

Disruption of bladder epithelium barrier function after spinal cord injury

Gerard Apodaca,¹ Susanna Kiss,² Wily Ruiz,²
Susan Meyers,² Mark Zeidel,¹ and Lori Birder^{2,3}

¹Laboratory of Epithelial Cell Biology, Department of Cell Biology and Physiology,

²Renal-Electrolyte Division, Department of Medicine, and ³Department of Pharmacology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Submitted 7 October 2002; accepted in final form 7 January 2003

Apodaca, Gerard, Susanna Kiss, Wily Ruiz, Susan Meyers, Mark Zeidel, and Lori Birder. Disruption of bladder epithelium barrier function after spinal cord injury. *Am J Physiol Renal Physiol* 284: F966–F976, 2003. First published January 14, 2003; 10.1152/ajprenal.00359.2002.—Neural-epithelial interactions are hypothesized to play an important role in bladder function. We determined whether spinal cord injury (SCI) altered several indicators of urinary bladder epithelium barrier function, including continuity of the surface umbrella cell layer, transepithelial resistance (TER), and urea and water permeability. Within 2 h of SCI, significant changes in uroepithelium were noted, including disruption of the surface umbrella cells and an ~50% decrease in TER. By 24 h, TER reached a minimum and was accompanied by significant increases in water and urea permeability. Regeneration of the surface uroepithelium was accomplished by 14 days after SCI and was accompanied by a return to normal TER and urea and water permeabilities. This early disruption of the uroepithelial permeability and accompanying changes in uroepithelial morphology were prevented by pretreatment with hexamethonium (a blocker of ganglion transmission), indicating involvement of sympathetic or parasympathetic input to the urinary bladder. In addition, prior treatment with capsaicin worsened the effect of SCI on uroepithelial permeability, suggesting that capsaicin-sensitive afferents may play a protective role in the process. These results demonstrate that SCI results in a significant disruption of the urinary bladder uroepithelium and that these changes may be mediated in part by an interaction with bladder nerves.

uroepithelium; urea and water permeability; bladder nerves

THE STORAGE FUNCTION of the bladder depends on the presence of a barrier that prevents urine from being reabsorbed into the bloodstream and regulates the expulsion of urine from the body (14, 16, 17, 23, 34). During bladder filling, the outlet, which includes the bladder neck and the smooth and striated muscles of the urethra, remains closed and the smooth muscle of the bladder remains relaxed (14, 16, 17). The uroepithelium, which lines the mucosal surface of the bladder, forms the permeability barrier, which retains the urine and prevents the paracellular leakage of solutes

and ions across the outermost, umbrella cell layer. Characteristics of the barrier include a high transepithelial resistance (TER) and low permeability to urea and water (27, 34). Intravesical pressure remains low during filling, and increased urine volume can be accommodated by unfolding of the mucosa and accompanying increases in the surface of the umbrella cell layer (51). When the bladder reaches capacity and voiding is initiated, the muscles of the outlet relax, and urine is expelled by contractions of the smooth detrusor muscle in the wall of the bladder.

The interplay between the outlet and the bladder is under control of the central nervous system as well as sympathetic, parasympathetic, and somatic nerves that innervate the detrusor muscle and outlet in a reciprocal fashion (14, 16, 17). Bladder innervation can be disrupted as a result of trauma or disease (14, 16, 17). For example, rostral lumbar spinal cord transection eliminates voluntary control of voiding and is accompanied by uncoordinated bladder and external urethral sphincter activity, termed detrusor-sphincter dyssynergia (7, 8, 10, 15, 16, 55). An additional consequence of spinal cord injury (SCI) is a high intravesical bladder pressure, which can lead to damage and malfunction of the upper urinary tract and hypertrophy of the smooth muscle mass (56). The latter is accompanied by enormous enlargement of the urinary bladder (32, 56). Although a great deal of attention has focused on alterations in the detrusor muscle and its innervation after SCI, much less is understood about changes in the uroepithelium and its associated barrier function that might accompany SCI.

An emerging body of literature indicates reciprocal communication between the neuronal system and the uroepithelium. The urinary bladder is innervated by several types of bladder afferents (A δ - and C fibers), some of which are found in the muscle layers and some just below and within the uroepithelium (5, 19, 33). Significantly, epithelium-associated afferent nerves are sensitive to chemicals released from uroepithelial cells, including nitric oxide (NO), prostaglandins, and

Address for reprint requests and other correspondence: G. Apodaca, Renal Div., 982 Scaife Hall, University of Pittsburgh, 3550 Terrace St., Pittsburgh, PA 15261 (E-mail: gla6@pitt.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

ATP (2, 30, 36). The latter binds to P2X₃ purinergic receptors on afferent nerves and is likely to play an important role in detrusor contraction as well as nociception (40, 53). In turn, uroepithelial cells respond to various chemicals and neurotransmitters, including acetylcholine, norepinephrine, ATP, calcitonin gene-related peptide, and substance P (3–6). Changes elicited in the uroepithelium by a variety of stimuli include the release of mediators such as NO and ATP and increases in intracellular Ca²⁺ (3, 5, 6, 18, 48, 53). Whether neurotransmitters affect parameters associated with uroepithelial barrier function, such as maintenance of a high TER or low water and urea permeabilities, is not well understood, but treatment with stress hormones, including norepinephrine, can lead to disruption of tight junctions and loss of umbrella cells (52).

To examine the relation between the nervous system and the uroepithelium, we have studied the effects of SCI on uroepithelial cell morphology as well as barrier function. We observed that SCI was accompanied by a rapid loss of barrier function, and these acute changes were prevented by pretreatment with the ganglion transmission blocker hexamethonium, whereas capsaicin potentiated the effects of SCI. Normal barrier function was observed 14–28 days after SCI. Our results indicate that neurotransmitter release shortly after SCI may play an important role in disrupting epithelial barrier function.

MATERIALS AND METHODS

Materials and animals. Unless specified otherwise, all chemicals were obtained from Sigma (St. Louis, MO) and were of reagent grade or better. [¹⁴C]urea and [³H]water were obtained from American Radiolabeled Chemicals (St. Louis, MO). Female Sprague-Dawley rats weighing 275–350 g were fed a standard diet (Lab Diet 5P00, PMI Nutritional, Brentwood, MO) with free access to water before and after SCI. All animal studies were carried out with the approval of the University of Pittsburgh Animal Care and Use Committee, and animals were maintained according to the standards set forth in the American Physiological Society's "Guide for the Care and Use of Animals."

Rat model of SCI. Rats were anesthetized with halothane [2% (vol/vol) in oxygen], and after an epidural injection of lidocaine (Xylocaine, 5 μ l) a laminectomy was aseptically performed at the T₈–T₉ spinal level. Gelfoam (Fisher) was placed between the cut ends of the spinal cord, and the muscle and skin were sutured. The animals were allowed to recover in the absence of anesthesia for 2 h, 1 day, 3 days, or 24 days. Sham-operated animals were placed under anesthesia, and the bone and muscle surrounding T₈–T₉ were removed, but the spinal cord was not transected. Sham-operated and SCI rats were given prophylactic antibiotic treatment (ampicillin, Polyflex, 100 mg/kg im daily) for 1 wk after the procedure and allowed free access to food and water. During the first 7–10 days after transection, urine was eliminated from the bladder by manual compression two to three times a day. After this time, spinal reflexes developed that allowed for autonomic control of micturition. To provide a control for effects of bladder distension that might follow acute spinalization, some spinal-intact animals were placed under continuous halothane anesthesia (which suppresses

bladder reflexes) for 2.5 h before euthanasia and tissue removal. This amount of time allowed accumulation of urine volumes that were similar to those observed 2 h after SCI. In one animal with SCI, urine volume was 3 ml. The data for this animal were discarded, inasmuch as it was likely that the bladder was overdistended. In some experiments, animals were treated with hexamethonium (50 mg/kg iv; Sigma) 0.5 h before SCI. Other animals were injected with 5 μ l of PBS beneath the dura of the spinal cord (via a 30-gauge needle) into the underlying gray matter, and the needle was removed without transection of the spinal cord. After incisions were sutured, the animals were allowed to recover for 2 h in the absence of anesthesia. In addition, a small number of animals ($n = 4$) were pretreated with capsaicin (100 mg/kg sc; Sigma) in 10% (vol/vol) ethanol-10% (vol/vol) Tween 80 in normal saline 4 days before further experimentation. Desensitization was confirmed by measuring the number of defensive forelimb eye-wipe movements in response to 100 μ g/ml capsaicin solution (11). Control animals were given vehicle [10% (vol/vol) ethanol-10% (vol/vol) Tween 80 in normal saline] not containing capsaicin.

