0022-5347/01/1651-0235/0
THE JOURNAL OF UROLOGY®
Vol. 165, 235–239, January 2001
Printed in U.S.A.

ROLE OF AFFERENT NEURONS IN STRESS INDUCED DEGENERATIVE CHANGES OF THE BLADDER

FERIHA ERCAN, SULE OKTAY AND NURAY ERIN

From the Departments of Histology-Embryology, and Pharmacology and Clinical Pharmacology, Marmara University, Medical Faculty, Istanbul, Turkey

ABSTRACT

Purpose: We investigated the role of afferent C fibers in morphological changes of the rat bladder during stress.

Materials and Methods: Wistar albino rats were exposed to cold immobilization stress. Different routes of capsaicin administration before cold immobilization stress were studied. Capsaicin was given to neonates, around the vagus (perivagal) or celiac (periceliac), or perivagal plus periceliac. From each group samples of bladder were randomly chosen for morphological evaluation using electron microscopy.

Results: Stress exposure led to pathological changes, including an increased number of mast cells, degenerated urothelium and dilated tight junctions, in the bladder. Capsaicin given neonatally and around the vagal and celiac ganglia prevented these stress induced degenerative bladder changes.

Conclusions: Activation of capsaicin sensitive afferent neurons locally and centrally may be involved in stress related pathological changes in the rat bladder.

KEY WORDS: capsaicin, stress, bladder

The importance of stress related disturbances in normal homeostasis and on the pathogenesis of various diseases is well established.[1] Release of corticotropin-releasing hormone (CRH) from the paraventricular nuclei of the hypothalamus and other nuclei of the medulla, as well as stimulation of catecholaminergic neurons of the locus caeruleus are principal mediators of the behavioral, neuroendocrine and autonomic response to stress. Reciprocal neuronal connections exist between CRH and noradrenergic neurons which have cross regulated functions. Peripheral components of the stress response include the hypothalamic-pituitary-adrenal axis, efferent sympathetic/adrenomedullary system[2] and afferent neuronal systems. Activation of afferent fibers seems important in stress induced aggravation of some diseases, such as interstitial cystitis. Exposure to various stress conditions induces mast cell proliferation and activation with urothelial damage in the bladder.[3, 4] During stress neuropeptides, such as substance P, are released from primary afferent fibers and activate mast cells in the bladder. Specifically, neonatal denervation of afferent fibers with capsaicin reduces stress induced activation of mast cells in the bladder.[4] Recently, we demonstrated that cold restraint stress, which produces gastric mucosal lesions that mimic stress ulcers in patients, also leads to epithelial damage and mast cell proliferation in the rat bladder.[3] Furthermore, using this model we recently found that after capsaicin treatment caused systemic or local denervation of the afferent fibers in neonatal rats or vagus nerve and celiac ganglia of adult animals, gastric lesion formation due to cold restraint stress was prevented completely.[5]

In the present study we examined the effects of systemic or local capsaicin treatment on microscopic changes in the bladder induced by cold restraint stress. Although afferent fibers of vagus and celiac ganglia are not thought to innervate the bladder, vagal efferent fibers, which can be regulated with afferent input from viscera, integrating in the celiac ganglia can influence bladder function.[6] In agreement with neuronal interconnections, we now report that denervation of vagal and celiac afferent fibers with application of capsaicin perineurally and systemically prevents degenerative effects of cold restraint stress on the bladder.

Accepted for publication July 20, 2000.

MATERIALS AND METHODS

Newborn and adult 200 to 270 gm. Wistar albino rats were used in this study. A dose of 10 mg./ml. capsaicin was dissolved in a vehicle containing 10% ethanol, 10% Tween-80 and 80% physiological saline.

Experimental groups. Rats were divided into starvation and stress groups. The starvation group included 5 animals not exposed to stress or treated with capsaicin but were fasted for 48 hours and used as controls to determine the effect of stress exposure. These animals had free access to water. The stress group included neonatal—50 mg./kg. capsaicin in 8 rats or vehicle in 8, perivagal—1 mg. capsaicin in 8 or vehicle in 6, periceliac—1 mg. capsaicin in 9 or vehicle in 7 and perivagal plus periceliac—1 mg. capsaicin in 5 or vehicle in 5.

