Morphological Plasticity in Efferent Pathways to the Urinary Bladder of the Rat Following Urethral Obstruction

William D. Steers, Jonathan Ciambotti, Susan Erdman, and William C. de Groat

Departments of Pharmacology and Behavioral Neuroscience, and Division of Urological Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Partial urethral ligation in female Wistar rats produces changes in the neural control of the lower urinary tract including bladder hyperactivity and facilitation of a spinal micturition reflex pathway. To gain insight into the mechanisms underlying these changes, axonal tracing studies were conducted to examine the postganglionic efferent limb of the micturition reflex pathway which originates in the major pelvic ganglion (MPG). Forty microliters of the tracer Fluoro-Gold (4%) were injected into the right side of the bladder in urethral-obstructed ($n = 10$) and control ($n = 4$) rats 6 weeks after urethral ligation or sham surgery. As a control Fast blue (40 μ l, 5%) was injected into the colon to label neurons in the MPG innervating the intestine.

Obstructed rats exhibited a 6-fold increase ($p < 0.001$) in bladder weight (0.848 gm) compared to controls (0.148 gm). A significant increase ($p < 0.001$) in the size of labeled bladder postganglionic neurons in the MPG was noted in obstructed rats (576.4 μ m², $n = 4$) as compared to controls (299.6 μ m²). However, labeled, colon postganglionic neurons in the MPG in obstructed (312.9 μ m²) rats were not enlarged compared to controls (359.4 μ m²). Neuronal hypertrophy was not associated with a change in the number of labeled MPG neurons in control and obstructed groups. Capsaicin (150 mg/kg s.c. in divided doses) ($n = 3$) or unilateral pelvic nerve transection prior to urethral ligation ($n = 3$) did not prevent the enlargement of the bladder or of the postganglionic neurons, suggesting that neither capsaicin-sensitive afferents nor parasympathetic preganglionic efferent activity was essential for neuronal hypertrophy. Therefore, it is speculated that the neuronal changes induced by urethral obstruction are mediated by a peripheral mechanism such as an increased production of trophic factors in the bladder.

The parasympathetic excitatory innervation to the urinary bladder is derived from autonomic ganglion cells in the pelvic plexus, which in turn receive inputs from sacral preganglionic axons in the pelvic nerve (Langworthy, 1965; Purinton et al., 1973, 1976; Baljet and Drukker, 1980; Hulsebosch and Coggeshall, 1982;

Tabatabai et al., 1986; Steers et al., 1988; Keast et al., 1989; Mallory et al., 1989). In many species the pelvic plexus occurs as a complex, diffuse network of interconnected ganglia; however, the counterpart of this plexus in the rat exists primarily as a single major pelvic ganglion (MPG) (Langworthy, 1965; Baljet and Drukker, 1980; Papka et al., 1987; Keast et al., 1989). Due to this anatomical simplicity the rat has been very useful for neuroanatomical (Hulsebosch and Coggeshall, 1982; Papka et al., 1987; Keast and de Groat, 1989) and electrophysiological studies of neural control of the urinary bladder (Purinton et al., 1973; Steers and de Groat, 1988; Mallory et al., 1989).

In the rat (Mallory et al., 1989), as in the cat (de Groat, 1975), 2 distinct, parasympathetic reflex pathways to the bladder have been identified. In the normal rat a long-latency (120–150 msec) spinobulbospinal reflex pathway which passes through a relay center in the rostral pons has a major role in the control of bladder function (Noto et al., 1988). A shorter-latency (40–60 msec) spinal reflex occurs infrequently in normal rats, but is very prominent in paraplegic rats, where it appears to mediate automatic micturition (Mallory et al., 1989).