Measurements of water permeability, urea permeability, and TER. Water permeability, urea permeability, and TER were measured as described previously (24, 34). Briefly, after the treatments described above and at the designated time points, the animals were euthanized (by inhalation of medical-grade CO₂ followed by thoracotomy) and the bladder was placed immediately into Ringer solution (in mM: 111.2 NaCl, 25 NaHCO₃, 4.8 KCl, 2 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, and 11.1 glucose) maintained at 37°C and constantly bubbled with 95% O₂-5% CO₂ to maintain pH at 7.4. The tissue was placed on a stretching rack with the epithelium facing downward. A ring with a 0.73-cm² opening and surrounded by sharp metal pins was placed under the tissue and lifted up to spear the overlying tissue. Excess tissue surrounding the ring was removed with tissue scissors, and the ring and its associated bladder tissue were mounted between two halves of a modified Ussing chamber. The chambers, filled with Ringer solution, were maintained at 37°C, and the hemichambers were constantly stirred. The membranes were allowed to stabilize for 1 h before electrical measurements of TER to determine epithelial membrane integrity, and radiolabeled water and urea were added to measure permeability. Significant changes in TER were assessed by *t*-test.

¹⁴C-labeled (0.25 μ Ci/ml) urea and ³H-labeled (1 μ Ci/ml) water were added to the apical (luminal) side of the membrane, and both hemichambers were sampled (2 \times 100 μ l per hemichamber) at 15-min intervals throughout the experiment. After 1 h of baseline measurements, the ionophore nystatin (185 μ M) was added to the apical side to increase permeability across the apical membrane. To determine the contribution of unstirred layers to the measured permeabilities, the apical membrane barrier was ablated by addition of 100 μ l of Triton X-100 1 h after addition of nystatin. In all experiments, addition of nystatin and Triton X-100 abolished TER. Fluxes were calculated as described previously (24, 34). Significant changes in permeability were assessed by *t*-test.

Scanning electron microscopy of uroepithelium. At the designated time points, rats were euthanized as described above, and the bladder was rapidly excised, cut along the dorsal margin, and placed in a fixative containing 2.0% (vol/vol) glutaraldehyde and 2.0% (wt/vol) paraformaldehyde in 100 mM sodium cacodylate, pH 7.4, 1 mM CaCl₂, and 0.5 mM MgCl₂ for 2–4 h at 4°C. The fixed tissue was washed for 15 min in 100 mM sodium cacodylate buffer, pH 7.4, and then treated with 1% (wt/vol) OsO₄ in 100 mM sodium cacodylate buffer, pH 7.4, for 60 min at 4°C. After several rinses with

distilled water, the tissue was cut into small blocks (~0.5-mm cubes), and the samples were washed three times over 45 min in PBS and then dehydrated for 15 min in a graded series of ethanol: 30, 50, 70, and 95% (vol/vol in water). The samples were then incubated three times for 45 min in absolute ethanol. The dehydrated samples were critical point dried, sputter coated with gold-palladium, and viewed in a Jeol JSM T300 scanning electron microscope at 20 kV. Images were captured on Kodak type 52 film, scanned on a Linotype-Hell Saphir Ultra II scanner, contrast corrected in Photoshop 6.0 (Adobe), assembled in Freehand 10.0 (Macromedia, San Francisco, CA), and output on a dye sublimation printer (model 8650PS, Kodak, Rochester, NY). All images are representative of similar results obtained from the bladders of at least three animals.

Transmission electron microscopy of uroepithelium. Bladder tissue was fixed and osmicated as described above and then cut into small blocks. After several water rinses, the samples were stained en bloc overnight with 0.5% uranyl acetate in water. Samples were dehydrated in a graded series of ethanol, embedded in epoxy resin (LX-112, Ladd), and sectioned with a diamond knife (Diatome), silver to pale gold in color, were mounted on butvar-coated copper grids, contrasted with uranyl acetate and lead citrate, and viewed at 80 kV in a Jeol 100 CX electron microscope. Images were printed and processed as described above. All images are representative of similar results obtained from the bladders of at least three animals.

RESULTS

SCI is accompanied by decreases in TER, water permeability, and urea permeability. The uroepithelium forms a barrier that is characterized by its high resistance to ion flow and low permeabilities to water and urea (24, 28, 34). One indication of barrier function is TER, which measures the ability of the epithelium to impede paracellular (across the junctions) and transcellular (across the cell) ion flow. In umbrella cells, the combined low levels of paracellular ion flow and modest transcellular ion flow result in a high TER.

As controls, the TER was measured in animals that underwent no prior experimental manipulation. Sham controls were placed under anesthesia, and bone around the spinal cord was removed, but the spinal cord was not transected; then the animals were allowed to recover for up to 28 days. Distension controls were allowed to fill their bladders for 2.5 h but were unable to void because of anesthesia. The volume of urine in animals 2 h after SCI was 1.63 ± 0.4 ml, and in distension controls the urine volume was 1.53 ± 0.05 ml after 2.5 h. The TER values for all these controls were essentially identical (i.e., $1,802 \pm 101 \Omega \cdot \text{cm}^2$; Fig. 1A) and, as such, were grouped and are collectively referred to as "controls" in Figs. 1 and 4. This value is lower than those measured in rabbits but similar to our previous observations and may represent species variation, differences in the methods of measurement, or differences in the diet of the animals (24, 28, 34).

To assess the impact of SCI on TER, the spinal cord of anesthetized rats was transected at T₈-T₉. The animals were allowed to recover for 2 h, 24 h, 3 days, 14 days, or 28 days, and then the bladder was excised and TER of the organ tissue mounted in Ussing chambers

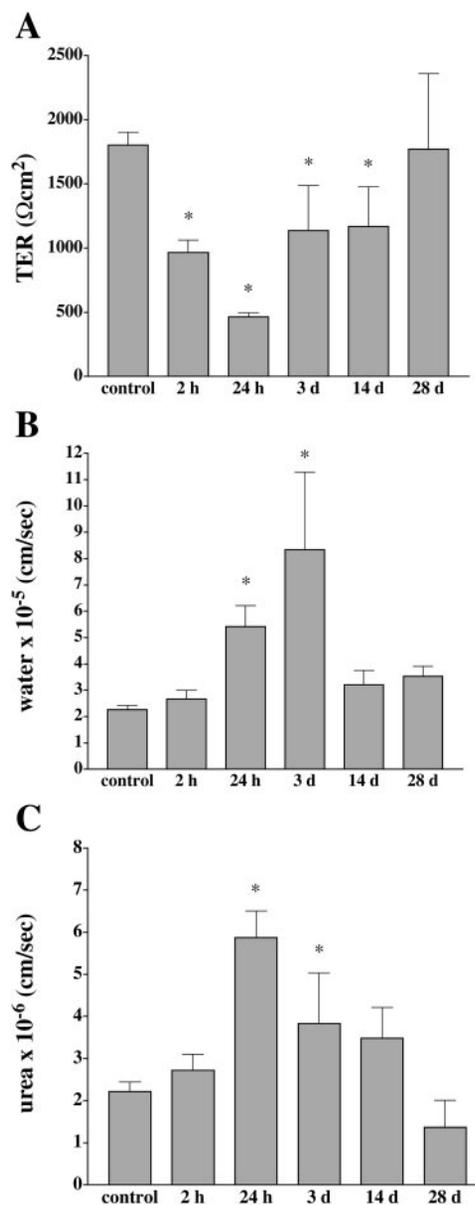


Fig. 1. Effect of spinal cord injury (SCI) on transepithelial resistance (TER, A), water permeability (B), and urea permeability (C). Control bladders or bladders from animals 2 h–28 days after SCI were mounted in Ussing chambers, and TER, water permeability, and urea permeability were monitored. Values are means \pm SE from 4–32 bladders. * $P < 0.05$ relative to control.