In the neonatal group capsaicin was administered intraperitoneally to pups day 2 after birth. Animals were exposed to stress at around 3 months of age. A drop of capsaicin solution was instilled into 1 eye of each rat and the wiping movements were counted to verify afferent denervation as reported previously.[7] If wiping movements were less than 5 per minute, animals were scored as denervated. Rats treated with perivagal capsaicin were pretreated with 1 mg./kg. atropine sulfate intraperitoneally and then anesthetized with ether. Through a midline neck incision the cervical vagi were exposed and freed from the carotid artery for a distance of 2 to 3 mm. Parafilm was placed around the nerve, and a cotton wool soaked in capsaicin or vehicle was placed around each vagus nerve for 30 minutes.

For periceliac capsaicin application a midline laparotomy was performed. The stomach and spleen were deflected to the right of the animal. Through a small incision in the surrounding connective tissue the celiac-superior mesenteric ganglion complex was exposed and a cotton wool soaked in capsaicin or vehicle was applied for 30 minutes. Animals were studied at

10 to 12 days after perineural capsaicin or vehicle treatment. In all stress groups animals were fasted for 48 hours but allowed free access to water. Following fasting rats were put into restraint cages for 3 hours at 6C in a refrigerator where a thermometer was kept to control temperature. The stress test was performed in the morning. Animals were sacrificed under ether anesthesia, and specimens were prepared for morphological observation.

Morphological preparation. Specimens were fixed in 4% phosphate buffered glutaraldehyde (0.13 M., pH 7.4) for 4 hours and post-fixed with 1% osmium tetroxide. Specimens were dehydrated in graded alcohol series and embedded in Epon 812. Sections were cut with an ultramicrotome, and semithin sections were stained with 1% toluidine blue and examined under a photomicroscope. Thin sections were stained with uranyl acetate and lead citrate, and examined under a transmission electron microscope. Changes in mast cell numbers were determined by double-blind counting of mast cells of 5 to 7 random slides from each group at 400× magnification.

To determine cellular damage as lesion index 4 criteria were used, including presence (1) or absence (0) of urothelial damage, loose tight junctions, formation of vacuoles and increase in lysosomes in 5 to 7 slices taken randomly. Each criterion was assessed with a rating of 1 or 0, giving a maximum total rating of 4 for each slide. For example, if only loose tight junctions and increase in vacuole formation were evident the tissue was scored as 2. Data were analyzed using 1-way analysis of variance followed by Dunnett's multiple comparisons test to determine the differences between capsaicin treated groups. Differences between capsaicin and vehicle treated groups, for example perivagal capsaicin and perivagal vehicle, were analyzed using unpaired Student's t test. Data were expressed as mean plus or minus standard error.

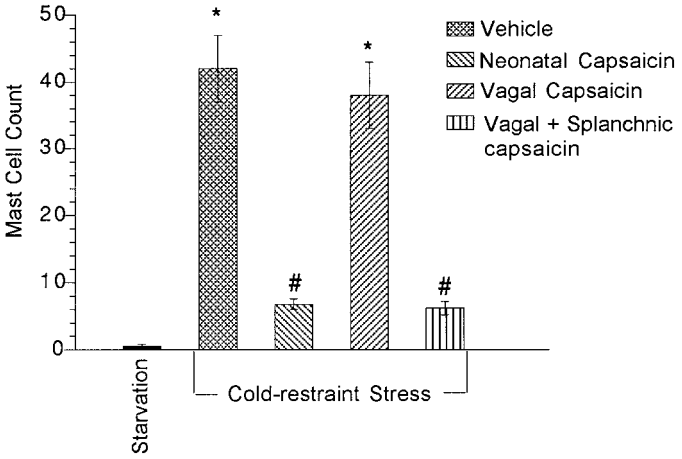

FIG. 2. Changes in mast cell count. Effects of stress and capsaicin treatment on mast cell count was determined by counting mast cells in lamina propia and urothelium of 5 to 7 random slices from each group. Significant increase in mean mast cell number was detected following ischemia. Asterisk denotes significant difference from starvation group at $p < 0.01$. Neonatal and vagal plus celiac capsaicin treatment prevented stress induced increase in mast cell number. Pound sign indicates significant difference from vehicle group at $p < 0.05$.

