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Capsaicin Induces Degeneration of Cutaneous Autonomic Nerve Fibers

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Abstract

Objective—To determine the effects of topical application of capsaicin on cutaneous autonomic nerves.

Methods—Thirty-two healthy subjects underwent occlusive application of 0.1% capsaicin cream (or placebo) for 48 hours. Subjects were followed for 6 months with serial assessments of sudomotor, vasomotor, pilomotor and sensory function with simultaneous assessment of innervation through skin biopsies.

Results—There were reductions in sudomotor, vasomotor, pilomotor and sensory function in capsaicin- treated subjects ($p < 0.01$ vs. placebo). Sensory function declined more rapidly than autonomic function; reaching a nadir by day 6 while autonomic function reached a nadir by day 16. There were reductions in sudomotor, vasomotor, pilomotor and sensory nerve fiber densities in capsaicin treated subjects ($p < 0.01$ vs. placebo). Intra-epidermal nerve fiber density declined maximally by 6 days while autonomic nerve fiber densities reached maximal degeneration by day 16. Conversely, autonomic nerves generally regenerated more rapidly than sensory nerves, requiring 40–50 days to return to baseline levels while sensory fibers required 140–150 days to return to baseline.

Interpretation—Topical capsaicin leads to degeneration of sudomotor, vasomotor and pilomotor nerves accompanied by impairment of sudomotor, vasomotor and pilomotor function. These results suggest the susceptibility and/or pathophysiologic mechanisms of nerve damage may differ between autonomic and sensory nerve fibers treated with capsaicin and enhances the capsaicin model for the study of disease modifying agents. The data suggest caution should be taken when topical capsaicin is applied to skin surfaces at risk for ulceration, particularly in neuropathic conditions characterized by sensory and autonomic impairment.

Keywords

Capsaicin; Autonomic Neuropathy; Nerve Degeneration

The vanilloid, capsaicin, the pungent component in hot chili peppers, is widely used as a topical agent to treat neuropathic and musculoskeletal pain and itch.¹ Capsaicin is an agonist of the transient receptor potential vanilloid type 1 (TRPV1) receptor, a non-selective, cation channel that functions as integrator of noxious chemical and physical stimuli. TRPV1 is expressed in small and medium diameter nociceptor sensory neurons although recent reports

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have suggested a more widespread distribution.^{2,3} Studies reveal TRPV1 plays a role in visceral sensation. It is expressed in visceral afferent fibers and influences visceral tissue homeostasis via efferent release of peptides such as CGRP and SP.^{4–8}

Topical application of capsaicin results in burning pain mediated by discharges in C polymodal and A δ mechano-heat nociceptors due to TRPV1 receptor activation. Physiological desensitization of nociceptor neurons follows the initial activation, however, with repeated application^{9–11} neural degeneration occurs. The desensitization and subsequent degeneration may underlie the analgesic effects of capsaicin. Furthermore, some investigators have suggested that nerve regeneration following capsaicin induced degeneration may provide a measure of efficacy in disease modifying peripheral neuropathy therapeutic trials.^{11,12}

All reports of capsaicin induced nerve degeneration in humans have examined small sensory neurons.^{9–11} Since TRPV1 receptors are present in visceral afferents in humans^{13–15} and other species,^{4–8} we hypothesized that topical application of capsaicin would lead to degeneration of cutaneous autonomic nerves. Such degeneration would not only extend our understanding of the cutaneous innervation but, if present, may have safety implications given the widespread use of topical capsaicin in patients with neuropathic pain.¹ Furthermore, the knowledge that another population of neurons degenerate and regenerate would provide an additional dimension to this model for the assessment of the therapeutic efficacy of disease modifying agents for peripheral neuropathy.

PATIENTS AND METHODS

Subjects

Thirty-two healthy subjects ages 20–52 with a mean age of 31.4 years were recruited for the study. Subjects were excluded from participation if they had evidence by history or exam of neuropathy, a history of medical disease, current use of medications (except oral contraceptives), tobacco or excessive alcohol use. The Beth Israel Deaconess Institutional Review Board approved the studies and all subjects signed an informed consent.

