Plasticity of Na^+ channels in afferent neurones innervating rat urinary bladder following spinal cord injury

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1. Whole-cell patch-clamp recordings in combination with axonal tracing techniques were used to investigate the effects of chronic spinal cord injury on the electrical properties of dorsal root ganglion neurones innervating the urinary bladder or colon of the adult rat.

2. In spinal intact animals, the majority (73–74%) of bladder and colon neurones which were small in size exhibited high-threshold humped spikes mediated by tetrodotoxin (TTX)-resistant Na^+ channels, whereas large neurones had low-threshold narrow spikes mediated by TTX-sensitive Na^+ channels.

3. In chronic spinal transected animals, 60% of bladder afferent neurones exhibited TTX-sensitive low-threshold spikes. The average diameter and input capacitance of the cells were significantly larger than those of cells obtained from spinal intact animals.

4. In bladder afferent neurones from chronic spinal transected rats, the density of TTX-resistant Na^+ currents significantly decreased from 60·5 to 17·9 pA pF^{-1}, whereas that of TTX-sensitive currents increased from 32·1 to 80·6 pA pF^{-1}.

5. These changes in action potential and Na^+ current characteristics were not detected in colon afferent neurones following spinal cord injury.

6. The results indicate that spinal cord injury increases bladder afferent neurone excitability by shifting the expression of Na^+ channels from a high-threshold TTX-resistant type to a low-threshold TTX-sensitive type. This change in properties may occur in response to alterations in neurotrophic signals originating in the hypertrophied bladder.

Damage to the spinal cord rostral to the lumbosacral level can induce marked changes in the neural control of the lower urinary tract. Following spinal cord injury which interrupts the normal supraspinal pathway regulating micturition, the urinary bladder is initially areflexic, but then over the course of several weeks becomes hyper-reflexic and hypertrophic (de Groat *et al.* 1990; Kruse, Belton & de Groat, 1993). The bladder hypertrophy is due to increased bladder work which occurs in response to urethral sphincter spasticity and increased urethral outlet resistance. Electrophysiological studies in cats (de Groat *et al.* 1990) revealed that bladder hyper-reflexia is at least in part caused by changes in the afferent limb of the micturition reflex, since unmyelinated C fibre bladder afferents which are mechano-insensitive in normal animals become mechano-sensitive in the paraplegic animals. In contrast, colon function which is normally mediated by a spinal reflex pathway is less affected following spinal cord injury (de Groat & Krier, 1978).

In spinal transected rats, dorsal root ganglion (DRG) neurones innervating the hypertrophic bladder also undergo hypertrophy exhibiting a 50% increase in cross-sectional area of the cell body (Kruse, Bray & de Groat, 1995). Since urinary diversion prevented bladder and afferent neurone hypertrophy (Kruse *et al.* 1995) and since partial urethral obstruction induced bladder and neuronal hypertrophy (Steers, Ciambotti, Etzel, Erdman & de Groat, 1991*a*), it appears that changes in a target organ can influence the properties of neurones innervating that organ.

However, little is known about mechanisms that underlie the functional changes in bladder afferents induced by spinal cord injury. Therefore we have evaluated the influence of spinal cord injury and neural–target organ interactions on the electrical properties of afferent neurones innervating the urinary bladder or colon. In contrast to the marked changes in bladder function after spinal cord injury, colonic function is less affected since defecation is normally mediated by a spinal reflex pathway that is not interrupted by spinal cord transection (de Groat & Krier, 1978). Therefore, colon afferent neurones served as controls for comparison with bladder afferent neurones. We have previously reported that patch clamp recordings can be used in combination with retrograde axonal tracing methods to examine the ionic mechanisms in identified populations of afferent and autonomic neurones (Yoshimura, White, Weight & de Groat, 1994). Using this method we found that the majority of bladder afferent neurones were relatively inexcitable due to

high-threshold action potentials mediated by tetrodotoxin (TTX)-resistant Na^+ channels (Yoshimura, White, Weight & de Groat, 1996). In this study we report that chronic spinal cord injury enhances the electrical excitability of bladder afferent neurones by increasing the expression of low-threshold TTX-sensitive Na^+ channels.