was measured. We observed a significant decrease in TER as early as 2 h after SCI ($965 \pm 98 \Omega \cdot \text{cm}^2$, $P < 0.05$) relative to the control group of animals described above (Fig. 1A). The largest decrease in TER was observed at 24 h, when TER was $466 \pm 28 \Omega \cdot \text{cm}^2$. However, by 3 days after SCI, TER was beginning to recover, and, by 28 days, TER approached control levels of $1,771 \pm 590 \Omega \cdot \text{cm}^2$ (Fig. 1A).

Next, we measured the impact of SCI on water and urea permeability. Controls identical to those used in the TER analysis were employed in these studies. As we reported previously, water and urea permeabilities were low in control tissue: $\sim 2.3 \times 10^{-5}$ or 2.2×10^{-6}

cm/s, respectively (24, 34). Water permeability remained near control levels after 2 h but significantly increased ~ 2.5 -fold above controls after 24 h (Fig. 1B). At 3 days, water permeability was increased 3.7-fold above controls (Fig. 1B). Water permeability decreased to near control levels after 14 days and remained at these levels up to 28 days after SCI (Fig. 1B). Urea permeability followed a similar pattern. At 2 h after SCI, there was little alteration in urea permeability; however, by 24 h a 2.7-fold increase in urea permeability was observed (Fig. 1B). Urea permeability was elevated at 24 h and after 3 days but was not significantly different from controls 14 or 28 days after SCI (Fig. 1B).

SCI is accompanied by disruption of the uroepithelium. Scanning and transmission electron microscopy (SEM and TEM, respectively) were used to determine whether the functional changes in the uroepithelium of SCI animals were accompanied by morphological changes in the uroepithelium of these animals. The ultrastructure of umbrella cells from sham-operated animals (control) is shown in Figs. 2A and 3A. Identical morphology was observed in untreated animals and distension controls and is not shown. When examined

by SEM, the umbrella cells of these control animals were large (~ 40 – 50 μm across) and polyhedral in shape, and the surface membrane was folded in a parallel array (Fig. 2A). A thin tight junction complex circumscribed the perimeter of each cell (Fig. 2B). In thin-section electron micrographs, the uroepithelium contained an upper umbrella cell layer, two to three intermediate cell layers, and a basal cell layer that rested on the underlying connective tissue (Fig. 3A). Junctional complexes were observed at higher magnification (arrow, Fig. 3B).

The morphology of the uroepithelium was significantly altered by SCI. In addition to normal-appearing areas of uroepithelium, patches of small cells were noted 2 h after SCI (boxed area in Fig. 2C and higher-magnification view in Fig. 2D). These latter areas consisted of disorganized patches of cells that were 10–25 μm long and exhibited a relatively smooth surface (Fig. 2D). A junctional ring was observed at the periphery of many, but not all, of these cells. Because of their small size and morphology, it is likely that they represent underlying intermediate or basal cells that were exposed as a result of umbrella cell layer injury. Analysis of these areas by TEM confirmed disruption of the

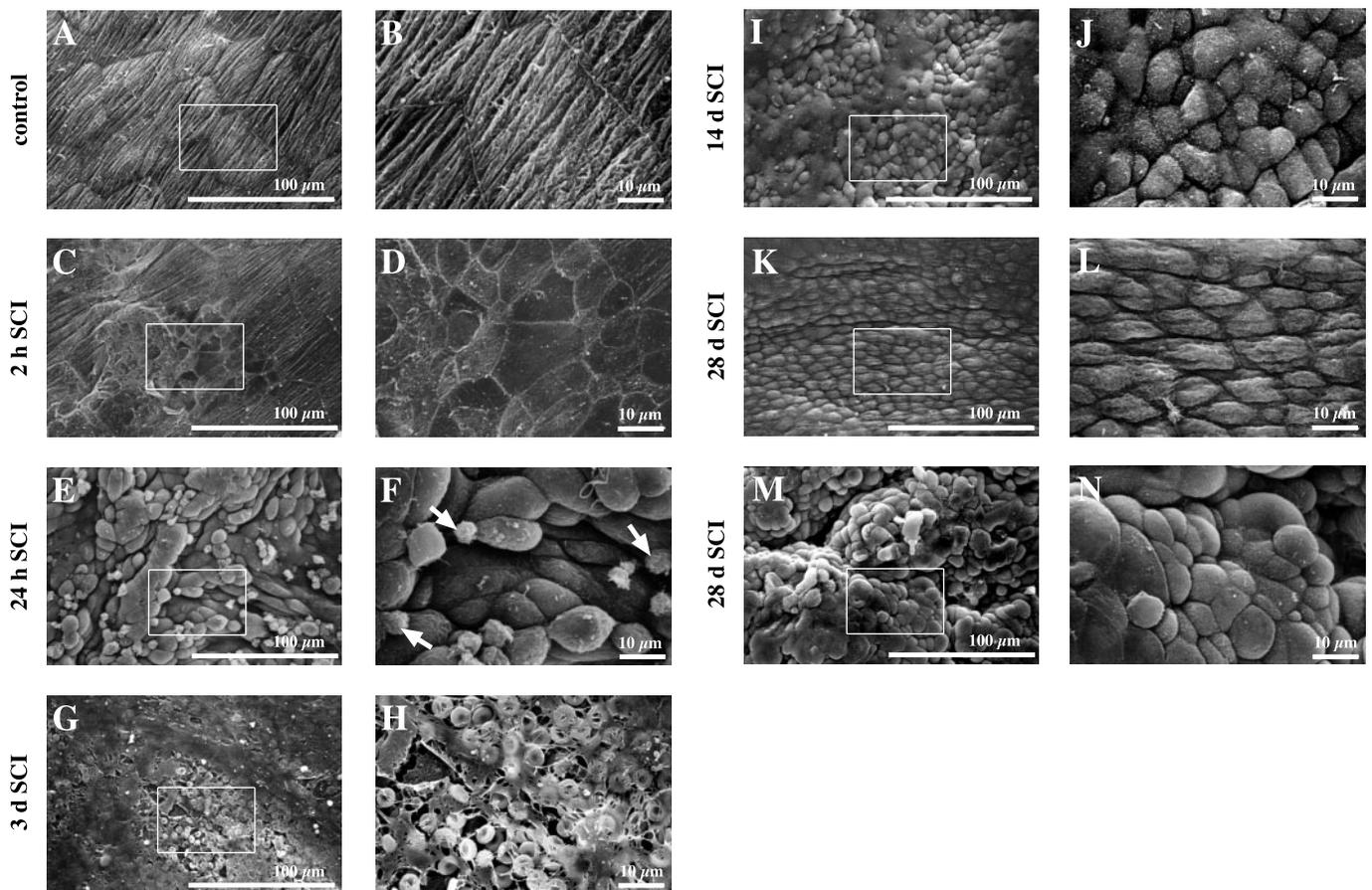


Fig. 2. Scanning electron microscopy (SEM) analysis of bladder uroepithelium from control and SCI animals. Bladders from sham-treated animals (A and B) or from animals 2 h (C and D), 24 h (E and F), 3 days (G and H), 14 days (I and J), or 28 days (K–N) after SCI were fixed, processed, and then analyzed by SEM. A, C, E, G, I, K, and M, lower-magnification views of representative areas; B, D, F, H, J, L, and N, higher-magnification views of boxed area in micrograph at left. Arrows in F, small shrunken cells associated with epithelium.

