

Capsaicin-mediated Denervation of Sensory Neurons Promotes Mammary Tumor Metastasis to Lung and Heart

NURAY ERIN^{1,2}, PHILIP J. BOYER^{2,4}, ROBERT H. BONNEAU³,
GARY A. CLAWSON^{1,2} and DANNY R. WELCH^{1,2,5,6}

¹*Jake Gittlen Cancer Research Institute,*

²*Department of Pathology and*

³*Department of Microbiology and Immunology,*

The Pennsylvania State University College of Medicine, Hershey, PA, U.S.A.

⁴*Current Address: Department of Pathology, University of Texas Southwestern, Dallas, TX;*

⁵*Current Address: Department of Pathology and Comprehensive Cancer Center,
University of Alabama at Birmingham, Birmingham, AL, U.S.A.*

Abstract. Capsaicin specifically activates or destroys small diameter nociceptive sensory neurons that contain the capsaicin receptor, also called vanilloid receptor 1. Neurons sensitive to capsaicin mediate inflammatory pain and are important targets for management of chronic pain. These neurons also regulate local tissue homeostasis, inflammation, healing and development, especially under conditions of psychological stress. Stress contributes to increased cancer recurrence and metastasis through as yet undefined mechanisms. Likewise, activity of capsaicin-sensitive neurons is altered by pathological conditions that may lead to metastatic growth (e.g. stress). Therefore, we examined effects of a treatment that induces sensory nerve denervation on breast cancer metastases. Systemic denervation of sensory neurons caused by treatment with 125 mg/kg capsaicin resulted in significantly more lung and cardiac metastases in adult mice injected orthotopically with syngeneic 4T1 mammary carcinoma cells than was observed in vehicle-treated controls. Heart metastases, normally very rare, occurred as pericardial nodules, intra-myocardial nodules, or combined pericardial-myocardial lesions. Since the rate of

primary tumor growth was unaffected, effects on metastases appear to be host tissue-specific. Although preliminary, these observations provide one possible explanation for resistance of cardiac tissue to tumor involvement and highlight contributions of host tissue, including sensory neurons, in the efficiency of cancer metastasis.

Cancer survival decreases in patients experiencing various psychosocial stresses (1), suggesting a potential role for the central nervous system in progression of malignancy. Specifically, stress-induced changes in neuroendocrine and immune functions may contribute to cancer mortality (2). Stress not only alters the neuroendocrine system, but also modifies sensory nerve function. Small diameter, capsaicin-sensitive sensory nerve fibers (CSFs) can especially contribute to stress-induced inflammatory disorders, such as aseptic cystitis (3). In addition to their sensory function, CSFs also act locally by releasing vasoactive peptides, such as Substance P (SP) and calcitonin-gene-related peptide (CGRP). These peptides, in turn, regulate blood flow and can modulate the cellular immune system (4, 5). The role(s) of CSFs in cancer metastasis, however, has not been studied extensively, although patients with sensory neuropathies have been reported to have an increased incidence of various types of malignancies (6).

The present study was designed to test the hypothesis that CSFs influence the capacity of cancer cells to metastasize. A neurotoxic dose of capsaicin was used to inactivate CSF. Capsaicin, the pungent ingredient of hot peppers, selectively activates or destroys CSFs, depending upon the dose, time and route of application (7). The specificity of capsaicin is provided by the selective expression of the capsaicin receptor, vanilloid receptor 1 (VR1) on CSFs (8). Excitatory

Abbreviations: CGRP, calcitonin gene-regulated peptide; CSF, capsaicin-sensitive sensory nerve fibre; SP, substance P; VR1, vanilloid receptor 1.

Correspondence to: Danny R. Welch, Department of Pathology and Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, U.S.A. e-mail: DWELCH@path.uab.edu

Key Words: Capsaicin, denervation, sensory neurons, mammary tumor, metastasis.