METHODS

Animal preparation

Experiments were performed on spinal intact and spinal transected adult female Sprague–Dawley rats (150–250 g). Care and handling of animals were in accordance with institutional guidelines and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. In chronic spinal transected rats, the spinal cord was cut at the level of T8–T9 under halothane anaesthesia as described previously (Kruse *et al.* 1993). The space between the retracted ends of the spinal cord was packed with Gelfoam and the incision was sutured. After spinal transection, animals recovered quickly and were then maintained in shallow cages lined with Alpha Dri (Shepherd Speciality Paper, Inc., Kalamazoo, MI, USA) with free access to water and food. During the first 7–10 days after the transection when the bladder was areflexic and the animals were in complete urinary retention, urine was eliminated from the bladder by manual compression 2–3 times a day; after this period the bladder was emptied once a day. An antibiotic (ampicillin, 3 mg per animal per day) was given subcutaneously for 3–5 days after the operation. Urinary tract infection was checked for by noting the clarity of the urine when the bladder was manually emptied. When cloudy urine was observed, which was rare, an antibiotic was administered for several days until the urine cleared. With this postoperative care, the overall survival rate following spinal cord transection was higher than 90% and animal weight was normal at the end of the 4 week survival period. The populations of DRG neurones which innervate the urinary bladder and colon were labelled by retrograde axonal transport of a fluorescent dye, Fast Blue (4% w/v; Polyloy, Gross Umstadt, Germany) injected into the wall of the bladder or colon in halothane anaesthetized animals 7–10 days prior to the dissociation (Keast & de Groat, 1992). The dye was injected with a 28 G needle at three to six sites on the dorsal surface of the organs (5–6 μl per site, total volume of 20–30 μl). Each injection site was washed with saline to minimize contamination of adjacent organs with the dye. Particular care was taken to avoid injections into the lumens, major blood vessels, or overlying fascial layers to minimize non-specific labelling due to dye leakage (Keast & de Groat, 1989, 1992; Steers, Ciambotti, Erdman & de Groat, 1990; Steers *et al.* 1991*a*). Animals were killed by overdose of ketamine (200–250 mg kg^{-1} I.M.).

Cell dissociation

Freshly dissociated neurones from DRG were prepared from ketamine anaesthetized (100–125 mg kg^{-1}, I.M.) animals as described previously (Yoshimura *et al.* 1994, 1996). Briefly, L6 and S1 DRGs were dissected from spinal intact and chronic spinal transected rats, and then dissociated in a shaking bath for 25 min at 35 °C with 5 ml of Dulbecco's modified Eagle's medium containing trypsin (0·3 mg ml^{-1}), collagenase (1 mg ml^{-1}) and deoxyribonuclease (0·1 mg ml^{-1}). Trypsin inhibitor was then added to neutralize the activity of trypsin.

Electrical recording

Dye-labelled primary afferent neurones that innervate the urinary bladder and colon were identified using an inverted phase contrast microscope (Nikon, Tokyo) with fluorescence attachments (UV-1A filter; excitation wave length, 365 nm). Gigaohm-seal whole-cell recordings were performed at room temperature (20–22 °C) on each freshly dissociated labelled neurone in a culture dish which usually contained two to five labelled cells among a few hundred unlabelled neurones. The number of labelled cells per dish was not obviously different in spinal intact and spinal transected animals. The internal solution used during current-clamp recording of action potentials contained (mM): 140 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 10 Hepes and 2 Mg-ATP; adjusted to pH 7·4 with KOH; 310 mosmol l^{-1}. Patch electrodes had resistances of 1–4 MΩ when filled with the internal solution. Neurones were superfused at a flow rate of 1·5 ml min^{-1} with an external solution containing (mM): 150 NaCl, 5 KCl, 2·5 $CaCl_2$, 1 $MgCl_2$, 10 Hepes and 10 D-glucose; adjusted to pH 7·4 with NaOH; 340 mosmol l^{-1}. All recordings were made with an Axopatch-1D patch clamp amplifier (Axon Instruments), and data were acquired and analysed by pCLAMP software (Axon Instruments). Cell membrane capacitances were obtained by reading the value for whole-cell input capacitance neutralization directly from the amplifier. Durations of action potentials were measured at 50% of the spike amplitude. In current clamp recordings, data are presented from neurones that exhibited resting membrane potentials greater than −45 mV and action potentials that overshot 0 mV. TTX was applied to neurones by injection into the external solution. Recordings of currents and action potentials were conducted on separate populations of cells because it was necessary to alter the internal and external solutions to isolate and properly clamp the Na^+ currents. In voltage clamp recordings, the filter was set to −3 dB at 2000 Hz. Leak currents were subtracted by a *P*/4 pulse protocol and the series resistance was compensated by 50–60%. For the isolation of Na^+ currents, the external solution contained (mM): 100 NaCl, 45 tetraethylammonium chloride, 0·03 $CaCl_2$, 10 Hepes, 10 $MgCl_2$ and 10 D-glucose; adjusted to pH 7·4 with NaOH; 340 mosmol l^{-1}. The pipette solution contained (mM): 115 CsCl, 25 NaCl, 10 Hepes, 2 $MgCl_2$, 1 $CaCl_2$ and 11 EGTA; adjusted to pH 7·4 with CsOH; 310 mosmol l^{-1}. All data are expressed as means ± S.E.M. The data were analysed by the Mann–Whitney *U* test. A level of $P < 0·05$ was considered to be statistically significant.