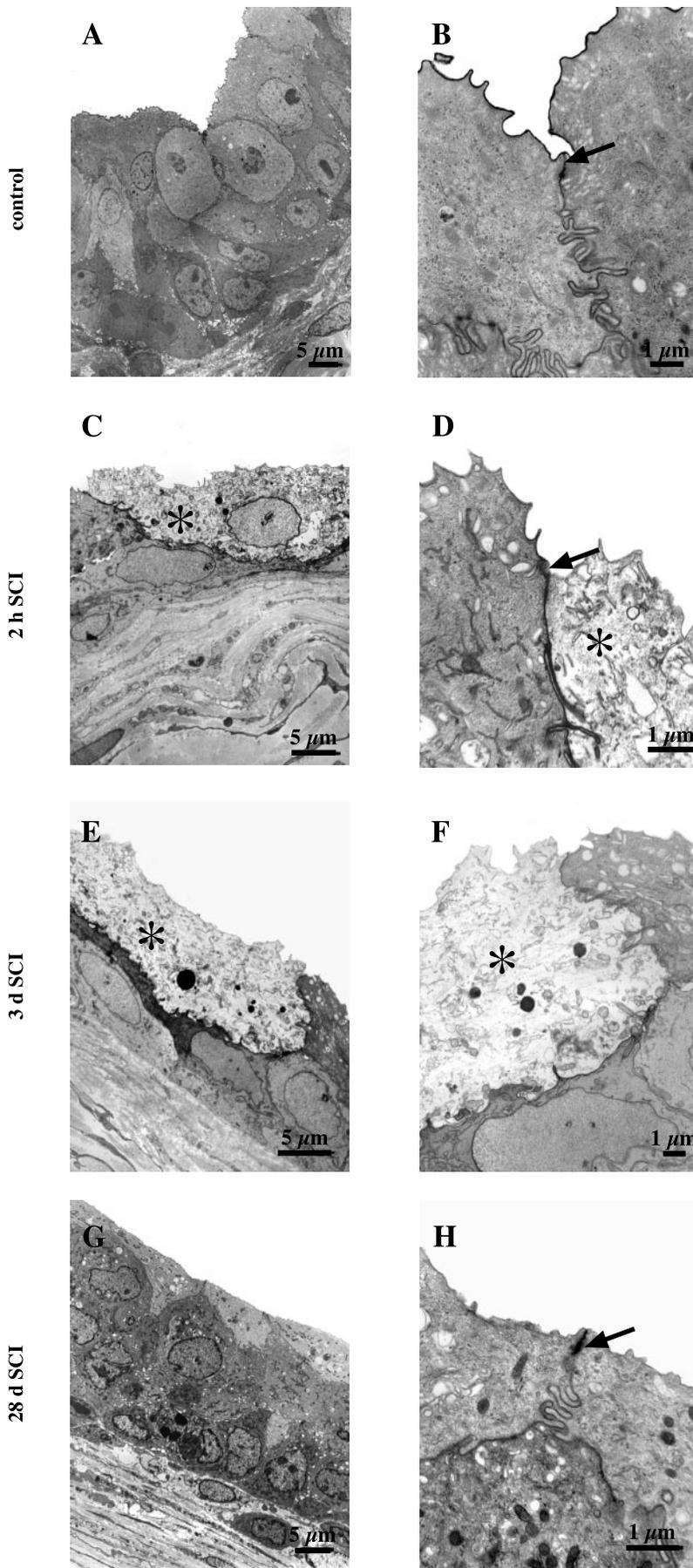


Fig. 3. Transmission electron microscopy (TEM) analysis of bladder uroepithelium from control and SCI animals. Bladders from sham-treated animals (A and B) or from animals 2 h (C and D), 3 days (E and F), or 28 days (G and H) after SCI were fixed, processed, and then analyzed by TEM. A, C, E, and G, lower-magnification views of representative areas; B, D, F, and H, higher-magnification views. Arrows, position of junctional complexes in B, D, and H. Distinct junctional complexes were not observed in F. *, Cells that have lost their cytoplasmic density.

uroepithelium. Instead of several cell layers, the uroepithelium in these areas often consisted of only one or two cell layers (Fig. 3C). In addition, the cells lining the surface of these areas sometimes appeared necrotic and lacked an electron-dense cytoplasm, apparently the result of plasma membrane disruption. Such a cell is shown in Fig. 3, C and D.

Changes were noted at other time points as well. At 24 h after SCI, the surface uroepithelium appeared significantly disorganized. Instead of the flat regular appearance of the umbrella cells in control animals, the cells lining much of the bladder of these SCI animals appeared small (10–20 μm long) and had a bumpy, cobblestone appearance (Fig. 2F). The surface of the cells was relatively smooth. Small shrunken-looking membranous structures (arrows, Fig. 2F) were also observed and appeared to be associated with surface lining cells and might represent necrotic uroepithelial cells. At 3–4 days after SCI, a frank hematuria was noted in many of the animals. Consistent with this observation, we observed areas of the uroepithelium that contained denuded or necrotic uroepithelium (Fig. 2H). Adjacent areas appeared less disrupted. When examined by TEM, these adjacent areas consisted of a few cell layers, and the surface cells often lacked cytoplasmic density, appeared necrotic, and had pycnotic nuclei (Fig. 3, E and F).

However, 2 wk after SCI, the uroepithelium appeared to regenerate. The epithelium was continuous, had a cobblestone appearance, and consisted of small surface cells (10–20 μm long) with a rough appearance (Fig. 2, I and J). At 28 days after SCI, the uroepithelium in many animals had significantly recovered. The epithelium was continuous and relatively flat, and surface folds were apparent (Fig. 2, K and L). Although similar to control epithelium, it was not identical, inasmuch as the cells in SCI animals appeared significantly smaller than in controls and were 10–20 μm long. When examined by TEM, the epithelium of the SCI animals consisted of several cell layers (Fig. 3G), and prominent junctional complexes were noted between adjacent cells (arrow in Fig. 3H). Some of the 28-day post-SCI animals developed large stones in their bladders. The nature of these stones is unknown, but they did not appear to involve bacterial infection, inasmuch as the urine was clear and there was no evidence of cystitis. The uroepithelium from these animals was morphologically altered and was reminiscent of 24-h samples. The cells were small, had a cobblestone appearance, and appeared fairly smooth (Fig. 2, M and N).

