Proliferation and repair of guinea pig tracheal epithelium after neuropeptide depletion and injury in vivo

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Kim, John S., Valerie S. McKiniss, Kimberly Adams, and Steven R. White. Proliferation and repair of guinea pig tracheal epithelium after neuropeptide depletion and injury in vivo. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L1235–L1241, 1997.—Neuropeptides stimulate airway epithelial cell proliferation and migration in vitro, but the role of neuropeptides in the repair of the epithelium after injury in vivo is not clear. We studied epithelial proliferation and repair in 83 male Hartley guinea pigs. Animals received capsaicin weekly for 3 wk to deplete airway neuropeptides. One week later, the dorsal aspect of the trachea was injured with a metal stylette. Animals were killed 1 to 72 h later, after which epithelial cell proliferation was assessed for the presence of proliferating cell nuclear antigen (PCNA). PCNA labeling was <3% in noninjured animals. PCNA labeling increased substantially in the first 72 h after injury in control animals but was significantly decreased in capsaicin-treated animals within and adjacent to the site of injury. PCNA labeling increased opposite to the injury site in both control and capsaicin animals over the first 72 h. We conclude that neuropeptide depletion significantly attenuates both epithelial cell proliferation and repair in the first 72 h after mechanical injury to the trachea. However, neuropeptide depletion did not slow the ultimate repair of tracheal mucosal injury. Proliferation of epithelial cells in response to injury occurs throughout the airway, even away from the injury site.

Methods

Chronic capsaicin treatment to deplete neuropeptides from sensory nerves. Institutional approval for the use of guinea pigs in the study was given by The University of Chicago Animal Care and Use Committee. Capsaicin treatment was used to deplete sensory nerve endings of neuropeptides (7, 16, 19). There is >90% depletion of the neuropeptides with this technique as measured by physiological responses in guinea pig lungs (8, 16, 19) and by the depletion of tachykinins from guinea pig lungs (8, 16). Seventy-four male Hartley guinea pigs (weight 500–700 g, age 45–60 days old) were anesthetized with 1–2 ml from a solution of 6.5 ml of 100 mg/ml ketamine and 8.7 ml of 20 mg/ml xylazine (KX solution) intramuscularly, which was divided into each limb. Animals were pretreated with 10 mg/ml aminophylline intraperitoneally and 0.2 to 0.5 mg/ml terbutaline subcutaneously before each capsaicin treatment. Capsaicin was dissolved in 80% normal saline, 10% ethanol, and 10% Tween 80 at a concentration of 50 mg/ml. Animals received 50 mg/kg on day 1, 100 mg/kg on day 2, and 200 mg/kg on day 15 via subcutaneous injection. Control animals received the capsaicin solution without capsaicin. Animals received low-flow O2 and were monitored continuously for 4 h after each treatment. Cardio-pulmonary resuscitation was performed on all animals that experienced cardiac or pulmonary arrest. Mortality of capsaicin pretreatment was 20%.

THE AIRWAY EPITHELIUM is a target of inflammatory and physical stimuli in obstructive diseases such as asthma, cystic fibrosis, and bronchopulmonary dysplasia. In addition to being the first layer of physical defense, the epithelium regulates fluid and ion transport to the airway lumen, mucociliary clearance, and airway diameter. Injury to the epithelium is a common finding in pathological studies of patients with asthma, even when the clinical state of disease is judged “mild” (14, 15). Repair of a damaged epithelium is a necessary part of the restoration of the airway to its normal state after an exacerbation of asthma. Such repair generally involves several steps, including migration and spreading of epithelial cells at the margin of the injury into the damaged region and proliferation of new epithelial cells (9–11). Each step can be modulated actively by growth factors secreted by constitutive cells within the airway, such as fibroblasts (22), or depressed by mediators secreted by inflammatory cells (18, 27).

Neuropeptides are localized to sensory nerves and neuroepithelial bodies within the airway mucosa and are in a strategic location to participate in the repair of a damaged epithelium (1, 2). We have previously shown that calcitonin gene-related peptide (CGRP), the tachykinins neurokinin A and substance P, and the bombesin-related peptides gastrin-releasing peptide and neuropeptide B stimulate proliferation and/or migration of airway epithelial cells (12, 13, 20, 22). We have also demonstrated previously that the bombesin-related peptides, though they may be mitoattractants, do not enhance the initial 24 h of wound repair in a confluent layer of cultured epithelial cells. The actual physiological significance of the neuropeptides in airway epithelial repair thus is unknown.