RESULTS

There were no pathological changes (lesion index 0) in bladders of rats starved for 48 hours. Mast cells were not activated and mean mast cell number was 0.4 ± 0.25 (figs. 1 and 2).

Vehicle treated stress groups. In all vehicle treated groups (intragastric, perivagal, periceliac or perivagal plus periceliac) mast cell numbers in the mucosa and between urothelial cells were significantly increased. Mean mast cell numbers were not significantly different among the vehicle treated groups. Thus, mean mast cell number was calculated from the cumulative results of all vehicle groups and was 42.2 ± 4.98 ($p < 0.01$ compared to the 48-hour starvation group) (figs. 3, *A* and 2). Disrupted urothelium, increased vacuoles in the cytoplasm, loose tight junctions and electron dense materials in the intercellular area were observed (lesion index 4) (fig. 3, *B*). Most mast cells in the mucosa and urothelium were degranulated.

Neonatal capsaicin treated stress group. Capsaicin treatment of neonates completely prevented the increase in mast cell number (mean 6.16 ± 0.9, fig. 2) and degenerative changes. In this group regular urothelium and tight junctions were observed (lesion index 0) (fig. 4).

Periceliac capsaicin treated stress group. Disrupted urothelium and loose tight junctions were prominent. Severe losses of urothelium were observed in most samples. Since most mast cells were in the urothelial area in other groups, we were not able to determine mast cells which had migrated into the urothelium and compare mean mast cell number in this group. Thus, it is possible that mast cells may fall apart with the urothelium as a result of severe urothelial degeneration. Mean mast cell number in the mucosa was 1.7 ± 0.8 but most mast cells were activated (lesion index 4) (fig. 5).

Perivagal capsaicin treated stress group. Similar to the vehicle treated group, increased mast cells (mean 38.4 ± 5.3, fig. 2) were also seen following treatment with capsaicin perivagally (fig. 6, *A*). However, most mast cells were not

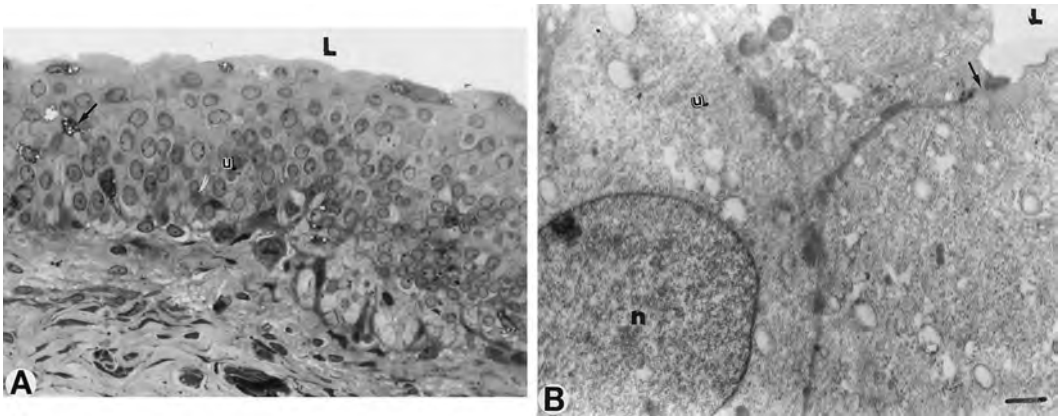

FIG. 1. Effects of starvation on bladder morphology. Regular urothelium (u) and lamina propria with normal tight junctions (arrow). *A*, toluidine blue, reduced from ×132. *B*, transmission electron micrograph. Bar equals 2 μm. L, lumen. n, nucleus.

