural input and neurotransmitter release, 2) only slightly depresses bladder muscle contractility and 3) does not impair afferent information to supraspinal sensory structures (see Maggi and Meli, 1986). One problem with these studies, at least for our initial purposes, is that in each case the urethra was in some manner occluded. Since the result of urethral blockade is the absence of fluid emission, this setup does not effectively reproduce the physiological micturition process. Therefore, experiments were initially performed in animals whose urethra was unligated. This allowed study of not only the contractile behavior of the detrusor in response to distension, but function of the sphincter in fluid emission as well.
5.0.2. Materials and Methods

Male Sprague Dawley rats (Harlan), 220-250 g, were used for all experiments. Animals were housed in groups of 4 per cage at a controlled temperature of 23° C on a 12 hour light/dark cycle. Purina lab chow and water were supplied ad libitum where applicable. Four experimental conditions were compared: control, diabetic, galactose fed, and sucrose fed. Rats were made diabetic by a single injection of streptozocin (U.S. Biochemical) (65 mg/kg i.p.) after an overnight fast. Approximately 5 days post-injection, a plasma sample was obtained by tail vein puncture, and the glucose level was estimated from the peroxide produced by glucose oxidase (Sigma) (Dahlqvist, 1968). Animals with plasma glucose levels exceeding 300 mg/dl were assumed to be diabetic, and only these rats were used for further experimentation. Galactose treated rats received the sugar (U.S. Biochemical) as 50% of their diet, the sugar being mixed in appropriate proportions with pulverized Purina rat chow and supplied ad libitum. Plasma galactose levels were estimated from the peroxide produced by galactose oxidase (Sigma) (Dahlqvist, 1968). Reversibility studies were performed by removing galactose from the chow for 4 weeks. Sucrose treated rats were fed 5% sucrose (E M Science) in their drinking water and received unmodified rat chow and water ad libitum. The period of treatment for all experiments was 4-5 weeks.

In contrast to in vitro procedures, in vivo cystometrography is modified by the neuronal input to the urinary bladder. The methods used for in vivo cystometrography were adapted from those of Maggi,
Santicioli and Meli (1986). The urinary bladder of urethane anesthetized rats was exposed through a midline incision in the abdomen. A cannula connected to an infusion pump and pressure transducer was inserted into the bladder dome and secured with a purse string suture. The body temperature of the animal was maintained at 37° C with a heat lamp, while warmed Tyrode's buffer dripped on the exposed organ. After an equilibration period of 30 minutes, warmed buffer was infused into the organ at 0.05 ml/min. Pressure changes were continuously measured via a Grass polygraph. Each bladder was used to generate at least three cystometrograms of approximately 30 minute duration, and the results were averaged. The volume to first contraction, contraction frequency, contraction duration and intercontraction interval are defined in Figure 7.

In some cases, animals were treated with capsaicin, phentolamine or reserpine prior to in vivo cystometrographic evaluation. Only control rats were subjected to capsaicin and phentolamine pretreatment, whereas all groups (control, diabetic and diuretic) received reserpine. A single 50 mg/kg s.c. injection of capsaicin (Sigma) in a vehicle of normal saline, Tween 80 and 95%-ethanol (8:1:1/v:v:v) was given 6-7 days before cystometrographic analysis. Phentolamine (Sigma) was administered, 1 mg/kg, into the tail vein of an anesthetized rat approximately 15 minutes prior to cystometrographic analysis. Reserpine (Sigma), 10 mg/kg, in a 5.5% glucose solution was administered s.c. 24 hours prior to cystometrographic analysis.

The following statistical tests were performed. When multiple
comparisons were made amongst treatment groups, Tukey's multiple range test was performed on ranked data. When multiple treatment groups were compared to the control group only, Dunnett's test was employed. The Student's t test was used in all other circumstances. The level of significance was set at p<0.05 for all tests.

5.0.3. Results

From the various parameters measured in the in vivo cystometry, three patterns of results were observed (Table 4, Figure 8). Compared to control, the galactose and sucrose treated groups were characterized by a large volume of fluid required to elicit first contraction, low contraction frequency, small reduction in contraction duration and a small increase in intercontraction interval. Sucrose fed rats also displayed a significantly greater peak height than the control group. The diabetic group was similar to control for the volume to first contraction, was similar to diuretic groups for reductions in contraction frequency, but had a much longer intercontraction interval and shorter contraction duration than any of the other groups. Peak height for the diabetic group was not significantly different from control. The effects of removal of galactose treatment on bladder function were also studied. In vivo cystometry of these bladders demonstrated a return of some of the measured parameters to control levels, the most significant change being a 68% reduction in volume of fluid required to initiate the first contraction (Table 4).

Chemically induced neuropathies were produced to see if bladder changes occur similar to those observed in diabetic animals. Capsaicin
administration produces a specific sensory neuropathy in the urinary bladder (see Maggi and Meli, 1986). When compared to controls, capsaicin treatment resulted in a very large volume to first contraction, a decreased contraction frequency, no change in the duration of contractions, and lengthened intervals between contractions (Table 5). Reserpine administration, which amongst other effects, depletes sympathetic neurons of norepinephrine, reduced the volume to first contraction, decreased contraction frequency, reduced duration of contractions, and lengthened the intervals between contractions (Table 5). The effects of reserpine, which may involve alpha- and beta-adrenoceptor function, were compared with those of the peripheral alpha-adrenergic antagonist, phentolamine. Phentolamine administration also produced a reduction of volume to first contraction and a decrease in contraction time. However, in contrast to reserpine, the contraction frequency was slightly increased and the intercontraction interval reduced (Table 5).

Since the pattern of the in vivo cystometrograms from diabetic animals resembled that from reserpine treated animals more closely than from other groups, additional experiments were performed using this condition. Reserpine pretreatment had smaller effects in the diabetic animals, while it produced quantitatively similar changes in galactose and sucrose treated animals as compared with controls for all parameters except volume to first contraction (Table 6).
Figure 7. Schematic representation of an in vivo cystometrogram. The figure defines the following parameters: A = volume of fluid required to initiate first contraction, B = time between contractions, C = contraction duration, D = peak height. Average frequency of contractions is the total number of contractions divided by the duration of the observation.
Figure 8. Representative in vivo cystometrographic tracings from control and treated rat bladders (unligated). Shown above are typical cystometrograms derived from control (CON), diabetic (DIA), sucrose fed (SUC) and capsaicin treated (CAP) rats.
Table 4
In vivo cystometrographic parameters (unligated) from treated and control rats

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>DIABETIC</th>
<th>GALACTOSE</th>
<th>SUCROSE</th>
<th>REV-GALAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol to 1st contract (ml)</td>
<td>0.30 ± 0.05</td>
<td>0.30 ± 0.06</td>
<td>0.60 ± 0.12*</td>
<td>0.51 ± 0.07</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>Contraction freq. (contr/min)</td>
<td>0.57 ± 0.05</td>
<td>0.36 ± 0.07</td>
<td>0.39 ± 0.10</td>
<td>0.32 ± 0.03</td>
<td>0.51 ± 0.14</td>
</tr>
<tr>
<td>Contraction duration (min)</td>
<td>0.54 ± 0.07</td>
<td>0.18 ± 0.01*</td>
<td>0.31 ± 0.03</td>
<td>0.44 ± 0.06</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>Intercontraction interval (min)</td>
<td>1.37 ± 0.19</td>
<td>3.53 ± 0.78*</td>
<td>1.96 ± 0.39</td>
<td>2.14 ± 0.32</td>
<td>2.18 ± 0.73</td>
</tr>
<tr>
<td>Peak height (cmHg)</td>
<td>3.16 ± 0.23</td>
<td>3.31 ± 0.27</td>
<td>N.D.</td>
<td>4.80 ± 0.39*</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

In vivo cystometrography was performed on diabetic rats (n=5) or rats treated for 4 weeks with galactose (n=4) or sucrose (n=4). A second group of galactose fed rats (n=4) were removed from treatment for four weeks prior to analysis. * indicates the value differs significantly from control (n=6) (p<0.05). N.D. indicates value not determined.
Table 5
Effects of neurotoxic and antagonistic agents on in vivo cystometrography in control rats (unligated)

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>RESERPINE</th>
<th>PHENTOLAMINE</th>
<th>CAPSAICIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>VoI to 1st contract (ml)</td>
<td>0.30 ± 0.05</td>
<td>0.10 ± 0.02*</td>
<td>0.18 ± 0.04</td>
<td>0.66 ± 0.09*</td>
</tr>
<tr>
<td>Contraction freq. (contr/min)</td>
<td>0.57 ± 0.05</td>
<td>0.29 ± 0.01*</td>
<td>0.66 ± 0.07</td>
<td>0.31 ± 0.09*</td>
</tr>
<tr>
<td>Contraction duration (min)</td>
<td>0.54 ± 0.07</td>
<td>0.18 ± 0.02</td>
<td>0.24 ± 0.01*</td>
<td>0.58 ± 0.26</td>
</tr>
<tr>
<td>Intercontraction interval (min)</td>
<td>1.37 ± 0.19</td>
<td>3.73 ± 0.29*</td>
<td>1.29 ± 0.15</td>
<td>2.74 ± 0.39*</td>
</tr>
</tbody>
</table>

In vivo cystometrography was performed for control rats treated with reserpine (n=3), phentolamine (n=4) or capsaicin (n=3) and compared with untreated controls (n=6). * indicates the value differs significantly from control (n=6) (p<0.05).
5.0.4. Discussion

In contrast to in vitro preparations, in vivo cystometrography permits assessment of the neural control of the intact urinary bladder. In these studies, the increased volume to first contraction and the decrease in contraction frequency in galactose and sucrose treated animals suggest a less responsive organ (Table 4). The bladder hyporeactivity may be the result of the physical alterations in the organ described in the previous chapter of this dissertation (see Chapter IV). Data from the sugar fed animals are comparable to those derived from capsaicin treated rats. Capsaicin has been shown to deplete substance P from sensory neurons and produce sensory deprivation (see Buck and Burks, 1983; Maggi and Meli, 1986). However, rather than being neurogenic in origin, the apparent sensory deficit in sucrose and galactose treated animals is attributed in these studies to alterations in the physical properties of the organ. Diabetic rats appear to deviate from the diuretic models in the in vivo preparations. Unlike sugar fed animals, the volume of fluid required to elicit the first contraction in the diabetic animals was the same as in control rats. This result does not correlate with the prediction from in vitro cystometrographic data described in Chapter IV, i.e., a tendency for reduced sensation which would increase the volume to first contraction like the diuretic models. Therefore some change in the parasympathetic, sympathetic, non-adrenergic-non-cholinergic or sensory innervation may be responsible for the hyperreactivity of the large capacity, diabetic bladder.
Phentolamine administration produced qualitatively similar changes, compared to reserpine, in the volume to first contraction and contraction duration but not in contraction frequency or intercontraction interval (Table 5). Some of the differences in the cystometrographic parameters between the two may be due to the fact that phentolamine is selective for blockade of alpha-adrenoceptors whereas reserpine affects neurotransmission at both alpha- and beta-adrenoceptors. Furthermore, there is also the possibility that the effects of reserpine on bladder function are not restricted to peripheral depletion of sympathetic neurotransmitters. Reserpine depletes stores of catecholamines, as well as serotonin, in many tissues, including the brain. Activation of central adrenergic mechanisms in rats by peripheral L-DOPA administration has been shown to influence urinary bladder control by the production of a hyperreactive bladder response (Sillen et al., 1979). It is possible that reserpine may deplete the central adrenergic neurons which mediate bladder contraction. This may explain the observed reduction in contraction frequency and increased intercontraction interval which occurs with reserpine, but not phentolamine, treatment.
Table 6  
Effects of reserpinization on in vivo cystometrography in treated and control rats

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>GALACTOSE</th>
<th>SUCROSE</th>
<th>DIABETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol to 1st contraction (ml)</td>
<td>67%*</td>
<td>38%†</td>
<td>37%†</td>
<td>24%†</td>
</tr>
<tr>
<td>Contraction frequency (contr/min)</td>
<td>49%*</td>
<td>67%†</td>
<td>60%**</td>
<td>15%†</td>
</tr>
<tr>
<td>Contraction duration (min)</td>
<td>66%†</td>
<td>43%†</td>
<td>45%†</td>
<td>7%†</td>
</tr>
<tr>
<td>Intercontraction interval (min)</td>
<td>62%*</td>
<td>68%**</td>
<td>68%**</td>
<td>27%†</td>
</tr>
</tbody>
</table>

Diabetic rats (n=3), controls (n=3), and rats treated with galactose (n=3) or sucrose (n=4) for 4 weeks were injected with reserpine 24 hours prior to cystometrography. Results are expressed as percent change from non-reserpinized values (shown in Table 4). * indicates the value differs significantly from non-reserpinized value for that group (p<0.05).
Alternatively, or perhaps concurrently, reserpine might affect a descending inhibitory serotonergic pathway which reportedly stimulates the activity of the sacral parasympathetic nucleus (Kuru, 1965; DeGroat, Booth, Mihe and Roppolo, 1982). By contrast, phentolamine administration produces effects which can be explained solely on the basis of peripheral alpha-adrenergic blockade. Evidence for a diminished urethral resistance is probably best reflected in the decreased contraction duration although the decline in sphincter competence and, hence, ease of expulsion, would be reflected in other parameters, as well. Alpha-adrenergic antagonism at the level of the parasympathetic ganglia would be expected to facilitate urination which would be characterized by increased contraction frequency, diminished contraction interval and reduced volume to first contraction. The enhanced parasympathetic response in the bladder body coupled with a reduction in urethral pressure in the base thereby act in concert to enhance micturition by increasing the efficiency of expulsion.