We postulated that neuropeptides are involved in the repair of a damaged airway epithelium. We studied the role of neuropeptides in airway epithelial repair by depleting airway neuropeptides by chronic capsaicin treatment in guinea pigs. The tracheal mucosa then was injured with a metal stylette. Epithelial cell proliferation was assessed 1 h to 72 h later by immunohistochemical staining of tracheal sections for the presence of proliferating cell nuclear antigen (PCNA), a marker of proliferation past late G1 phase (4, 23). We demonstrate that neuropeptide depletion significantly attenuates both epithelial cell proliferation seen in the first 72 h after mechanical injury to the trachea and the repair of the injury site. However, neuropeptide depletion does not slow the ultimate repair of tracheal mucosal injury. Proliferation of epithelial cells in response to injury occurs throughout the airway, even away from the injury site.
Injury of guinea pig tracheal mucosa. Five to seven days after the last capsaicin treatment, guinea pigs were anesthetized with 0.5 ml im of KX solution. After ensuring proper anesthesia, animals were intubated with a pediatric otoscope (Welch-Allen), and the airways were visualized directly. A 1.5-mm-diameter stainless steel probe with a flattened scraper tip was introduced 2 cm into the trachea, and the scraper was oriented to the dorsal aspect (overlying the smooth muscle of the trachea). While the probe was torqued, it was pulled back along the tracheal rings to remove the epithelium. This was repeated a total of two to three times, the specimen then was removed, and the animal was allowed to recover. Animals were monitored for 4 h after the procedure and then daily for general health until they were killed. Animals received a standard diet and water ad libitum from time of recovery from anesthesia to death. Mortality due to the procedure was <10%.

Preparation of guinea pig tracheae. Animals were killed by intramuscular injection of 1 ml of KX solution followed by placement in a 100% CO₂ chamber for 6–10 min. The midcervical trachea was dissected free and was placed in 10% Formalin for <24 h. Tracheal segments were cut transversely into 1- to 2-mm segments, embedded in paraffin blocks, and cut in 6-µm sections. For each tissue section, injuries that were found over the dorsal aspect were evaluated. Injuries had to be seen on at least two sections on each slide. If no injury was found on the dorsal aspect of any section, the animal was disqualified. One section from each animal was stained with hematoxylin and eosin for evaluation of injury morphometry.

Measurement of epithelial injury. Microscope images of stained tracheal specimens were photographed using a Sony Iris charge-coupled device camera (Sony, Rolling Meadows, IL) on a Nikon light microscope. Video images were digitized using a Power Macintosh 6100 AV computer (Apple Computer, Cupertino, CA) and video monitor software (Apple Computer). Analysis of each image was performed using National Institutes of Health Image software (Wayne Rasband, National Institutes of Health, Bethesda, MD; available on the Internet at http://rsb.info.nih.gov/nih-image). Calibration at each magnification for all measurements was done using a hemacytometer as a standard. With this method, each of the distances could be measured to ±0.1 µm at ×40 magnification and to ±0.02 µm at ×200 magnification.

The margin of each injury was defined visually, and the length of the injury between the margins was measured along the basement membrane. For wounds in the acute and 24-h groups, the margins were sharp and easily distinguished. For wounds in the 48- and 72-h groups, the margins were less distinct due to infill of cells in an epithelium of lower height. In most animals in the 48- and 72-h groups, at least some completely denuded basement membrane remained within the injury site. The total area of the epithelial cells within the injury site was measured by tracing on the image, and the index of remaining/regenerating epithelium was defined as the area of the epithelial cells within the injury site (in mm²) per length of the injury (in mm).

The height of the adjacent normal epithelium on either side of the injury (in µm) and the opposite wall of the trachea was measured using the same images. This was done ~100–200 µm from the defined margin of the injury. Three measurements on either side were averaged and were coded as LE for left-side epithelium and RE for right-side epithelium. The average of LE and RE height was multiplied by the mucosal length to calculate the total area of the mucosal epithelial injury (in mm²). To determine if the plane of the tracheal cross section was perpendicular to the long axis of the trachea, the average of the LE and RE heights was compared with the height of the epithelium on the opposite tracheal wall. This difference was <20% for all tracheal sections examined. Because the cosine of 0.20 is 0.980, it was determined that no further correction was required to adjust for the plane of cross section.