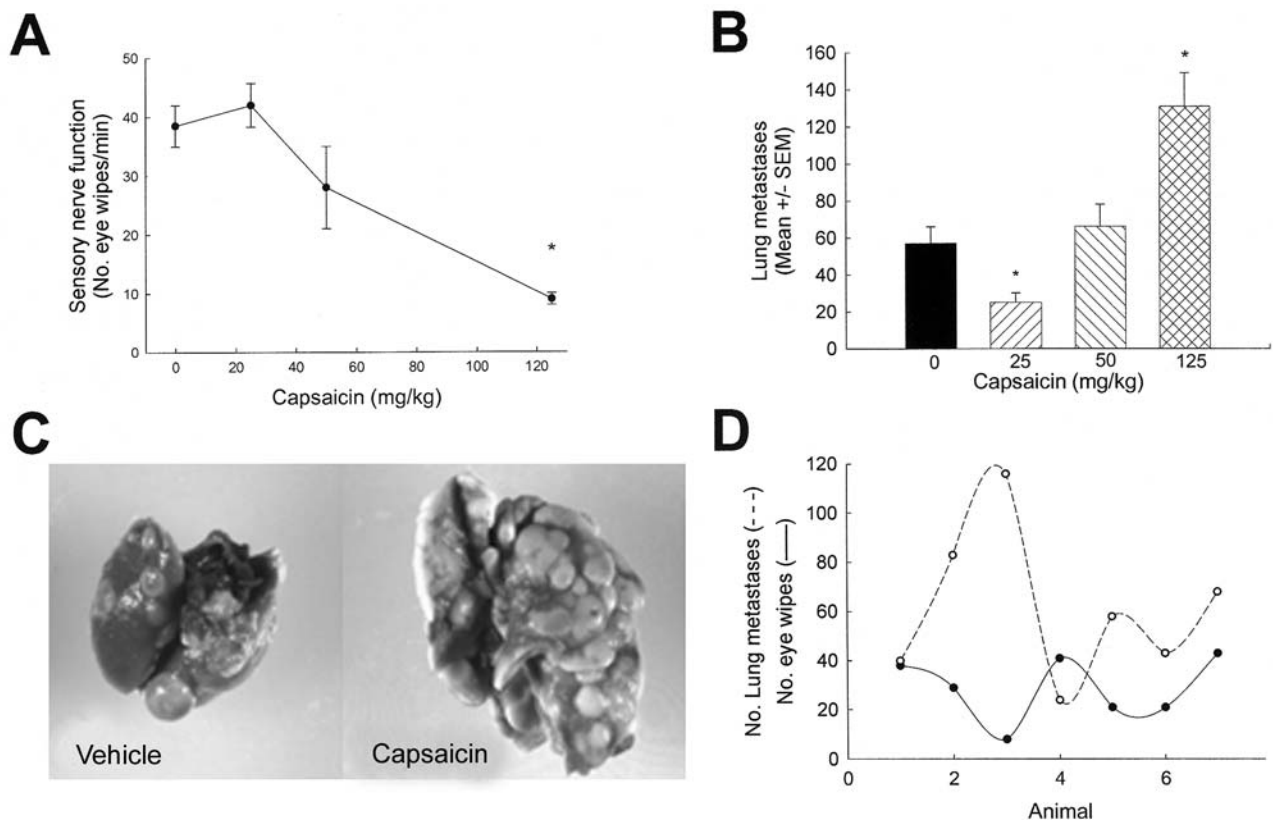


Figure 1. Capsaicin treatment causes sensory nerve denervation in adult (4-6 wk) BALB/c female mice. Panel A shows dose-dependent inactivation of sensory neurons as assessed by altered corneal sensitivity to capsaicin. Panels B shows a dose-dependent change in metastatic potential to lung. Panel C shows representative lungs from vehicle only and capsaicin-treated mice. Note that there is both an increased number and size of lung metastases. Panel D shows results from a single experiment depicting a correlation between corneal response and formation of lung metastases in animals treated with 50 mg/kg capsaicin. Results are expressed as mean±SEM. *p<0.05, significantly different than vehicle-treated group.

and neurotoxic effects of capsaicin have been used to demonstrate the role of CSFs in neurogenic inflammation, pain sensation and regulation of the immune system (9). In the studies reported here, we administered neurotoxic doses of capsaicin and examined the effects on the metastatic efficiency and distribution of the 4T1 murine breast carcinoma cell line (10, 11).

Materials and Methods

Animals and chemicals. Virus- and pathogen-free female BALB/c mice, 4-5 weeks old, were purchased from Harlan Sprague-Dawley (Indianapolis, IN, USA) and kept under controlled diet and 12h light-dark cycle. All mice were provided food (Purina Rodent Chow) and water *ad libitum*. All protocols were approved and performed under the supervision of the Penn State University College of Medicine Institutional Animal Care and Use Committee.

Capsaicin (10 mg/ml; M-2028, Sigma Chemicals, St. Louis, MO, USA) was dissolved in 10% ethanol, 10% Tween-80 and 80% sterile water to administer a concentration of 125 mg/kg. Subsequent dilutions were made in sterile water. Capsaicin was administered

into subcutaneous fat tissues of the neck in two injections (50 mg/kg and 75 mg/kg) 24 h apart under ketamine/xylazine anesthesia (80 mg/kg;15 mg/kg *i.m.*). Atropine (5 mg/kg, *i.p.*) was administered immediately before capsaicin injection to prevent acute cardiopulmonary effects of excessively released sensory mediators. This dose of capsaicin was previously reported to inactivate sensory neurons by decreasing neuropeptide levels for 4-6 wk (12).