Testing Protocol

A 50×80 mm area was demarcated on the right volar forearm. The area was divided into discrete testing regions (Fig 1). Subjects were evaluated on days 1, 3, 6, 9, 16, 30, 58, 100 and 150 (tests were completed on days 1, 3, 6 and 9 without variation and within ± 2 days on days 16 and 30, and within ± 3 days on 58, 100 and 150).

Capsaicin or Placebo Application

After the first day of testing, an occlusive bandage containing 2.4 g of 0.1% capsaicin cream (Chattem Inc., Chattanooga, TN) or placebo cream was applied to the right volar forearm. The bandage was applied for 24 hours with reapplication on day 2 for another 24 hours.¹¹ Subjects were block randomized in a blinded fashion to capsaicin or placebo treatment, with a total of 20 subjects receiving capsaicin, and twelve receiving placebo.

Skin Biopsies

All patients underwent 3-mm punch skin biopsies at the volar aspect of the forearm. Subjects had a total of 5 skin biopsies performed during the course of the study: 4 biopsies in the region of capsaicin/placebo application, and 1 on the contralateral forearm as a control site (termed baseline biopsy). Subjects were randomly assigned to biopsies on days 3, 6, 30, 100 or 6, 16, 58, 150 (10 in each group for capsaicin and 6 for placebo subjects). On the 1st biopsy day, both the right and the left forearm were biopsied. The remaining 3 biopsies were

performed only on the right arm adjacent to the original biopsy sites.¹¹ Specimens were fixed and stained with protein gene product 9.5 (1:1000, rabbit anti PGP 9.5, Chemicon International Inc) and co-stained with either the cutaneous vasculature marker, platelet endothelial adhesion molecule CD31 (1:4000, mouse anti CD31, Dako Inc) or the sympathetic adrenergic marker tyrosine hydroxylase (1:500, rabbit anti TH, Sigma Inc) labeled with fluorescein isothiocyanate, using previously described techniques for confocal microscopy.¹⁶ Digital images were obtained by confocal microscope (Zeiss LSM Pascal Exciter, Germany) with a 10× or 20× objective lens at the appropriate wavelengths for cyanine 2, 3.18 and 5.18 fluorophores. Z-stack images (1–2 μm thick optical sections) were acquired at successive levels through the tissue sections and were projected in three dimensional images.

Sudomotor

Quantitative sudomotor axon reflex testing (QSART)—The quantitative sudomotor axon reflex was assessed by iontophoresing acetylcholine solution (10%) into the skin with a 2 mA stimulus for 5 minutes by standard procedure at each visit.¹⁷ Results were quantified by area under the curve analysis for the first 15 minutes after iontophoresis.

Sweat gland nerve fiber density—Sweat gland nerve fiber density (SGNFD) was calculated using the PGP 9.5 stained tissue sections by light microscopy. Sweat glands for all biopsies were imaged using a 6.2 megapixel Pixelink camera at 20× resolution and counted using a manual intercept density technique as previously described.¹⁸ Briefly, the images of the sweat gland were outlined and a standardized grid of circles 10 μm in diameter spaced 50 μm apart horizontally, and offset 25 μm vertically was placed over the sweat gland. Nerve fibers that intersect the grid of circles were counted. The numbers of circles intersected out of the total number of circles present within the outlined area of interest (the percent intercept density) were reported for each sweat gland.

Vasomotor

Nociceptive axon reflex flare response—The C-fiber mediated axon-reflex was measured using the laser Doppler flowmetry technique after acetylcholine iontophoresis using standard methodologies.¹⁹ Endothelial and C-fiber-mediated vasodilation were provoked by acetylcholine (Penta, Fairfield, NJ; 1% in sterile water) using anodal current. The Periflux 5000 Master Laser Doppler (Perimed, Jarfalla, Sweden) was used to measure cutaneous blood flow.¹⁹ The tests were performed at ambient room temperatures between 23.7 and 25.6°C.