Correlation of PCNA and BrdU staining in guinea pig tracheal epithelium. In all tracheal injury experiments, proliferation was assessed by the percentage of cells labeling with PCNA, which is present in proliferating cells from the G1 phase through the M phases (4, 23, 24). In selected experiments, the correlation between PCNA labeling and uptake of BrdU, a thymidine analog that is incorporated during S phase (5, 26), was determined. Five animals were sham instrumented. Twenty-four hours later, animals received 25 mg/kg BrdU in saline by intraperitoneal injection. Animals were killed after an additional 24 h (48 h after sham instrumentation). Serial tracheal sections were stained for PCNA and BrdU labeling. On each slide, 200 cells in the posterior membrane and 200 cells in the opposite wall were counted from the same tracheal ring for both PCNA and BrdU labeling. Materials. Capsaicin, NaCl, and Tween 80 were obtained from Sigma Chemical (St. Louis, MO). Xylazine was obtained from Butler (Columbus, OH), and ketamine was obtained from Mallinckrodt Veterinary (Mundelein, IL). Ethanol and 10% Formalin were obtained from Fisher Scientific (Pittsburgh, PA). Terbutaline was obtained from Ciba-Geigy (Basel, Switzerland). Aminophylline was obtained from Abbott Laboratories (North Chicago, IL). Mouse anti-PCNA and anti-BrdU antibodies were obtained from Dako (Carpenteria, CA).

Data analysis. Cell counts are expressed as means ± SE. Comparisons between multiple groups were made by repeated-measures analysis of variance; when significant differences were found, further comparisons were made either by Fisher’s least significant difference test or by a nonpaired t-test. Comparisons between two groups were made by Student’s t-test. Bonferroni’s correction was made as appropriate for multiple comparisons. Differences were considered significant when P was <0.05.

RESULTS

A total of 83 animals were used in this study. Of these, 10 died during capsaicin treatment. Tracheae
from six of these animals were collected and analyzed for PCNA labeling. The remaining four were not used. Four animals died after tracheal injury but before they were to be killed according to the protocol, and these were excluded from analysis. The remaining animals were included in the data analysis.

Airway morphology after injury. Tracheal injuries could be found in each animal killed 1 h after injury in the control (n = 7) and capsaicin-treated (n = 6) groups. Representative photomicrographs of the acute tracheal mucosal injury are shown in Fig. 1. Immediately after injury, the wound margins were sharp, and little injury to the submucosa was seen on light microscopy. In most sections, little epithelium remained, but in a few, small numbers of basal epithelial cells could occasionally be found, demonstrating that epithelial removal was not absolute. The area of the remaining epithelium within the injury site was 9 ± 4% of predicted area in control animals and 12 ± 3% of predicted area in capsaicin-treated animals.

After 48–72 h, mucosal injuries in control animals demonstrated substantial repair, with ingrowth of epithelial cells from either margin and layering of flattened epithelial cells. The area of the regenerating epithelium in the repairing section was substantially greater than that seen at 1 h after injury. In some sections, a central area devoid of epithelium or a discernable, sharp wound edge remained at 48 h, but in most animals at 72 h after injury, the entire mucosal injury site was covered with flattened epithelium. The area of the regenerating epithelium within the margins of the mucosal injury increased quickly over 72 h in both groups (Fig. 2). Although epithelial regeneration in capsaicin-treated animals lagged behind that of control animals in the first 48 h, both groups were similar at 72 h (Fig. 2). By 1 wk after injury, little evidence of damage could be found at the injury site (Fig. 1).

PCNA staining in uninjured epithelium of sham- and capsaicin-treated animals. Capsaicin treatment itself did not stimulate epithelial cell proliferation. In two animals receiving a single dose of capsaicin, PCNA labeling was 0.2 and 3.1%. In two animals receiving two doses of capsaicin, PCNA labeling in epithelial cells was 1.9 and 2.5%. In two animals receiving three doses of capsaicin, PCNA labeling in epithelial cells was 1.4 and 0.0%. These values were not different from PCNA labeling in the opposite airway wall of control animals 1 h after injury (2.3 ± 0.3% of all cells; n = 7).