The efficacy of nerve inactivation was evaluated by the eye-wipe test (12). Briefly, a drop of dilute (0.1%) capsaicin was put onto one eye and wiping movements were counted. Exposure to a denervating dose of capsaicin caused loss of corneal sensitivity, indicating inactivation of sensory neurons. While control animals wiped their eyes repeatedly (~40 times), capsaicin-treated animals responded with fewer wipes in a dose-dependent manner (Figure 1A). Lower doses of capsaicin were also tested as internal controls.

Metastasis assay. 4T1 cells murine mammary carcinoma cells were utilized. The cell line was derived from a spontaneous tumor in a BALB/c mouse (10). Cells (1×10^5) were injected into a right axillary mammary pad 7-21 days after capsaicin or vehicle treatment. Longer times were used to assess the impact of sensory nerve regeneration on metastasis. Three doses of capsaicin (25, 50 and 125 mg/kg) were tested. Experimental groups consisted of 5-

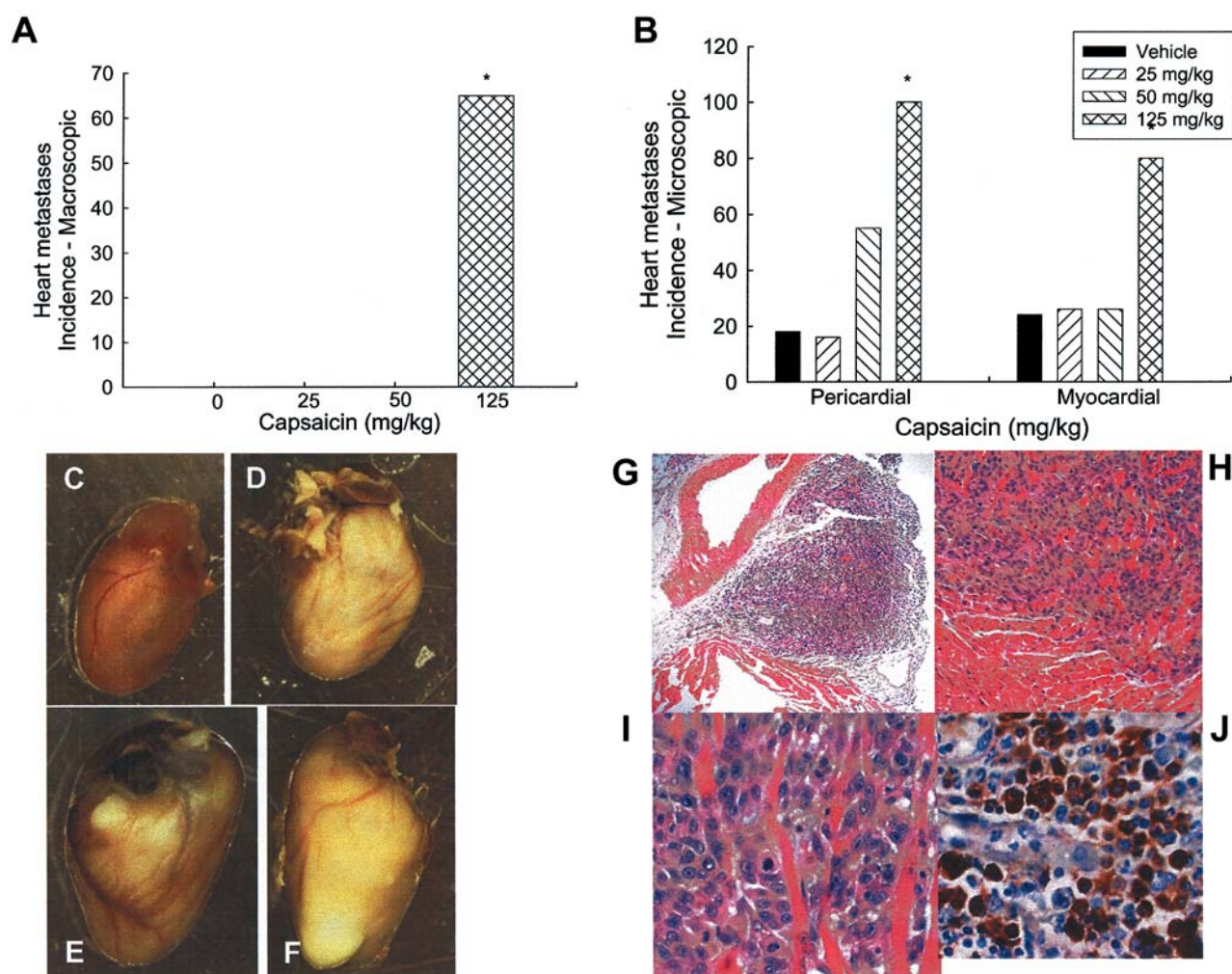


Figure 2. Incidence of heart metastases increases after sensory-nerve denervation with 125 mg/kg capsaicin. Panel A shows dose-dependent changes in the incidence of macroscopic heart metastases following capsaicin treatment. * $p < 0.05$, significantly different from vehicle-treated group. Panel B. Horizontally cut 3-4 mm heart sections were examined under the light microscope. Metastases were measured as presence or absence of pericardial or myocardial infiltration by carcinoma cells. Macroscopic appearance of hearts from mouse treated with vehicle (Panel C) or capsaicin (Panels D-E). Note: In some mice, the heart was enlarged and nodules representing metastatic aggregates were discernable. Panels F-J show examples of mammary tumors in the pericardium (G) and myocardium (H-J) by H&E staining. Most neoplastic cells were positive by immunoperoxidase for cytokeratin. Note that endothelial cells and neutrophils in the blood vessel (left-center of image J) are negative.

