Gene Expression in Skin, Muscle, and Dorsal Root Ganglion after Plantar Incision in the Rat

Christina M. Spofford, M.D., Ph.D.,* Timothy J. Brennan, M.D., Ph.D.†

ABSTRACT

Background: Treating postoperative pain remains a significant challenge for perioperative medicine. Recent studies have shown that nerve growth factor is up-regulated and contributes to incisional pain. To date, few studies have examined expression of other neurotrophin-related mediators that may contribute to the development and/or maintenance of incisional pain.

Methods: Male Sprague-Dawley rats underwent a plantar incision, and pain behaviors were examined (n = 6). In a separate group of rats, expression of neurotrophin factors were studied. At various times after incision (n = 4) or sham surgery (n = 4), the skin, muscle, and dorsal root ganglia were harvested and total RNA isolated. Real-time reverse transcription polymerase chain reaction was performed and the fold change in gene expression was analyzed using significance analysis of microarrays.

Results: Several genes were changed (P < 0.05) as early as 1 h after incision. Expression of artemin and nerve growth factor were increased in both incised skin and muscle. Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-5 were all down-regulated in the skin but up-regulated in the muscle 48 h after incision. Few genes changed in the dorsal root ganglion. Most changes in expression occurred in the first 48 h after incision, a timeframe when pain behavior was the greatest.

Conclusion: Surgical incision is associated with pain-related gene expression changes in skin, muscle, and, to a lesser extent, dorsal root ganglion. The gene expression profile provides clues as to mediators that are involved in peripheral sensitization and pain transmission after surgical incision and also suggest mechanisms for resolution of postoperative pain when more persistent pain syndromes like neuropathic pain continue.

NEW analgesic therapies devoid of serious side effects are needed to advance perioperative pain management. In effort to develop new therapies, we must discover factors potentially contributing to incisional pain, evaluate their time course in relation to pain, and understand how these substances affect nociception. Several mediators such as nerve growth factor (NGF), complement, prostaglandins, and lactic acid have been evaluated and likely contribute to nociception after incision in animal models.

Evaluation of pain mediators from surgical incisions in patients is restricted to serum and wound exudates. However, in animal models, gene expression profiles can be studied in injured tissues and dorsal root ganglion (DRG) innervating those tissues. Array technology or targeted gene expression profiles have been used to evaluate the patterns of nociceptive mediators in preclinical neuropathic pain models like sciatic nerve transection, nerve root ligation, and spinal nerve transection, but not in incisional pain.

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What We Already Know about This Topic

- Inflammatory product and neurotrophin genes are upregulated in peripheral tissues, including peripheral nerves in animal models of chronic inflammation or nerve injury, but a survey of such genes after incisional injury has not been performed.

What This Article Tells Us That Is New

- Using a targeted array for 84 genes often altered in pain states, incisional injury was shown in rats to increase proinflammatory interleukins in skin as well as nerve growth factor and artemin in skin and muscle.
- The pattern of gene upregulation observed would be expected to result in and contribute to hypersensitivity and pain after incisional surgery.

NEW analgesic therapies devoid of serious side effects are needed to advance perioperative pain management. In effort to develop new therapies, we must discover factors potentially contributing to incisional pain, evaluate their time course in relation to pain, and understand how these substances affect nociception. Several mediators such as nerve growth factor (NGF), complement, prostaglandins, and lactic acid have been evaluated and likely contribute to nociception after incision in animal models.

Evaluation of pain mediators from surgical incisions in patients is restricted to serum and wound exudates. However, in animal models, gene expression profiles can be studied in injured tissues and dorsal root ganglion (DRG) innervating those tissues. Array technology or targeted gene expression profiles have been used to evaluate the patterns of nociceptive mediators in preclinical neuropathic pain models like sciatic nerve transection, nerve root ligation, and spinal nerve transection, but not in incisional pain.