The short-latency spinal reflex pathway is also more prominent in other pathological conditions, such as urethral obstruction (Steers and de Groat, 1988). A rat model for bladder outlet obstruction produced by partial urethral ligation (Uvelius and Mattiasson, 1984; Malmgren et al., 1987; Steers and de Groat, 1988) has demonstrated a number of anatomical and functional changes similar to those observed in patients with severe bladder outlet obstruction. Obstruction in both species produces detrusor smooth muscle hypertrophy and hyperplasia, as well as increases in micturition volume, voiding pressure, and residual urine (Sibley, 1984, 1985; Uvelius and Mattiasson, 1984; Gosling et al., 1986; Levin and Wein, 1986; Malmgren et al., 1987; Steers and de Groat, 1988). Urethral obstruction also induces bladder hyperactivity, resulting in urinary frequency and detrusor hyperreflexia (Andersen, 1976; Chalfin and Bradley, 1982; Sibley, 1984, 1985; Malmgren et al., 1987; Mattiasson et al., 1987; Steers and de Groat, 1988). It has been speculated that the hyperactivity in obstructed animals may be mediated by the facilitated spinal micturition reflex mechanism (Chalfin and Bradley, 1982; de Groat, 1987; Steers and de Groat, 1988).

The present study was undertaken to determine whether morphological changes in the bladder innervation accompany the functional and electrophysiological changes observed following outlet obstruction. It is known that obstruction of the small intestine in the guinea pig causes hypertrophy of the intestinal smooth muscle as well as enlargement of neurons in the myenteric plexus (Gabella, 1984). In the present study an increase in

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Correspondence should be addressed to Dr. William D. Steers, at his present address: Department of Urology, Box 422, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

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the size of postganglionic neurons innervating the urinary bladder of the adult female rat was detected following chronic partial urethral obstruction. Preliminary results of this study have been presented as an abstract (Steers et al., 1988).

Materials and Methods

Surgery. Fourteen female Wistar rats (300–500 gm) 90–100 d old were used in this investigation. A modification of the technique described by Uvelius and Mattiasson (1984) (Steers and de Groat, 1988) was used to create partial bladder outlet obstruction. Under halothane anesthesia, the skin was shaved, treated with an iodine/alcohol mixture, and a low midline incision was performed. The urethra was freed from surrounding connective tissue just cephalad to the pubic symphysis. The urethral diameter was reduced to 1 mm by tying 2 4-0 nylon sutures around the urethra, including an extraluminally positioned 1-mm-diameter polyethylene tubing. The tubing was then removed, and the abdominal incision was reapproximated using 2 layers of interrupted nylon sutures. Control rats underwent sham surgery in which the urethra was circumferentially dissected, but not ligated. Throughout the study rats were maintained on tetracycline (6.5 mg/ml) administered in their drinking water to prevent urinary tract infections. Experiments were also conducted in animals with peripheral nerve lesions. Seven rats were pretreated with capsaicin under halothane anesthesia prior to the urethral ligation. Capsaicin was administered in divided doses of 50 mg/kg, s.c., in the morning and evening of day 1 and then once the following day (total dose 150 mg/kg, s.c.). Prior to injections of axonal tracer substances, an eye wipe test (Gamse, 1982) was performed to judge the efficacy of capsaicin treatment. The test consisted of applying a drop of capsaicin (100 μ g/ml) to the rat's eye and comparing the number of eye wipes to untreated animals. Previous tracing studies that examined the afferent innervation to the urinary tract revealed long-lasting (8 weeks) effects of capsaicin on neural pathways (W. C. Steers, J. Ciambotti, and W. C. de Groat, unpublished observation) which extended beyond the time period of the present study. In 3 animals a unilateral parasympathetic decentralization of the bladder was performed by removing a 10-mm section of the left pelvic nerve central to the MPG at the time of urethral ligation.

Dye injection for axonal tracing. Rats were anesthetized with halothane 6 weeks following urethral obstruction or sham pelvic surgery, and the urinary bladder was exposed by a midline abdominal incision. A suspension of Fluoro-Gold (FG, 4% wt/vol; Fluorochrome Inc., Englewood, CO) was injected into the right bladder wall in 4 control and 10 obstructed rats (including 3 obstructed, capsaicin-treated). Bilateral FG injections were made in the unilaterally parasympathetically decentralized preparations (3 obstructed). In addition, a suspension of Fast blue (FB, 5% wt/vol; Polyloy, Gross Umstat, FRG) was injected into the ventral wall of the colon lying behind the uterus in 2 control and 2 obstructed rats.