Vasomotor density—Confocal images of cutaneous vasculature and associated nerves, stained with PGP 9.5 and CD31 were studied. The linear length of capillaries and adjacent nerve fibers in each section of each biopsy was determined by manual tracing with results expressed as μm of nerve per mm of capillary.

Pilomotor

The pilomotor response was assessed by electrical stimulation of pilomotor arrector muscles with a 2 mA current for 5 minutes over a 1cm diameter region. Silicone material (GmbH&Co, Ettlingen, Germany) was placed over the area for 5 minutes post-stimulation. The stimulated erect hair follicles formed indentations in the silicone material and were quantified by size, number and location. Total magnitude of piloerection was determined by calculating the sum of ½ the spherical volume of each hair follicle ($\frac{2}{3}\pi r^3$) in the 3cm diameter circle of testing.

Pilomotor density—Confocal images of arrector pili muscles, stained with PGP 9.5 and TH were studied. The greatest linear width of each arrector pili muscle was determined and the number of crossing nerve fibers at that site counted with results expressed as the number of crossing fibers per mm diameter of arrector pili muscle.

Sensory

Quantitative sensory testing—Testing included heat, cold, and heat-pain perception thresholds at each visit. Testing was performed with ascending (heat) or descending (cold) methods of limits using standard protocols.²⁰ Thermal stimuli were applied using a 16×16 mm thermode (Medoc TSA II Neurosensory Analyzer). The results of 4 individual trials were averaged. Patient instructions were standardized with cueing cards. A temperature maximum of 52°C was set to prevent thermal injury.²⁰

Intra-epidermal nerve fiber density—All patients underwent IENF density counting by a physician blinded to treatment allocation and results were expressed as a linear density (number of fibers per millimeter) as previously described.²¹

Statistical Analysis

Statistical analysis of results was performed using Systat 11 (Systat Software Inc, San Jose, CA). Data are presented as mean $\pm$ standard deviation. Repeated measures ANOVA with post-hoc analysis (with Bonferroni correction) was performed for variables. A *p* value <0.05 was used to define statistical significance for all data sets, with all results compared to placebo. Differences in both degeneration and regeneration rates between nerve fiber types were analyzed with a bivariate regression model using the Mann-Whitney *U* test, with a *p* value <0.05 considered significant. Successful regeneration was defined as a non-significant change from baseline.

RESULTS

A total of 32 subjects were studied; 20 treated with capsaicin (mean age 31 ± 7 years; 55% female) and 12 with placebo (mean age 28 ± 6 years; 50% female). Capsaicin treated subjects reported peak discomfort of $5.5 \pm 1.6/10$ (on a 0–10 modified Likert scale) 30–120 minutes after capsaicin application. Pain was reduced to $2.6 \pm 0.9/10$ with application of a cold compress in ~50% of subjects. Placebo treated subjects reported a mean pain score of $2.1 \pm 0.7/10$. All capsaicin and placebo treated subjects tolerated the procedure. All subjects completed testing through day 58; 19 (of 20) capsaicin and 10 (of 12) placebo subjects completed testing through day 150.

Sudomotor

Structure—A total of 362 sweat glands were analyzed from 157 skin biopsies; 235 sweat glands from capsaicin-treated and 127 sweat glands from placebo-treated tissue. There was a decrease in SGNFD in the capsaicin-treated group by day 9 ($P < 0.05$) with regeneration to baseline levels by day 58 ($p < 0.05$; Fig 2A). The maximal decrease in SGNFD occurred by day 16 ($p < 0.01$). Day 16 images of sweat gland innervation from representative capsaicin- and placebo-treated biopsy sites are shown in Figure 3A and B.

Function—There was a decrease in axon reflex mediated sweating measured by QSART by day 9 after capsaicin application ($p < 0.05$) with return to baseline by day 30 ($p < 0.01$) (Fig 2B). The maximal reduction in axon reflex mediated sweat production occurred by day 16 ($p < 0.005$ vs. placebo) (Fig 2B, 3D). A functional “overshoot” of sweat production was seen on days 100 and 150 ($p < 0.05$ days 100 and 150 vs. placebo).