The cystometrograms derived from diabetic bladders are obviously different from those obtained from diuretic models. If we try to rationalize this difference on the basis of a dysfunction of neurogenic origin, the cystometrographic profile of the diabetic rat shows all of the characteristics of loss of sympathetic tone except for the absence of a decrease in volume to first contraction (Table 4). However, since this parameter was increased by diuresis, when the diabetic rats are compared to the diuresed controls (sucrose treatment), the volume to first contraction shows a change characteristic of loss of sympathetic tone (Table 4). In the bladder neck, alpha adrenoceptors are
responsible for the maintenance of sympathetic tone as demonstrated by diminished urethral resistance after administration of alpha-adrenoceptor antagonists such as phentolamine (Norlen, 1977; Tulloch, 1975). Beta-2 adrenoceptor activation has been shown to be responsible for inducing relaxation of the bladder body by suppressing the onset of spontaneous contractions (Maggi and Meli, 1982). Thus, a decrease in sympathetic tone to the urinary bladder would result in a decrease in the neurogenic tone of the sphincter smooth muscle and/or a decrease in bladder body relaxation. Such changes are manifested in the in vivo cystometrogram of a reserpinized rat as a decrease in volume required to initiate first contraction, a decrease in contraction frequency, a reduction in contraction duration and an increase in intercontraction volume (Table 5,6). The concept that diabetic bladders are reduced in sympathetic tone was further strengthened by results of experiments in which control and treated rats were pretreated with reserpine prior to cystometrographic analysis. The patency of sympathetic nervous control in the bladders of diuretic models does not appear to be changed; i.e., all in vivo cystometrographic parameters were altered after reserpinization to a similar magnitude as controls (Table 6). The lack of a significant effect on volume to initiate first contraction was taken as an indication of changes in the physical or compliance properties of the diuresed organ. By contrast, reserpinization of the diabetic animals did not result in any significant change in the in vivo cystometrographic profile. Hence, there appears to be a pre-existing loss of sympathetic tone in the diabetic bladder which is expressed by a failure of a significant reserpine effect in these organs.
Other factors which may contribute to, or perhaps be responsible for, the proposed sympathetic deprivation in the diabetic rat include non-neurogenic loss of bladder neck tone and enhanced parasympathetic or other excitatory influences. Concomitant with a reduced sexual function in the male diabetic rat is an atrophy of sexual organs (Cusan, Belanger, Seguin and Labrie, 1980). The possibility exists that a decrease in the size of the prostate gland may partially relieve the sphincter of its competence, resulting in the facilitation of fluid expulsion. Also, the fact that reserpine does not affect the in vivo cystometrogram of diabetic animals may not necessarily be due to a lack of sympathetic influences but rather a predominance of parasympathetic control or perhaps other excitatory neurotransmission. Furthermore, enhanced sensory nerve function, such as might occur due to disruption of Loop II pathways, is also consistent with these results as are changes in the inhibitory influences of central Loop I structures.

Galactose treatment did not appear to induce an autonomic neuropathy analogous to the diabetic condition. In vitro cystometrographic data from the previous chapter suggested that enhanced diuresis which accompanies galactosemia is responsible for alterations in the compliance properties of the organ, resulting in the hyporreactivity observed in the in vivo cystometrograms. To further dismiss the possibility of galactose inducing a permanent neurologic lesion, a group of galactose treated animals were removed from their diet for a period of four weeks. Bladder weights of tissues derived from these animals were not different from control, although the fluid capacity of these organs remained elevated. In vivo cystometrography further supports the
lack of a specific neurological effect since most of the measured parameters for rats removed from the galactose diet returned to control values (Table 4). It is interesting to note that after four weeks of marked diuresis bladder function can return to normal. In fact, the volume to first contraction appears to be less than control, which may represent a supersensitivity to fluid infusion. Based upon these data, we conclude that the 4 week galactose treated rat is not a useful model for autonomic neuropathy as monitored by urinary bladder dysfunction.

The lack of a correlation between galactosemic and diabetic bladder function in our system is supported by several related findings. First of all, the basic mechanism(s) responsible for the induction of galactosemic vs. diabetic neuropathy do not appear to be as similar as once thought. Polyol pathway metabolites cause sciatic nerve hyperhydration in galactosemia but not STZ-induced diabetes (Willars, Lambourne and Tomlinson, 1987). Furthermore, a reduction in ouabain-sensitive ATPase activity in diabetic sciatic nerves has been observed, whereas the same parameter was significantly increased in galactosemic animals (Lambourne, Tomlinson, Brown and Willars, 1987). On a functional level, the limited usefulness of galactosemia as a model for certain diabetic complications has recently been described by Skau and Cacini (1987). The density of membrane-bound acetylcholinesterase was reduced in skeletal muscle and atria of diabetic rats but found to be normal in galactosemic rats. Furthermore, the carbachol dose-response curve was normal in galactosemic rat bladders, whereas in diabetic tissue the curve was shifted to the left. On the basis of these and other data, it was concluded that although galactosemia is an acceptable
model for sugar cataract formation, it is not adequate for other diabetic complications.
5.1. Micturition Profiles: Ligated Urethra

5.1.1 Introduction

The similarities between in vivo cystometrographic profiles from reserpinized and diabetic rats suggest an impairment in sympathetic regulation of these bladders. A decrease in sympathetic input to the urinary bladder would be expected to result in a decrease in neurogenic tone of the sphincter smooth muscle which would facilitate expulsion and/or result in a decrease in bladder body relaxation which would produce hyperreflexia. One in vivo method to distinguish the sympatholytic effects on each component of the bladder is cystometry after ligation of the urethra. This would eliminate the contribution of the bladder base to the cystometrographic profile and permit focus on the role of the bladder body in the functional changes observed in the diabetic rat bladder.

The dissimilarities between the in vivo cystometrographic profiles from reserpinized and phentolamine treated bladders suggest that reserpine may be affecting an additional, perhaps non-peripheral adrenergic or serotonergic site. Since the effects of reserpine mimic those of diabetes, it would be useful to examine more closely the possibility of changes in the spinal and supraspinal control of bladder function in treated rats. The presence of a central monoaminergic component of rat bladder function was examined by Sillen et al. (1979). They found that central monoaminergic stimulation after L-DOPA administration resulted in a hyperreactive bladder response characterized by a high intravesicular pressure and more prominent
detrusor contractions. These effects are believed to occur through the activation of dopamine receptors in the pontine-mesencephalic region of the rat brain and to be mediated peripherally by the pelvic nerves (Sillen, Rubenson and Hjalmas, 1981). Hence, the use of a ligated urethral system, coupled with analysis of the effects of central monoaminergic stimulation, might provide additional insight into higher levels of organ control which might be altered in the diabetic rat. Clinically, diabetic neuropathy is known to affect the central as well as the peripheral nervous system (Rudy and Muehlner, 1941). Several studies have demonstrated diabetes-induced alterations of central nervous function. STZ-induced diabetes of one week duration was found to temporarily alter cerebral acetylcholine control of the rat cardiovascular apparatus (Squadrito, Trimarchi, Lupica, Magri, Costa, Brezenoff and Caputi, 1986). The adrenergic innervation of major cerebral arteries was found to decrease, possibly altering blood brain barrier permeability (Wesselmann, Konkol, Leo and Harder, 1987). Finally, diabetes appears to produce a differential effect on the sensitivity of central dopamine receptor subtypes which mediate diabetes-induced polydypsia (Lewis, Keresztury, Walker, Cook, Milesen and Mailman, 1987).

5.1.2 Materials and methods

Male Sprague Dawley rats (Harlan) were used for all experiments. Animals were housed in groups of 4 per cage at a controlled temperature of 23°C on a 12 hour light/dark cycle. Purina lab chow and water were supplied ad libitum. Three experimental conditions were compared:
control, diabetic and sucrose fed. Rats were made diabetic by a single injection of streptozotocin (U.S. Biochemical) (65 mg/kg, i.p.) after an overnight fast. Rats were determined to be diabetic if urine glucose was present using Lilly TestTape. Urine glucose levels from diabetic rats were typically in excess of 0.1%. Sucrose treated rats were fed 5% sucrose (E M Science) in their drinking water and received unmodified rat chow and water ad libitum. Neither control nor sucrose fed rats tested positive for urine glucose. The period of treatment for all experiments was 4-5 weeks.

Cystometrography was performed essentially as described previously for the unligated urethra studies with modifications. Rats were anesthetized with urethane (1.2 g/kg, s.c.) and a midline incision was made to expose the urinary bladder. A cannula connected to an infusion pump and Statham pressure transducer was inserted into the bladder dome and secured with a purse string suture. Another thread was tied around the distal urethra to prevent fluid expulsion. The body temperature of the animal was maintained at 37°C with a heat lamp, while warmed Tyrode's buffer dripped on the exposed organ. After an equilibration period of approximately 15 minutes, buffer was infused into the organ at the rate of 0.05 ml/min for 15 minutes. During infusion, the bladder intravesicular pressure increased and a series of rhythmic contractions were observed. Pressure changes were continuously measured via a Grass polygraph connected to an IBM computer. The average pressure was calculated by taking the area under the curve at 5 second intervals. After the cystometrography was complete, the bladder was emptied of buffer and allowed to rest for approximately 15 minutes. An injection
of L-DOPA (Sigma) (100 mg/kg, i.p.) was made and a second cystometrogram performed. Parameters including the average pressure, time to first contraction, contraction frequency, contraction duration, intercontraction interval and maximum contraction were measured. The effects of haloperidol (McNeil) (0.5 mg/kg, i.p.) on the hyperactivity produced by L-DOPA were assessed for several animals. Injection of the dopamine antagonist was made during the resting period after the post-L-DOPA cystometrogram. After approximately 15 minutes, another cystometrogram was performed. In some cases, control rats were treated with neurotoxic agents to assess their effects in this system. Capsaicin (Sigma) was administered as a single 50 mg/kg s.c. injection in a vehicle of normal saline, Tween 80 and 95%-ethanol (8:1:1/v:v:v) 8 days prior to cystometrographic analysis. Acrylamide (MCB Reagents) was administered (100 mg/kg, p.o.) every 3 days for a total of 3 doses. Cystometrographic analysis was performed either the day immediately following the last treatment or a week from this date.
5.1.3 Results

The cystometrograms obtained from urethra-ligated control rats were characterized by an increase in pressure upon infusion followed by a series of numerous, brief contractions of increasing peak height (Figure 9). By contrast, the cystometrogram of the sucrose fed rat was characterized by a minimal rise in pressure, followed by only 1 or 2 contractions of relatively sustained duration (Figure 9). The cystometrogram of the diabetic animal demonstrated a pattern in between that of the control and sucrose fed rats (Figure 9). The average intravesicular pressure in the sucrose fed rats was less than that of control and diabetic rats (Table 7). The volume of fluid required to initiate the first contraction was nearly the same as the value derived from unligated urethral preparations and was again increased in the case of the sucrose fed bladder, although insignificantly. However, the volume to first contraction for the diabetic organ was again comparable to control. In this preparation, however, there were no significant differences between contraction duration for any of the groups as there was when the urethra was unligated. The sucrose fed, but not the diabetic rat bladder demonstrated a significantly lower contraction frequency and intercontraction interval. The maximum contraction height produced by diabetic rat bladders was greater than that for sucrose fed or control organs.
Figure 9. Representative in vivo cystometrographic tracings from control and treated rat bladders (ligated). Shown above are typical cystometrograms from control (CON), diabetic (DIA), sucrose fed (SUC) and capsaicin treated (CAP) rats.
Table 7
In vivo cystometrographic parameters (ligated) from treated and control rats

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>SUCROSE</th>
<th>DIABETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average pressure</td>
<td>2.33 ± 0.39</td>
<td>1.19 ± 0.24</td>
<td>2.22 ± 0.32</td>
</tr>
<tr>
<td>cmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vol. 1st contract.</td>
<td>0.34 ± 0.07</td>
<td>0.47 ± 0.12</td>
<td>0.33 ± 0.05</td>
</tr>
<tr>
<td>ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contraction freq.</td>
<td>0.85 ± 0.22</td>
<td>0.23 ± 0.06*</td>
<td>0.45 ± 0.13</td>
</tr>
<tr>
<td>contr/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contract. duration</td>
<td>0.86 ± 0.22</td>
<td>1.08 ± 0.27</td>
<td>1.01 ± 0.23</td>
</tr>
<tr>
<td>min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercontraction</td>
<td>0.89 ± 0.21</td>
<td>4.00 ± 1.22*</td>
<td>1.86 ± 0.78</td>
</tr>
<tr>
<td>interval (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum contract.</td>
<td>5.25 ± 0.51</td>
<td>5.14 ± 1.02</td>
<td>7.76 ± 0.26</td>
</tr>
<tr>
<td>(cmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In vivo cystometrography was performed in urethra ligated control (n=6), sucrose fed (n=5) and diabetic rats (n=4). Values represent mean ± S.E.M. * indicates the value differs significantly from control (p<0.05).
The administration of 100 mg/kg L-DOPA to control rats resulted in a hyperreactive bladder characterized by an increase in average pressure, a reduction in volume to first contraction and a reduction in contraction frequency which was accompanied by a significant increase in contraction duration and a slight increase in intercontraction interval (Table 8). The maximum peak height also increased slightly. By contrast, although the sucrose fed rat did demonstrate a number of changes qualitatively similar to control, L-DOPA induced changes in contraction frequency and intercontraction interval were in opposite directions to those observed in controls. This may be due to the fact that the control bladder has reached its limit of responsiveness, whereas the sucrose fed organ, originally very hyporesponsive, can elicit a greater effect. The effects of L-DOPA on the diabetic rat bladder were typically less pronounced. Interestingly, shortly after L-DOPA administration, 83% of the diabetic bladders responded with a contraction even before infusion was begun. This effect occurred to a much lesser extent in the other two groups. Administration of haloperidol (0.5 mg/kg, i.p.) after L-DOPA produced a significant reduction in the average pressure in sucrose fed (80%) and diabetic (70%) rat bladder cystometrograms. However, haloperidol (0.5 mg/kg, i.p.) did not affect nicotine (Sigma) (20 ug, i.v.) induced bladder contraction, which supports the role of central dopaminergic receptors in this hyperreactive state.