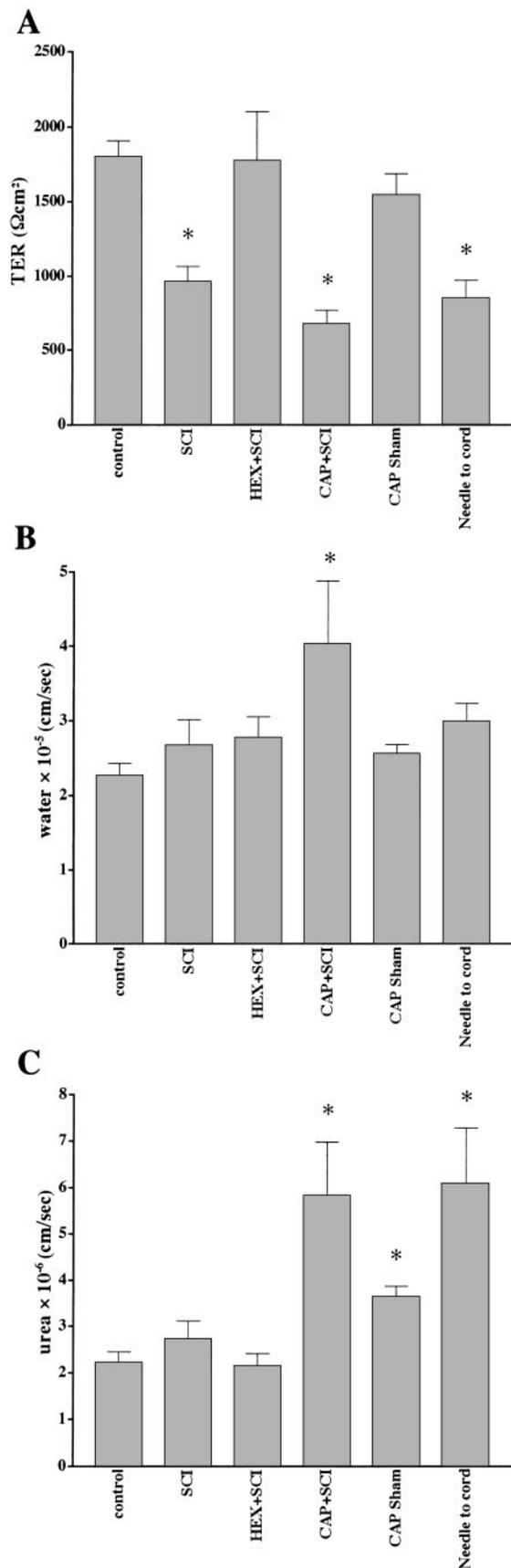
Effect of hexamethonium and capsaicin on acute (2 h) effects of SCI. We were surprised to find significant changes in TER and the morphology of the uroepithelium 2 h after SCI. To better understand the underlying cause of these acute changes, we assessed whether blockers of neurotransmission could prevent the acute SCI-dependent effects on TER as well as urea and water permeability. Pretreatment with hexamethonium, a blocker of ganglionic transmission, prevented the decrease in TER observed in 2-h post-SCI animals

(Fig. 4A). No significant effect on water or urea permeability was noted in these animals (Fig. 4, B and C). The protective effects of hexamethonium were not observed 24 h after SCI, and the values for TER and urea and water permeability were similar to those of untreated SCI animals (data not shown).

Pretreatment with capsaicin, which suppresses C-fiber input from the lower urinary tract (9, 11, 43), potentiated the effect of SCI. TER was lower in these animals than in untreated SCI animals (Fig. 4A), and there was a significant increase in water and urea permeability (Fig. 4, B and C). Vehicle alone had no effect on these treatments (data not shown), and capsaicin treatment in the absence of SCI had no significant effect on TER or water permeability but marginally increased urea permeability (Fig. 4).

To evaluate whether acute stimulation of neural pathways may lead to changes in uroepithelial barrier function, we inserted a needle beneath the spinal cord dura and injected PBS (in place of lidocaine) into the gray matter. The effect of this acute stimulation or perturbation on urothelial TER, water permeability, and urea permeability was assessed after 2 h. Acute insertion of a needle beneath the dura alone without spinal cord transection caused a significant decrease in TER (Fig. 4A) and a significant increase in urea permeability (Fig. 4C). However, this treatment had no effect on water permeability (Fig. 4B).

SEM analysis was used to assess the effects of these treatments on uroepithelial morphology. The umbrella cell layer of the hexamethonium-treated SCI animals lacked any obvious disruptions (cf. Fig. 5, A and B, with 2-h post-SCI tissue in Fig. 2, C and D). Continuous junctional rings were observed at the periphery of each cell, consistent with the TER results described above. When examined by TEM, the cells appeared normal (data not shown). One change was noted: the umbrella cells of these animals were smaller in diameter than those of untreated control animals, and their apical surface appeared to be highly wrinkled. The presence of plaques and hinge regions confirmed that these were umbrella cells. Bladders from hexamethonium- and sham-treated animals appeared normal (Fig. 5, C and D). The surface architecture of the umbrella cells of capsaicin-pretreated SCI animals appeared fairly intact. The umbrella cells were large and had a continuous tight junction band surrounding each cell, and no discontinuities in the umbrella cell layer were observed (Fig. 5, E and F). However, the surface of the cells appeared relatively smooth, with only a few surface folds. The umbrella cell architecture of sham-operated, capsaicin-pretreated animals appeared normal (Fig. 5, G and H). The surface of umbrella cells from animals in which a needle was inserted into the spinal cord appeared smooth and resembled the surface of umbrella cells 2 h after SCI (Fig. 2, C and D). There were no obvious discontinuities between the cells; however, the diameter of the umbrella cells was highly variable, and patches of large and small cells were noted.



DISCUSSION

SCI is accompanied by a number of well-described changes in normal bladder function: loss of voluntary control of micturition, bladder hyperactivity, bladder/sphincter dyssynergia, development of spinal cord reflexes (including unusual reflexes that are triggered by cold-water instillation or perineum stimulation), increased intravesical pressure, and bladder hypertrophy (21, 56). In experimental animal models, a hemorrhagic cystitis is often noted early after SCI, and in patients, SCI is well correlated with an increased incidence of bacterial cystitis and cancer (37, 39, 54). The effects of SCI on the uroepithelium, which forms the barrier between the urine and the underlying tissue, are not well understood.

Disruption of the uroepithelium in response to SCI. Many SCI patients exhibit mucosal ulceration and irritation (22). Using a well-described rat model system, we have observed that SCI is accompanied by considerable changes in uroepithelial morphology and barrier function. Within 2 h of SCI, we observed areas of disrupted uroepithelium that lacked surface umbrella cells, exposing the underlying intermediate cell layer. Consistent with this disruption, we noted that the TER of the uroepithelium was significantly decreased. Although this decrease could reflect increased ion transport, we believe that the morphology points to a disruption of tight junctions and cell-cell contact. In contrast, we noted only minor alterations in water and urea permeability. It could be that the underlying intermediate cells provide a reasonable barrier to water and urea at this early point after SCI. We observed what appeared to be the formation of tight junctions in the exposed intermediate cells, which is consistent with our recent observations that selective injury of the umbrella cell layer is accompanied by a rapid differentiation of the intermediate cell layer, including expression of uroplakin III and formation of tight junctions (23). However, it is possible that the tight junctions formed at these early stages are not as impermeable to ion flow as those found in differentiated umbrella cells.

A decrease in TER and a corresponding increase in water and urea permeability were observed 24 h after SCI. The uroepithelium at this point lacked umbrella cells in large areas and was generally covered by what appeared to be small intermediate and/or basal cells. By 3–4 days after SCI, the animals often had a hemorrhagic cystitis, which correlated with disruptions in the uroepithelium, significant decreases in TER, and

Fig. 4. Effect of hexamethonium and capsaicin on acute changes in TER (A), water permeability (B), and urea permeability (C) after SCI. Bladders were obtained from animals as follows: control, 2 h after SCI (SCI), pretreated with hexamethonium before SCI (HEX + SCI), pretreated with capsaicin before SCI (CAP + SCI), capsaicin pretreated and then sham operated (CAP Sham), or injected with PBS beneath the dura of the spinal cord (Needle to cord). Bladders were mounted in Ussing chambers, and TER, water permeability, and urea permeability were monitored. Data for control and SCI are from Fig. 1. Values are means \pm SE from 4–32 bladders. * $P < 0.05$ relative to control.