Vasomotor

Structure—A total of 84 skin biopsies were analyzed for vasomotor nerve fiber density; 54 from capsaicin treated and 30 from placebo treated tissue. There was a decrease in vascular nerve fiber density by day 3 ($p<0.05$) with regeneration to baseline levels by day 58 ($p<0.05$) (Fig 2C). The maximal decrease occurred by day 16 ($p<0.01$). Day 16 images of vasomotor innervation from representative capsaicin- and placebo-treated biopsy sites are shown in Figure 3A and B.

Function—There was a decrease in the indirect vasomotor response to acetylcholine iontophoresis by day 3 after capsaicin application ($p<0.005$) with return to baseline by day 100 ($p<0.05$) (See Fig 2D). The maximal decrease occurred by day 16 ($p<0.001$) (See Fig 2D, 4D).

Pilomotor

Structure—A total of 312 arrector pili muscles were analyzed from 157 skin biopsies; 198 from capsaicin-treated and 114 from placebo-treated tissue. There was a decrease in pilomotor nerve fiber density in the capsaicin-treated group by day 9 with regeneration to baseline levels by day 58 ($p<0.05$) (Fig 2E). The maximal decrease occurred by day 16 ($p<0.01$). Day 16 images of arrector pili innervation from representative capsaicin and placebo treated biopsy sites are shown in Figure 5A and B.

Function—At baseline, the total number of erect hair follicles on silicone impressions was similar in capsaicin- and placebo-treated subjects. There was a decrease in the magnitude of piloerection, analyzed by volumetric analysis of the silicone impressions, by day 9 after capsaicin application ($p<0.01$) with return to baseline function by day 58 ($p<0.05$) (Fig 2E). The maximal decrease occurred on day 16 ($p<0.001$, Fig 4D, 5F). Day 16 silicone impression images of representative control and capsaicin-treated subjects are shown in Figure 5D, 5E.

Sensory

Structure—One hundred fifty-seven skin biopsies were performed during the course of the study, 99 from capsaicin-treated and 58 from placebo-treated subjects. There was a decrease in IENFD by day 3 ($p<0.001$) with regeneration to baseline levels by day 100 ($p<0.05$) (Fig 2G). The maximal decrease occurred on day 6 ($p<0.001$). Day 16 images of IENFD from representative capsaicin- and placebo-treated biopsy sites are shown in Figure 6A and B.

Function—In the capsaicin treated group, there was a decrease by day 3 in the heat detection threshold ($p<0.01$); cold detection threshold ($p<0.05$) and heat pain detection ($p<0.01$, Fig 2H). Return to baseline sensory function for heat detection threshold occurred by day 100, ($p<0.05$); cold detection threshold by day 58, ($p<0.05$); and heat pain detection by day 100 ($p<0.05$) (Fig 2H). The maximal decrease occurred on day 16 ($p<0.01$ all thermal tests, Fig 2H).

Rates of Degeneration and Regeneration

Measures of sensory structure and function decreased maximally by days 6 to 9, while measures of autonomic function did not achieve maximal degeneration until day 16 (Fig 7A). Conversely, autonomic nerves regenerated more rapidly than sensory nerves, requiring only 40–50 days to return to baseline levels, with the exception of vasomotor structure and function that required 80–90 days as seen in Figure 7B. Sensory fibers required 140–150 days for maximal regeneration, with the exception of cold detection which required 80–90 days to return to baseline (Fig 7B).

DISCUSSION

This study demonstrates that topical capsaicin, applied in a 48 hour occlusive dressing, leads to cutaneous autonomic nerve fiber injury with associated functional impairment. Capsaicin was previously shown to cause reversible changes in sensory nerve fiber structure and function.^{9–11} The present data show that capsaicin application also causes degeneration of sudomotor, vasomotor and pilomotor nerves that is accompanied by parallel changes of sudomotor, vasomotor and pilomotor function. The autonomic nerves tend to degenerate more gradually and recover more rapidly than sensory fibers. These results suggest the nerve susceptibility and/or pathophysiologic mechanisms of nerve damage may differ between autonomic and sensory nerve fibers treated with capsaicin.