11 mice each. Mice were euthanized 23-33 days after tumor cell inoculation. At necropsy, all tissues were harvested and examined for macroscopic metastases. Lung and heart were removed together and stored in a mixture of Bouin's fixative and neutral buffered formalin (1:5 v/v) at room temperature. Lung metastases were quantified as previously reported (13). Hearts were step sectioned horizontally to maximize surface area examined per section. Presence or absence of macroscopic lesions was recorded and the sections were then embedded in paraffin. Sections from several levels of each paraffin block were stained with hematoxylin and eosin and examined microscopically. For each heart, the presence or absence of metastases was determined. Tumor location was recorded as being present in the pericardium, the myocardium, or both. Primary tumors and metastases were immunostained with

cytokeratin AE 1,3 (Boehringer-Mannheim, CT, USA) using the avidin-biotin peroxidase technique with diaminobenzidine visualization.

Statistics. One-way analysis of variance was used to compare differences in the numbers of lung metastases. Differences between incidences of metastases were compared by using a z-test. P values less than 0.05 were considered significant.

Results

Capsaicin-induced sensory nerve denervation increased lung and heart metastasis. Female BALB/c mice were treated with three doses of capsaicin. A dose-dependent inactivation of CSFs

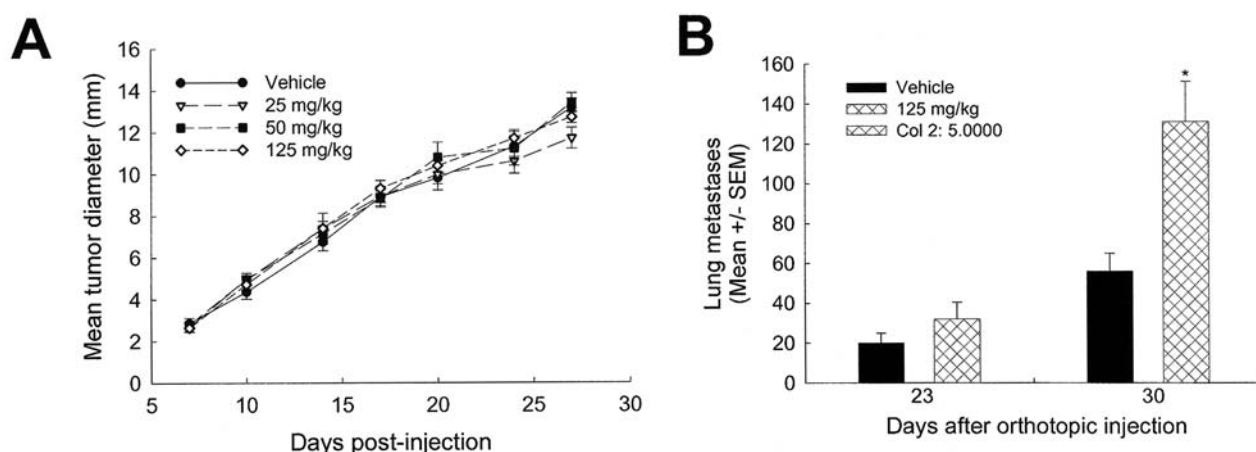


Figure 3. Primary tumor growth was unaffected by sensory-nerve denervation with capsaicin (Panel A). In a separate experiment, lung metastases were counted 23 and 30 days after orthotopic tumor cell injection in vehicle-only or capsaicin-treated mice. Panel B shows that the number of lung metastases 23 days after tumor inoculation were not different, while vehicle- and capsaicin-treated groups were significantly ($p < 0.05$) different at 30 days. Data = mean \pm SEM.

activity was observed (Figure 1A), as measured by corneal sensitivity. Evidence of denervation was observed in mice treated with 125 mg/kg capsaicin, the dose that significantly increased the number of lung metastases per animal (Figure 1, Panels B and C). Corneal sensitivity at the 50 mg/kg dose was variable, *i.e.*, causing complete loss of corneal response in one animal (# 1), partial response in mouse # 2 and the remaining mice unaffected. Cells (4T1, 1×10^5) were injected one week after capsaicin treatment so that capsaicin would be cleared (14) and so that direct effects on tumor cells would not complicate interpretation. Interestingly, in a limited number of mice examined, mice treated with 50 mg/kg capsaicin generally showed an inverse correlation between number of lung metastases and loss of corneal sensitivity (Figure 1, Panel D, mice #2 and #3). The mice having lowest nerve function (#2 and #3) had microscopic pericardial and myocardial metastases. At low doses (25 mg/kg), capsaicin did not inhibit corneal response, but caused a statistically significant reduction in lung metastases (Figure 1B).