The effects of two neurotoxic agents were also assessed in this system. Eight days after capsaicin (50 mg/kg) administration, cystometrograms from treated rats were characterized by their low
average pressure and the absence of contractions (Table 9). Administration of 100 mg/kg L-DOPA did not increase the intravesicular pressure or produce contractions in the bladders from these animals. Another substance which produces urinary bladder dysfunction is acrylamide. This effect occurs in conjunction with peripheral distal neuropathy which is characterized by hind limb abnormalities such as foot dragging. These effects were observed in treated animals one day after cessation of the treatment regimen. When acrylamide treated rat bladder function was examined at this time, the initial cystometrogram was characterized by a low average pressure and the absence of contractions (Table 9). Administration of 100 mg/kg L-DOPA to these rats was without effect. Topical application of acetylcholine or nicotine injection were capable of eliciting transient, irregular contractions.
Table 8
Effects of L-DOPA on in vivo cystometrography (ligated) in treated and control rats

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>SUCROSE</th>
<th>DIABETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average pressure (cmHg)</td>
<td>29%**</td>
<td>51%**</td>
<td>27%**</td>
</tr>
<tr>
<td>Vol to 1st contraction (ml)</td>
<td>50%**</td>
<td>45%</td>
<td>69%*</td>
</tr>
<tr>
<td>Contraction frequency (contr/min)</td>
<td>46%†</td>
<td>69%**</td>
<td>19%†</td>
</tr>
<tr>
<td>Contraction duration (min)</td>
<td>53%**</td>
<td>53%</td>
<td>4%†</td>
</tr>
<tr>
<td>Intercontraction interval (min)</td>
<td>11%†</td>
<td>88%**</td>
<td>25%†</td>
</tr>
<tr>
<td>Maximum contraction</td>
<td>14%†</td>
<td>15%†</td>
<td>2%†</td>
</tr>
</tbody>
</table>

The effects of 100 mg/kg L-DOPA administration on in vivo cystometrographic parameters was examined in control (n=6), sucrose fed (n=5) and diabetic rats (n=4). * indicates the value differs significantly from non-L-DOPA treated value for that group shown in Table 7 (p<0.05).
Table 9
Effects of neurotoxic agents on in vivo cystometrography in control rats (ligated)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ORGAN WT (mg)</th>
<th>AVG PRESS. (cmHg)</th>
<th>AVG. CON. #</th>
<th>AVG PRESS. (cmHg)</th>
<th>AVG. CON. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>97 ± 6</td>
<td>2.33 ± 0.39</td>
<td>12.8 ± 3.3</td>
<td>3.27 ± 0.28</td>
<td>6.8 ± 1.5</td>
</tr>
<tr>
<td>CAP</td>
<td>133 ± 2*</td>
<td>0.56 ± 0.06*</td>
<td>0</td>
<td>0.44 ± 0.03*</td>
<td>0</td>
</tr>
<tr>
<td>ACR (DAY 1)</td>
<td>138 ± 10*</td>
<td>0.56 ± 0.06*</td>
<td>0</td>
<td>0.49 ± 0.11*</td>
<td>0</td>
</tr>
<tr>
<td>ACR (DAY 7)</td>
<td>154 ± 9*</td>
<td>1.08 ± 0.41</td>
<td>2.7 ± 1.7*</td>
<td>1.71 ± 0.21*</td>
<td>9.7 ± 5.2</td>
</tr>
</tbody>
</table>

The effects of 50 mg/kg capsaicin (n=2) were assessed 8 days after administration to control rats. Acrylamide was administered 100 mg/kg in three doses over a period of 2 weeks. The effects on generation of average intravesicular pressure (avg press.) and average number of contractions (avg. con. #) were examined on the day following the last treatment (n=3) or 7 days following the last treatment (n=3). Values represent mean ± S.E.M. The data were compared with control (n=6) using Student's two tailed t-test for unpaired data. * indicates significant difference from control at p<0.05.
However, when bladder function was assessed 7 days after administration of the final dose of acrylamide, the responsiveness of the bladder appeared to be returning towards control (Table 9). The initial cystometrograms obtained from animals which were allowed to recover from acrylamide toxicity demonstrated an average pressure not significantly different from control and the presence of contractions. L-DOPA administration did elicit an effect, qualitatively similar to control, although the average pressure and the number of contractions were still diminished.

5.1.4 Discussion

The use of a ligated urethral cystometrographic preparation provides further insight into the in vivo function of the diabetic urinary bladder. The system differs from the non-ligated setup in that bladder pressure, and hence, bladder wall strain, is not relieved by micturition but rather increases with continuous fluid infusion. Fluid induced distension of the bladder wall with a consequent increase in sensory receptor discharge triggers the sacral parasympathetic outflow to the pelvic ganglia where detrusor muscle contraction is activated (Maggi and Meli, 1983). The consequence of urethra ligation is apparent in the cystometrogram of control rats (Figure 9). Urethra-ligated bladders typically produce a series of contractions which increase in amplitude with continued infusion. This may be the result of a facilitory mechanism(s) reinforcing cholinergic neurotransmission at the level of the pelvic ganglia (DeGroat and Booth, 1980).
The hyperreactive bladder produced by the administration of L-DOPA is believed to occur as a result of its enzymatic conversion to dopamine which then activates receptors in the pontine-mesencephalic region (Sillen et al., 1979). Dopamine has not been shown to exert direct effects on dopaminergic receptors in bladder smooth muscle but at high doses can activate alpha receptors producing contractions (Benson, Raezer, Anderson, Saunders and Corriere, 1976). The amelioration of the effects of L-DOPA by haloperidol in these experiments and those reported by others support the idea of dopamine receptor stimulation (Sillen et al., 1979). Stimulation of the micturition center results in activation of efferent pathways which promote bladder contraction via pelvic nerve stimulation. Since the micturition process is a reflex phenomenon, it is characterized not only by efferent output, but also afferent nervous input. The importance of sensory nerve competence is apparent by the inability of L-DOPA to stimulate a response in capsaicin treated rat bladders. Likewise, an effect could not be elicited from acrylamide treated rat bladders when examined the day subsequent to the final dose of neurotoxin. The effects of acrylamide on urinary bladder function have really not been studied except for a few descriptions of gross bladder distension in acrylamide intoxicated animals (McCollister, Oyen and Rowe, 1964; Fullerton and Barnes, 1966). Distension was reportedly unaccompanied by abnormalities in the myelinated nerve fibers of affected bladders, although this does not eliminate the possibility of pathological changes in unmyelinated nerve fibers (Fullerton and Barnes, 1966).
The neuropathic effects of acrylamide are most commonly observed in motor and sensory somatic nerve dysfunction in the distal limbs; however, alterations in the autonomic sympathetic nerves controlling the mesenteric vascular beds have been reported (Post and McLeod, 1977).

The neurotoxic effects of acrylamide are believed to be due to an alteration of axonal transport (for review see Miller and Spencer, 1985). Interestingly, capsaicin is also believed to produce its neurotoxic effect by inhibition of axonal transport (Taylor, Pierau and Szolcsanyi, 1985; Miller, Buck, Sipes, Yamamura and Burks, 1982; Otten, Lorez and Businger, 1983). Furthermore, one of the underlying causes of diabetic neuropathy is believed to be axonal transport dysfunction (Tomlinson et al., 1987; MacLean, 1987; Schmidt et al., 1985; and Willars et al., 1986). A comparison between the retrograde axonal transport of proteins in the sciatic nerve of diabetic and acrylamide treated rats has revealed some similarities between the two neuropathies (Logan and McLean, 1988). Of the four proteins whose transport was affected by diabetes, three were also affected by acrylamide treatment. However, the dissimilarities which exist in the profile of proteins affected by the two conditions may be responsible for the marked functional differences observed in the bladders from these animals. The relatively rapid onset and reversibility of the effects of these neurotoxins on bladder function are consistent with abnormalities in axonal transport processes rather than structural damage. It is conceivable that both autonomic motor and visceral sensory nerves may be responsible for the bladder dysfunction associated with acrylamide intoxication such as was proposed to occur in the diabetic condition.
However, unlike the diabetic rat, there appears to be more evidence in favor of a sensory nerve dysfunction. The earliest reported changes after acrylamide administration are an increase in threshold and a diminished response of muscle spindle endings, effects which occurred even before abnormalities in motor fibers (Goldstein, 1985; LeQuesne, 1980). In our preparation, administration of acetylcholine (topically) or nicotine (topically or i.v.) was capable of inducing bladder contractions. Although the sensitivity to these cholinergic agonists was not determined, the results do suggest some competence of motor nerve structures. As has been reported for other forms of neuronal dysfunction associated with acrylamide, the effects on bladder function appear to be reversible. Hence, 7 days after administration of the last acrylamide dose, bladder function appeared to be returning to normal. This relatively rapid reversibility of effects further supports the hypothesis of a neuronal defect unrelated to a structural loss of nerves.

Cystometrographic data from the ligated urethra experiments support the contention that the sucrose fed rat bladder is large capacity and hypotonic. Chronic diuresis is believed to be responsible for the increased volume to first contraction as well as the reduction in contraction frequency observed in the sucrose fed rat bladder (Table 7). The reduction in number of contractions is somewhat surprising considering the fact that the urethra is ligated and that a constant stimulus should produce a series of contractions of increasing peak height as was observed in the control cystometrograms. However, this was not the case. Rather, the cystometrogram of sucrose fed rats was
characterized by the appearance of one or two contractions of rather long duration which were followed by a return of pressure to baseline. Apparently, the sucrose fed bladder has adapted in some way to suppress the appearance of rhythmic contractions. Structural changes in the diuresed organ undoubtedly play a major role in the production of bladder quiescence. Increased compliance of the bladder, which was described in Chapter IV, presumably facilitates storage of increased fluid volumes characteristic of the diuretic state. The increased capacity of the organ suggests that larger fluid volumes are required to produce that critical value of strain which is necessary to trigger micturition. In addition to these physical phenomena, we might also speculate the presence of a neurological component in the hyporreactivity of the sucrose fed bladder. Spontaneous bladder contractions have also been found to be suppressed in vivo after administration of beta-2 adrenoceptor agonists in urethra ligated rats (Maggi and Meli, 1982). By contrast, administration of beta-adrenoceptor blocking agents, such as propranolol, increased the spontaneous motility of the bladder triggered by progressive filling of the organ. However, beta-adrenoceptor stimulation does not appear to increase bladder capacity except at high filling volumes. These data suggest the possibility of increased sympathetic input to the diuretic urinary bladder consistent with its hypotonicity. An increase in sympathetic nerve activity in the sucrose fed organ would provide the functional advantage of facilitating the storage of larger than normal urine volumes.
In contrast to the sucrose fed rat bladder which was described as large capacity and hypotonic, the diabetic rat bladder appears to be large capacity but hypertonic. This statement is based upon the observation that the values for the various in vivo parameters derived from urethra ligated diabetic rats seem to fall between those from control and sucrose fed rats. The cystometrograph which most closely approximates that of the diabetic rat is the hyperreactive, diuresed bladder i.e. the sucrose fed bladder after L-DOPA administration. A pre-existing hyperreactivity in the diabetic bladder is suggested by the minimal effect of L-DOPA administration on cystometrographic parameters (Table 8) and by the appearance of contractions immediately after L-DOPA injection (prior to infusion). The hyporreactivity of the sucrose fed bladder was speculated to partially result from an increase in sympathetically mediated relaxation. The hyperactivity of the diabetic rat bladder might be explained by defect in sympathetic neuronal regulation. These data are consistent with those of the previous section in which similarities between the effects of reserpine and diabetes in the unligated cystometrograph led to the hypothesis of a decline in sympathetic activity in the bladder.

The site(s) of the proposed sympathetic lesion(s) in the diabetic rat bladder have yet to be resolved. A diabetes-induced loss of response in central adrenergic areas is unlikely. Since the pontine-mesencephalic region mediates bladder contraction, dysfunction in the area would produce hypotonicity or atonicity. The result would be a cystometrogram characterized by a low pressure and an absence of contractions accompanied, perhaps, by a supersensitivity to dopaminergic stimulation.
Rather, these studies indicate full functional capacity of the central monoaminergic center. However, we cannot dismiss the possibility of altered regulation of the activity of this locale by central inhibitory pathways e.g. cortical influences. The apparent conversion of L-DOPA to dopamine suggests the presence of intact presynaptic nerve terminals and the resultant response implies functional postjunctional dopaminergic nerves. Sites where a loss of sympathetic input would be characterized by bladder hyperreactivity observed in the urethral-ligated diabetic bladder preparation include the parasympathetic ganglia and the detrusor smooth muscle itself. The final area where sympathetic dysfunction might be relevant is the bladder base where loss of sympathetically mediated competence could result in facilitation of urine expulsion. However, the persistence of hyperreactive bladder response in the ligated urethra preparations suggests that if sympathetic dysfunction does occur in the diabetic bladder base, it plays a minor role in the overall organ dysfunction.

Substantial evidence exists for autonomic neuropathy as a cause of dysfunction of the urinary bladder of diabetic patients. Eighty-three percent of diabetic patients with somatic neuropathy were also shown to have neurogenic bladder (Ellenberg, 1980). In addition to the atonic bladder which is most characteristic of human diabetic bladder dysfunction, detrusor hyperreflexia, which is characterized by a detrusor reflex contraction which cannot be suppressed by the patient, is also encountered in the diabetic population. These functional changes are not unlike those observed in the in vivo cystometrograms of diabetic rats, which suggests that this may be a model for the clinical
condition. Human diabetic detrusor hyperreflexia has been attributed to alterations in cortical or spinal regulatory tracts (Bradley, 1980). The primary neuronal dysfunction which is believed to cause changes in the cystometrographic profile of the diabetic rat bladder is a loss of sympathetic nervous input. Studies have also shown impairment in sympathetic nervous system activity in diabetic patients with autonomic neuropathy although not in the urinary bladder (Watkins and Edmonds, 1983).