RESULTS

Spinal intact rat

Based on current clamp recordings, the neurones were divided into two populations according to the sensitivity of their action potentials to TTX. In spinal intact animals, the most common population of bladder and colon afferent neurones (greater than 70%) exhibited long duration action potentials (8·9 ± 0·5 ms, $n = 22$ bladder neurones and 8·6 ± 0·4 ms, $n = 23$ colon neurones) that were resistant to TTX at a concentration up to 6 μM (Fig. 1*A* and *C*). The action potentials were activated by depolarizing current pulses at mean thresholds of −20·4 ± 0·9 mV for bladder neurones and −21·6 ± 1·0 mV for colon neurones. This type of cell was small, with mean diameters of 23·6 ± 1·3 μm and 24·1 ± 1·4 μm for bladder and colon afferent cells, respectively. Approximately 25% of bladder and colon afferent neurones exhibited action potentials that were blocked by TTX (1 μM). These neurones were significantly ($P < 0·01$) larger (30·6 ± 1·8 μm, $n = 8$ bladder cells and 31·1 ± 1·7 μm, $n = 8$ colon cells) and had shorter ($P < 0·05$) duration action potentials (5·4 ± 0·5 ms and

5·6 ± 0·5 ms, respectively) that were activated at significantly ($P < 0·05$) lower thresholds (−26·1 ± 0·9 mV and −27·2 ± 1·0 mV, respectively). The mean cell diameter and cell input capacitances for the entire population of bladder and colon afferent neurones are shown in Table 1.

Voltage clamp recordings of Na^+ currents obtained from an additional group of cells revealed a similar correlation between cell size and sensitivity to TTX. Both TTX-resistant and TTX-sensitive Na^+ currents were identified in bladder and colon afferent neurones (Fig. 2*A* and *B*). Often a single neurone expressed both types of currents but invariably one of the two currents predominated. TTX-resistant Na^+ currents were prominent (85–100% of total Na^+ currents) in small bladder neurones (25·3 ± 1·4 μm, $n = 46$) and colon neurones (24·7 ± 1·2 μm, $n = 44$), whereas larger bladder neurones (31·5 ± 1·6 μm, $n = 14$) and colon neurones (32·1 ± 1·5 μm, $n = 15$) had TTX-sensitive currents comprising 60–100% of the total Na^+ currents (Figs 3*A* and *C*, and 4*A* and *C*). In the complete sample of bladder ($n = 60$) and colon ($n = 59$) afferent neurones from spinal intact rats, the mean reduction of peak amplitude of Na^+ currents by TTX was 17·6 ± 3·9 and 19·1 ± 4·0%, respectively. As noted in previous experiments (Yoshimura *et al.* 1996), activation and inactivation curves for TTX-resistant Na^+ currents were displaced to more depolarized levels by approximately 10 and 30 mV, respectively, in comparison with the TTX- sensitive currents in both bladder and colon neurones (Fig. 2*C* and *D*).

Spinal transected rat

Following spinal cord injury, the properties of colon neurones were not changed significantly, but those of bladder neurones were altered. The number of dye-labelled