High-dose of capsaicin (125 mg/kg) led to the formation of macroscopically discernible heart nodules, unobserved in any vehicle-treated animal or animals treated with lower doses of capsaicin (Figure 2, Panel A). The incidence of microscopic metastasis was also significantly higher in the same group (Figure 2, Panel B). There were no macroscopic metastases in vehicle-treated mice, although some showed microscopic tumor cells infiltrates into both pericardium and myocardium (Figure 2, Panel B). Macroscopic cardiac lesions were visible as whitening and enlargement of the heart with discernable nodularity (Figure 2, Panel D). Microscopically, neoplastic masses were identified in

pericardium (Figure 2, Panels G) and/or in myocardium (Figure 2, Panels H-J). As expected, cytokeratin immunoperoxidase evaluation of the locally growing tumor and metastases (Figure 2, Panel J) were immunoreactive.

Sensory-nerve denervation-induced enhancement of metastases is apparently not due to altered primary tumor growth or initial lodging of tumor cells. Tumor growth at an orthotopic site (*i.e.*, mammary fat pad) after capsaicin treatment was virtually identical to vehicle controls (Figure 3A). This result suggests that enhancement of metastasis was not simply due to a higher rates of tumor cell proliferation. Likewise, the number of lung metastases were not increased over controls on the 23rd day post tumor injection (Figure 3B). However, by day 30, marked increases in lung metastases were observed (Figure 3B). Similarly, no macroscopic heart metastases were observed at 23 days, while multiple cardiac metastases were evident on day 30. These findings suggest that enhancement of metastases is due to denervation-induced alterations in the secondary site microenvironment rather than to alterations in frequency of initial tumor cell lodging.

Metastatic potential returns to basal levels as sensory nerves regenerate. The denervating effect of capsaicin in adult animals is temporary (12). CSFs begin regenerating with a time-course dependent upon the tissue (12). To determine the effects of CSF regeneration on metastases, 4T1 cells were injected 7-23 days after capsaicin treatment (Figure 4A). The number and incidence of heart and lung metastases returned to baseline (*i.e.*, similar to vehicle-treated group) as the interval between capsaicin treatment