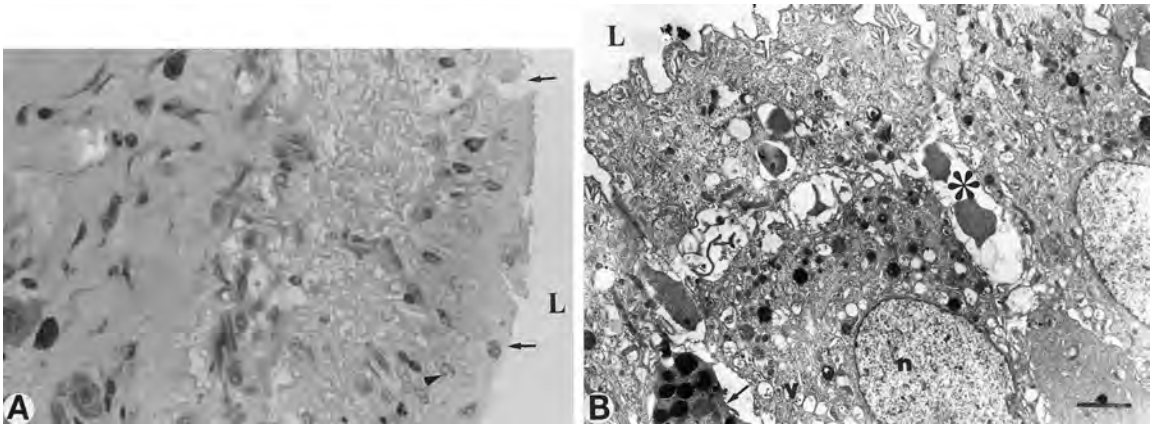

FIG. 3. Effects of stress on sham operated, vehicle treated group. *A*, loss of urothelial cells in some areas (arrow) and increased number of mast cells (arrowhead) in lamina propria and urothelium. Toluidine blue, reduced from ×132. *B*, transmission electron micrograph of bladder. Dilatation in interdigitations (asterisk), accumulation of electron dense material in area, vacuoles in cytoplasm (v) and increased number of mast cells between urothelial cells. Bar equals 2 μm. L, lumen. n, nucleus.

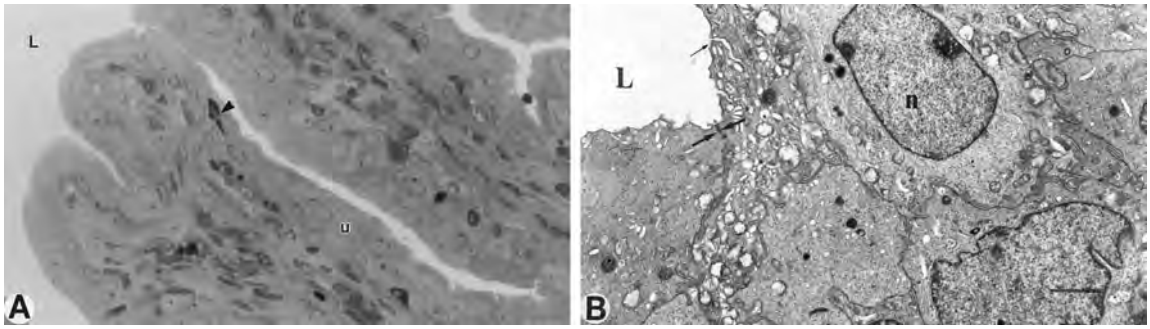

FIG. 4. Effects of stress on neonatal capsaicin treated group. *A*, regular urothelial cells (*u*) with decreased numbers of migrated mast cells (arrowhead). Toluidine blue, reduced from ×132. *B*, regular urothelial luminal surface surrounded by asymmetric unit membrane (large arrow) and tight junctions (small arrow) on transmission electron microscopy. Bar equals 2 μm. L, lumen. n, nucleus.