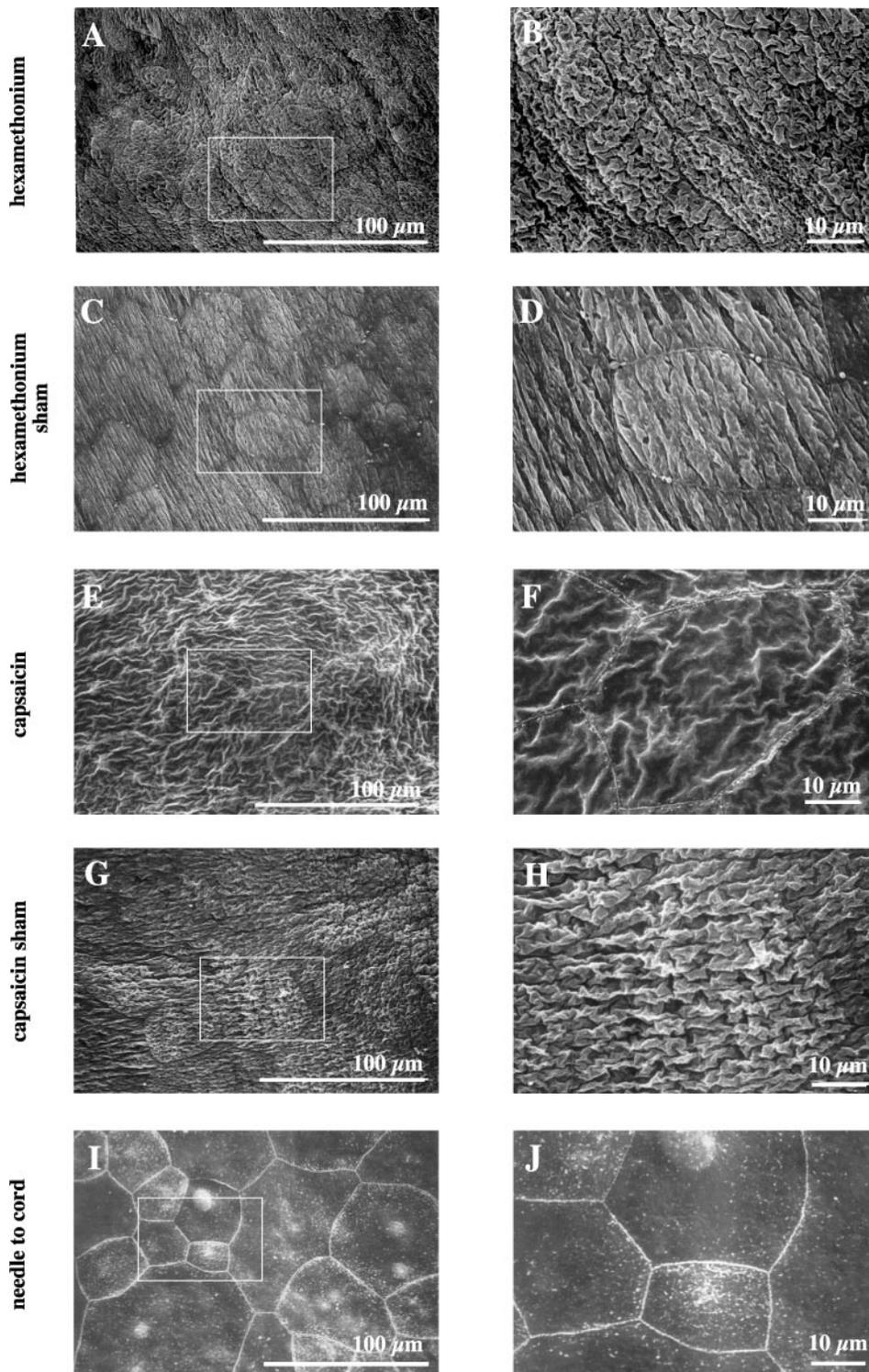


Fig. 5. SEM analysis of effects of hexamethonium, capsaicin, and nerve stimulation on acute changes in uroepithelial morphology after SCI. Bladders were obtained from animals as follows: pretreated with hexamethonium before SCI (*A* and *B*), hexamethonium pretreated and then sham operated (*C* and *D*), pretreated with capsaicin before SCI (*E* and *F*), capsaicin pretreated and then sham operated (*G* and *H*), and efferent nerve stimulated by needle placement into gray matter of spinal cord (*I* and *J*). *A*, *C*, *E*, *G*, and *I*, lower-magnification views of representative areas; *B*, *D*, *F*, *H*, and *J*, higher-magnification views of boxed area in micrograph at left.

an increase in water and urea permeability. The cause of this cystitis is unknown but is likely to reflect disruptions of the uroepithelium and vasculature and underlying inflammation provoked by infiltration of urine into the underlying tissue. Other possible factors are discussed below. By 4 wk after SCI, spinal reflexes were established, the animals could void involuntarily, and TER and water and urea permeability returned to

baseline. The bladder appeared to be hypertrophied during this time, and the uroepithelium appeared generally normal; however, the surface umbrella cells were significantly smaller. This may indicate increased turnover of surface lining cells.

Role of efferent nerves and other factors in epithelial injury. Several factors could contribute to the observed changes in the barrier function of the uroepithelium.

The ability of hexamethonium, a ganglionic blocking agent, to prevent the epithelial disruptions and the corresponding decrease in TER after acute SCI indicate that efferent pathways arising from the autonomic nervous system could be involved in the acute stages of this response. Stimulation of neural pathways by insertion of a needle beneath the dura of the spinal cord into the gray matter was also sufficient to change the surface morphology of the umbrella cells, decrease TER, and increase urea permeability. However, the effect was not identical, because disruption of cell-cell junctions was not observed, indicating that the lesion associated with neural stimulation was in the apical membrane or tight junction barrier. Although it is possible that a drop in blood pressure after spinal transection may contribute to the uroepithelial alterations, this is unlikely, inasmuch as hexamethonium also results in a drop in blood pressure, and treatment with hexamethonium before SCI reversed the acute effects of SCI. Hexamethonium might act by suppressing sympathetic or parasympathetic input to the urinary bladder.

Cutting the spinal cord could stimulate neural release of stress hormones, such as catecholamines, which would contribute to the uroepithelial alterations after acute SCI. Altered catecholamine levels in a number of tissues have been demonstrated to influence mucosal integrity (44, 50). Our data have revealed that intravesical application of norepinephrine can significantly reduce epithelial viability (shown by decrease in TER compared with vehicle controls, data not shown). In further support of an effect of catecholamines on epithelial function, other studies have shown that administration of stressors, such as norepinephrine, results in a desquamation of the bladder urothelium (52). Although the mechanism has not been identified, there is some suggestion that the effect on mucosal integrity may be due to alterations in the tight junction, which leads to desquamation of the epithelial cells (52). One possible trigger for these events may be through secretion of a chemical mediator from epithelial cells such as NO, which in excess concentrations has been demonstrated to alter mucosal integrity (29, 41, 42, 46). We previously showed that adrenergic agonists, including the α , β -agonist norepinephrine, can evoke NO release from urinary bladder uroepithelial cells (4, 6). Although further experiments are needed to establish a mechanism for these events, taken together, these data suggest a possible involvement of the sympathetic nervous system in uroepithelial damage after acute spinal injury.

In addition to the possible release of catecholamines from stimulated efferent nerves, other factors may contribute to the changes in uroepithelial barrier function. The submucosal tissue underlying the uroepithelium is populated by mast cells (12, 25). Intriguingly, mast cells in the gastrointestinal tract and in bladders are intimately associated with neuronal processes and can secrete their contents in response to neurotransmitters (20, 25, 35, 47). Thus SCI-dependent neurotransmitter release from efferent nerves could stimu-

late mast cell release of several mediators, including histamine, bradykinin, prostaglandin D₂, leukotriene C₄, and proteases, all of which could contribute to tissue damage and inflammation (20, 45). These and other inflammatory modulators released from other immune cells might be significant players in the loss of barrier function noted at 2 or 24 h after SCI and the cystitis we observed 3–4 days after SCI.