Capsaicin, TRPV1, visceral and cutaneous autonomic neurons

Capsaicin is a ligand of the transient receptor potential vanilloid type 1 receptor (TRPV1), a member of the transient receptor potential (TRP) family of ion channels. In addition to capsaicin, this non-selective cation channel is activated by noxious heat (>42 degrees centigrade), protons, exogenous ligands such as resiniferatoxin, and endogenous ligands including the lipid anandamide.²² TRPV1 channels are expressed predominantly on small and medium diameter sensory neurons, however, recent studies have suggested a more widespread distribution.³ There is evidence of TRPV1 expression on visceral sensory neurons including bladder, bronchopulmonary, colonic and cardiac afferents where they play a role in visceral sensation and, tissue homeostasis most likely through the release of tachykinins and CGRP.⁸ The present data, showing evidence of capsaicin mediated degeneration and functional impairment of cutaneous autonomic nerves, suggest that TRPV1 is expressed on cutaneous autonomic nerves too.

Trpv1 and thermoregulation – peripheral and central

Several lines of evidence support an important role for the TRPV1 receptor in thermoregulation. Acute administration of capsaicin or the ultra-potent TRPV1 agonist resiniferatoxin via oral and various systemic routes in a variety of species causes hypothermia. The sites mediating this hypothermic response have not been fully elucidated. Some studies suggest central sites, most likely the pre-optic hypothalamic nuclei because capsaicin produces hypothermia when injected directly into the pre-optic area of the hypothalamus,³¹ whereas others have proposed peripheral sites.^{32–34} Furthermore, acute delivery of TRPV1 antagonists, which are in development as analgesic agents, causes hyperthermia in experimental animals and in human subjects.³⁵ Body temperature in humans treated with TRPV1 antagonists may reach 40°C and last for several days.³⁵

Cutaneous sudomotor, vasomotor and pilomotor nerves mediate thermoregulatory homeostasis in humans and animals. Our results in humans, demonstrating capsaicin induced degeneration of these cutaneous autonomic thermoeffector nerves, with concomitant functional impairment, are consistent with the evidence supporting an important thermoregulatory role for the TRPV1 receptor.³⁶ Further studies are necessary to address whether more extensive, chronic, topical application or systemic delivery of TRPV1 receptor agonists has functional thermoregulatory effects humans.

Degeneration and regeneration differences

Our results reveal variability in the magnitude and rate of degeneration and regeneration in different populations of cutaneous nerve fibers. The mechanism of capsaicin induced neurodegeneration is not fully elucidated. TRPV1 mediated calcium influx³⁷ with subsequent glutamate release is most likely implicated.^{38,39} There is strong concordance between structural and functional changes in both sensory and autonomic neurons (Fig 7).

An exception to this concordance is the early decrease in the magnitude of the vasomotor flare that is most likely due to capsaicin induced depletion of peptides such as CGRP and substance P that occurs prior to vasomotor nerve degeneration (Fig 2D). The nerve degeneration rate is faster and magnitude greater in small sensory nerve fibers than autonomic fibers. This may be due to the relative depth of the autonomic fibers and variability in TRPV1 expression within different nerve fiber populations. At present, there are few comparative studies of TRPV1 expression in humans.^{13–15} In addition, other intrinsic differences among neuronal populations including differences in signal transduction may be implicated; TRPV1 activation of signal transduction pathways may play a role in capsaicin mediated neurotoxicity.³⁹ Further, cutaneous and autonomic neuronal populations may have different susceptibility to non-TRPV1 capsaicin-mediated neurotoxicity.

We also show there are differences in the time to and rate of regeneration of autonomic and sensory nerve fibers. Regeneration rate is more rapid in most autonomic nerve fibers than in sensory nerve fibers (Fig 7). The reason for these differences is not known. It is well established that the nerve growth factor and glial cell line-derived neurotrophic factor family, are differentially involved in the development and maintenance of sensory, sympathetic and parasympathetic neurons.^{40–42} Differential effects on cutaneous autonomic and sensory neuronal populations of growth factor and growth factor receptor expression, and growth factor signaling may be responsible.^{42–44} The demonstration that capsaicin causes extensive cutaneous autonomic nerve degeneration with different degeneration and regeneration rates of sensory and autonomic neurons enhances the capsaicin model for the study of disease study modifying agents. Furthermore, this model provides an accessible method for the study of the factors involved in degeneration and regeneration of human autonomic and sensory nerves.