PCNA labeling within the wound site after mechanical tracheal injury. In animals that did not receive capsaicin, there was evidence of epithelial cell proliferation that began 24 h after injury. PCNA labeling was substantial and could be demonstrated clearly (Fig. 3). PCNA labeling of epithelial cells within the mucosal injury site in control animals at 1 h after injury was 0.9 ± 0.6% (n = 7). PCNA labeling increased over time.
to a maximum at 48 h after injury (31.6 ± 9.4%; n = 7; Fig. 4). Even at 1 wk after injury, there was still evidence of epithelial cell proliferation, although no injury site could be seen: PCNA labeling in the mucosal layer at the posterior membrane was 7.7 ± 2.2% (n = 2).

In contrast, PCNA labeling within the injury site in capsaicin-treated animals was substantially less than in control animals. PCNA labeling was 0.3 ± 0.3% 1 h after injury (n = 6) and 6.5 ± 2.0% 48 h after injury (n = 8, P = 0.01 vs. control animals; Fig. 4). Differences in PCNA labeling between the control and capsaicin-treated groups also were significant 24 h (P = 0.04) and 72 h (P = 0.05) after injury. At 1 wk after injury in capsaicin-treated animals, there was evidence of epithelial cell proliferation, but no injury site was present. PCNA labeling in the mucosal layer at the posterior membrane was 7.3 ± 1.5% (n = 3; P = not significant vs. control animals).

PCNA labeling adjacent to the wound site after mechanical tracheal injury. As with labeling within the injury site, PCNA labeling in the 200 cells on either side adjacent to the injury site was increased in control animals after tracheal injury. PCNA labeling was 2.3 ± 0.5% 1 h after injury (n = 7) and 26.6 ± 7.5% 48 h after injury (n = 7; Fig. 5).

Fig. 2. Area of remaining/regenerating epithelium in tracheal sections after mucosal injury in control and capsaicin-treated guinea pigs. Area of regenerating epithelium is normalized to the length of the mucosal injury measured at the basement membrane. Epithelial removal is near complete at 1 h. Epithelial regeneration is substantial in both groups over 72 h, and control animals have somewhat more regeneration in the first 48 h. *P < 0.05 vs. 1 h time-point in same group. At day 2, P = 0.07 for regenerating area in control vs. capsaicin-treated animals.

Fig. 3. Epithelial cell proliferation after injury in guinea pig trachea: representative photomicrographs of sections in control and capsaicin-treated animals. Tissue sections were stained for proliferating cell nuclear antigen (PCNA; brown nuclei) by immunohistochemistry and were counterstained with hematoxylin (purple nuclei) as described in METHODS. A: tracheal section collected 48 h after injury in a control animal that demonstrates healing epithelium. Large percentage of epithelial cells have an appreciable concentration of PCNA. In this example, original wound edge is seen clearly. Original magnification, ×400. B: tracheal section collected 48 h after injury in a capsaicin-treated animal that demonstrated less PCNA staining. Most nuclei are purple, and few brown nuclei are seen. Original magnification, ×400.

Fig. 4. PCNA staining within the injury site for control and capsaicin-treated animals. For each group there is increased PCNA staining within the injury site, with substantially more staining in control animals. In control animals, n = 7 for all time points except n = 6 for 72 h. In capsaicin-treated animals, n = 6 for 1 h, n = 7 for 24 h, and n = 8 for 48 and 72 h.
In capsaicin-treated animals, PCNA labeling was increased after injury but was less pronounced than in control animals, as with labeling within the injury site. PCNA labeling adjacent to the injury site for capsaicin-treated animals was 1.1 ± 0.6% 1 h after injury (n = 6) and 8.0 ± 3.0% 48 h after injury (n = 8, P = 0.05 vs. control animals; Fig. 4). Differences in PCNA labeling adjacent to the wound site at other time points was less in capsaicin-treated group than in control animals, although these differences were not statistically significant (Fig. 5).

PCNA staining opposite to the wound site after mechanical tracheal injury. PCNA labeling opposite the injury site for control animals and capsaicin-treated animals increased over time to a peak at 72 h (Fig. 6). PCNA labeling was increased at 1 wk after injury in the opposite wall of the trachea. There was no significant difference between the two groups of animals at any time point (Fig. 6).