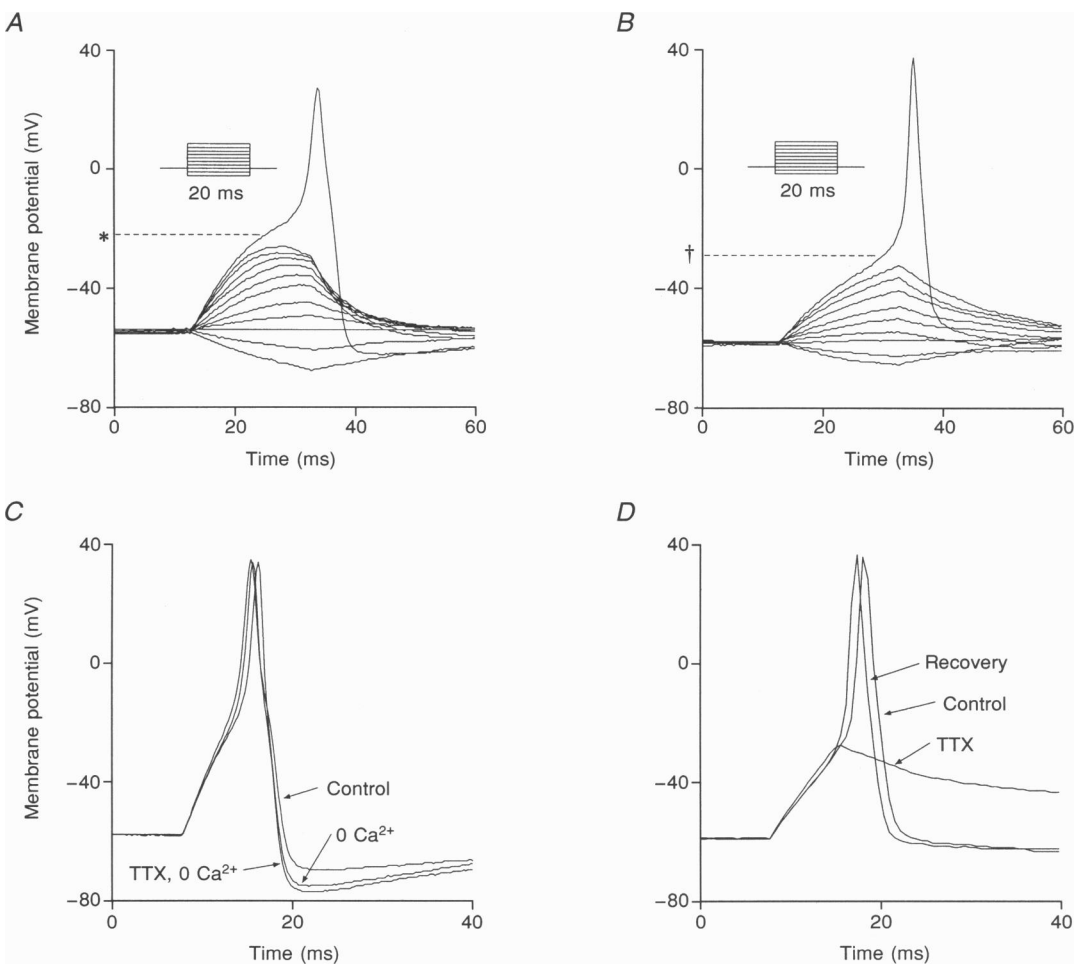

Figure 1. Action potentials in dorsal root ganglion neurones innervating the urinary bladder

A, depolarizing current pulses elicited a humped action potential in a bladder neurone from a spinal intact rat (spike duration, 6·8 ms; * spike threshold, −22 mV; resting membrane potential, −57 mV). *B*, action potential in a neurone from a spinal transected rat had a shorter duration (3·9 ms) and occurred at a lower threshold (†, −29 mV; resting membrane potential, −59 mV). *C*, in the same neurone as in *A*, tetrodotoxin (TTX) up to 6 μM and the removal of extracellular Ca^{2+} ions (0 Ca^{2+}) did not block the action potential while inflection on the repolarizing phase of the spike was suppressed by the removal of extracellular Ca^{2+}. *D*, in the same neurone as in *B*, TTX at a concentration of 1 μM reversibly blocked the action potential. The pulse protocols are shown in the insets of *A* and *B*.

bladder afferent cells per animal was not different between spinal intact (37·3 ± 2·3 cells per animal, $n = 4$) and spinal transected rats (31·3 ± 1·7 cells per animal, $n = 4$). A significantly larger percentage (60% *versus* 27%) of bladder neurones exhibited TTX-sensitive action potentials ($n = 30$) and the mean spike duration (5·3 ± 0·7 ms) was shorter (31%) and the mean threshold for spike activation (−25·5 ± 0·9 mV) was lower (21%) than measurements in intact animals (Fig. 1*B* and *D*, Table 1). Diameter and cell input capacitance in bladder cells from spinal transected rats significantly ($P < 0·01$) increased to 31·6 ± 2·8 μm and 39·6 ± 2·4 pF, respectively (Table 1).

The alteration in pharmacological and electrophysiological properties of action potentials in bladder afferent neurones following spinal cord injury was also reflected in changes in