Unlike the human disease, there was no evidence of an absence of sensory nerve function in cystometrographic analysis of diabetic rat bladders. Rather than diminished sensation, the short term diabetic rat bladders are apparently more sensitive to fluid infusion. This may be the result of some intravesicular irritation or an alteration in spinal afferent pathways. Recent evidence has shown that sensory nerves are affected by diabetes at levels above the organ. A loss of sensory input to the dorsal horn of the spinal cord, as indicated by a significant decrease in substance P innervation, was observed in alloxan diabetic rats (Gorio, Di Giulio, Mannavola, Tenconi and Mantegazza, 1987). These data support the presence of diabetes-induced changes in peripheral, as well as central, sensory pathways. The presence of sensory nerve hyperreactivity, perhaps in preface to dysfunction, is also a possibility since our diabetic model is short term.

In addition to the dysfunction commonly associated with the bladder body, disorders of the internal sphincter have been reported as well; in fact, the frequency of large, atonic bladders was greater in cases of
disorders of the internal sphincter. However, a review of clinical studies, as well as our own experiments, shows the bladder body to be the primary site of diabetes-induced neurological dysfunction. The identity of the neurological alteration, however, has yet to be resolved.
CHAPTER VI

EFFECTS OF DIURESIS AND DIABETES ON THE RAT URINARY BLADDER:

IN VITRO FUNCTION

6.1. Introduction

The main conclusion from the preceding chapter was that a defect in the sympathetic nervous innervation of the bladder may be responsible for the altered micturition profile observed in diabetic rats. However, in vivo functional data become difficult to interpret because of the multiplicity of neuronal types innervating the organ as well as the presence of reflex arcs. For example, what appears to be a loss of sympathetic nervous function in the bladder might actually be an increase in the activity of parasympathetic, non-adrenergic-non-cholinergic or sensory nerves. Therefore, in order to focus on the specific neuronal dysfunction in the diabetic bladder body and the base, in vitro functional studies were performed. At this stage, in vitro techniques provide the advantages which were described as disadvantages in the preface to the in vivo studies. That is, in vitro studies permit analysis of isolated tissue responsiveness to, most importantly for our purposes, sympathomimetic agents, in addition to parasympathomimetic and non-adrenergic-non-cholinergic substances.
These methodologies allow the assessment of each aspect of autonomic, as well as non-autonomic nervous function on an isolated region of the bladder without the complications associated with the whole animal.

The majority of studies which have examined functional changes in diabetic bladder strip responsiveness to autonomic agents (Lincoln et al., 1984; Longhurst and Belis, 1986; Kolta et al., 1985; and Uvelius, 1986). However, these studies suffer from several shortcomings. First of all, there is a significant degree of variability amongst laboratories in bladder responsiveness to adrenergic and cholinergic agonists. Secondly, studies of diabetes induced changes in non-autonomic bladder neurotransmitters are limited. Support for the possibility of such an effect comes from observations of an increase in atropine resistance of transmurally stimulated isolated human bladder muscle derived from functionally impaired organs (Sjogren, Andersson, Husted, Mattiasson and Moller-Madsen, 1982). Furthermore, reports of increased PGI₂ production by diabetic rat bladders (Jeremy et al., 1986) and elevated bladder levels of VIP in diabetic Chinese hamsters (Diani et al., 1985) have not been correlated with functional changes. There are also several disadvantages of using strip preparations (Levin, Memberg, Ruggieri and Wein, 1986). Since the muscle layers of the bladder run in multiple directions, bladder strips do not contain a uniform arrangement of muscle fibers. The autonomic innervation of the bladder is also not uniform; hence, bladder strips taken from different locations do not necessarily represent the entire organ. The bladder does not function in a linear direction, and it has not been established that length-tension studies on isolated strips can be directly
correlated with volume-pressure characteristics of the intact urinary bladder. Furthermore, unlike skeletal muscle which has a well defined optimum length, the bladder can operate efficiently over a range of volumes. Therefore, no single bladder strip length can be considered normal operating length. The final shortcoming of previous analyses of diabetic bladder function is that, in most cases, proper controls were not included. The first chapter of this dissertation described structural changes in diabetic and diuretic rat bladders which may contribute to altered organ function in response to agonist stimulation. Of three studies using an intact bladder preparation (Carpenter, 1983; Kolta et al., 1985; and Moss et al., 1987), only one included a control for diuresis (Carpenter, 1983). Hence, the in vitro whole bladder system was utilized so as to provide a physiologically relevant system in which to study the effects of diabetes and diuresis on organ function in response to cholinergic, adrenergic and non-adrenergic-non-cholinergic stimulation.

6.2. Methods

General. Male Sprague Dawley rats (Harlan), 220-250 g were used for all experiments. Animals were housed in groups of four per cage at a controlled temperature of 23°C on a 12 hour light/dark cycle. Purina lab chow and water were supplied ad libitum. Three experimental conditions were compared: control, diabetic and sucrose fed. Rats were made diabetic by a single injection of streptozocin (Sigma) (65 mg/kg, i.p.) after an overnight fast. Diabetes was assessed at the time of sacrifice using Lilly Tes-Tape to measure urine glucose levels. All
animals which tested positive for diabetes had urine glucose levels greater than 0.5%. Sucrose treated rats were fed Purina rat chow and water containing 5% sucrose (E and M Science) ad libitum. This treatment produces diuresis equivalent to that of the diabetic group but does not cause metabolic changes. Neither sucrose fed nor control rats tested positive for urine glucose. The period of treatment for all experiments was 4-5 weeks. Sympathectomized male Sprague-Dawley rats, weighing approximately 500 grams, were treated with 6-hydroxydopamine (Sigma) (100 mg/kg, s.c., dissolved in saline containing 0.1% ascorbic acid) at 5 days of age.

The in vitro whole bladder preparation was adapted from the techniques of Levin and Wein (1982). Rats were killed by cervical dislocation, and a midline incision was made to expose the urinary bladder. The ureters were ligated and sectioned below the ligatures. The bladder base was then severed at the level of the pelvic bone. After emptying the bladder completely, the isolated, intact organ was maintained at 37°C in an aerated (95% O₂/5% CO₂) tissue bath containing Tyrode's buffer. The urethra was secured to a cannula which was connected with an apparatus for measuring isometric changes in pressure or isotonic changes in bladder volume. Before testing drug effects, cystometrography was performed to study compliance properties of the bladder. After a 30 minute equilibration period, cystometrography was performed by infusing Tyrode's buffer into the organ at a rate of 0.05 ml/min while continuously monitoring pressure via a pressure transducer connected to a Grass polygraph.
**In vitro bladder studies.** The whole bladder preparation was used in one of three systems: isometric pressure increases, isotonic expulsion, or isotonic relaxation. Measurements of pressure increases and of expulsion were performed with bladders filled with buffer at two different volumes: 0.5 ml and 3/4 of the bladder capacity. The protocol using bladders filled to 0.5 ml provides information regarding responses when the smooth muscle is minimally stretched. The 3/4 capacity volume was determined from the cystometrogram and varied among the bladders. Data from this protocol provide information regarding responses of stretched smooth muscle. This is important because diuresis causes the excretion of large fluid volumes which would be approximated by filling to 3/4 of bladder capacity. Once filled with the appropriate volume of fluid, bladders were allowed to equilibrate until baseline pressure was obtained. Dose response curves were obtained by adding drugs in a cumulative manner. For pressure responses, the cannula connected the bladder to a pressure transducer. For expulsion responses, the cannula connected the bladder to an empty 2 ml glass pipet which was fastened horizontally at a level approximately 2 inches above the bladder. Drug responsiveness was determined by measuring the movement of fluid into the pipet.

The measurement of whole bladder relaxation in vitro was performed as for the fluid expulsion studies. However, the pipet was filled with buffer and carefully placed at a level above the bladder such that resistance of the tubing was sufficient to prevent gravity induced flow of fluid into the bladder. Response to addition of drugs was analyzed by measuring movement of fluid from the pipet into the organ.
To measure the responsiveness of the bladder base in vitro, a single section of the bladder smooth muscle below the ureters and above the external sphincter was excised as a ring. Rings were mounted on stainless steel z-shaped hooks and placed in a tissue bath containing aerated Tyrode’s buffer. Isometric force displacement was measured with a Grass transducer connected to a Grass polygraph. Each ring was hung under 500 mg tension. After mounting, tissues were allowed to equilibrate for 30 minutes. KCl (200 mM) was added at the end of each experiment to determine maximum contractile response.

Neurochemical studies. Choline acetyltransferase (CAT) was assayed by the method of Fonnum (1975). Norepinephrine (NE) analysis was carried out using HPLC according to the method of Lincoln et al. 1984. The amount of ongoing sympathetic activity in vivo was estimated from the extent of NE depletion induced by inhibition of tyrosine hydroxylase activity with alpha-methyl-p-tyrosine (AMPT) (Sigma). (Spector, Sjoerdsma and Udenfriend, 1965). AMPT (250 mg/kg, i.p.) was administered in two separate doses given four hours apart. Animals were sacrificed 8 hours after the initial dose, and tissue levels of NE analyzed as above. For each of these procedures, the bladder body was analyzed separately from the bladder base. The bladder base consisted of the region beginning at the level of the ureters and extending rostrally to include the outlet and proximal portion of the urethra. The internal sphincter was dissected free from the external sphincter. Heart and adrenal glands were also analyzed for comparative purposes. When NE levels were determined, only the ventricles were used, whereas for analysis of CAT activity, only the atria were used. Whole adrenal
glands from the various groups were dissected free of fat and analyzed separately for NE content by the method of McKay (personal communication).

NE uptake was determined by measuring the amount of $^3$H-(D,L)-NE (New England Nuclear) which accumulated in bladder body tissue after incubation according to the method of Uretsky (personal communication). The procedure was as follows. The bladder body was dissected from the rat, minced into fine pieces with scissors and weighed. The tissue was then placed in an incubation vial and 5 ml of incubation buffer added. The incubation buffer consisted of a Krebs-bicarbonate mixture including 10 uM pargyline and 10 uM hydrocortisone. Non-specific uptake was determined by the inclusion of 10 uM desipramine in the buffer of parallel tissues. Bladder tissue was then dispersed using a Pasteur pipet. After addition of 0.02 uCl to each sample, vials were placed in a shaking water bath at 37°C for 15 minutes. After this time, vial contents were transferred to 10 ml tubes and centrifuged at 2800 rpm for 10 minutes. The supernatant was discarded and the pellet washed with 5 ml cold buffer. After decanting the wash buffer, the pellet was resuspended in 2 ml 0.1 N perchloric acid and allowed to set at room temperature for 48 hours. At this time, the suspensions were again centrifuged and the supernatant withdrawn for counting by liquid scintillation. The tissue to medium ratio for each sample was calculated by dividing the cpm of specific NE uptake (cpm total - cpm DMI) per 1 gram of tissue by the cpm per ml of medium.

Statistical analysis. Results are expressed as mean ± SEM. All
multiple comparisons were performed using Tukey's multiple comparison
test at p<0.05. Student's t test was used in all other cases at p<0.05.

**Drugs.** The following drugs were used (from Sigma unless indicated
otherwise): \(\alpha,\beta\text{-CH}_2\text{-ATP, aminophylline, bethanechol, capsaicin,}
desipramine (USV), 5-hydroxytryptamine, hydrocortisone, isoproterenol,
sodium nitroprusside, \((-\text{-})\text{-norepinephrine, pargyline, phenylephrine,}
\text{PGF}_2\alpha, \text{substance P and vasoactive intestinal peptide (abbreviated VIP).}

6.3. Results

6.3.1. Adrenergic effects

**Functional effects.** Functional responses to adrenergic agonists were
examined using the in vitro whole bladder preparation. Phenylephrine has
been shown to elicit contractions in bladder body strips (Longhurst and
Belis, 1986; Uvelius, 1986; Kolta et al., 1985) but did not produce any
significant effects on pressure generation or fluid expulsion in
concentrations up to 0.1 mM in whole bladders from any group whether
filled with 0.5 ml or 3/4 capacity. Thus, the whole bladder body of
this strain of rats does not respond to alpha-1 adrenoceptor activation.
Function of beta-adrenoceptors of the bladder body was studied by
measuring the relaxation response to isoproterenol. Isoproterenol
cumulative dose response curves were generated for control bladders;
however, a significant lack of reproducibility was found between the
initial and the subsequent dose response curve (Figure 10). The maximum
response to isoproterenol was decreased by approximately 75% in the
second dose response curve although the sensitivity ($EC_{50}$) was unchanged. For this reason, and because the time for isoproterenol-induced relaxation was found to be rather slow, only the relaxation response to a maximum dose of isoproterenol (500 uM) was assessed in subsequent experiments. The maximum relaxation volume in response to 500 uM isoproterenol in sucrose-fed and diabetic rat bladders was approximately eight-fold greater than that of control bladders (Table 10) and approximately half the amount of time was required by treated bladders to reach 50% of the total relaxation volume (Figure 11). The exaggerated responsiveness was compared to a well established model for adrenergic denervation supersensitivity. The maximum isoproterenol relaxation volume of bladders derived from sympathectomized rats was also significantly greater than control although there was no difference in organ weight between the two groups. When data from diuretic, sucrose fed and adrenergically denervated groups were normalized to either bladder weight or capacity, equivalent results were obtained for all three groups.
Experiments were performed to determine whether stimulation of the relaxation response at a level beyond the receptors also produces an exaggerated effect. The maximum aminophylline (500 uM) relaxation response was four-fold greater than control in diabetic but only 2.5-fold greater in sucrose fed bladders (Table 10). Specificity of the super-responsive relaxation was determined by comparison with stimulation of relaxation mechanisms not associated with beta-adrenoceptors. There were no significant differences between treatment and control responses to nitroprusside (Table 10) which produces relaxation via generation of cGMP. Administration of VIP, a peptide that relaxes some strip preparations via cAMP mediated effects, did not induce a significant relaxation in bladders derived from any of the groups in concentrations up to 0.1 uM (data not shown).
Figure 10. Cumulative dose-response relationship for isoproterenol-induced relaxation in control bladders. An initial dose response curve (●) was generated in bladders from control rats (n=3) by measuring the volume of fluid taken into the organ after each isoproterenol dose. After washing and re-equilibration, a second dose response curve was generated (▲) in the same tissues. * indicates significant difference between the relaxation response for the two dose response curves.
Figure 11. Isoproterenol-induced relaxation time-response for treated and control bladders. A single maximum dose of isoproterenol (0.5 mM) was added to control ● (n=6), diuretic ■ (n=3) and diabetic ▲ (n=4) bladders and the amount of fluid taken into the organ measured at 2 minute time intervals.
Table 10
Isotonic relaxation response to various agents in treated and untreated bladders