Multiple injections (8–10 for each dye, 4–5 μ l vol) into the wall of the bladder and colon were made using a Hamilton syringe with a 30-gauge needle for a total volume of 40 μ l. The large volume was used to maximize labeling of bladder neurons in obstructed animals in whom the size of the bladder was markedly increased. The needle was kept in place for several seconds following each injection, after which the area was gently swabbed with a cotton applicator and rinsed with 0.9% saline. Intraluminal or vascular injections were avoided in these animals. However, in 2 normal female rats, 40 μ l of FG was injected into the bladder lumen to document that MPG neurons were not labeled in this manner. Five to 10 d after dye injections the animals were deeply anesthetized with halothane, and the ganglia were removed and placed into 4% buffered formalin for 4 h. The bladder was removed, blotted dry, and weighed.

MPGs containing FG- and FB-labeled cells were then washed in PBS and equilibrated with successive PBS solutions containing 10, 20, and 30% sucrose for cryoprotection. Cryostat sections (14 μ m) were cut and mounted on gelatinized slides, dehydrated using a series of alcohols, mounted in glycerin, and cover-slipped. FG- and FB-labeled MPG preparations were viewed using a Leitz Ploempak A filter (UV excitation at 340–380 nm wavelength).

Analysis of labeled cells. Cell counts were obtained by examining every fourth tissue section to avoid double counting of cells. Based on previous literature (Kanerva and Teravainen, 1972; Papka et al., 1987), structures with diameters less than 13 μ m, which in this study roughly corresponded to areas less than 150 μ m², were considered to be neuron fragments

or small intensely fluorescent (SIF) cells and were not included in the measurements of postganglionic perikarya. Morphometric analysis was performed on neurons distributed throughout the MPG. Approximately 200 labeled neurons in each MPG were analyzed.

Computerized image processing was used to quantify cell sizes and shapes at a magnification of 200 $\times$. Video images were obtained through a DAGE-MTI 70 series camera and then analyzed with an Olympus Cue-2 Image Processor, using the Olympus Morphology Image Analyzer software. Manual thresholding was performed to construct an image identical to that viewed under UV light (FG, FB). Morphometric determinants for irregular 2-dimensional image processing were used to analyze cell size since neurons were occasionally pleomorphic. Interconversion of radii and areas was possible only for symmetrical (ellipsoid) neurons. Manual analysis of several hundred neurons in each group resulted in a measurement slightly larger (10%) than computer calculated values. A diameter was calculated from the projected lengths of the neuron at 0, 90, and 135°, and mean radius was determined as projections from the center of gravity of each cell at 6 different angles (Fig. 1). Cross-sectional area was calculated from the total number of black pixels. The shape factor was calculated from the formula $(\text{area}/p^2) \times 4$, where p = total perimeter (1 = circle and 0 = straight line). Anisotropy as a measure of unevenness corresponded to the ratio of the projected diameter at 90° divided by projected diameter at 0°.

Differences in morphometric parameters were analyzed with the Wilcoxon Matched-Pairs Signed-Ranks Test using SPSS/PC+ software. All morphometric data are presented as a mean $\pm$ SD.

Results

Urinary bladder

The bladders of the obstructed rats, including those that had also undergone capsaicin pretreatment or unilateral pelvic nerve transection, were grossly enlarged, containing volumes at the time of perfusion of 4–15 ml versus 0.4–0.6 ml in control animals. Bladder weights for the obstructed group were significantly heavier (mean 0.848 ± 0.2731 gm, $p < 0.001$) compared to controls (mean 0.148 ± 0.0243 gm).