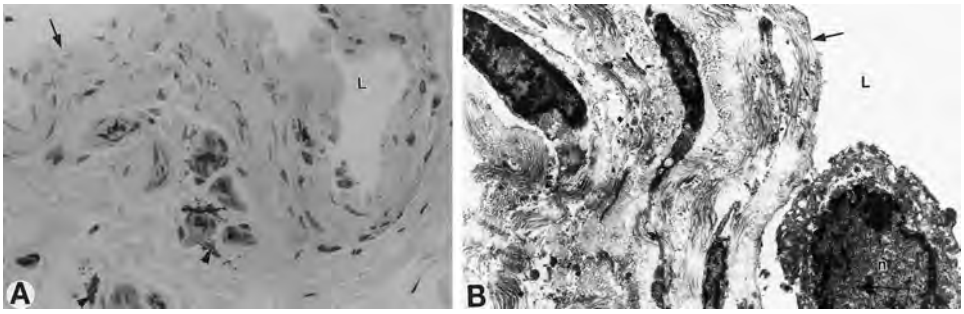

FIG. 5. Effects of stress on periceliac capsaicin treated group. Massive loss of urothelial cells (arrow) and degranulated mast cells (arrowhead) in lamina propria. *A*, toluidine blue, reduced from ×132. *B*, transmission electron micrograph of bladder. Bar equals 2 μm. L, lumen. n, nucleus.

degranulated. Some urothelial cells showed loose connections at the apical surface but in general tight junctions showed regular morphology. Increases in vacuole formation and lysosomes were not detected (lesion index 1) (fig. 6, *B*).

Perivagal plus periceliac capsaicin treated stress group. Results from this group were similar to the neonatal capsaicin treated group. Mast cells in the mucosa and between urothelial cells were decreased (mean 6.16 ± 1.4, figs. 2 and 7, *A*), while regular urothelium and tight junctions were observed (lesion index 0) (fig. 7, *B*).

DISCUSSION

Sensory innervation of the bladder by afferent fibers containing neurokinins A, B and substance P is important in normal physiological functions such as control of voiding.[8] Similarly, these afferent fibers may be involved in diseases such as interstitial cystitis.[9] It has been reported that denervation of these afferent fibers with neonatal capsaicin treatment prevents mast cell activation resulting from 30 minutes of immobilization stress.[4] In our study exposure to a more vigorous stress (cold immobilization) led to urothelial damage and increased numbers of mast cells, which were prevented by capsaicin treatment neonatally and perineurally (perivagal and/or periceliac).

We hypothesize that the protective effect of neonatal capsaicin treatment was due to peripheral and central depletion of substance P and subsequent inhibition of mast cell activity. Administration of capsaicin to newborn animals causes irreversible degeneration and subsequent loss of primary sensory neurons.[10,11] Specifically, neonatal capsaicin de-

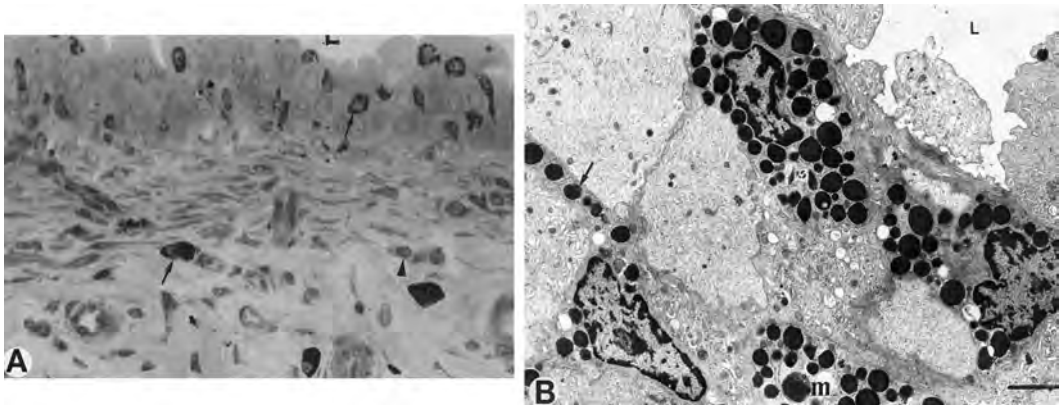

FIG. 6. Effects of stress on perivagal capsaicin treated group. *A*, increased numbers of migrated mast cells (arrow) into urothelial cells, mast cells and polymorph leucocyte (arrowhead) in lamina propria. Toluidine blue, reduced from ×132. *B*, mast cell granules (arrow) between urothelial cells on transmission electron micrography. Bar equals 2 μm. L, lumen. m, mast cell.