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment</th>
<th>n</th>
<th>Expansion volume</th>
<th></th>
<th>% capacity</th>
<th>ml/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ml</td>
<td></td>
<td></td>
<td>ml/g</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>CON</td>
<td>8</td>
<td>0.36 ± 0.08</td>
<td>26.5 ± 6.2</td>
<td>3.84 ± 1.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SUC</td>
<td>4</td>
<td>2.66 ± 0.56*</td>
<td>80.5 ± 14.5*</td>
<td>14.85 ± 3.28*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIA</td>
<td>5</td>
<td>2.64 ± 0.27*</td>
<td>88.7 ± 5.26*</td>
<td>18.07 ± 2.27*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SYMP</td>
<td>3</td>
<td>1.25 ± 0.13*</td>
<td>88.6 ± 8.01*</td>
<td>11.67 ± 1.14*</td>
<td></td>
</tr>
<tr>
<td>Aminophylline</td>
<td>CON</td>
<td>4</td>
<td>0.51 ± 0.08</td>
<td>36.6 ± 6.1</td>
<td>5.29 ± 0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SUC</td>
<td>5</td>
<td>1.33 ± 0.47</td>
<td>43.9 ± 15.6</td>
<td>9.29 ± 3.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIA</td>
<td>5</td>
<td>2.17 ± 0.44*</td>
<td>72.1 ± 12.1*</td>
<td>12.23 ± 2.53*</td>
<td></td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>CON</td>
<td>5</td>
<td>0.53 ± 0.11</td>
<td>32.6 ± 7.11</td>
<td>5.16 ± 1.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SUC</td>
<td>5</td>
<td>0.70 ± 0.25</td>
<td>23.2 ± 8.20</td>
<td>3.94 ± 1.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIA</td>
<td>6</td>
<td>0.85 ± 0.27</td>
<td>31.1 ± 9.95</td>
<td>4.50 ± 1.34</td>
<td></td>
</tr>
</tbody>
</table>

The responses to isoproterenol (500 μM), aminophylline (500 μM) and nitroprusside (500 μM) were assessed in control (CON), sucrose fed (SUC) and diabetic (DIA) rat bladders. Relaxation response to isoproterenol only was measured in bladders from sympathectomized rats (SYMP). The data is expressed in total volume of fluid taken up into the bladder after drug administration (ml), the percent of organ capacity (determined from in vitro cystometry) that this volume represents (% capacity), and as a ratio of the volume to the weight of the bladder (ml/g). Values represent mean ± S.E.M. * indicates significant difference from control p<0.05.
Functional response of the bladder base to adrenergic agonists was determined by measuring isometric tension generation in response to adrenergic stimulation in proximal urethral ring preparations. Although the sensitivity to norepinephrine was not different between the groups, rings from sucrose-fed and diabetic rats exhibited a 36% greater maximum response to the transmitter compared to control (Figure 12). A similar response was observed with phenylephrine (Figure 13). The amount of tension produced in response to KCl was not significantly different between rings derived from control (800 ± 96 mg), sucrose fed (753 ± 63 mg) or diabetic (825 ± 87 mg) bladders. Neither was there a significant difference in the weight of urethral rings obtained from control (6.87 ± 0.55 mg), sucrose fed (7.58 ± 0.40 mg) or diabetic (6.70 ± 0.86 mg) bladders.

Neurochemical effects. Norepinephrine levels were used as a marker of presynaptic adrenergic innervation in the bladder body and base regions. The concentration of NE in the bladder body of sucrose-fed and diabetic rats was decreased by 57% and 22%, respectively. The total amount of NE in the bladder body of sucrose fed and diabetic rats was decreased by 70% and 46%, respectively (Table 11). To investigate in vivo sympathetic nervous activity in the bladder body, AMPT (250 mg/kg, i.p.) was administered in two separate doses over 8 hours. A significant reduction in bladder body NE content was observed only in control tissues (Table 12).
The entire bladder base region from the diabetic bladder weighed significantly less than that from sucrose fed or control rats (Table 11). No significant differences between any groups were found in norepinephrine levels in the bladder base region (Table 11) nor was a significant amount of NE turnover observed in this region for any of the treatment groups (Table 12).

To better assess the competence of sympathetic nerves innervating the bladder body of treated rats, $^{3}$H-NE uptake was measured. There is no significant difference in the total amount of $^{3}$H-NE accumulated by bladders from any of the groups when data are expressed as dpm/region. However, since the bladder weights of treated rats increased, a decrease in total $^{3}$H-NE accumulation is obtained when expressed as dpm/g tissue. However, desipramine (10 uM) reduced the total $^{3}$H-NE accumulated in bladder body tissues derived from sucrose fed and control but not diabetic tissues (Figure 14). Furthermore, when expressed in terms of a tissue to medium ratio, the values from control ($0.87 \pm 0.17$) and sucrose fed ($0.85 \pm 0.13$) organs were significantly greater than ratios from diabetic tissues ($0.22 \pm 0.09$).
Diabetic rat ventricles weighed significantly less than the control and sucrose fed organs (Table 11). Total NE content of the diabetic rat heart was approximately 60% of control and sucrose fed levels although there was no difference when expressed on a weight basis (Table 11). Depletion of NE after AMPT administration occurred in the hearts of all groups but to a significantly lesser extent in diabetic rat tissue (Table 12).

There was no significant difference in the weight of diabetic adrenal glands compared to control (Table 11). However, glands from sucrose fed animals were significantly larger than control, probably as a result of undissected fat. Total NE content and NE concentration were significantly lower in glands from diabetic rats but not sucrose fed compared to control (Table 11).
Figure 12. Cumulative norepinephrine dose response curves in proximal urethral rings from treated and control rats. Data shown are mean ± SEM of the isometric contractile response to drug expressed as percent of maximum KCL contraction in tissues from control ● (n=6), diuretic ■ (n=6) and diabetic ▲ (n=6) rats.
Figure 13. Cumulative phenylephrine dose response curves in proximal urethral rings from treated and control rats. Data shown are mean ± SEM of the isometric contractile response to drug expressed as percent of maximum KCL contraction in tissues from control \( \bullet \) (n=6), diuretic \( \blacksquare \) (n=6) and diabetic \( \triangle \) (n=6) rats.
Table 11
Analysis of NE levels in tissues from treated and control rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>ORGAN WT.(mg)</th>
<th>ngNE/region</th>
<th>ngNE/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BLADDER BODY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>15</td>
<td>106 ± 4</td>
<td>37.0 ± 1.8</td>
<td>381 ± 25</td>
</tr>
<tr>
<td>SUC</td>
<td>6</td>
<td>160 ± 6*</td>
<td>16.7 ± 3.3b</td>
<td>110 ± 25a</td>
</tr>
<tr>
<td>DIA</td>
<td>10</td>
<td>154 ± 8*</td>
<td>29.1 ± 2.7a</td>
<td>206 ± 25a</td>
</tr>
<tr>
<td><strong>BLADDER BASE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>13</td>
<td>22.9 ± 1.0</td>
<td>82.5 ± 8.9</td>
<td>3408 ± 361</td>
</tr>
<tr>
<td>SUC</td>
<td>6</td>
<td>22.6 ± 1.1</td>
<td>77.3 ± 7.4</td>
<td>3343 ± 240</td>
</tr>
<tr>
<td>DIA</td>
<td>10</td>
<td>18.1 ± 1.3*</td>
<td>80.2 ± 7.6</td>
<td>4644 ± 361</td>
</tr>
<tr>
<td><strong>HEART</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>9</td>
<td>0.99 ± 0.03</td>
<td>776 ± 67</td>
<td>822 ± 45</td>
</tr>
<tr>
<td>SUC</td>
<td>4</td>
<td>0.97 ± 0.02</td>
<td>641 ± 113</td>
<td>651 ± 107</td>
</tr>
<tr>
<td>DIA</td>
<td>7</td>
<td>0.57 ± 0.03*</td>
<td>431 ± 30*</td>
<td>770 ± 78</td>
</tr>
<tr>
<td><strong>ADRENAL GLAND</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>30</td>
<td>20.6 ± 0.7</td>
<td>166 ± 9</td>
<td>8257 ± 608</td>
</tr>
<tr>
<td>SUC</td>
<td>12</td>
<td>23.8 ± 0.7*</td>
<td>160 ± 14</td>
<td>6756 ± 590</td>
</tr>
<tr>
<td>DIA</td>
<td>17</td>
<td>21.6 ± 0.8</td>
<td>101 ± 5*</td>
<td>4732 ± 230*</td>
</tr>
</tbody>
</table>

NE levels were determined by HPLC in tissues derived from control (CON), sucrose fed (SUC) and diabetic (DIA) rats. Values represent mean ± S.E.M. * indicates significant difference from control p<0.05.
Table 12
Reduction in NE levels in treated and control rat tissues after AMPT administration

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CONTROL</th>
<th>SUCROSE</th>
<th>DIABETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder body</td>
<td>46%*</td>
<td>6%</td>
<td>3%</td>
</tr>
<tr>
<td>Bladder base</td>
<td>0%</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Heart</td>
<td>29%*</td>
<td>21%</td>
<td>13%</td>
</tr>
</tbody>
</table>

Tissue NE levels were measured by HPLC 8 hours after administration of AMPT (250 mg/kg, i.p. x 2 doses). Results were compared to groups which had not received AMPT on the basis of ng/g NE (see Table 11). * indicates significant difference from untreated NE levels p<0.05.
Figure 14. Norepinephrine uptake in bladder body tissue from treated and control rats. The total amount of \(^{3}\)H-NE accumulated by control (n=5), sucrose fed (n=3) and diabetic bladders (n=5) in the absence of the uptake-1 blocker, desipramine (DMI) is expressed as dpm per organ. The presence of DMI (10 uM) defines non-specific \(^{3}\)H-NE uptake in control (n=4), sucrose fed (n=2) and diabetic bladders (n=5). When expressed as dpm per gram of tissue, the total uptake for sucrose fed and diabetic bladders are both less than control, although the proportion of specific binding remains less in the diabetic bladder.
6.3.2. Cholinergic effects

**Functional effects.** Functional responsiveness to cholinergic agonists was compared for bladders from control, diabetic and diuresed rats. This was determined by two methods using an in vitro whole bladder preparation: expulsion of intravesicular fluid (isotonic) and generation of intravesicular pressure (isometric). In bladders from diabetic animals, bethanechol-induced fluid expulsion from bladders initially containing 0.5 ml of buffer exhibited a significant reduction in sensitivity (higher EC$_{50}$ value) and a diminished total expulsion volume; i.e., bladders did not empty completely (Figure 15). Bladders from sucrose treated rats also exhibited a diminution in these parameters. Bethanechol induced expulsion was also performed using bladders filled to 3/4 of capacity. The volume of fluid representing 3/4 organ capacity was significantly greater in sucrose-treated and diabetic than in control bladders (see Chapter IV). In organs filled to 3/4 of capacity, the sensitivity to bethanechol was reduced in bladders of sucrose treated and diabetic animals; however, all bladders were able to completely empty (Figure 16). By contrast, in the isometric system, bethanechol responses were nearly identical for treated and control bladders filled with either 0.5 ml (Figure 17) or to 3/4 capacity (Figure 18).
Figure 15. Isotonic expulsion dose response relationship for bethanechol: 0.5 ml. Data shown are mean ± SEM for the volume of buffer expelled from control • (n=6), diuretic ▲ (n=5) and diabetic ■ (n=5) rat bladders filled with 0.5 ml fluid. * indicates significant difference from control p<0.05.
Figure 16. Isotonic expulsion dose response relationship for bethanechol: 3/4 capacity. Data shown are mean ± SEM for the percent of total buffer expelled from control • (n=4), diuretic ■ (n=3) and diabetic ▲ (n=3) rat bladders filled to 3/4 capacity. * indicates significant difference from control p<0.05.
Figure 17. Isometric pressure generation dose-response relationship for bethanechol: 0.5 ml. Data shown are mean ± SEM of the pressure response from control • (n=7), diuretic ▲ (n=6) and diabetic ■ (n=6) rat bladders filled with 0.5 ml buffer.
Figure 18. Isometric pressure generation dose-response relationship for bethanechol: 3/4 capacity. Data shown are mean ± SEM of the pressure response from control ● (n=7), diuretic ▲ (n=6) and diabetic ■ (n=6) rat bladders filled to 3/4 capacity.
Responses to KCl, a nonspecific contractile agent, were also compared using the isometric (pressure) system. No significant differences between groups were observed in pressure responses to 100 mM KCl in bladders filled 0.5 ml or to 3/4 capacity, respectively, for control (4.54 ± 0.45, 3.77 ± 0.23 cmHg), diabetic (4.77 ± 0.45, 3.92 ± 0.21 cmHg) or sucrose fed bladders (4.29 ± 0.63, 3.37 ± 0.72 cmHg).