Postganglionic neurons

No statistically significant difference was detected in the average number (per MPG) of FG-labeled bladder neurons obtained by examining every fourth section in obstructed (273 ± 86.3) and control (264 ± 74.4) animals. The location of bladder neurons within the MPG was similar in control and obstructed rats, with the predominance of labeled cells residing near the origin of the postganglionic bladder nerves (ventral half of MPG), although many labeled neurons were widely distributed throughout the ganglia (Keast et al., 1989). Proximal axons of FG-labeled neurons appeared to be better labeled in obstructed versus control rats, but these axons were not included in measurements of neuronal size. However, the fluorescence of FG-labeled bladder neurons appeared to be more variable and less intense in obstructed animals than in controls.

Bladder neurons in obstructed rats were significantly larger than in control rats (Figs. 2, A, B; 3, A, B; Table 1). Because distinct nuclei were seen only in FG-labeled cells with areas greater than 150 μ m², the use of this value as the lower limit for morphometric analysis of bladder neurons was validated. In obstructed animals the mean cross-sectional area for FG-labeled bladder postganglionic neurons (576.4 ± 264.04 μ m², $n = 4$) (Figs. 2B and 3B) was significantly ($p < 0.001$) increased in comparison to mean area (299.6 ± 118.32 μ m², $n = 4$) (Fig. 3A, Table 1) in the control group, as were values for diameters and radii (Fig. 4, A, B, Table 1).

Neuronal enlargement did not occur in all populations of postganglionic neurons in the MPG since morphometric parameters for FB-labeled colonic neurons in obstructed rats (Figs.

Figure 1. Determination of morphometric parameters from vector analysis of predetermined angles from the center of an object.

5B, 6B, Table 1) were not significantly different than in controls (Figs. 5A, 6A, Table 1). Occasional FB-labeled colon cells with areas between 95 and 150 μm^2 contained distinct nuclei; therefore, the lower limit for the cross-sectional areas of colon neurons was set at 95 μm^2 .

Although bladder neurons in obstructed animals were larger than in control rats, the overall shape of these cells was similar. The shape factor for obstructed bladder neurons was 0.81 ± 0.091 with an anisotropy value (measure of unevenness) of 1.04 ± 0.262 , compared to 0.80 ± 0.09 and 1.14 ± 0.293 for controls. To examine the influence of preganglionic efferent input on the hypertrophy of bladder neurons, unilateral pelvic nerve transection was performed at the time of urethral ligation. Following pelvic nerve transection in combination with urethral obstruction, FG-labeled bladder neurons ipsilateral to the denervation exhibited a significant increase ($p < 0.001$) in cell size parameters, including area ($518.3 \pm 208.84 \mu\text{m}^2$, $n = 2$) (Fig. 7A, Table 1), compared to controls. However, these parameters were not statistically different from those of neurons in obstructed neurally intact rats. In 1 of the 2 obstructed, decentralized rats the contralaterally labeled neurons were significantly larger (mean area $718 \mu\text{m}^2$), while in the second rat mean area for the intact side was the same as that on the denervated side.

Capsaicin pretreatment did not prevent the enlargement of bladder neurons in the MPG following obstruction (area $557 \pm 209.3 \mu\text{m}^2$, $n = 3$) (Fig. 7B, Table 1). However, the intensity of neuronal labeling in capsaicin-treated rats appeared to be less than in controls or even untreated obstructed rats. It should

Figure 2. Photomicrographs of FG-labeled bladder neurons in the MPG from a control and an obstructed female rat. A, Labeled bladder neurons in control rat. B, Labeled bladder neurons in an obstructed rat 6 weeks after partial urethral ligation. Note marked increase in bladder neuron size while unlabeled background cells in A and B are similar in size. Arrowhead depicts labeled neuron with filling of proximal axon. Calibration bars in A and B correspond to 50 μm .

also be noted that capsaicin treatment plus obstruction resulted in a 60% mortality compared to a 20% mortality in obstructed animals over a 6 week period and a 40% mortality due to capsaicin pretreatment alone. Autopsies revealed that capsaicin