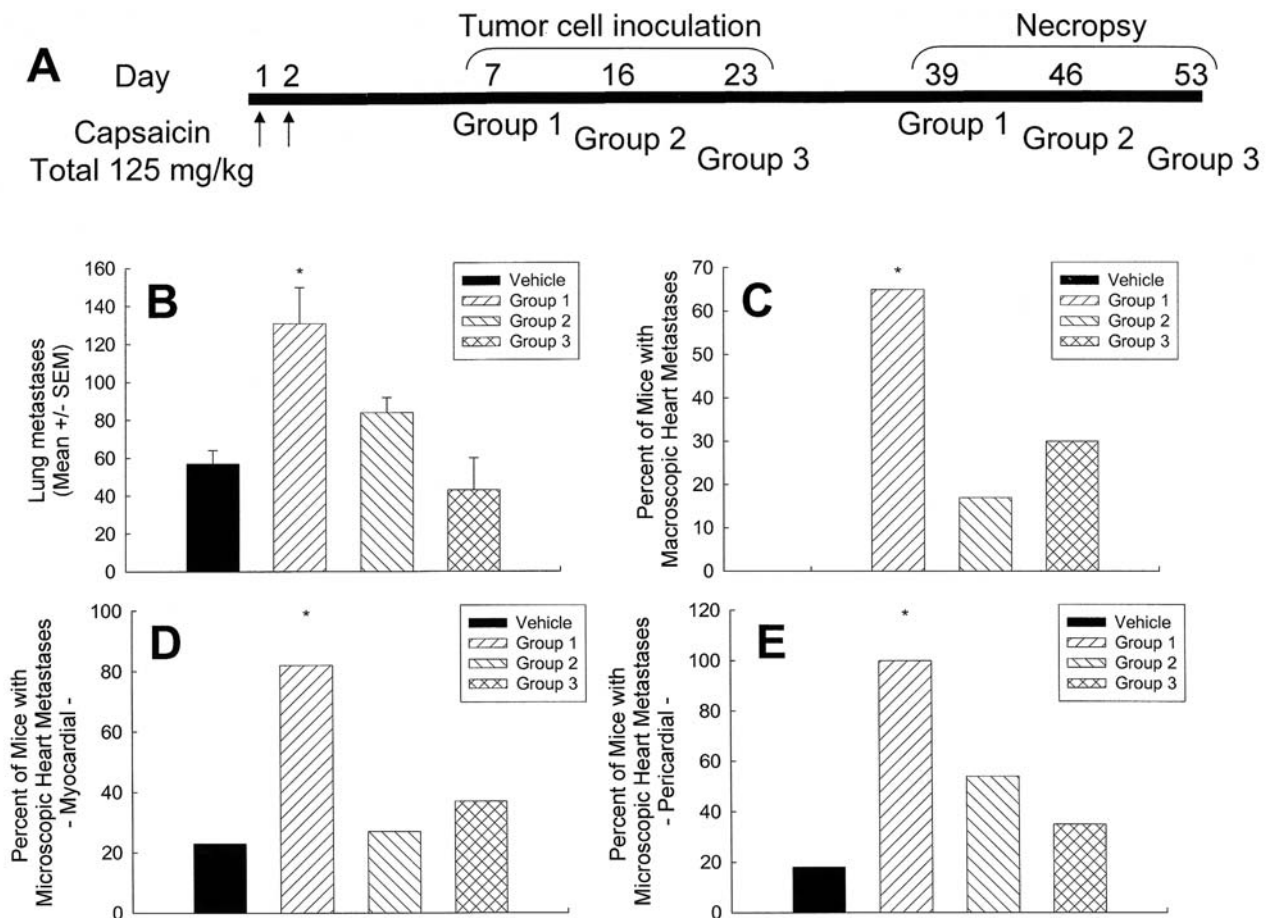


Figure 4. Capsaicin-enhanced metastasis to lung and heart is reversible. Panel A is a schematic depiction of treatment and necropsy schedules. 4T1 cells were injected 7-23 days after capsaicin injection. Panel B shows number of lung metastases from different treatment groups. Panels C-E illustrate changes in the incidence of macroscopic (C) and microscopic cardiac metastases in the myocardium (D) and pericardium (E). Data = mean ± SEM. * $p < 0.05$, significantly different than vehicle-treated group.

and 4T1 cell inoculation increased (Figures 4B-E). Metastases to heart and lung were only statistically different when tumor cells were injected one week following capsaicin-induced denervation.

Discussion

Using a mouse mammary tumor model, we demonstrated, for the first time, that capsaicin-sensitive nociceptive neurons may be involved in modulating metastases to the heart and lung. We observed that systemic inactivation of CSF markedly increased metastasis to both organs. Macroscopic and microscopic heart metastases were seen in all denervated mice 4-5 wk after orthotopic injection of the 4T1 cell line while growth of tumor in the mammary fat pad was unaffected.

These findings are significant because metastasis to heart is extremely rare. Capsaicin-enhanced metastasis is not due to direct effects on carcinoma cells since capsaicin is cleared within 48 h of injection (14), long before we injected mammary tumor cells. Capsaicin specifically activates or inactivates CSFs (depending on dose) by depletion of neuropeptides such as SP and CGRP. Neurotoxic effects are reversible in adult mice (12). As expected, metastatic potential returned to baseline levels when CSFs were allowed to regenerate (*i.e.*, 3 wk after capsaicin treatment (12)). Several unique characteristics of CSFs mediate their specialized functions, as well as sensitivity to capsaicin. These characteristics include expression of capsaicin/VR1 receptors (8, 9) and production of neuropeptides that localize specifically in CSFs (4). CSF cell bodies reside at dorsal root ganglia. Afferent nerve fibers extend centrally to the dorsal

horn. Peripheral projections terminate at various tissues, e.g. skin, lung and vessels. Activation of these CSF by local inflammation or ischemia releases neuropeptides at both local and central nerve endings (4, 15). Of these neuropeptides, SP, CGRP and neurokinin A are present in sensory nerve endings of both heart and lung. Neuropeptide levels decrease after exposure to high doses of capsaicin (4, 8, 16-20). Compared to most other tissues, lung and heart are abundantly innervated by CSFs (21-24). CSFs, for example, are more abundant in carotid arteries innervating heart than in arteries supplying abdomen, limbs and head (24). Thus, it is not surprising that major capsaicin-induced effects on metastasis were in heart and lung. Taken together, the facts compel the hypothesis that CSFs (*via* local release of mediators) play a major role in cardiac and pulmonary tissues to modulate local responses to metastatic growth.

Neuropeptides released from CSF nerve terminals are also involved in local inflammatory responses (e.g., asthma (25) and tissue injury). SP and CGRP cause vasodilation; increase vascular permeability; induce extravasation of plasma proteins and leukocytes (4); stimulate mitogenesis of fibroblasts and endothelial cells; and can induce angiogenesis (26, 27). SP reportedly enhances breast cancer growth and metastases (28). Considering these observations, we expected diminished metastasis following denervation by capsaicin treatment. However, we observed the opposite metastasis to vital organs increased. The conclusion, therefore, is that CSF-derived neuropeptides in lung and heart are not directly promoting tumor growth. On the contrary, CSF-derived neuropeptides may prevent growth at metastatic tumor cells by altering the local microenvironment.