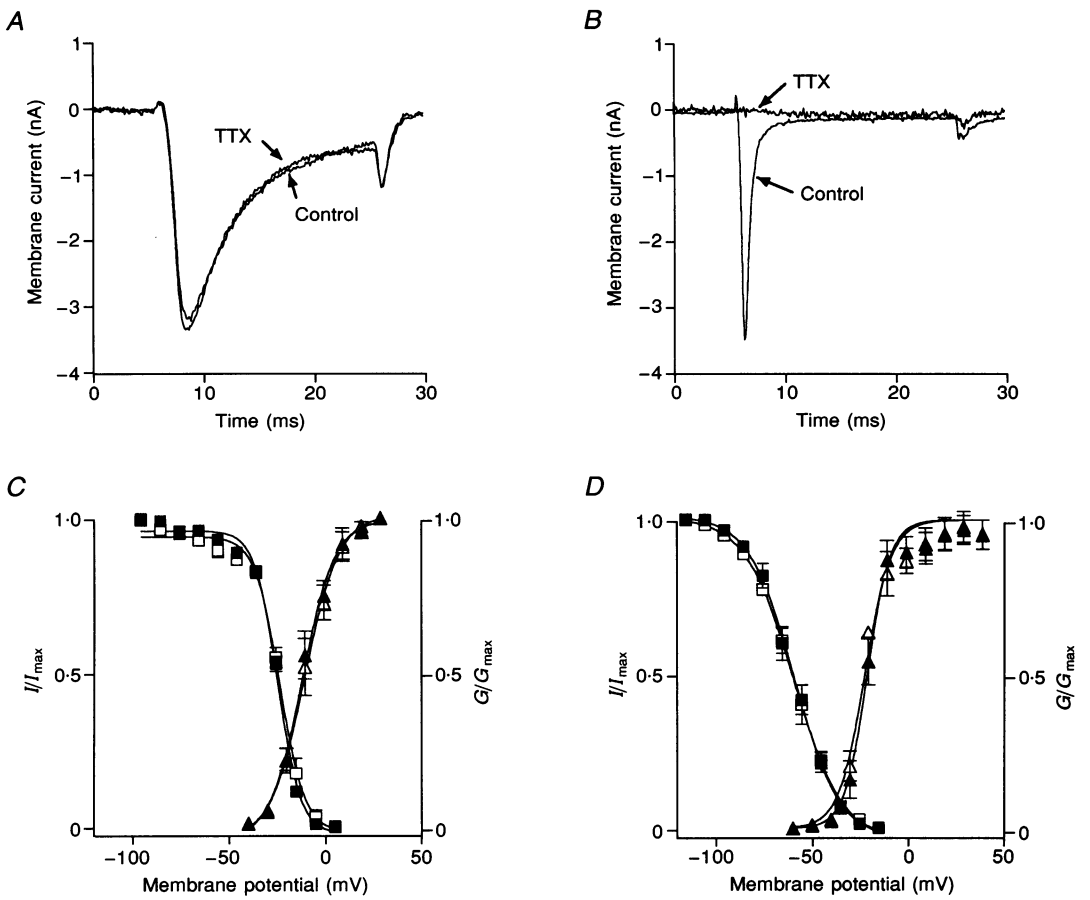

Figure 2. Tetrodotoxin (TTX) sensitivity and voltage dependence of Na^+ currents in dorsal root ganglion neurones innervating the urinary bladder

A, superimposed traces of Na^+ currents elicited by depolarizing voltage steps to +5 mV from the holding potential of −70 mV in the absence (control) and presence of 2 μM TTX in a neurone from a spinal intact rat. *B*, superimposed traces of Na^+ currents elicited by depolarizing voltage steps to −10 mV from the holding potential of −70 mV in the absence (control) and presence of 2 μM TTX in a neurone from a spinal transected rat. *C*, activation and inactivation characteristics of TTX-resistant Na^+ currents ($n = 8$). The inactivation curves of spinal intact (□) and spinal transected animals (■) are plotted as the normalized peak currents (I/I_{max}) *versus* the prepulse potential. The activation curves of neurones from spinal intact (△) and spinal transected animals (▲) are plotted as the relative Na^+ conductance (G/G_{max}) *versus* the command potential. The continuous lines represent non-linear least squares fit to the Boltzmann equation. The V_h and slope factor (k) values for activation of TTX-resistant currents were −11·4 mV and 8·5 mV, respectively, in spinal intact animals and −10·3 mV and 9·1 mV, respectively, in spinal transected animals. The inactivation curve for TTX-resistant currents in spinal intact and spinal transected rats had V_h of −24·2 mV with k of 5·6 mV and V_h of −23·0 mV with k of 6·2 mV, respectively. *D*, activation and inactivation characteristics of TTX-sensitive Na^+ currents. The V_h and k values for activation of TTX-sensitive currents were −20·6 mV and 6·1 mV, respectively, in spinal intact animals (△) and −21·9 mV and 7·0 mV, respectively, in spinal transected animals (▲). The inactivation curve for TTX-sensitive currents in spinal intact (□) and spinal transected rats (■) had V_h of −59·0 mV with k of 11·5 mV and V_h of −59·3 mV with k of 12·3 mV, respectively. The properties of Na^+ currents in colon neurones were not significantly different from those in bladder neurones.

Table 1. Membrane characteristics of bladder and colon afferent neurones in spinal intact and spinal transected rats

	Bladder		Colon	
	Intact	Transected	Intact	Transected
Diameter (μm)	25·1 ± 2·8 (90)	31·6 ± 2·8** (88)	24·8 ± 3·0 (90)	26·6 ± 3·3 (81)
Input capacitance (pF)	27·2 ± 3·8 (90)	39·6 ± 2·4** (88)	28·0 ± 3·9 (90)	27·8 ± 2·4 (81)
Spike threshold (mV)	−21·1 ± 1·6 (30)	−25·5 ± 0·9** (30)	−21·1 ± 1·2 (31)	−22·6 ± 1·3 (28)
Spike duration (ms)	7·7 ± 1·1 (30)	5·3 ± 0·7* (30)	7·9 ± 1·2 (31)	7·2 ± 1·0 (28)
Na^+ current threshold (mV)	−26·5 ± 1·4 (60)	−38·9 ± 1·1** (58)	−27·6 ± 2·0 (59)	−27·1 ± 1·7 (53)
Na^+ current density (pA pF^{-1})				
TTX resistant	60·5 ± 5·5 (60)	17·9 ± 9·2* (58)	64·6 ± 7·0 (59)	61·7 ± 8·1 (53)
TTX sensitive	32·1 ± 9·5 (60)	80·6 ± 10·1** (58)	38·1 ± 8·8 (59)	41·6 ± 6·1 (53)

Values are means ± S.E.M. *$P < 0.05$ and **$P < 0.01$ indicate differences between values obtained in neurones from spinal intact and spinal transected groups of animals. Values of n are given in parentheses.