Correlation of PCNA and BrdU staining. These experiments demonstrated that PCNA labeling correlated well to a second index of proliferation, incorporation of BrdU. In five animals that were sham instrumented and had no tracheal injury, PCNA and BrdU staining were similar in both the posterior membrane and in the opposite wall. PCNA labeling was 1.2 ± 0.6% in the posterior membrane epithelial layer and 0.9 ± 0.6% in the opposite wall epithelium. BrdU labeling was 0.3 ± 0.2% in the posterior membrane epithelial layer (P = 0.18 vs. PCNA labeling) and 0.2 ± 0.1% in the opposite wall epithelium (P = 0.25 vs. PCNA labeling). In two additional animals in which epithelial cell proliferation was assessed 48 h after injury, PCNA and BrdU labeling again were similar. Within the injury site, PCNA staining was 13.5 ± 0.4% and BrdU labeling was 10.8 ± 0.9% of all cells. In the adjacent site, PCNA labeling was 9.9 ± 0.9% and BrdU labeling was 6.5 ± 0.5% of all cells. In the tracheal mucosa opposite to the wound site, PCNA labeling was 7.3 ± 2.8% and BrdU labeling was 7.0 ± 2.0%.

DISCUSSION

The objective of this study was to determine if neuropeptides are involved in the proliferation phase of the repair of a damaged airway epithelium in vivo. We depleted airway neuropeptides from guinea pigs by pretreatment with capsaicin. Guinea pig airways have substantial sensory innervation both in distal lung (17) and in the trachea (6, 17), and capsaicin pretreatment is a well-established method for neuropeptide depletion in this species (8, 16, 19). The trachea of the animal was injured by mechanical abrasion with a metal stylette, and the animals were killed at various time points within the first week after injury. Repair of the epithelium was followed by simple morphological measurements and PCNA labeling within, adjacent to, and opposite the site of injury. Our data demonstrate that prior neuropeptide depletion significantly attenuates epithelial cell proliferation seen in the first 72 h after mechanical injury to the trachea and attenuates somewhat the repair of the injury site. However, whereas neuropeptide depletion modestly slowed the ultimate repair of the tracheal mucosal injury as measured by the regeneration of the epithelium within the mucosal injury, by the third day epithelial regeneration was near equal in both control and capsaicin-treated animals (Fig. 2). Proliferation of epithelial cells in response to injury occurred throughout the airway section, even away from the site of injury (Figs. 4–6).

Fig. 5. PCNA staining adjacent to injury site for control and capsaicin-treated animals. There is increased PCNA staining in the epithelium immediately adjacent to injury site in both groups of animals. There is significantly greater PCNA staining in control group at 48 h compared with capsaicin-treated group. In control animals, n = 7 for all time points except n = 6 for 72 h. In capsaicin-treated animals, n = 6 for 1 h, n = 7 for 24 h, and n = 8 for 48 and 72 h.

Fig. 6. PCNA staining opposite to the injury site for control and capsaicin-treated animals. There is increased PCNA staining in the opposite airway wall after injury in both groups of animals. There is no significant difference between control and capsaicin-treated animals at any time point. In control animals, n = 7 for all time points except n = 6 for 72 h. In capsaicin-treated animals, n = 6 for 1 h, n = 7 for 24 h, and n = 8 for 48 and 72 h.
It is important to note that epithelial cell proliferation was attenuated but was not abolished by prior neuropeptide depletion. PCNA labeling within and adjacent to the injured epithelium was increased in capsaicin-treated animals, even though the magnitude of the increase was substantially less than for control animals (Figs. 4-6). Epithelial cell proliferation occurring in capsaicin-treated animals was uniform throughout the epithelial mucosa in a given tracheal ring, as evidenced by the relative uniform increase in PCNA labeling in these animals in the regions within, adjacent to, and opposite the injury site. This suggests that a different mechanism not dependent on neuropeptide release had a more regional effect on epithelial cell proliferation. In contrast, cell proliferation in control animals was highest closest to the injury site, although proliferation was increased throughout the tracheal ring. This suggests that neuropeptide-stimulated proliferation was most active closest to the injury and was additive to whatever neuropeptide-independent regional signal was present. The mechanisms by which neuropeptide-independent regional signals stimulate epithelial cell proliferation were not delineated in the present study.

In both groups of animals, cell proliferation was highest in the first 24-48 h after injury at and adjacent to the injury site. This is similar to data seen in studies of hamsters (9-11) and rats (21). Cell proliferation in guinea pig tracheal epithelium began to decrease at 72 h and was substantially lower 1 wk after injury, which is also consistent with models generated in other animals.