Although the breach in barrier function and cystitis observed 1–3 days after SCI is likely the downstream consequence of the initial disruption of the barrier, some of the damage could have also resulted from several factors, including overdistension of the bladder, a result of the loss of voluntary control of voiding. To guard against this, the bladder was manually compressed several times a day after the SCI, and when the bladders were excised from animals 1 or 3 days after SCI, they rarely appeared to be obviously overdistended. However, we cannot rule out that SCI induced some overdistension that may have potentiated or prolonged the barrier disruption. Although catheterization may have reduced some of these effects, we did not evaluate this procedure because of possible inflammatory changes or damage to the urothelium, which might result from placement of the catheter. Moreover, catheterization does not prevent cystitis in human patients (39). Finally, stress associated with SCI could cause increased catecholamine levels or delay healing as a result of the inability of SCI animals to obtain food and water. The latter was unlikely to be the case, inasmuch as SCI had no obvious effect on their consumption of food or water or their ability to defecate (unpublished observations).

Possible protective role of afferent pathways. Although activation of efferent pathways may have a detrimental effect on barrier function, afferent pathways may have a protective role. The neurotoxin capsaicin was used to evaluate the involvement of capsaicin-sensitive small-diameter afferents (A δ - and C fibers) in the changes in ultrastructure and permeability of the bladder mucosa after an acute SCI. These changes were not prevented by capsaicin pretreatment, which desensitizes bladder afferents (13, 31). Capsaicin pretreatment enhanced the susceptibility of the mucosa to injury by further decreasing the TER compared with untreated SCI animals or capsaicin-treated controls. These results are consistent with reports that suggest a contribution by capsaicin-sensitive nerves, which are sensitive to bladder distension and are located adjacent to the bladder mucosa, to mucosal protection in various tissues after injury or inflammation (1, 26). Studies suggest that this protective effect may be due to substance P/calcitonin gene-related peptide content in capsaicin-sensitive bladder nerves (38, 49). Although there was no significant difference in the surface architecture between these treated animals and treated controls, dilation of extracellular spaces or alterations in tight junctions leading to increased permeability may not be evident using the present morphological approaches. Thus further studies are

needed to examine in more detail the mechanism for these alterations in mucosal integrity.

Summary. Our results indicate that, in addition to affecting the detrusor muscle and its innervation, SCI also leads to a rapid disruption of the uroepithelial barrier. This was manifested in a loss of cell-cell interactions, decreases in TER, and increases in water and urea permeability. The present understanding is that SCI, which leads to loss of neural activity below the level of the lesion, blocks neurotransmitter release from transected nerves. However, our results indicate otherwise. The acute effects of SCI on uroepithelial function could be blocked by pretreatment with hexamethonium, indicating that release of neurotransmitters by bladder efferent nerves was at least partially responsible for the disruption of the uroepithelium. In addition, capsaicin treatment magnified the effects of SCI, indicating that capsaicin-sensitive afferent nerves may play a protective role in the process. Although the neurotransmitters and/or inflammatory mediators that disrupt barrier function are not well understood, our observations indicate that the nerves that innervate the bladder may play an important role in regulating the barrier function of the uroepithelium. By modulating the release of neurotransmitters and/or inflammatory mediators, it may be possible to stem the disruption of the uroepithelium that accompanies SCI and other inflammatory conditions, such as interstitial cystitis.

We thank W. C. de Groat for critical comments and suggestions.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants RO1-DK-54425 (to G. Apodaca), RO1-DK-54824 and RO1-DK-57284 (to L. A. Birder), and DK-48217 (to M. Zeidel).

REFERENCES

1. **Abdel-Salam OM, Debrecini A, Mozsik G, and Szolcsanyi J.** Capsaicin-sensitive afferent sensory nerves in modulating gastric mucosal defense against noxious agents. *J Physiol Paris* 93: 443–454, 1999.
2. **Andersson KE and Persson K.** Nitric oxide synthase and the lower urinary tract: possible implications for physiology and pathophysiology. *Scand J Urol Nephrol Suppl* 15: 43–53, 1995.
3. **Birder LA.** Involvement of the urinary bladder urothelium in signaling in the lower urinary tract. *Proc West Pharmacol Soc* 44: 85–86, 2001.
4. **Birder LA, Apodaca G, de Groat WC, and Kanai AJ.** Adrenergic and capsaicin-evoked nitric oxide release from urothelium and afferent nerves in urinary bladder. *Am J Physiol Renal Physiol* 275: F226–F229, 1998.
5. **Birder LA, Kanai AJ, de Groat WC, Kiss S, Nealen M, Burke N, Dineley K, Watkins S, Reynolds I, and Caterina MJ.** Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. *Proc Natl Acad Sci USA* 98: 13396–13401, 2001.
6. **Birder LA, Nealen M, Kiss S, de Groat WC, Caterina MJ, Wang E, Apodaca G, and Kanai AJ.** β -Adrenoceptor agonists stimulate endothelial nitric oxide synthase in rat urinary bladder urothelial cells. *J Neurosci* 22: 8063–8070, 2002.
7. **Chancellor MB and Blaivas JG.** Spinal cord injury. In: *Practical Neuro-Urology: Genitourinary Complications in Neurologic Disease*, edited by Chancellor MB and Blaivas JG. Stoneham, MA: Butterworth Heinemann, 1995, p. 99–118.
8. **Chancellor MB and Blaivas JG.** Spinal cord injury. In: *Atlas of Urodynamics*, edited by Blaivas JG and Chancellor MB. Philadelphia, PA: Williams & Wilkins, 1996, p. 160–173.
9. **Chancellor MB and de Groat WC.** Intravesical capsaicin and resiniferatoxin therapy: spicing up the ways to treat the overactive bladder. *J Urol* 162: 3–11, 1999.
10. **Chancellor MB, Rivas DA, Huang B, Kelly G, and Salzman SK.** Micturition patterns after spinal trauma as a measure of autonomic functional recovery. *J Urol* 151: 250–254, 1994.
11. **Cheng CL, Ma MP, and de Groat WC.** Effects of capsaicin on micturition and associated reflexes in rats. *Am J Physiol Regul Integr Comp Physiol* 265: R132–R138, 1993.
12. **Christmas TJ and Rode J.** Characteristics of mast cells in normal bladder, bacterial cystitis, and interstitial cystitis. *Br J Urol* 68: 473–478, 1991.
13. **Craft RM, Cohen SM, and Porreca F.** Long-lasting desensitization of bladder afferents following intravesical resiniferatoxin and capsaicin in the rat. *Pain* 61: 317–323, 1995.
14. **De Groat WC.** Anatomy of the central neural pathways controlling the lower urinary tract. *Eur Urol* 1: 1–5, 1994.
15. **De Groat WC.** A neurologic basis for the overactive bladder. *Urology* 50, Suppl 6A: 36–52, 1997.
16. **De Groat WC, Booth AM, and Yoshimura N.** Neurophysiology of micturition and its modification in animal models of human disease. In: *The Autonomic Nervous System: Nervous Control of the Urogenital System*, edited by Maggi CA. London, UK: Harwood, 1993, p. 227–290.
17. **De Groat WC and Yoshimura N.** Pharmacology of the lower urinary tract. *Annu Rev Pharmacol Toxicol* 41: 690–721, 2001.
18. **Ferguson DR, Kennedy I, and Burton TJ.** ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes: a possible sensory mechanism. *J Physiol* 505: 503–511, 1997.
19. **Gabella G and Davis C.** Distribution of afferent axons in the bladder of rats. *J Neurocytol* 27: 141–145, 1998.
20. **Galli SJ.** New concepts about the mast cell. *N Engl J Med* 328: 257–265, 1993.
21. **Geirsson G, Fall M, and Lindstrom S.** The ice water test, a simple and valuable supplement to routine cystometry. *Br J Urol* 71: 681–685, 1993.
22. **Janzen J, Bersch U, Pietsch-Breitfeld B, Pressler H, Michel D, and Bultmann B.** Urinary bladder biopsies in spinal cord-injured patients. *Spinal Cord* 39: 568–570, 2001.
23. **Lavelle J, Meyers S, Ramage R, Bastacky S, Doty D, Apodaca G, and Zeidel M.** Bladder permeability barrier: recovery from selective injury of surface epithelial cells. *Am J Physiol Renal Physiol* 283: F242–F253, 2002.
24. **Lavelle JP, Apodaca G, Meyers SA, Ruiz WG, and Zeidel ML.** Disruption of the guinea pig urinary bladder permeability barrier in noninfectious cystitis. *Am J Physiol Renal Physiol* 274: F205–F214, 1998.
25. **Letourneau R, Pang X, Sant GR, and Theoharides TC.** Intragranular activation of bladder mast cells and their association with nerve processes in interstitial cystitis. *Br J Urol* 77: 41–54, 1996.
26. **Leung FW.** Role of capsaicin-sensitive afferent nerves in mucosal injury and injury-induced hyperemia in rat colon. *Am J Physiol Gastrointest Liver Physiol* 262: G332–G337, 1992.
27. **Lewis SA and de Moura JLC.** Incorporation of cytoplasmic vesicles into apical membrane of mammalian urinary bladder epithelium. *Nature* 297: 685–689, 1982.
28. **Lewis SA and Diamond JM.** Na^+ transport by rabbit urinary bladder, a tight epithelium. *J Membr Biol* 28: 1–40, 1976.
29. **Lundberg JON, Hellstrom PM, Lundberg JM, and Alving K.** Greatly increased luminal nitric oxide in ulcerative colitis. *Lancet* 344: 1673–1674, 1994.
30. **Maggi CA, Giuliani S, Conte B, Furio M, Santicioli P, Meli P, Gragnani L, and Meli A.** Prostanoids modulate reflex micturition acting through capsaicin-sensitive afferents. *Eur J Pharmacol* 145: 105–112, 1988.
31. **Maggi CA, Patacchini R, Tramontana M, Amann R, Giuliani S, and Santicioli P.** Similarities and differences in the action of resiniferatoxin and capsaicin on central and peripheral endings of primary sensory neurons. *Neuroscience* 37: 531–539, 1990.