Na^+ currents. The proportion of the peak inward current sensitive to TTX increased from 17·6 ± 3·9% to 70·6 ± 4·78% ($n = 58$ cells). Likewise, the density of the TTX-sensitive Na^+ current in bladder cells significantly increased from 32·1 ± 9·5 to 80·6 ± 10·1 pA pF^{-1}; while TTX-resistant current density decreased from 60·5 ± 5·5 to 17·9 ± 9·2 pA pF^{-1} after spinalization (Table 1). In addition, Na^+ currents in bladder afferent neurones from spinal transected rats were elicited at a lower threshold (−38·9 ± 1·1 mV, $n = 30$) than those in neurones from spinal intact rats (−26·5 ± 1·4 mV) (Table 1). TTX-sensitive currents were detected across the entire range of cell sizes (Fig. 3*B*) in samples from spinal transected animals in comparison with the more restricted distribution to the larger cells noted in tissues removed from normal animals (Fig. 3*A*). In addition to the shift in TTX sensitivity of

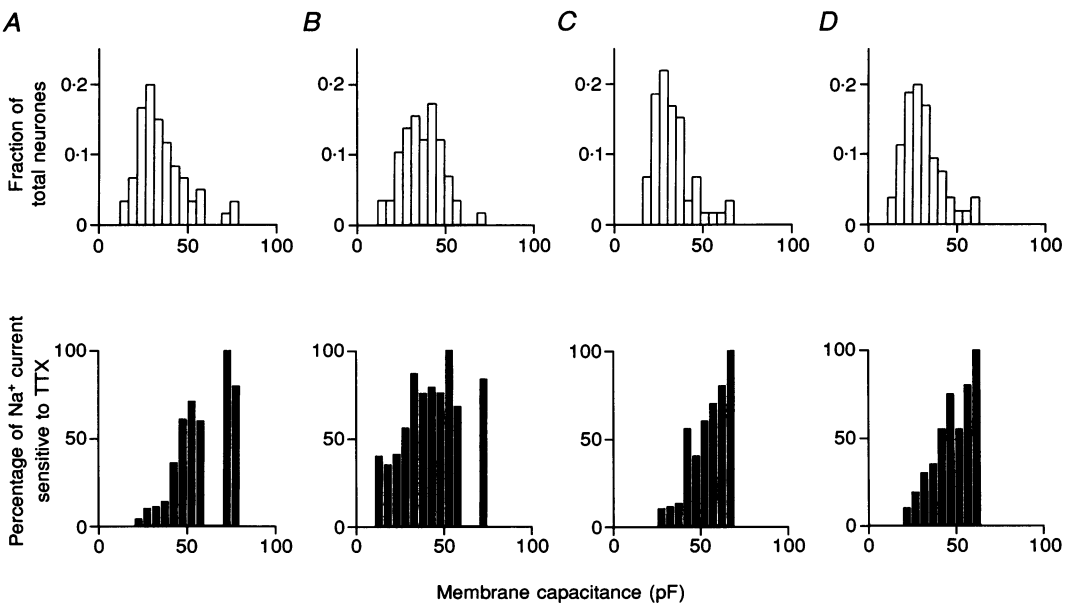

Figure 3. Cell size distribution and expression of tetrodotoxin (TTX)-sensitive Na^+ currents in bladder and colon afferent neurones from spinal intact and spinal transected rats

A, bladder afferent neurones from spinal intact animals ($n = 60$). *B*, bladder afferent neurones from spinal transected animals ($n = 58$). *C*, colon afferent neurones from spinal intact animals ($n = 59$). *D*, colon afferent neurones from spinal transected animals ($n = 53$). Upper histograms, cell size distribution indicated by membrane capacitance; bin width is 5 pF capacitance. Open bars represent the fraction of neurones in each bin relative to the total number of neurones examined. Note that a shift in cell size distribution toward the right occurred in bladder neurones from spinal transected animals (*B*). Lower histograms, percentage of the total Na^+ current that was TTX sensitive *versus* membrane capacitance; bin width 5 pF. Filled bars indicate the average percentage of the total Na^+ current inhibited by TTX (2 μM) for all neurones of each bin.