Capsaicin and the nociceptor axon reflex

Prior studies have demonstrated impaired neurogenic inflammation following capsaicin induced desensitization.²³ The present data extend those findings by showing impaired sudomotor axon reflex and nociceptor axon reflex responses, their structural basis, and the time course of the reversible change in small sensory and autonomic structure and function. These findings may have clinical implications. Activation of the terminal branches of the sympathetic and nociceptor fibers results in axon reflex mediated sweating, vasodilation and other features of neurogenic inflammation. Due to distal peripheral nerve degeneration, these protective responses are impaired in diabetic and other small fiber neuropathies.^{24–26} Impaired neurovascular function, attenuated neurogenic inflammation, dry skin due to impaired sudomotor function and loss of sensation play a central role in the predisposition of diabetic patients to foot ulceration and amputation;^{27,28} a peripheral neuropathy in individuals with diabetes leads to a 7-fold increase in the risk of foot ulceration.^{29,30} The present data demonstrate that topical application of capsaicin under an occlusive dressing provides a human model of diminished sympathetic function, neurogenic inflammation and neurovascular function in addition to, as previously shown, attenuated sensation. These data suggest that caution should be taken when topical capsaicin is applied to skin surfaces at risk for ulceration, particularly in neuropathic conditions characterized by baseline sensory and autonomic impairment.¹¹

Clinical Implications

Our study, in which 0.1% capsaicin was applied in an occlusive dressing for 48 hours, does not represent the typical clinical use of capsaicin when prescribed for neuropathic pain. However, in a study that more closely reflects low concentration capsaicin use in the clinical setting, a similar degree (~80%) of epidermal denervation was seen after three weeks of topical capsaicin (0.075%) applied to the volar forearm four times per day.⁹ Subepidermal

nerve fiber density, assessed semi-quantitatively in this study, was decreased. Whereas capsaicin (8%), recently approved for the treatment of post-herpetic neuralgia, when applied to the inner thigh at the recommended dose (a single application for 60 minutes) produced ~60% epidermal denervation.⁴⁵ Subepidermal nerve fiber density was not assessed in this study. It is likely that capsaicin induced degeneration and dysfunction is dependent upon several factors that include the capsaicin concentration, frequency and duration of exposure, application site, and presence or absence of neuropathy. Future studies, which more closely reflect the clinical use of capsaicin, should be performed to examine the effects of capsaicin on intraepidermal and subepidermal nerve structure and function.

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Fig 1.

Outline of the test paradigm on the forearm. The capsaicin treated area is divided into quadrants for structural and functional assessments: laser Doppler flowmetry to measure cutaneous vasomotor function, quantitative sensory testing (QST) to measure sensory function, silicone impressions to measure pilomotor function, quantitative sudomotor axon reflex testing (QSART) to measure sudomotor function and skin biopsies to quantify autonomic and sensory nerve fiber density.

Fig 2.

The change over time in cutaneous autonomic and sensory nerve fiber structure (Panels A, C, E, G) and function (Panels B, D, F, H) in capsaicin treated (closed black circles) and placebo treated subjects (open white circles). The results are expressed as percent of baseline for all panels. All results (A-H) were significant at $p < 0.01$ compared to placebo treated subjects (ANOVA). A decrease in measures of structure and function is seen between days 3–16 after capsaicin treatment. There are no corresponding changes in the placebo-treated group. Increases in heat and heat-pain thresholds (Panel G) implies a greater temperature is required to reach threshold.

Fig 3.