32. **Mimata H, Satoh F, Tanigawa T, Nomura Y, and Ogawa J.** Changes of rat urinary bladder during acute phase of spinal cord injury. *Urol Int* 51: 89–93, 1993.
33. **Morrison J.** The activation of bladder wall afferent nerves. *Exp Physiol* 84: 131–136, 1999.
34. **Negrete HO, Lavelle JP, Berg J, Lewis SA, and Zeidel ML.** Permeability properties of the intact mammalian bladder epithelium. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F886–F894, 1996.
35. **Newton B, Dahlstrom A, Enerback L, and Ahlman H.** Suggestive evidence for a direct innervation of mucosal mast cells. *Neuroscience* 10: 565–570, 1983.
36. **Pandita RK and Andersson KE.** Intravesical adenosine triphosphate stimulates the micturition reflex in awake, freely moving rats. *J Urol* 168: 1230–1234, 2002.
37. **Pannek J.** Transitional cell carcinoma in patients with spinal cord injury: a high-risk malignancy. *Urology* 59: 240–244, 2002.
38. **Reinshagen M, Flamig G, Ernst S, Geerling I, Wong H, Walsh JH, Eysselein VE, and Adler G.** Calcitonin gene-related peptide mediates the protective effect of sensory nerves in a model of colonic injury. *J Pharmacol Exp Ther* 286: 657–661, 1998.
39. **Ronald A.** The etiology of urinary tract infection: traditional and emerging pathogens. *Am J Med* 113, Suppl 1A: 14S–19S, 2002.
40. **Rong W, Spyer KM, and Burnstock G.** Activation and desensitization of low- and high-threshold afferent fibers mediated by P2X receptors in the mouse urinary bladder. *J Physiol* 541: 591–600, 2002.
41. **Saito M, Wada K, Kamisaki Y, and Miyagawa I.** Effect of ischemia-reperfusion on contractile function of rat urinary bladder: role of nitric oxide. *Life Sci* 62: PL149–PL156, 1998.
42. **Salzman AL.** Nitric oxide in the gut. *New Horiz* 3: 352–364, 1995.
43. **Santiciolo P, Maggi CA, and Meli A.** The effect of capsaicin pretreatment on cystometry of urethane-anesthetized rats. *J Urol* 133: 700–703, 1985.
44. **Sautner T, Wesseley C, Riegler M, Sedivy R, Gotzinger P, Losert U, Roth E, Jakesz K, and Fugger R.** Early effects of catecholamine therapy on mucosal integrity, intestinal blood flow and oxygen metabolism in porcine endotoxin shock. *Ann Surg* 228: 239–248, 1998.
45. **Serafin WE and Austen KF.** Mediators of immediate hypersensitivity reactions. *N Engl J Med* 317: 30–34, 1987.
46. **Southey A, Tanaka S, Murakami T, Miyoshi H, Ishizuka T, Sugiura M, Kawashima K, and Sugita T.** Pathophysiological role of nitric oxide in rat experimental colitis. *Int J Immunopharmacol* 19: 669–676, 1997.
47. **Stead RH, Dixon MF, Bramwell NH, Riddell RH, and Bienenstock J.** Mast cells are closely apposed to nerves in the human gastrointestinal mucosa. *Gastroenterology* 97: 575–585, 1989.
48. **Sun Y, Keay S, DeDeyne PG, and Chai TC.** Augmented stretch-activated adenosine triphosphate released from bladder uroepithelial cells in patients with interstitial cystitis. *J Urol* 166: 1951–1956, 2001.
49. **Szolcsanyi J and Bartho L.** Capsaicin-sensitive afferents and their role in protection: an update. *J Physiol Paris* 95: 181–188, 2001.
50. **Tepperman BL and Whittle BJ.** Comparison of the effect of NPY and noradrenaline on rat gastric mucosal blood flow and integrity. *Br J Pharmacol* 102: 95–100, 1991.
51. **Truschel ST, Wang E, Ruiz WG, Leung SM, Rojas R, Lavelle J, Zeidel M, Stoffer D, and Apodaca G.** Stretch-regulated exocytosis/endocytosis in bladder umbrella cells. *Mol Biol Cell* 13: 830–846, 2002.
52. **Veranic P and Jezernik K.** The response of junctional complexes to induced desquamation in mouse bladder urothelium. *Biol Cell* 92: 105–113, 2000.
53. **Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, Ford AP, and Burnstock G.** P2X₃ knockout mice reveal a major sensory role of urothelially released ATP. *J Neurosci* 21: 5670–5677, 2001.
54. **Wall BM, Dmochowski RR, Malecha M, Mangold T, Bobal MA, and Cooke CR.** Inducible nitric oxide synthase in bladder of spinal cord injury patients with a chronic indwelling catheter. *J Urol* 165: 1457–1461, 2002.
55. **Wein AJ.** Neuromuscular dysfunction of the lower urinary tract. In: *Campbell's Urology*, edited by Walsh PC, Retik AB, Stamey TA, and Vaughan JED. Philadelphia, PA: Saunders, 1992, p. 573–642.
56. **Yoshimura N.** Bladder afferent pathway and spinal cord injury: possible mechanisms inducing hyperreflexia of the urinary bladder. *Prog Neurobiol* 57: 583–606, 1999.