bladder neurones, the neurones which still exhibited a predominance of TTX-resistant Na^+ currents after spinal cord injury also exhibited an increase in the proportion of the total Na^+ current that was TTX sensitive. In bladder neurones from spinal intact rats (Fig. 4*A*) and colon neurones from spinal intact (Fig. 4*C*) and spinal transected rats (Fig. 4*D*), the expression of TTX-sensitive Na^+ currents exhibited a bimodal pattern (either less than 20% or over 60%). On the other hand, bladder neurones from spinal transected rats showed no obvious pattern in the expression of TTX-sensitive Na^+ currents; and in the population of neurones exhibiting predominantly TTX-resistant Na^+ currents, the TTX-sensitive component increased to 45% of the total Na^+ current (Fig. 4*B*). Inactivation and activation characteristics of TTX-resistant and TTX-sensitive Na^+ currents in bladder afferent neurones were not altered following spinal cord injury (Fig. 2*C* and *D*). Action potentials or Na^+ currents in colon afferent neurones were not changed after spinal cord injury (Figs 3*C* and *D*, and 4*C* and *D*; Table 1).

DISCUSSION

The present results indicate that spinal cord injury in the adult rat can increase the electrical excitability of a specific population of visceral primary afferent neurones by changing the ionic mechanisms underlying the action potential. In normal animals, the majority of bladder afferent neurones exhibited high-threshold action potentials mediated by TTX-resistant Na^+ channels; but following spinal cord injury the majority of cells exhibited low-threshold, TTX-sensitive action potentials and Na^+ channels. Since the numbers of dye-labelled bladder neurones were not different in spinal intact and spinal transected animals, it is unlikely that the shift in cell sizes or Na^+ channel expression after spinal cord injury was due to loss of a specific neuronal population. Moreover, these changes were not observed in a different population of cells innervating the colon despite the same experimental protocol. Therefore, it seems reasonable to assume that spinal cord injury specifically induces a switch in Na^+ channel expression in afferent

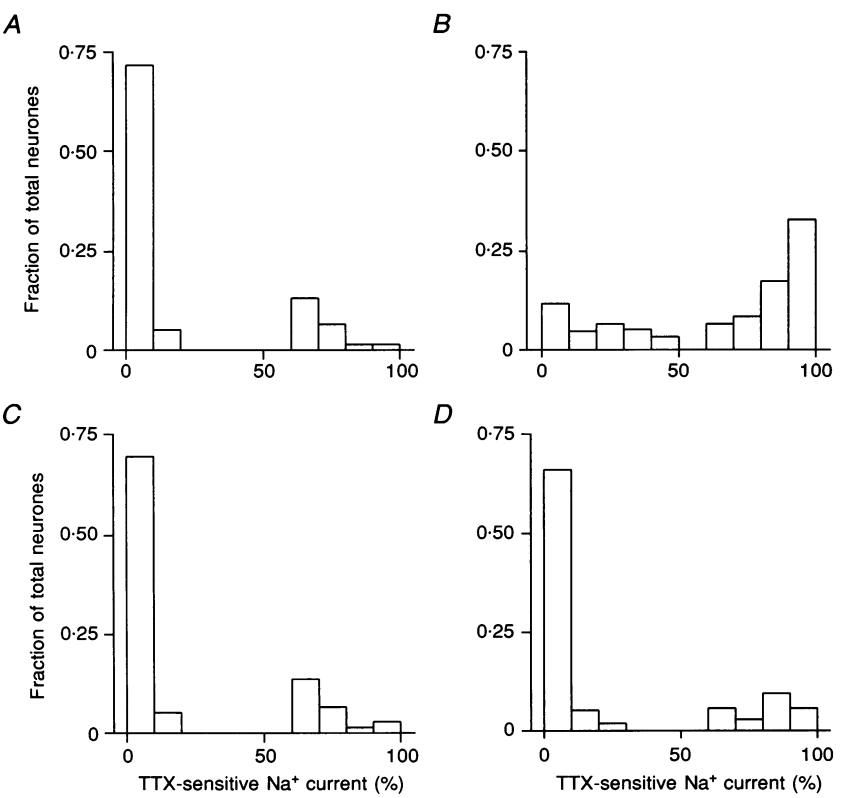

Figure 4. The classification of bladder and colon afferent neurones from spinal intact and spinal transected rats based on the magnitude of tetrodotoxin (TTX)-sensitive Na^+ currents

A, bladder afferent neurones from spinal intact animals ($n = 60$). *B*, bladder afferent neurones from spinal transected animals ($n = 58$). *C*, colon afferent neurones from spinal intact animals ($n = 59$). *D*, colon afferent neurones from spinal transected animals ($n = 53$). Abscissa, percentage of the total Na^+ current that was TTX sensitive. Abscissa divided into 10 bins (10% for each bin). Ordinate, the fraction of the total population of cells exhibiting the corresponding percentage of TTX-sensitive Na^+ current. Note that bladder neurones from spinal intact animals (*A*) and colon neurones from both types of animals (*C* and *D*) exhibited a bimodal pattern in which the TTX-sensitive current was either more than 60% or less than 20% of the total Na^+ current while bladder neurones from spinal transected animals (*B*) exhibited no obvious pattern in the expression of TTX-sensitive Na^+ currents.

neurones innervating the urinary bladder. This conclusion is also supported by the finding that some bladder afferent neurones that retained a predominance of TTX-resistant channels exhibited a graded increase in the expression of TTX-sensitive Na^+ currents after spinal cord injury. Since the two types of Na^+ channels are produced by different genes (Akopian, Sivilotti & Wood, 1996; Sangameswaran *et al.* 1996), the effects of spinal cord injury might be due to changes in gene expression.