**Neurochemical effects.** CAT activity was used as a marker for presynaptic cholinergic innervation in bladder body and base regions of treated rats (Table 13). In the bladder body, sucrose treatment caused an increase in total enzyme activity but no change in concentration due to increased organ weight. Diabetes-induced effects resulted in only a minor increase in total CAT activity which was smaller than the increase in organ weight, resulting in a net decrease in enzyme concentration. Total CAT activity was about ten-fold greater in the bladder body of all groups compared with the bladder base, although the concentration in the base is nearly 60% that in the body. No significant differences between groups were observed in CAT activity for tissues derived from bladder base or atria.

**6.3.3. Non-adrenergic-non-cholinergic effects**

Several endogenous substances have been proposed to be the "missing" neurotransmitter responsible for the atropine-insensitive component of pelvic nerve stimulation. These include prostaglandins and purinergic compounds. Both sucrose-treated and diabetic bladders demonstrated a two-fold increase in maximum response to PGF$_{2\alpha}$ compared to control (Figure 19). In all bladders examined, regardless of treatment, the
PGF$_2\alpha$ dose-response curve was biphasic with the appearance of the second phase occurring uniformly at a prostaglandin concentration of approximately 4 uM. The cumulative dose response curve to $\alpha,\beta$-CH$_2$-ATP was also biphasic in treated and untreated tissues (Figure 20). The secondary phase of the dose response curve appeared uniformly at a purine concentration of approximately 0.5 uM. There were no significant differences in bladder responsiveness to this agent between groups for the initial (Table 14) or secondary phase (see Figure 20). Nor were there any significant differences between groups in response to other excitatory transmitters such as serotonin or substance P (Table 14). Capsaicin, which releases substance P, (0.1 uM), did not produce a significant difference in maximum pressure generated in diabetic bladders as compared with control (Table 14).
Figure 19. Isometric pressure generation dose-response relationship for PGF$_2\alpha$. Data shown are mean ± SEM of the pressure response from control ● (n=4), diuretic ■ (n=6) and diabetic ▲ (n=4) bladders filled with 0.5 ml buffer. * indicates significant difference from control p<0.05.
Figure 20. Isometric pressure generation dose-response relationship for α,β-methylene-ATP. Data shown are mean ± SEM of the pressure response from control ● (n=7), diuretic ■ (n=4) and diabetic ▲ (n=3) bladders filled with 0.5 ml buffer. * indicates significant difference from control p<0.05.
**Table 13**  
CAT activity in treated and control rat tissues

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>ORGAN WT. (mg)</th>
<th>nmol/mg/hr</th>
<th>nmol/region/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BLADDER BODY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>14</td>
<td>101 ± 4</td>
<td>0.34 ± 0.03</td>
<td>34.72 ± 3.1</td>
</tr>
<tr>
<td>SUC</td>
<td>6</td>
<td>161 ± 4*</td>
<td>0.31 ± 0.05</td>
<td>48.62 ± 1.7*</td>
</tr>
<tr>
<td>DIA</td>
<td>8</td>
<td>158 ± 7*</td>
<td>0.27 ± 0.02</td>
<td>37.05 ± 4.7</td>
</tr>
<tr>
<td><strong>BLADDER BASE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>14</td>
<td>18.9 ± 0.78</td>
<td>0.21 ± 0.01</td>
<td>4.10 ± 0.42</td>
</tr>
<tr>
<td>SUC</td>
<td>6</td>
<td>21.7 ± 1.46</td>
<td>0.20 ± 0.02</td>
<td>4.47 ± 0.62</td>
</tr>
<tr>
<td>DIA</td>
<td>8</td>
<td>15.0 ± 0.92*</td>
<td>0.22 ± 0.02</td>
<td>3.21 ± 0.41</td>
</tr>
<tr>
<td><strong>ATRIA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>14</td>
<td>56.7 ± 1.79</td>
<td>0.30 ± 0.02</td>
<td>16.57 ± 1.11</td>
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<tr>
<td>SUC</td>
<td>6</td>
<td>59.8 ± 4.90</td>
<td>0.31 ± 0.02</td>
<td>19.24 ± 1.96</td>
</tr>
<tr>
<td>DIA</td>
<td>8</td>
<td>55.1 ± 4.32</td>
<td>0.35 ± 0.03</td>
<td>20.63 ± 1.49</td>
</tr>
</tbody>
</table>

Choline acetyltransferase activity was measured in tissues derived from control (CON), sucrose fed (SUC) and diabetic (DIA) rats. Values represent mean ± S.E.M. * indicates significant difference from control p<0.05.
Table 14
Treated and control bladder isometric responsiveness to several NANC agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment</th>
<th>n</th>
<th>pD2</th>
<th>Maximum pressure (cmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α,β-CH2-ATP</td>
<td>control</td>
<td>7</td>
<td>7.08 ± 0.06</td>
<td>1.08 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>sucrose</td>
<td>4</td>
<td>6.89 ± 0.12</td>
<td>1.07 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>diabetic</td>
<td>3</td>
<td>7.26 ± 0.07</td>
<td>0.94 ± 0.23</td>
</tr>
<tr>
<td>serotonin</td>
<td>control</td>
<td>6</td>
<td>6.40 ± 0.13</td>
<td>0.68 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>sucrose</td>
<td>3</td>
<td>6.59 ± 0.29</td>
<td>0.59 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>diabetic</td>
<td>5</td>
<td>6.32 ± 0.17</td>
<td>0.71 ± 0.11</td>
</tr>
<tr>
<td>substance P</td>
<td>control</td>
<td>6</td>
<td>8.34 ± 0.22</td>
<td>1.85 ± 0.34</td>
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<tr>
<td></td>
<td>sucrose</td>
<td>4</td>
<td>8.61 ± 0.24</td>
<td>2.16 ± 0.40</td>
</tr>
<tr>
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<td>5</td>
<td>7.81 ± 0.09</td>
<td>2.37 ± 0.52</td>
</tr>
<tr>
<td>capsaicin</td>
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<tr>
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<td>diabetic</td>
<td>4</td>
<td>-</td>
<td>1.66 ± 0.22</td>
</tr>
</tbody>
</table>

Cumulative dose response curves were generated for the non-adrenergic-non-cholinergic agents listed above in bladders from control and treated rats. pD2 values were determined from -log EC50 obtained by graphical analysis. Capsaicin was administered in a single dose (0.1 uM) and only the maximum pressure assessed. No significant differences were from control were found for any of the treatment groups p<0.05.
6.4. Discussion

The work presented here utilizes several important innovations in the study of urinary bladder dysfunction. One is the use of intact, functional bladders in vitro which allows assessment of both the isotonic and isometric properties of the organ. Another is the use of a diuretic control group. Because diuresis produces changes in urinary bladder structure and function (see Chapters IV and V), use of such controls is crucial in order to distinguish between alterations produced by diabetes-induced metabolic changes on nerve or muscle function from those caused by processing larger than normal urine volumes. Furthermore, changes in the in vitro function of the diabetic and diuretic urinary bladders were correlated with the presence or absence of changes in presynaptic markers of cholinergic and adrenergic nerve activity. A major conclusion from our study is that the postsynaptic changes in bladder function observed in the streptozotocin treated rat can be attributed to diuresis which accompanies the diabetic state. However, pre-synaptic nervous alterations are apparently unique to the diabetic condition and may represent metabolically induced dysfunction.

Adrenergic effects. Functional studies. Beta-adrenoceptor mediated relaxation in the in vitro whole bladder preparation appears to be very susceptible to isoproterenol-induced desensitization (Figure 10). Beta-adrenoceptor desensitization is a well recognized phenomenon, that has been explained by several different mechanisms (Stiles, Caron and Lefkowitz, 1984, Triggle, 1980). For example, Su et al. (1980) suggest that catecholamine-selective desensitization of a number of cell types
occurs by a two step procedure in which receptor uncoupling from adenylate cyclase is followed by eventual loss of beta-adrenoceptors from stimulated cells. In our system, when a second cumulative dose response curve was generated for isoproterenol, the maximum relaxation response was diminished although the EC$_{50}$ did not appear to change. The decreased maximum responsiveness may be the result of a loss of beta-adrenoceptors, although the possible contribution of changes in receptor coupling or other post-receptor events cannot be dismissed. Regardless of the mechanism, as a result of the rapid desensitization in our system, only the maximum dose of isoproterenol was utilized to study beta-adrenoceptor mediated relaxation in diabetic and diuretic bladders.

The largest change in receptor related effects was an exaggerated beta-adrenoceptor mediated relaxation response in sucrose fed and diabetic rat bladders. This conclusion is based the more rapid effect of isoproterenol in treated compared to control bladders and the large increase in maximum relaxation response in sucrose fed and diabetic bladders (Figure 11, Table 10). The increase in responsiveness persisted even after accounting for the increased size and capacity of diabetic and treated organs (Table 10). A supersensitivity to adrenergic agonists, including isoproterenol, in the bladder body of adult rats is produced by repeated administration of 6-hydroxydopamine (Ekstrom, 1979). We produced such a denervation supersensitivity in bladders from animals which were treated neonatally with 6-hydroxydopamine (Table 10). The magnitude of the exaggerated response was the same as that observed for bladders from diabetic rats and sucrose treated rats. Thus, we conclude that diuresis produces a
bladder characterized by supersensitivity to relaxation responses induced by beta-adrenoceptor stimulation. An enhanced isoproterenol relaxation response was also reported in whole, intact bladders from 6 week diabetic rats although such an effect was not observed in bladder body strips (Kolta et al., 1985).

Beta-adrenoceptor mediated relaxation occurs through the generation of cyclic AMP. Levels of cAMP can be increased, and, hence, relaxation may ensue via inhibition of cyclic nucleotide phosphodiesterase, the enzyme which catalyzes the hydrolysis of cAMP to its inactive metabolite 5'-AMP. Administration of the phosphodiesterase inhibitor, aminophylline, produced a four-fold increase in relaxation response in diabetic bladders but only a 2.5-fold increase in sucrose fed organs (Table 10). These data suggest the presence of post-junctional beta-adrenoceptor supersensitivity in treated bladders. However, phosphodiesterase inhibition can also affect an increase in cyclic GMP as well. The lack of a significant difference between the nitroprusside effects in any of the bladders suggests that cyclic GMP mediated relaxation is not altered by diuresis or diabetes. Furthermore, VIP, which also stimulates cAMP production through activation of its own receptor, had no effect in any bladder, which suggests a beta-adrenoceptor specific change. A number of mechanisms have been proposed to account for increases in postjunctional receptor sensitivity including increased receptor density (Fleming, 1976). In smooth muscle, however, changes in membrane polarization and altered calcium permeability and/or binding appear to be the most important mechanisms underlying sensitivity changes. Rather than a receptor-related cause
for the increased responsiveness to isoproterenol, non-specific changes in the treated bladders may be responsible for the exaggerated effects. The increased compliance in diabetic and diuresed bladders described in Chapter IV, which occurs due to structural changes, might cause these organs to relax more than control. However, we would therefore expect all relaxatory agents to produce the same exaggerated effects in treated bladders, which is apparently not the case. Furthermore, the differential relaxation responses in treated organs are probably not due to potency differences between the compounds since there was no significant difference between maximum relaxation response to aminophylline, isoproterenol and nitroprusside in untreated bladders. There are experimental data which support the specificity of increases in receptor and post-receptor related events as a result of presynaptic alteration. For example, an enhanced formation of cyclic AMP in response to catecholamine stimulation has been reported for several tissues whose adrenergic innervation had been destroyed by 6-hydroxydopamine (Pik and Wallemann, 1977; Huang, Ho and Daly, 1973). In addition, the activities of both adenyl cyclase and phosphodiesterase were found to increase after muscle denervation (Pacifici, Pellegrino, Maffei and Beconcini, 1978). Hence, the post-junctional super-responsiveness of diuretic and diabetic bladders may occur as the result of presynaptic alterations.

Although a marked beta-adrenoceptor response was observed in the bladder body, alpha-adrenoceptor stimulation of this region did not produce any significant effects. The absence of a contractile response in our system is inconsistent with results obtained in other studies.
Bladder body strips from male Sprague-Dawley rats have been shown to respond to phenylephrine with contraction (Kolta et al., 1985). Whole bladder preparations from the same (Kolta et al., 1985) and different strains of rats (personal communication) also respond to alpha-adrenergic stimulation, the only difference being that in the experiments of Kolta et al. (1985), the lumen of the bladder was open to the tissue bath. Hence, the lack of a phenylephrine response in our system may be related to a lack of bladder body alpha receptors (suggested by the drug effectiveness in a similar setup but different rat strain) or the inability of drug to reach the receptor (suggested by drug effectiveness in the same rat strain but different setup). Furthermore, the absence of an alpha-adrenoceptor mediated effect in the sucrose fed and diabetic bladders suggests that neither diuresis nor diabetes affects this aspect of sympathetic innervation, which is consistent with the findings of Uvelius (1986). This is important because parasympathetic denervation has been shown to promote alpha-adrenergic activity in the urinary bladder, thereby providing a compensatory expulsion mechanism (Sundin et al., 1977).

A second site of enhanced adrenoceptor responsiveness was observed in the bladder base of diabetic and sucrose fed rat bladders. Cumulative dose-response curves generated for NE and phenylephrine in proximal urethral rings of control and treated bladders demonstrated a modest, but reproducible increase in maximum contractile response without changes in sensitivity (EC50). However, it appears that the increased diabetic bladder body responsiveness is not due to metabolic alterations in nerve or muscle function. We propose rather that this enhanced
responsiveness is an adaptation of the tissue to the maintenance of continence when the bladder is handling large volumes of urine associated with diuresis. Our data are consistent with those of Kolta et al. (1985) who also demonstrated a significant increase in phenylephrine responsiveness in the 6 week diabetic rat bladder body.