Table 1. Morphometric parameters for MPG neurons

	Controls ($n = 4$)	OBST ($n = 4$)	DC ($n = 3$)	CAPS + OBST ($n = 3$)
Bladder neurons				
Area (μm^2)	300 ± 118	$576 \pm 264^*$	$518 \pm 209^*$	$557 \pm 209^*$
Mean radius (μm)	9.4 ± 1.9	$13.1 \pm 3.1^*$		
Maximum diameter (μm)	25.4 ± 3.6	$31.9 \pm 6.8^*$		
Mean diameter (μm)	20.0 ± 3.0	$27.3 \pm 5.5^*$		
Colon neurons				
Area (μm^2)	359 ± 147	313 ± 161		
Mean radius (μm)	9.7 ± 2.1	9.4 ± 2.4		

* $p < 0.001$. Values, mean $\pm$ SD. CAPS, capsaicin treated; OBST, urethral obstruction; DC, decentralization.

Figure 3. Distributions of areas for FG-labeled bladder neurons in the MPG of control and obstructed rats. *A*, Area distribution for 600 bladder cells in control rats ($n = 4$). The mean area was $299.6 \mu\text{m}^2$. *B*, Area distribution for 950 bladder cells in obstructed rats ($n = 4$). The mean area was $576.4 \mu\text{m}^2$. The difference in these 2 populations was significant ($p < 0.001$). Note the rightward shift of the histogram in *B* with the presence of neurons greater than $1500 \mu\text{m}^2$.

treatment plus urethral ligation resulted in urinary retention in 3 of 4 animals that died (24 hr–1 week).

Discussion

The present experiments revealed that the hypertrophy and hyperactivity of the urinary bladder that occur following urethral obstruction in the adult rat are accompanied by hypertrophy of the efferent neurons innervating the urinary bladder. These observations raise obvious questions regarding the mechanisms and functional significance of the neural changes, particularly the possible link between the neuronal hypertrophy and increased target organ size.

Urethral ligation produced a 7-fold increase in bladder weight and capacity and a $>90\%$ increase in the mean cross-sectional area of neurons projecting to the bladder. This percentage increase in neuronal size is relatively large, approximating the maximal increase seen in other ganglia, such as the superior cervical ganglion, during postnatal development or due to the administration of exogenous nerve growth factor (Hendry and Campbell, 1976). A similar increase in the size of neurons in the myenteric plexus has also been noted in response to obstruction and hypertrophy of the guinea pig small intestine (Gabella, 1984).

In theory, a number of factors could contribute to the increase in neuronal size; however, the present observations appear to exclude some of these factors. For example, it is unlikely that

circulating humoral substances or the release of local nonspecific inflammatory mediators was responsible for the neuronal hypertrophy, since colonic neurons also present within the MPG did not change in size following obstruction. Urethral obstruction and overdistention of the bladder could also produce axonal degeneration in the bladder (Elbadawi et al., 1989) followed by neuronal death and expansion of the terminal fields of the surviving neurons. This might lead to an increase in neuronal size by 2 mechanisms: (1) a selective loss of small cells or (2) enlargement of the surviving cells in response to expansion of their terminal fields. Axonal degeneration and subsequent regeneration in the bladder following outlet obstruction in the rabbit has been noted in electron microscopic studies (Elbadawi et al., 1989). However, it seems unlikely that neuronal death in the MPG occurred in the present experiments since the average number of labeled neurons in the MPG was not changed following obstruction.