Various mechanisms might be involved in these changes in electrical properties including direct damage to ascending primary axons in the spinal cord or alterations in trophic signals arising from the central or peripheral targets of the primary afferent neurones. The first mechanism seems unlikely since bladder and colon afferents should be equally affected by direct axonal damage and therefore both would exhibit the electrical changes, when in fact only bladder neurones were altered. In addition, the spinal projections of most bladder and colon afferent neurones should be localized below the level of the spinal transection (Keast & de Groat, 1992) and therefore not be directly damaged. Thus the role of trophic factors in the afferent plasticity seems more likely since substances such as nerve growth factor (NGF) as well as antibodies to NGF are known to influence the electrical and chemical properties of sensory neurones (Aguayo & White, 1992).

How could spinal cord injury selectively change the trophic factor regulation of bladder afferent neurones? Previous studies revealed that spinal cord injury in the rat induces uncoordinated bladder and urethral sphincter activity, which increases urethral resistance and bladder work, eventually leading to bladder hypertrophy (Kruse *et al.* 1993, 1995). NGF and other trophic factors increase in hypertrophied bladders (Steers *et al.* 1991*a*; Buttyan, Jacobs, Blaivas & Levine, 1992); and afferent and efferent neurones innervating the bladder also undergo hypertrophy (Steers *et al.* 1990, 1991*a*; Kruse *et al.* 1995), which appears to be mediated in part by NGF (Steers, Kolbeck, Creedon & Tuttle, 1991*b*). An increase in the average size of bladder afferent neurones was also noted in the present studies. However, morphological and functional changes were not observed in colon afferent neurones in spinal transected animals. Since colonic function, which is normally controlled by a spinal reflex pathway, is less affected by spinal cord injury (de Groat & Krier, 1978), it is not unexpected that the properties of colon afferents were unchanged in the paraplegic animals.

Although NGF has been implicated in the morphological changes in bladder neurones following urethral obstruction (Steers *et al.* 1991*a*), it seems unlikely that NGF mediates the changes in Na^+ channels noted in the present experiments since NGF promotes the expression of TTX-resistant Na^+ channels in small DRG cells (Aguayo & White, 1992). When cultured DRG cells are maintained in the absence of NGF, TTX-resistant action potentials are replaced by TTX-sensitive action potentials. Since this is opposite to the changes noted in the present study, other trophic signals such as basic fibroblast growth factor (bFGF), which increase in the hypertrophied bladder (Buttyan *et al.* 1992), may initiate the changes in electrical properties of the cells.

The physiological significance of the changes in the electrical excitability of the DRG cell body is uncertain. However, it has been reported that TTX-resistant Na^+ currents occur in C fibres of human sensory nerves (Quasthoff, Grosskreutz, Schröder, Schneider & Grafe, 1995). TTX-resistant Na^+ conductances in slow conducting C fibres are also involved in spinal nociceptive transmission (Jeftinija, 1994). Thus, it is possible that the changes in the perikarya of bladder afferent neurones also occur at the afferent receptors in the bladder wall and thereby alter the afferent signals triggered during bladder filling.

Small-diameter DRG cells with TTX-resistant Na^+ currents are normally sensitive to capsaicin (Arbuckle & Docherty, 1995). In the cat, capsaicin-sensitive C fibre bladder afferents which normally have high thresholds to mechanical stimuli and are unresponsive to bladder distension (Jänig & Koltzenburg, 1990) become responsive after spinal cord injury and are the principal triggers for initiating voiding and bladder hyper-reflexia in the paraplegic animal (de Groat *et al.* 1990). Capsaicin-sensitive, C fibre bladder afferents also contribute to bladder hyperactivity in patients with various neurological disorders such as multiple sclerosis (Fowler, Beck, Gerard, Betts & Fowler, 1994) and Parkinson's disease (Geirsson, Fall & Lindström, 1993), as well as in spinal cord injured patients (Geirsson, Fall & Sullivan, 1995). Thus, it is tempting to speculate that the changes in the electrical excitability of bladder afferents similar to those detected in the present experiments may underlie the emergence of neurogenic bladder hyperactivity and incontinence that occur in various human disorders.

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