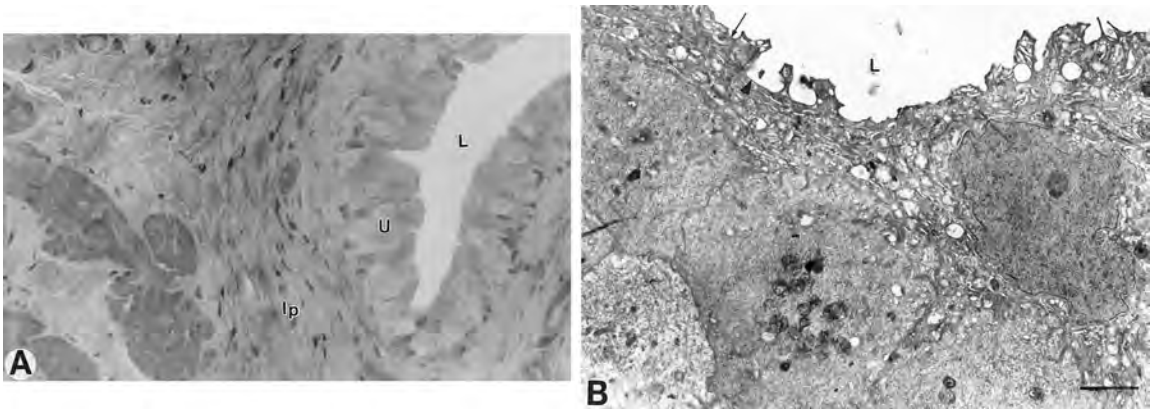

FIG. 7. Effects of stress on perivagal and periceliac capsaicin treated group. Regular urothelium (u), lamina propria (lp) and asymmetric unit membrane covered luminal surface (arrow). *A*, toluidine blue, reduced from ×132. *B*, transmission electron micrograph of bladder. Bar equals 2 μm. Arrowhead denotes fusiform vesicles. L, lumen.

pletes afferent fibers that contain substance P in the skin, mucosa, stellate ganglia, hepatic duct, myocardium, lungs, trachea and bladder.[12] Substance P was decreased by 84% in the bladder.[13] Release of substance P from afferent fibers causes vasodilatation and plasma extravasation. Exposure to various stressful conditions, including cold restraint stress, induces mast cell proliferation and activation.[3,4]

Substance P activates and causes release of mast cell mediators, including histamine and proteases, which can enhance vasodilatation and tissue destruction,[12] which are pathological features of interstitial cystitis. Increased levels of substance P are found in interstitial cystitis, and stress may be a triggering factor. In patients with interstitial cystitis mast cell numbers are also increased adjacent to substance P containing fibers,[9] and the mast cells are more sensitive to IgE and substance P. Therefore, it is believed that in interstitial cystitis some of the symptoms and microscopic findings are due to the release of mast cell mediators. Cytokines and tryptase released from mast cells can cause direct tissue damage and pain.[14] We recently demonstrated that peripheral injection of a substance P receptor antagonist CP 99994 prevented degenerative changes in the bladder induced by stress.[15]