The labeling of a similar number of MPG neurons by injection of tracer into the normal and obstructed/enlarged urinary bladder has additional implications. First, it appears that uptake and axonal transport of tracer were not affected by obstruction. Second, the number of postganglionic axonal projections accessing tracer within the bladder must be maintained, otherwise tracer would have labeled a smaller number of axons and thus fewer cells. This is at odds with histological and ultrastructural studies in the obstructed pig (Gosling et al., 1987) and human bladder

Figure 4. Distributions of mean radii for FG-labeled bladder neurons in the MPG of control and obstructed rats. *A*, Control population with a mean radius of 9.4 μm . *B*, Obstructed population has a mean radius of 13.1 μm . The difference in these 2 populations was significant ($p < 0.001$). Distributions are not identical to those seen for areas since a lower limit for area was used to define a neuron, while the mean radius was independently calculated from radii generated at 6 different angles and not used to directly calculate cell area.

(Gosling et al., 1986) which revealed a decreased density of axons within the smooth muscle. However, traditional morphometric analysis of bladder smooth muscle and neural components may be inapplicable because of the complex interlacing smooth muscle arrangement and considerable variability in the density and pattern of distribution of nerve fibers in the bladder (Elbadawi and Meyer, 1989). In one particularly detailed study (Elbadawi et al., 1989) early axonal degeneration in the partially obstructed bladder of the rabbit was followed by axonal regeneration with axonal sprouting.

Increased synaptic input to the bladder postganglionic neurons is also a possible factor in the initiation of neuronal hypertrophy, since previous studies have revealed a hyperreflexia in the obstructed bladder of the rat (Malmgren et al., 1987; Steers and de Groat, 1988). Alterations in synaptic input or neural firing have been implicated in changes in neuronal morphology at other sites in the nervous system (Gall et al., 1979; Pysh and Weiss, 1979). However, the present experiment would exclude centrally generated neural activity as a mediator of the hypertrophy, since unilateral transection of the pelvic nerve, which would destroy the sacral preganglionic parasympathetic efferent input as well as the sacral afferent input to the MPG, did not produce a significant change in the neuronal hypertrophy. Destruction of small-diameter bladder afferents with the neurotoxin capsaicin also did not change the neuronal response to obstruction.

It seems likely, therefore, that partial urethral obstruction and subsequent hypertrophy of the bladder smooth muscle lead to an increase in the levels of local trophic factors within the target organ. It is known that neurotrophic factors serve as retrograde messengers between target tissues and innervating neurons (Heumann et al., 1984). These factors are thought to modulate neuronal survival, differentiation, process outgrowth, synapse formation, and transmitter synthesis (Thoenen and Edgar, 1985; Lander, 1987). Recent evidence suggests that these factors are essential not only during development, but also as regulators in adult neuroplasticity (Gordon, 1983; Purves et al., 1988; Voyvodic, 1989). One potential trophic factor is NGF, which promotes the survival and growth of sensory and catecholamine-containing sympathetic neurons (Thoenen and Edgar, 1985; Thoenen et al., 1985). Preliminary data indicate that obstructed rat bladders produce about 10 times as much NGF per whole bladder as controls (Creedon et al., 1989). Since the mean number of neurons in the MPG following urethral ligation is unchanged, efferent neurons may access 10–20 times as much NGF due to increased bladder size and NGF content.

Although NGF is the best characterized of the neurotrophic factors, not all peripheral neurons respond to this substance (Thoenen et al., 1985). For example, in the ciliary ganglion, which consists primarily of parasympathetic cholinergic neurons, NGF promotes neurite extension but does not promote neuronal survival (Collins and Dawson, 1983). Thus, other neu-

Figure 5. Photomicrographs of FG-labeled bladder and FB-labeled colon neurons in MPG from a control and an obstructed rat. *A*, Four FG-labeled bladder cells (arrowheads) and 8 FB-labeled colon cells (arrows) in MPG from a control rat. *B*, Three bladder and 7 colon neurons in an obstructed rat. While bladder neurons were generally larger in size than in controls, colon neurons were similar in size to those in controls, as graphically displayed in Figure 6. Calibration bars in *A* and *B* correspond to 50 μ m.