Much of what is known about epithelium repair comes from studies in which the epithelium is injured and repair followed histologically over time. Early events in repair of a damaged epithelium and mucosal layer include cell spreading, migration, and proliferation (9-11). Adjacent stem cells initiate proliferation and migrate into the denuded area. These cells then flatten and spread over the site of injury. A multilayered sheet forms over the wound, and then differentiation into a single layer with specific cell types begins. A similar pattern of repair was seen in tracheal sections after injury in the present study (Fig. 1). Cell culture models of airway epithelial wound repair suggest that in small wounds created by mechanical injury, repair involves migration but not necessarily proliferation of cells (12, 28). In contrast, in larger wounds, both migration and proliferation are required (28). We did not specifically assess migration in this study. The size of the injuries created, however, would predict that cell proliferation is required, and this was seen in our experiments.

Past studies have shown a possible role for the neuropeptides in airway epithelial repair but have involved in vitro studies using cultured epithelial cells. We have demonstrated previously that CGRP and the tachykinin neurokinin A stimulated both proliferation and migration of guinea pig tracheal epithelial cells in culture (13, 20, 25). The physiological significance of these findings had not been established. The present study demonstrates that these sensory nerve-derived peptides also stimulate epithelial cell proliferation in vivo, and that prior depletion of these peptides impairs proliferation.

There are limitations to the present study. One major limitation is the inconsistency of the degree of injury. Due to the technical aspect of intubating a small airway and using a blind approach for wound creation, an injury of consistent width within the airway circumference could not be created reliably. Thus more sophisticated morphometric measurements could not be generated. It is reasonable to assume that larger mucosal wounds would close less quickly than smaller ones, and this in turn may influence the degree of proliferation within and adjacent to the injury site. However, differences in PCNA labeling between control and capsaicin-treated animals were large, and this difference is unlikely to be explained by differences in injury length.

A second limitation in this study is the inability to assess the contributions of each of the individual neuropeptides secreted by sensory nerves and neuroendocrine cells on airway epithelial repair. Chronic capsaicin treatment has been used to deplete sensory nerve endings of neuropeptides (7, 19). There is >90% depletion of the neuropeptides with this technique as measured by physiological responses in guinea pig lungs (19). The goal of this study was to assess whether neuropeptides in general had a role in airway epithelial repair, and this was answered effectively by the depletion of all neuropeptides. The potential role of individual neuropeptides, alone or in combination, was beyond the scope of the present study because the dosing and administration of receptor antagonists, particularly in combination and for a period of up to 1 wk, has not been well established in guinea pigs. Our results will require further testing to determine which specific neuropeptides are responsible for epithelial cell proliferation after injury.

An additional limitation relates to the phases of repair normally seen in a regenerating airway epithelium. As noted by Keenan et al. (9-11), proliferation is but one phase of repair. Cell migration into the injury site is required early in repair, and differentiation of new epithelial cells into required subtypes (e.g., ciliated columnar cells, secretory cells) is required in the later phase of repair. Our study examined proliferation only. It is possible that neuropeptide depletion had some effect on either migration or differentiation in vivo. Indeed, epithelial regeneration was slower in capsaicin-treated animals compared with control animals in the first 48 h after injury, although this difference was abolished by 72 h (Fig. 3). Given that the airway morphology in capsaicin-treated animals was similar to that seen in control animals 1 wk after injury, it is certainly possible that the process of differentiation and late repair does not require neuropeptides. Such studies remain to be done.

A final limitation concerns whether capsaicin treatment alters the baseline phenotype and physiology of the tracheal epithelium. In our experiments, there was no evidence at the level of light microscopy that the
epithelial cells themselves were altered by capsaicin treatment. Baseline PCNA labeling was similar in both control and capsaicin-treated animals 1 h after mucosal injury at all sites examined, suggesting that capsaicin pretreatment did not alter baseline (immediate postinjury) proliferation.

In summary, we conclude that neuropeptide depletion significantly attenuates epithelial cell proliferation seen in the first 72 h after mechanical injury to the trachea and attenuates the repair of the injury site. However, neuropeptide depletion may not slow the ultimate repair of tracheal mucosal injury. Proliferation of epithelial cells in response to injury occurs throughout the airway, even away from the site of injury.

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