One way that CSF might alter the local tissue is regulation of local immune responses. CSF-secreted SP can increase immunoglobulin secretion (29), cytokine production and secretion (30) and induce B-cell differentiation (31). SP also augments Con-A-induced lymphocyte proliferation *in vitro* (29) and enhances human T-lymphocyte proliferation (32). Capsaicin treatment of neonatal rats inhibits NK cell activity in spleen and peripheral blood for up to 3 months (33). Hence, it is possible that altered immune cell function following depletion of neuropeptides allows metastatic growth. We did not detect significant changes in the numbers of circulating CD4⁺ and CD8⁺-positive T-cells or *in vitro* T- and NK-cell function following CSFs inactivation (data not shown). Yet, it is still possible that altered localized immune response may influence metastatic growth in capsaicin-treated mice. Further studies will be required to clarify potential mechanisms.

In mice treated with 25 mg/kg capsaicin, lung metastasis decreased markedly compared to the vehicle controls. This opposite effect may be due to CSF sensitization since it is known that low doses of capsaicin elicit powerful excitatory

effects on CSFs (12). Activation of CSFs by local inflammatory mediators, some of which act through capsaicin receptors, sensitizes these neurons (34) and increases neuropeptide content at nerve endings (35). These characteristics are consistent with our hypothesis that activated CSFs may protect against metastatic tumor cell growth.

Besides the implications of our findings with regard to tumor cell response at local tissue microenvironments, there are implications with regard to the recent targeting of capsaicin (VR1) receptors in the treatment of chronic pain (8). Given the unexpected enhancement of metastasis, clinical trials involving VR1 antagonists for pain treatment of cancer patients should proceed cautiously until further experiments are performed to assess the generalization of our findings.

In summary, our study suggests that capsaicin-sensitive sensory neurons may play a role in preventing metastatic growth, particularly in heart and lung. Further elucidation of mechanisms underlying sensory neuron-mediated defense against metastases could provide novel therapeutic targets for the prevention and/or elimination of metastases.

Acknowledgements

We would like to thank Heidi Young and D. Michael Turner for their outstanding technical assistance. We also thank members of the Welch lab for stimulating discussions. This study was supported by grants from the National Foundation for Cancer Research-Center for Metastasis Research, the U.S. Public Health Service (CA87728 to DRW and AI 49719 to RB) and the U.S. Army Medical Research and Materiel Command (DAMD17-02-1-0541 to DRW). This project was also funded, in part, under a grant with the Pennsylvania Department of Health. The Department specifically disclaims responsibility for any analyses, interpretations or conclusions.

References

- 1 Spiegel D: Effects of psychotherapy on cancer survival. *Nature Rev Cancer* 2: 383-389, 2002.
- 2 Spiegel D, Sephton SE, Terr AI *et al*: Effects of psychosocial treatment in prolonging cancer survival may be mediated by neuroimmune pathways. *Ann N Y Acad Sci* 840: 674-683, 1998.
- 3 Ercan F, Oktay S and Erin N: Role of afferent neurons in stress induced degenerative changes of the bladder. *J Urol* 165: 235-239, 2001.
- 4 Holzer P: Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neurosci* 24: 739-768, 1988.
- 5 Girolomoni G and Tigelaar RE: Capsaicin-sensitive primary sensory neurons are potent modulators of murine delayed-type hypersensitivity reactions. *J Immunol* 145: 1105-1112, 1990.
- 6 Camerlingo M, Nemni R, Ferraro B *et al*: Malignancy and sensory neuropathy of unexplained cause: a prospective study of 51 patients. *Arch Neurol* 55: 981-984, 1998.
- 7 Hiura A: Neuroanatomical effects of capsaicin on the primary afferent neurons. *Arch Histol Cytol* 63: 199-215, 2000.