Figure 6. Distributions of areas of FB-labeled colon neurons in the MPG of control and obstructed rats. *A*, A total of 150 labeled colon cells in control rats ($n = 2$) with a mean area of $359.4 \mu\text{m}^2$. *B*, A total of 200 labeled colon neurons in obstructed rats ($n = 2$) with mean area of $312.9 \mu\text{m}^2$. Mean cross-sectional areas were not significantly different in the 2 groups.

retrophic substances such as basic fibroblast growth factor may have a more prominent influence on the growth and survival of peripheral cholinergic neurons (Tuttle and Pilar, 1986; Vaca et al., 1989).

Since a substantial portion of the MPG postganglionic neurons innervating the rat urinary bladder are cholinergic rather than adrenergic (Chesher, 1967; Alm and Elmer, 1975; Papka et al., 1987), it is possible that other factors as well as NGF may be responsible for the neuronal hypertrophy following urethral obstruction. In this regard it is noteworthy that hormones can also influence the growth and survival of peripheral neurons. For example, testosterone causes hypertrophy of MPG cells (Hervonen et al., 1972), and neonatal castration produces loss of catecholamine fluorescence (Partanen and Hervonen, 1979). Furthermore, the effects of testosterone in other ganglia appear to be mediated indirectly by an up-regulation of NGF synthesis in the target organs (Ishii and Shooter, 1975).

The functional significance of the neuronal hypertrophy observed in this study is uncertain. However, it seems reasonable that a larger target organ requires an expansion of the efferent innervation. Because the number of ganglion cells innervating the bladder did not increase, each cell therefore must provide a more extensive axonal terminal field within the hypertrophied bladder wall. The maintenance of a larger terminal field might in turn require increased synthetic capacity in the cell body, which can only be achieved by an increased cell volume (Henneman et al., 1964). Further studies will be needed to determine whether morphological alterations in MPG neurons are also

correlated with physiological and neurochemical changes in the cells. It is already known that urethral obstruction facilitates certain reflex pathways to the urinary bladder (Steers and de Groat, 1988), changes the peptide content in bladder nerves (Andersson et al., 1988), and alters the responses of bladder smooth muscle to autonomic transmitters (Mattiasson et al., 1987; Speakman et al., 1987).

The results of the present investigation are consistent with the concept proposed by Purves and coworkers (1988) that the geometry of autonomic neurons in the adult is influenced by ongoing interactions with the tissues they innervate. Pertinent to the present investigation, Voyvodic (1989) examined the influence of the size of target tissue on postganglionic neuron dendritic and somal geometry. In a series of experiments in which partial denervation of the submandibular gland was used to increase relative target size, this investigator showed that ganglion cells in contact with the enlarged target developed more complex dendritic arborizations and an increase in a somal cross-sectional area. The conclusion of his study was that the morphology of postganglionic neurons is regulated in part by a retrograde signal derived from the peripheral target.

In summary, changes in urinary bladder size in the adult in response to functional demands may alter the supply of trophic material available to neurons innervating the bladder. Examination of the possible neurotrophic basis for this plasticity could provide a better understanding of reactive trophic factor regulation in the adult. This could eventually lead to the development of drugs that alter trophic mechanisms, which in turn

Figure 7. Distributions of cross-sectional areas of FG-labeled bladder neurons in MPG of obstructed rats that underwent either unilateral pelvic nerve transection or capsaicin treatment (150 mg/kg s.c. total dose) 6 weeks prior to analysis. *A*, A total of 412 labeled bladder neurons in the MPG ipsilateral to the decentralization had a mean area of $518.3 \mu\text{m}^2$ ($n = 3$), which is not significantly different from the intact-obstructed group. *B*, A total of 378 labeled bladder neurons from animals pretreated with capsaicin had a mean area of $557 \mu\text{m}^2$ ($n = 3$), which was not significantly different from untreated obstructed rats. Cross-sectional areas of bladder neurons in denervated and capsaicin-treated obstructed animals are significantly ($p < 0.001$) larger than areas of neurons from control unobstructed rats.

might be useful in preventing or reversing the bladder dysfunction associated with obstruction.

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