Adrenergic effects. Neurochemical studies. Enhanced post-junctional adrenergic receptor responsiveness in the bladder body and base of treated rats might occur as a consequence of altered presynaptic adrenergic input. Therefore, NE levels were measured in the bladders of treated and untreated rats as a presynaptic marker of adrenergic nerves. An analysis of NE levels in the bladder body of diabetic and sucrose fed rat bladders demonstrated a reduction in neurotransmitter concentration in treated tissues which was attributable, at least in part, to organ enlargement (Table 11). Total bladder body levels of NE also decreased in treated tissues. Hence, diabetes as well as diuresis, appears to be associated with a reduction in NE synthesis, storage and/or a loss of sympathetic nerves which may be associated with the postjunctional increase in bladder responsiveness to isoproterenol and aminophylline. The depletion of NE after administration of AMPT was used as an indication of in vivo sympathetic nerve activity. AMPT is a potent inhibitor of the rate limiting enzyme in NE synthesis, tyrosine hydroxylase. AMPT reduces the storage level of NE, and the rate of depletion of NE increases with the frequency of stimulation (Spector et al., 1965). A significant decrease in NE levels after AMPT administration was observed only in the bladder body of control rats (Table 12). This effect suggests the presence of viable sympathetic
nerve activity in this region. By contrast, the lack of a significant amount of transmitter turnover in the bladder body of treated rats suggests a reduction in sympathetic nerve activity which is consistent with the low levels of NE.

To determine if diminished NE levels and the absence of turnover in the bladder bodies of treated rats were due to a diuresis-induced loss of sympathetic neurons, uptake of $^{3}$H-NE was assessed. NE uptake is the most important factor involved in the termination of responses to stimulation of noradrenergic neurons (see Paton, 1976). The uptake of NE occurs by a carrier mediated process as defined by its structural specificity, saturability, temperature and ion dependence. The process can be blocked by uptake inhibitors such as desipramine. However, extraneuronal uptake may also occur by smooth muscle cells (see Gillespie, 1976). This process can be inhibited by various substances such as steroid hormones, cold and alkylamines, including phenoxybenzamine. Unlike neuronal uptake, extraneuronal uptake is unaffected by desipramine. Another site of extraneuronal uptake is connective tissue binding, which is, however, believed to be of little importance. Our data show no change in desipramine sensitive uptake of $^{3}$H-NE in the sucrose fed bladder body compared with control (Figure 14). This suggests that presynaptic adrenergic nerve terminals are intact and functionally capable. By contrast, although total NE accumulation remains unchanged, desipramine sensitive uptake of the neurotransmitter in the diabetic bladder body was decreased. This may occur because of a) a loss of presynaptic adrenergic nerve terminals, b) uptake of NE by carriers or newly synthesized components not affected by desipramine or
c) a dysfunction of the desipramine sensitive NE uptake system. Diabetes-induced proliferation of a desipramine insensitive uptake site might explain the apparent neuronal loss of NE uptake. However, similar to our studies, destruction of adrenergic nerve endings by pretreatment of rabbits with 6-hydroxydopamine did not influence total removal of NE by lung tissue but the extraneuronal uptake was found to be responsible for the loss of transmitter in the absence of nerve terminals (Iwasawa and Gillis, 1974).

Data obtained from analysis of NE levels and NE uptake studies support the existence of a difference between the presynaptic adrenergic innervation of diabetic and sucrose fed bladder body regions. The decreased neurotransmitter levels in the diabetic organ are postulated to result from a metabolically induced loss of adrenergic nerves or nerve function which is supported by evidence of a reduction in desipramine-sensitive uptake of NE. However, considering the short term of the diabetes, it might be more reasonable to assume that there is a defect in the uptake process itself. Much evidence supports the direct or indirect involvement of Na-K-ATPase in the uptake of norepinephrine by presynaptic terminals (Paton, 1976). The proposed dysfunction in presynaptic Na-K-ATPase activity in our system is supported by studies by Greene and Macway (1986) in which diminished enzyme activity was observed in 8 week STZ diabetic rat sciatic nerves and superior cervical gangia. This effect was correlated with reduced levels of myoinositol which is believed to contribute to the decrease in enzyme activity. Although decreased NE levels are also observed in the sucrose fed bladders, there is no evidence to support a loss of adrenergic nerves or
function since NE uptake is comparable to control. Therefore, there must be some other factor responsible for the alteration in NE levels. One possibility is the presynaptic regulation of NE by some local modulator, such as prostaglandins. This hypothesis will be addressed in more detail in a later section. A second possible explanation for the reduction in catecholamine levels might be a "fatigue" response of the adrenergic nerve. It was hypothesized in the previous chapter that the hypotonicity which is characteristic of the sucrose fed bladder might result from an increased sympathetic input. Neuronal stress produced by constant nerve stimulation has usually been associated with a reflex increase in neurotransmitter synthesis and, hence, neurotransmitter levels (see Kvetnansky, 1980). However, this does not appear to be a universal phenomenon. In certain systems, if a stressor is sufficiently severe, or protracted, then the rate of utilization of an amine may exceed synthesis, resulting in a net decline of amine concentrations (Anisman, Irwin, Bowers, Ahluwalia and Zacharko, 1987). In our own experiments, it is proposed that diuresis is such a stressor which might result in excessive transmitter utilization. Such a pre-existing decrease in amine concentration would make it difficult to assess changes in NE levels after administration of AMPT. The net result of these effects on the isolated organ, however, is expected to also be adrenergic supersensitivity. These data are consistent with studies in which the diminution of prejunctional neurotransmission induced by chronic reserpine induces increased post-junctional responsiveness in various smooth muscle tissues (Fleming, 1976). In vivo cystometrographic analysis supports the hypothesized increase in
sympathetic nerve function in the sucrose fed bladder (see Chapter V).

Both total NE levels and NE concentration measured in the bladder base of treated animals were not significantly different from control (Table 11). NE turnover did not occur in the bladder base of any of the groups (Table 12) presumably because of differences in the properties of the short adrenergic neurons which innervate this area and the long adrenergic neurons which innervate the bladder body (Alm and Elmer, 1975). These data are consistent with those of Swedin (1971), who reported definite discrepancies between the effects of AMPT on various peripheral NE stores. Whereas heart and submaxillary glands lost a significant amount of neurotransmitter in 10 hours, the vas deferens and the seminal vesicle retained about normal NE levels even 16 hours after repeated AMPT administration. The difference was postulated to be either the result of low impulse flow in the adrenergic nerves to the genital organs at rest or to a true difference in synthesis and storage mechanisms for NE in short adrenergic nerves. In our studies, it would not be expected that the bladder base, especially from diuresed animals, should receive low impulse flow, since urine continence depends upon alpha-adrenoceptor mediated contraction in this region. Nevertheless, because these phenomena may mask any neurochemical differences between groups, the possibility of a change in bladder base sympathetic nerve activity in diuresed or diabetic rats cannot be excluded.

Consistent with the findings of altered sympathetic input in the diabetic rat bladder body is the diminution of NE levels in the ventricles and adrenal glands of the same animals (Table 11). An
Insignificant amount of NE turnover was observed in the ventricles of diabetic rats in contrast to the organs from control and sucrose fed rats. Therefore, the noradrenergic dysfunction associated with the 4 week diabetic condition is apparently diffuse and affects a number of organs in STZ-treated rats. We might speculate that adrenergic nerve dysfunction in all tissues occurs by the same mechanism, which is either the cause or effect of altered NE uptake.

Alterations in the diabetic sympathetic nervous system have been described in clinical as well as experimental subjects. Sympathetic nerve failure in diabetics is believed to be responsible for a variety of symptomologies, principally of the cardiovascular system, some of which are believed to occur relatively early on in the disease (Watkins and Edmonds, 1983). Similar to our studies, a reduction in heart NE in STZ diabetic rats has been reported (Head and Berkowitz, 1977), as well as in genetically diabetic ob/ob mice (Giachetti, 1978) and human diabetics at autopsy (Newbauer and Christensen, 1976). Also, the reduction in bladder body and ventricular NE turnover corresponds to similar findings in pancreas, heart and adipose tissues from 8 week STZ diabetic rats (Yoshida et al., 1977). Furthermore, Jackson and Carrier (1983) demonstrated that 4–5 week STZ diabetic rats were normotensive and had lower heart rates compared to controls. These data support the presence of functional autonomic nervous changes in the intact short term diabetic rat in an organ other than the urinary bladder.

Cholinergic effects. Functional studies. Results from the isotonic expulsion system suggest that the efficiency of muscarinic receptor
mediated contraction is diminished in the bladder body of diabetic and diuresed animals. This is deduced from the impaired ability of these organs to expel 0.5 ml fluid in response to bethanechol administration (Figure 15). The effect is more pronounced in diabetic organs. In both cases, diuresis-related structural changes in the organs are probably responsible for the majority of their subsensitive response (Carpenter, 1983). However, when diabetic as well as sucrose fed bladders were filled to 3/4 of their capacity, which effectively stretches the bladder muscle to a greater degree, the organs were able to completely expel their contents (Figure 16). This phenomenon probably reflects smooth muscle adaptation which allows treated bladders to expel more efficiently at larger fluid volumes. Contractility of bladder smooth muscle is reported to be a function of the initial resting length of the muscle fibers and the state of their elasticity (Carpenter, 1968). The contractile force developed by normal rat bladders is a function of organ volume at the time a stimulus is applied. Maximum force for the normal rat bladder occurs when the volume is in the range of 0.5 ml, which corresponds to the maximum fiber length obtained by microscopy. It is conceivable that in diabetic and sucrose fed bladders, the increased mass of the bladder wall causes maximum fiber length and contractile force to occur at higher filling volumes. However, even though the expanded bladder was able to completely empty in this model, greater amounts of muscarinic agonist were needed because the decreased sensitivity observed in partially filled bladders persisted in expanded organs. The impairment in emptying, which was observed in the diabetic bladder filled with 0.5 ml buffer, may represent the initial stages of
parasympathetic dysfunction which progresses to the point that an expanded bladder cannot void completely in advanced stages of diabetic-induced bladder dysfunction. In a similar study, a reduction in postjunctional sensitivity to carbachol in diabetic and diuretic rat bladders was attributed to the alteration of contractile elements caused by overdistension (Carpenter, 1983).

The isotonic expulsion and isometric pressure models differ in the conclusions they provide regarding adapatation to diuresis. In the isotonic system, diuresed bladders exhibited a decreased sensitivity to bethanechol (Figure 15, 16). However, isometric analysis did not reveal any significant differences between any of the groups (Figure 17, 18). Perhaps the same diuresis-related changes in bladder structure (see Chapter IV) which increase organ compliance and facilitate urine storage (isometric response) also impair urine expulsion (isotonic response). Most data generated in detrusor muscle strips from diabetic subjects have employed isometric methods of analysis using muscle preload ranging from 0.5-1.0 gm tension (Longhurst and Belis, 1986, Lincoln et al., 1984). When the data from these experiments are expressed as total force generated (not normalized to weight of strips), no change is observed in diabetic bladders, which agrees with our data from the isometric system.

The increased bladder size which occurs in response to sucrose-feeding induced diuresis was accompanied by a parallel increase in CAT activity, which is considered a marker for presynaptic parasympathetic nerves (Table 13). The increased enzyme activity might either represent
a) an increased amount of enzyme per nerve terminal, b) an increased number of terminals or c) an increase in maximum activity of existing enzymes. In contrast, an increase in total CAT activity was not observed in diabetic rat bladders. Hence, there appears to be presynaptic failure of the diabetic organ to compensate for diuresis. An absence of diabetic cholinergic nerve proliferation in response to diuresis is one possible explanation for the reduction in CAT activity. However, if we consider the hypothesized effects of diabetes on presynaptic adrenergic nerve function discussed earlier, we might rather speculate that, again, a metabolic derangement, perhaps affecting a carrier mediated uptake system, may be responsible for the effect. For example, the choline utilized for transmitter synthesis is obtained from the diet and is taken up into cholinergic nerve terminals by a process which is dependent upon sodium and ATP (Cooper, Bloom and Roth, 1982). If this carrier were adversely affected, as is the NE uptake system, the resultant decrease in intraneuronal choline might thereby reduce CAT activity. An alternate, or, perhaps additional explanation for altered diabetic CAT levels is a metabolic effect of diabetes on the axonal transport of the enzyme, which is synthesized in the cell body. Impairment of orthograde axonal transport of CAT has been observed in the sciatic nerve of 3 week STZ diabetic rats (Mayer and Tomlinson, 1983b). Regardless of the molecular mechanism, these data may indicate the preliminary stage of a process that eventually results in a loss of parasympathetic nerve function. This may be the underlying cause for the inability to completely empty the urinary bladder characteristic of the advanced stages of human diabetic bladder disease. In our own
studies, presynaptic cholinergic alterations might be related to the
differences observed in peak heights obtained from in vivo unligated
cystometrograms (see Chapter V). Peak heights produced by sucrose fed
rat bladders were significantly higher than control or diabetic rats
suggesting, perhaps, the release of more neurotransmitter.

Neither bladder base nor atria CAT activity was found to differ
between groups (Table 13). The lack of an effect in the atria, as well
as the bladder base suggests that a generalized parasympathetic
dysfunction does not exist in the diabetic rat at this time. Rather,
where cholinergic deficit does appear to occur is in an area of
functional stress, such as the bladder body, where the increased fluid
expulsion associated with diuresis enhances the amount of
parasympathetic nerve firing.

5-Hydroxytryptamine. Serotonin is a monoaminergic compound which has
been shown to possess direct stimulatory action on the urinary bladder
(Vanov, 1965). Responsiveness to this agent was assessed for several
reasons. First of all, alterations in serotonergic responsiveness, along
with other transmitters, have been reported in the diabetic rat
intestine (Atan et al., 1987) and aorta (MacLeod and McNeill, 1985).
Also, the blunted functional response of in vivo diabetic rat bladders
to reserpine may be the result of a loss of serotonin competency rather
than sympathetic nervous function (see Chapter V). In the isometric in
vitro bladder preparation, however, neither the maximum response nor the
sensitivity to serotonin differed between groups (Table 14). Hence, if
serotonergic innervation is affected in the diabetic rat bladder, it
must be at the level of the ganglia where it is believed to exert a strong stimulatory effect (Vanov, 1965). However, a lack of serotonergic function at this site would be expected to reduce bladder activity, rather than enhance it, which contradicts the in vivo observations of a hyperreactive bladder described in Chapter V.