In the present study exposure to stress led to tissue destruction and an increased number of mast cells. Neonatal denervation of substance P containing fibers systemically prevented mast cell migration to the submucosa and urothelium, and tissue destruction. Therefore, substance P could be responsible for proliferation or migration of mast cells and urothelial damage during stress. On the other hand, denervation of the afferent fibers of the vagus prevented tissue destruction without affecting the increase in mast cells. Prevention of urothelial damage in this group is likely due to inhibition of mast cell activation. In the perivagal capsaicin treated group the number of degranulated mast cells was less than that of the vehicle treated group. This result also suggests that inhibition of the release of mediators in vagal afferents, such as substance P, is sufficient to prevent urothelial destruction induced by stress. In contrast, urothelial loss was more severe in animals denervated of celiac afferent fibers than in the vehicle group. Urothelial loss was so severe that it was not possible to determine migrated mast cells in the random slices. When we specifically looked for areas where the urothelium was intact, we did not detect an increase in the number of mast cells. However, it is possible that in the damaged parts mast cells might be broken apart with urothelium. Hence, it is difficult to determine whether activation of mast cells but not necessarily an increase in their number is sufficient for tissue damage. Interestingly, denervation of vagal and celiac afferent fibers at the same time prevented the increase in mast cell number and urothelial damage, suggesting a balancing interaction between vagus and celiac afferent fibers during stress. Furthermore, these results suggest that stress can be an etiological factor in interstitial cystitis when there are increases in mast cell number and activity.

Protective effects of treatment with capsaicin neonatally may also be due to inhibition of the central response to afferent input carried by visceral neurons, including vagal and celiac fibers, and inhibition of afferent response to cen-

tral input. Neonatal capsaicin caused a 90% reduction of unmyelinated fibers and decreased levels of substance P, cholecystokinin, vasoactive intestinal peptide and somatostatin in the dorsal horn of the spinal cord. This treatment also depleted substance P in the trigeminal nucleus, nucleus tractus solitarius and substantia gelatinosa.[12] Similarly, perineural application of capsaicin depleted substance P in the peripheral and central termini of sensory neurons.[16]

The nucleus tractus solitarius is a potentially important central nervous system site in the protective effect of afferent denervation during stress. It is the first order receiving area for visceral afferents and receives prominent projections from the paraventricular nucleus of the hypothalamus and locus caeruleus. These brain regions also project to the bladder[17,18] and participate in controlling autonomic functions. Substance P containing afferent fibers that innervate the nucleus tractus solitarius make synaptic contact with catecholaminergic neurons,[19] integrating information from vagal and splanchnic afferents.[20] It is also known that the bladder receives descending input from the paraventricular nucleus, dorsolateral tegmental nucleus and locus caeruleus, which are brain regions important in stress response[17] that have a direct connection with the nucleus tractus solitarius. Thus, denervation of afferent fibers in the nucleus tractus solitarius and other central areas by neonatal or perineural capsaicin could influence bladder function and explain why systemic or perivagal and periceliac capsaicin treatment protects the bladder from stress induced damage. Furthermore, our results demonstrate that activation of vagal afferents is more important than that of celiac afferents in the development of bladder damage following exposure to stress.

Peripheral mechanisms may also be involved in the protective effects of perivagal and periceliac treatment with capsaicin. The bladder is innervated by pelvic and hypogastric nerves which contain various proportions of parasympathetic, sympathetic and sensory nerve fibers.[21] It has been reported that postganglionic axons in hypogastric nerves also originate from the superior mesenteric and celiac ganglia,[22] which are innervated by vagal efferent fibers.[6] Thus, capsaicin treatment of vagus and celiac ganglia may directly influence bladder function.

CONCLUSIONS

Our study demonstrates that activation of capsaicin sensitive afferent fibers, not only of the bladder but also of other viscera has an important role in the pathogenesis of stress related bladder damage. Since denervation of vagal afferent fibers was protective, activation of vagal afferent fibers during stress may be more important than activation of splanchnic fibers in the development of stress induced bladder damage. Thus, the protective effect of systemic (neonatal) and perineural (vagus plus celiac) capsaicin treatment may be due to peripheral and central inhibition of afferent activity. Interaction among stress, substance P containing afferent fibers and mast cells may contribute to the etiology of some bladder diseases.