- 8 Caterina MJ and Julius D: The vanilloid receptor: A molecular gateway to the pain pathway. *Ann Rev Neurosci* 24: 487-517, 2001.
- 9 Bevan S and Szolcsanyi J: Sensory neuron-specific actions of capsaicin: mechanisms and applications. *Trends Pharmacol Sci* 11: 330-333, 1990.
- 10 Lelekakis M, Moseley JM, Martin TJ *et al*: A novel orthotopic model of breast cancer metastasis to bone. *Clin Exptl Metastasis* 17: 163-170, 1999.
- 11 Aslakson CJ and Miller FR: Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Res* 52: 1399-1405, 1992.
- 12 Buck SH and Burks TF: The neuropharmacology of capsaicin: review of some recent observations. *Pharmacol Rev* 38: 179-226, 1986.
- 13 Welch DR: Technical considerations for studying cancer metastasis *in vivo*. *Clin Exptl Metastasis* 15: 272-306, 1997.
- 14 Saria A, Skofitsch G and Lembeck F: Distribution of capsaicin in rat tissues after systemic administration. *J Pharm Pharmacol* 34: 273-275, 1982.
- 15 Benson CJ, Eckert SP and McCleskey EW: Acid-evoked currents in cardiac sensory neurons: A possible mediator of myocardial ischemic sensation. *Circ Res* 84: 921-928, 1999.
- 16 Franco-Cereceda A, Henke H, Lundberg JM *et al*: Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. *Peptides* 8: 399-410, 1987.
- 17 Chang Y, Hoover DB and Hancock JC: Endogenous tachykinins cause bradycardia by stimulating cholinergic neurons in the isolated guinea pig heart. *Am J Physiol Regul Integr Comp Physiol* 278: R1483-R1489, 2000.
- 18 Steele PA, Gibbins IL, Morris JL *et al*: Multiple populations of neuropeptide-containing intrinsic neurons in the guinea-pig heart. *Neurosci* 62: 241-250, 1994.
- 19 Geppetti P, Frilli S, Renzi D *et al*: Distribution of calcitonin gene-related peptide-like immunoreactivity in various rat tissues: correlation with substance P and other tachykinins and sensitivity to capsaicin. *Regul Pept* 23: 289-298, 1988.
- 20 Hoover DB. Release of substance P from isolated guinea pig heart. *In*: Henry J, Couture R, Cuello AC *et al*: (eds). *Substance P and Neurokinins*. New York: Springer 208-209, 1987.
- 21 Lundberg JM, Franco-Cereceda A, Hua X *et al*: Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. *Eur J Pharmacol* 108: 315-319, 1985.
- 22 Baluk P, Nadel JA and McDonald DM: Substance P-immunoreactive sensory axons in the rat respiratory tract: a quantitative study of their distribution and role in neurogenic inflammation. *J Comp Neurol* 319: 586-598, 1992.
- 23 Reinecke M, Weihe E and Forssmann WG: Substance P-immunoreactive nerve fibers in the heart. *Neurosci Lett* 20: 265-269, 1980.
- 24 Furness JB, Papka RE, Della NG *et al*: Substance P-like immunoreactivity in nerves associated with the vascular system of guinea-pigs. *Neurosci* 7: 447-459, 1982.
- 25 Solway J and Leff AR: Sensory neuropeptides and airway function. *J Appl Physiol* 71: 2077-2087, 1991.
- 26 Ziche M, Morbidelli L, Masini E *et al*: Nitric oxide mediates angiogenesis *in vivo* and endothelial cell growth and migration *in vitro* promoted by substance P. *J Clin Invest* 94: 2036-2044, 1994.
- 27 Haegerstrand A, Dalsgaard CJ, Jonzon B *et al*: Calcitonin gene-related peptide stimulates proliferation of human endothelial cells. *Proc Natl Acad Sci* 87: 3299-3303, 1990.
- 28 Singh D, Joshi DD, Hameed M *et al*: Increased expression of preprotachykinin-I and neurokinin receptors in human breast cancer cells: implications for bone marrow metastasis. *Proc Natl Acad Sci* 97: 388-393, 2000.
- 29 Santoni G, Perfumi MC, Spreghini E *et al*: Neurokinin type-1 receptor antagonist inhibits enhancement of T cell functions by substance P in normal and neuromanipulated capsaicin-treated rats. *J Neuroimmunol* 93: 15-25, 1999.
- 30 Rossi R and Johansson O: Cutaneous innervation and the role of neuronal peptides in cutaneous inflammation: a minireview. *Eur J Dermatol* 8: 299-306, 1998.
- 31 Pascual DW, Beagley KW, Kiyono H *et al*: Substance P promotes Peyer's patch and splenic B cell differentiation. *Adv Exp Med Biol* 371A: 55-59, 1995.
- 32 McGillis JP, Mitsuhashi M and Payan DG: Immunomodulation by tachykinin neuropeptides. *Ann N Y Acad Sci* 594: 85-94, 1990.
- 33 Santoni G, Perfumi M, Birarelli P *et al*: *In vivo* capsaicin treatment inhibits rat NK cell cytotoxic functions. *Immunopharmacol Immunotoxicol* 17: 511-528, 1995.
- 34 Treede RD, Meyer RA, Raja SN *et al*: Peripheral and central mechanisms of cutaneous hyperalgesia. *Prog Neurobiol* 38: 397-421, 1992.
- 35 Myers AC, Kajekar R and Udem BJ: Allergic inflammation-induced neuropeptide production in rapidly adapting afferent nerves in guinea pig airways. *Am J Physiol Lung Cell Mol Physiol* 282: L775-L781, 2002.

Received December 4, 2003
Accepted February 12, 2004