Prostaglandins. Bladder body responsiveness to \( \text{PGF}_2\alpha \) is enhanced by diuresis and diabetes, however, the meaning of this effect, in terms of organ function, is unclear (Figure 19). Although we have not measured prostanoid levels, increased PGI\(_2\) production has been demonstrated in 2 month STZ diabetic rat bladders, which was attributed to distension and hyperosmolar urine secondary to glycosuria (Jeremy et al., 1986). Despite the fact that the production of prostaglandins by, and their pharmacological actions on, isolated urinary bladders are established, their effects in the normal micturition process are unclear. In vivo studies suggest that arachidonic acid metabolites regulate micturition by enhancing the amplitude of rat bladder myogenic muscle contractions and hence, the discharge of vesicle afferents to the CNS (Maggi et al., 1984). If this is the case, the increased production and/or activation of prostaglandins in diuresed bladders may be a local mechanism by which the micturition response can be accentuated to allow for greater voiding frequency.

In the whole bladder preparation, \( \text{PGF}_2\alpha \) dose response curves were biphasic (Figure 19). The initial contractile response may be the result of a direct interaction of the prostaglandin with its receptor. The existence of specific prostaglandin receptors in bladder tissue have
been suggested from experiments using antagonists such as N-0614 (Khanna, Barbieri and McMichael, 1978). The identity of the secondary phase, which occurs uniformly at 10 uM in all groups, is unknown, but presumably represents the release or generation of other endogenous contractile substances. Support for this hypothesis is derived from studies utilizing the isolated guinea pig trachea, in which high concentrations of PGF$_{2\alpha}$ were found to release lipoxygenase-mediated leukotriene-like substances (Shikada, Yamamoto, Tanaka and Sakoda, 1987). PGF$_{2\alpha}$ has also been demonstrated to release acetylcholine from cholinergic nerve terminals in dog salivary glands (Hahn and Patil, 1972).

Since the most prominent changes in the sucrose fed and diabetic rat bladders occurred in the responsiveness of these organs to isoproterenol and PGF$_{2\alpha}$, it might be hypothesized that the two effects are interrelated. Prostaglandins elicit a wide variety of effects in various smooth muscles which appear to be primarily the result of a direct action, although in some preparations they may act on neuronal elements (Hedqvist, 1977). It is well established that prostaglandins of the E series, and more recently, PGI$_2$, are able to dose-dependently inhibit nerve stimulated release of NE in the heart and other organs (Wennmalm, Fitzgerald and Wennmalm, 1987). However, the effects of PGF$_{2\alpha}$, which is the second principal prostaglandin released from adrenergically innervated tissues, are less clear since the concentrations required to produce inhibition of NE release are high compared with the amounts actually released from stimulated tissues. The mechanism(s) by which prostaglandins may produce neuromodulation may involve presynaptic or
postsynaptic sites (Hedqvist, 1977). We might speculate, based upon our own studies, that the PGF$_{2\alpha}$ supersensitivity observed in diabetic and diuretic bladder bodies might either be the cause of the observed reduction in sympathetic nerve function in these organs, or the result. An increase in distension-induced production of prostaglandins, such as PGI$_2$ (Jeremy et al., 1986) or PGF$_{2\alpha}$, might feedback to inhibit sympathetic nerve activity. This hypothesis is supported by studies in which treatment of rats with indomethacin was found to increase NE turnover in submandibular gland, spleen and heart (Fredholm and Hedqvist, 1975). Alternatively, a decrease in sympathetic nervous function, due to some other, undefined cause, might result in the same exaggerated response in prostaglandins as was observed with adrenergic agonists. Sympathetic nerve stimulation has been shown to evoke synthesis of prostaglandins, such as PGI$_2$ and PGEs, which are believed to be linked to alpha- and beta-adrenoceptors (Hedqvist, 1977).

Prostaglandins have been shown not only to affect sympathetic neurotransmission, but parasympathetic neuronal function as well. However, the neuromodulatory effects of prostaglandins on parasympathetic nervous function are not as clear as for sympathetic nerves. As was mentioned previously, PGF$_{2\alpha}$ has been found to liberate acetylcholine from cholinergic nerve terminals in dog salivary glands (Hahn and Patil, 1972), and can enhance contractions induced by cholinergic nerve stimulation in the guinea pig and rabbit ileum and sphincter muscle of the iris (see Hedqvist, 1977). Although there is no experimental evidence for this, a prostaglandin-mediated enhancement of parasympathetic function in the diabetic and diuretic rat bladders could
be a means of organ adaptation to increased voiding. In conclusion, the increased production of prostaglandins in the diuretic organ may play a role in the observed alterations in parasympathetic or sympathetic nervous function.

Purinergic effects. The atropine-insensitive component of pelvic nerve stimulation has been studied by many investigators and determined to be non-cholinergic-non-adrenergic in nature. (review, Taira, 1972). The most likely candidate for the "missing" neurotransmitter thus far appears to be ATP (Burnstock et al., 1978) which is known to promote bladder contraction, preferentially in the body region (Levin et al., 1979). In isometric whole bladder studies, we found no significant difference between groups in responsiveness to the non-hydrolyzable analogue of ATP, $\alpha,\beta$-CH$_2$-ATP (Table 14, Figure 20). Each $\alpha,\beta$-CH$_2$-ATP dose response curve was characterized by a biphasic appearance (see Figure 20). The secondary phase may be the result of the release of other endogenous transmitters. At least one study demonstrated this phase to be indomethacin sensitive and suggested the role of prostaglandins in the production of the ATP-mediated contraction of the rat bladder (Andersson et al., 1980). However, indomethacin reduced the contractile response of $\beta,\delta$-CH$_2$-ATP by only 10-20% suggesting only a minor role for the prostaglandins in this system (Brown, Burnstock and Cocks, 1979). In our system, there was no significant difference observed in the maximum response of the secondary phase between groups. The fact that the secondary phase of the $\alpha,\beta$-CH$_2$-ATP dose response curve was not increased in the diabetic and sucrose fed bladders may be the result of either a lack of production of prostaglandins by the purine
analogue or may be the result of variable actions of prostaglandins on this tissue. That is, the prostaglandin(s) whose synthesis or release is stimulated by ATP (Andersson et al., 1980) may be different than those which are proposed to increase in response to PGF$_2\alpha$ in treated bladders. The absence of a significant decrease in purinergic sensitivity in our system as was described by Moss et al. (1987) may be related to the duration of diabetes, since our results were analyzed at 4 rather than 8 weeks, at which time significant effects were just beginning to be seen in their study.

**Peptidergic effects.** A variety of peptides are known to exert pharmacological effects upon isolated urinary bladder strips (Erspamer et al., 1981). Reports of VIP responsiveness in bladder strips of different species are inconsistent. VIP produces a small contractile effect and nerve potentiation in the guinea pig bladder (Johns, 1979) but reportedly inhibits motility in isolated bladder strips from rabbit, human and pig (see Maggi and Meli, 1986). In other studies of human tissues, however, no effect was seen (Erspamer, Ronzoni and Falconieri-Erspamer, 1981). The lack of a direct effect of VIP on the whole bladder preparation may result from an insufficiency of or inaccessibility to the peptide's receptors, although VIP fibers have been localized in the rat bladder wall (Mattiasson, 1985). Evidence also exists for a modulatory, rather than direct, action of VIP which perhaps was not measurable under these conditions (Maggi and Meli, 1986). Hence, the role of VIP in bladder smooth muscle relaxation has yet to be resolved. Thus far, our data do not support the hypothesis of an increased role for this transmitter in diabetic bladder relaxation as
has been suggested by Dail et al. who reported elevated levels of the peptide (1977).

A second peptide known to possess bladder activity is the tachykinin, substance P (Erspamer et al., 1981). Substance P is the neurotransmitter associated with bladder afferent nerves (Maggi and Meli, 1986). Administration of the specific sensory neurotoxin, capsaicin, to adult rats in vivo has been shown to deplete bladder sensory nerve substance P levels (Maggi et al., 1987). The initial contractile effect of topical capsaicin on the in vivo and in vitro organ has been attributed to a release of substance P from afferent nerves (Maggi et al., 1984; Maggi et al., 1985). As has been demonstrated by other researchers using bladder strips (Erspamer et al., 1981), in our whole bladder preparation, substance P was found to be a potent and effective contractile agent. There was no difference in responsiveness to substance P between the various groups in the isometric system (Table 14). More importantly, however, the magnitude of the capsaicin response suggests that short term diabetes does not alter substance P content in bladder sensory nerves. Thus, if substance P can be considered a marker for the integrity of sensory neurons, our data do not support the hypothesis of sensory nerve dysfunction being an early event in diabetic bladder dysfunction in the rat. These data are supported by in vivo data described in Chapter V which suggested an increased, rather than decreased sensitivity in the diabetic rat urinary bladder. However, these in vitro studies do not eliminate the possibility of sensory nerve changes at a level above the end organ.
In conclusion, the dramatic effects produced by diuresis demonstrate the importance of a diuretic control when using the STZ-treated rat as a model to study bladder dysfunction in diabetes. Changes in the diabetic bladder responsiveness to autonomic agonists such as bethanechol and isoproterenol occur not because of metabolically-induced alterations in nerve or muscle function, but presumably because of diuresis-related effects. Altered diabetic bladder responsiveness to non-autonomic agents, such as PGF$_2\alpha$, are also the result of diuresis-induced effects. These results indicate a common, non-specific, postjunctional mechanism by which the effects of these transmitters are modified e.g. changes in contractile properties, changes in ion properties, etc. (see Fleming, 1976). In contrast to the non-specificity of the postjunctional changes proposed in the diabetic rat bladder, prejunctional alterations in adrenergic and cholinergic nerve terminals appear to be unique to the diabetic animal. Defects in both NE uptake and CAT activity are postulated to occur because of a metabolic derangement of transport processes such as those driven by Na-K-ATPase. The development of prejunctional neuropathic changes in the autonomic innervation of the diabetic bladder, in conjunction with postjunctional alterations in the organ imposed by diuresis, are postulated to contribute to the functional changes observed in the short term diabetic rat bladder.
Although a number of the alterations in the short term diabetic rat bladder are the result of the diuresis which accompanies the condition, there does appear to be metabolically-induced neurological changes in the organ. The autonomic nervous changes which occur in our experimental animal model of diabetic bladder dysfunction do not completely parallel the alterations which have been reported in the human disorder. The initial stage of human diabetic bladder disease is attributed to sensory nerve dysfunction which results in a large capacity organ with reduced sensation. An increase in bladder capacity, however, was characteristic of organs from both diabetic and diuresed rats as determined by in vitro cystometrography. Furthermore, our data do not support the loss of afferent nervous function. In vivo cystometrographic studies demonstrate no similarities between the micturition profiles of diabetic and capsaicin pretreated rats. In vitro studies support these findings since capsaicin-induced contractions in diabetic rat bladders are the same as control suggesting the presence of similar amounts of the sensory neurotransmitter, substance P, in both organs.
The possibility of increased sensory nerve function, however, is not inconsistent with our in vivo cystometrographic data in which the diabetic bladder is characterized by its hyperreactivity.

The major neuropathic changes in the short term diabetic rat bladder may involve sympathetic nerves. In vivo cystometrographic studies suggest the diabetic bladder is large in capacity but hypertonic which is suggested by the reduction in the hyperreactive response of these organs to L-DOPA administration. Bladder sympathetic nerve dysfunction would be consistent with these observations and the proposed sympathetic deficit is supported by a lack of an effect of reserpine on the micturition profile of diabetic rats. Although diuresis also appears to alter the sympathetic input at the organ level, as determined by in vitro functional and neurochemical methods, certain presynaptic changes are unique to the diabetic bladders. This is apparent from in vivo studies and by the reduction in desipramine sensitive NE uptake into diabetic bladder body tissues. Similar effects are not apparent in the bladder base region, which suggests, perhaps, a differential effect of diabetes on the adrenergic nerves which innervate this region. Although disorders of the sympathetic nervous innervation of the human diabetic urinary bladder have not been described, sympathetic nervous dysfunction has been clinically reported in numerous other organs. In our own studies, alterations in the levels of NE in diabetic hearts and adrenal glands suggest a widespread sympathetic neuronal dysfunction in the short term STZ rat.
The secondary or decompensatory stage of human diabetic bladder dysfunction occurs as a result of altered parasympathetic motor nerve function. Although there is little evidence for such an effect in our in vivo cystometrographic studies of short term diabetic rats, there are discernable changes in presynaptic cholinergic markers of the bladder body. The absence of a compensatory increase in diabetic presynaptic cholinergic synthetic enzyme activity in response to diuresis suggests the early stage of neural dysfunction. Rather than postjunctional supersensitivity, diuresis-related cholinergic subsensitivity was observed in diabetic bladders. Structural changes in the diabetic organ, which create voiding problems at low fluid volumes, coupled with presynaptic loss of synthetic enzymes, might together produce the impairment in bladder emptying which is characteristic of later disease stages.

Based upon these data, we conclude that the 4 week STZ diabetic rat bladder has limited usefulness as a model for the most common form of human diabetic bladder dysfunction which is characterized by organ insensitivity and atonicity. The hyperreactivity exhibited by our model system might, however, extrapolate to the subtype of diabetic bladder disease in which the organs are characterized by their increased sensitivity and instability. However, diabetes-specific neurological alterations which did occur in the STZ-treated rat bladder were not of sufficient magnitude to warrant further use of this model for examination of the problem of diabetic bladder dysfunction.
LIST OF REFERENCES