REFERENCES

1. Chrousos, G. P. and Gold, P. W.: The concepts of stress system disorders: overview of behavioral and physical homeostasis. JAMA, **267:** 1244, 1992
2. Stratakis, C. A. and Chrouses, G. P.: Neuroendocrinology and pathophysiology of the stress system. Ann NY Acad Sci, **771:** 1, 1995
3. Ercan, F., San, T., Aydin, H. et al: The evaluation of mast cells on the rat urinary bladder under stress conditions. Marmara Med J, **10:** 185, 1996
4. Spanos, C., Pang, X., Ligris, K. et al: Stress-induced bladder mast cell activation: implications for interstitial cystitis. J Urol, **157:** 669, 1997
5. Erin, N., Ercan, F., Yegen, B. C. et al: The role of capsaicin sensitive nerves in gastric and hepatic injury induced by cold-restraint stress. Dig Dis Sci, in press, 2000
6. Berthoud, H. R. and Powley, T. L.: Characterization of vagal innervation to the rat celiac, suprarenal and mesenteric ganglia. J Auton Nerv Syst, **42:** 153, 1993
7. Gamse, R.: Capsaicin and nociception in the rat and mouse. Naunyn Schmiedeberg's Arch Pharmacol, **320:** 205, 1982
8. de Groat, W. C.: Neuropeptides in pelvic afferent pathways. Experientia, **43:** 801, 1987
9. Pang, X., Marchand, J., Sant, G. R. et al: Increased number of substance P positive nerve fibers in interstitial cystitis. Br J Urol, **75:** 744, 1995
10. Jancso, G., Kiraly, E. and Jansco-Gabor, A.: Pharmacologically-induced selective degeneration of chemosensitive primary sensory neurons. Nature, **270:** 741, 1977
11. Nagy, J. I., Hunt, S. P., Iversen, L. L. et al: Biochemical and anatomical observations on the degeneration of peptide-containing primary afferent neurons after neonatal capsaicin. Neuroscience, **6:** 1923, 1981
12. Buck, S. H. and Burks, T. F.: The neuropharmacology of capsaicin: review of some recent observations. Pharmacol Rev, **38:** 179, 1986
13. Holzer, P., Bucsics, A. and Lembeck, F.: Distribution of capsaicin-sensitive nerve fibers containing immunoreactive substance P in cutaneous and visceral tissues of the rat. Neurosci Lett, **31:** 253, 1982
14. Theoharides, T., Pang, X., Letourneau, R. et al: Interstitial cystitis: a neuroimmunoendocrine disorder. Ann NY Acad Sci, **840:** 619, 1998
15. Ercan, F., Hürdag, C., Akici, A. et al: The effect of substance P NK1 receptor antagonist CP 99994 on stress-induced microscopic damage in urinary bladder. Presented at the 11th European Anatomical Congress, Timisoara, Romania, September 10–13, 1998
16. Gamse, R., Petsche, U. and Jancso, G.: Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. Brain Res, **239:** 447, 1982
17. Nadelhaft, I., Vera, P. L., Miselis, R. R. et al: Central nervous system neurons labelled following the injection of pseudorabies virus into the rat urinary bladder. Neurosci Let, **143:** 271, 1992
18. Grijalva, C. W. and Novin, D.: The role of the hypothalamus and dorsavagal complex in gastrointestinal function and pathophysiology. Ann NY Acad Sci, **597:** 207, 1990
19. Kubota, Y., Takagi, H., Morishima, Y. et al: Synaptic interaction between catecholaminergic neurons and substance P-immunoreactive axons in the caudal part of the nucleus of the solitary tract of the rat: demonstrated by the electron microscopic mirror technique. Brain Res, **333:** 188, 1985
20. Barber, W. D. and Yuan, C.: Gastric vagal-splanchnic interactions in the brainstem of the cat. Brain Res, **487:** 1, 1989
21. Alm, P. and Elmer, M.: Adrenergic and cholinergic innervation of the rat urinary bladder. Acta Physiol Scand, **94:** 36, 1975
22. Messenger, J. P., Furness, J. B. and Trudrung, P.: Location of post ganglionic nerve cells whose axons enter nerves originating from prevertebral ganglia. Arch Histol Cytol, **57:** 405, 1994