The effects of capsaicin on sudomotor nerves. Panels A and B: The sweat gland (green) and associated innervation (red) in a Day 16 control (A) and capsaicin-treated (B) biopsy. Fewer nerve fibers are visible in the capsaicin treated compared to the control treated biopsy. Red bar (A & B) = 100 μ m. Panel C: The calculated sudomotor nerve fiber density in the control (black bar) and capsaicin (white bar) treated regions at day 16. There is a significant reduction in nerve fibers in the capsaicin treated area ($p < 0.01$). Panel D: The maximum change in sudomotor function measured by QSART. There is a significant decrease in sweat output in the capsaicin treated region ($p < 0.01$).

Fig 4.

The effects of capsaicin on vasomotor nerves. Panels A and B: The cutaneous vasculature stained by CD 31 (green) and the associated innervation (red) stained by PGP 9.5 in a representative Day 16 control (A) and capsaicin treated (B) biopsy. The epithelial border is seen at the top of the image (white arrowheads). Fewer nerve fibers (yellow arrows) are visible in the Day 16 capsaicin-treated than control biopsy. Panel C: The vasomotor density for the control- (black bar) and capsaicin-treated (white bar) regions at day 16. There is a significant nerve fiber reduction in the capsaicin treated area ($p < 0.01$). Panel D: Vasomotor function for the control- (black bar) and capsaicin-treated (white bar) regions at day 16. There is a significant axon reflex mediated flare response reduction in the capsaicin treated area ($p < 0.01$). Scale bar (A & B) = 200 μ m.

Fig 5.

The effects of capsaicin on pilomotor nerves. Panels A and B: An arrector pili muscle is seen with nerve fibers extending parallel to the muscle in a control (A) and capsaicin-treated (B) biopsy stained with PGP 9.5. Fewer nerve fibers are visible in the capsaicin-treated compared to control on Day 16. Scale bar (A & B) = 200 μ m. Panel C: Pilomotor nerve fiber density in the control regions (black bar) is significantly higher than the capsaicin treated regions (white bar) regions at day 16 ($p<0.01$). Panel D. Arrector pili, when electrically stimulated, cause piloerection (goose-bumps) that can be quantified with silicone impressions. The red circle surrounds a representative 'goose-bump' in a Day 16 control subject. Panel E: In a representative Day 16 capsaicin treated region, the stimulated arrector pili muscles form smaller impressions (red circle). Panel F: The mean area of piloerection is greater in control regions (black bar) compared to the capsaicin treated regions (white bar) ($p<0.01$).

Fig 6.

The effects of capsaicin on sensory nerves. Panels A and B: The epidermal innervation stained by PGP 9.5 (green) penetrating the epidermal layer (yellow arrow) in a representative Day 16 control (A) and capsaicin-treated (B) biopsy. Fewer nerve fibers (green) are visible in the Day 16 capsaicin-treated than control biopsy. Scale bar (A & B) = 100 μ m. Panel C: The intra-epidermal nerve fiber density (IENFD) for the control- (black bar) and capsaicin-treated (white bar) regions at day 16. There is a significant nerve fiber reduction in the capsaicin treated area ($p < 0.01$). (D) The thermal and thermal-pain stimulation thresholds for control (black bar) and capsaicin (white bar) treated regions. There is a reduction in cold detection thresholds and an increase in heat and heat-pain (HP) detection thresholds in the capsaicin-treated regions at day 16 compared to control treated regions. * $p < 0.05$.

Fig 7.

The rates of nerve fiber degeneration (A) and regeneration (B) by nerve fiber subtype. Panel A: The lines represent the percent change from baseline on day 1 to the day of maximal change. Sensory nerve fibers degenerate and sensory function decreases more rapidly than autonomic nerve fibers. Vasomotor function declines at a rate between that of sensory and autonomic nerve fibers most likely in part due to physiologic desensitization and neuropeptide depletion. Panel B: The lines represent the time required for nerve fiber subtypes to return to baseline levels from the day of maximal degeneration (return to baseline levels defined as a non-significant change from baseline). Structural regeneration and functional improvement is more rapid in autonomic nerve fibers than in sensory nerve fibers. Vasomotor nerves, vasomotor function and cold detection thresholds return to baseline at a rate between sensory and other autonomic nerve fiber types.