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Global expression patterns can be ascertained using high-density oligonucleotide microarrays, but suffer from false positives, and difficulty in determining the thresholds for biologic relevance. Recent meta-analysis of microarray studies in neuropathic and inflammatory pain models suggest that immune-related genes are highly regulated across different pain models and have also identified new candidate genes.14

Because sensory neurons from different tissues respond uniquely to injury,15 the study of tissue-specific expression of nociception-related genes will advance our understanding of postoperative pain. In the current study, we examined pain behaviors and gene expression after plantar incision of the rat hind paw. We hypothesized that neurotrophin expression would be modulated by surgical incision in incised tissues. We determined the time course of expression of 84 genes from the neurotrophin and inflammatory cytokine families in skin, muscle, and DRG using quantitative, real-time reverse transcription polymerase chain reaction (RT-PCR) using a commercially available, targeted array.

Materials and Methods

Animals
This study was approved by the Institutional Animal Care and Use Committee at the University of Iowa (Iowa City, Iowa). Male Sprague-Dawley adult rats (Harlan, Indianapolis, IN) weighing 275−300 g were housed two per cage with a 12-h light-dark cycle. Food and water were available ad libitum. All experiments, including incision, behavioral testing, tissue isolation, and processing, were carried out by the same person (CMS) to reduce interindividual total variability. A total of 46 rats were used for this study; the behavior testing used six rats and the gene expression analysis used eight rats per time-point (four rats per group). Data from all rats were included in the analysis.

Plantar Incision
The rat hind paw plantar incision model of postoperative pain was used as previously described.16 Briefly, rats were anesthetized with 2% isoflurane delivered via nose cone. The plantar aspect of the right hind paw was prepared with povidone iodine and a 1.0-cm longitudinal incision was made 0.5 cm from the end of the heel. The underlying flexor muscle was increased and incised longitudinally, allowing the muscle origin and insertion to remain intact. After hemostasis with gentle pressure, the skin was closed with two mattress sutures of 5-0 nylon on a FS-2 needle. Antibiotic ointment was applied to the incision immediately after surgery. Sutures of 5-0 nylon on a FS-2 needle. Antibiotic ointment was applied to the incision immediately after surgery. Sutures were removed on postoperative day 2. Another group of rats served as controls and underwent sham surgery in which all procedures were performed except they did not undergo incision.

Tissue Harvest
Gene expression was examined 1 h, 4 h, 1 day, 2 days, and 10 days after incision or sham surgery. The times when pain behaviors are greatest after incision, postoperative days (POD) 0−2, were chosen to evaluate changes in gene expression. In addition, a time when pain behaviors have resolved, 10 days after incision, was chosen to determine if the gene expression had recovered. To collect tissues for real time RT-PCR, rats were deeply anesthetized with isoflurane (4−5%), and right hind paw skin, muscle, and the right-sided L4 and L5 DRG from sham and incised rats were removed and immediately placed in RNA Later® solution (Ambion, Austin, TX). Tissues were stored at 4°C until further processing. Rats were then euthanized.

Behavioral Testing
In a separate group of rats, pain behaviors were tested through POD 10. We elected to use another group of rats in order to minimize any changes in gene expression that might be affected by testing responses to noxious stimuli. Rats were acclimated to the testing environment for 3 days. Baseline pain scores were measured and rats underwent plantar incision.

Guarding Pain
A cumulative pain score was used to assess nonevoked pain behaviors as described previously.17 Briefly, unrestrained rats were placed on a plastic mesh floor (8 × 8 mm). The incised and nonincised paws were viewed. Both paws of each animal were closely observed during a 1-min period repeated every 5 min for 1 h. Depending on the position in which each paw was observed during the majority of the 1-min scoring period, a 0, 1, or 2 was given. Full weight-bearing of the paw (score = 0) was present if the wound was blanched or distorted by the mesh. If the paw was completely off the mesh, a score of 2 was recorded. If the area of the wound touched the mesh gently without any blanching or distorting, a 1 was given. The sum of the 12 scores (0–24) obtained during the 1-h session for each paw was obtained. The difference between the scores from the incised paw and nonincised paw is the cumulative pain score for that 1-h period. This test was performed before evoked tests were undertaken to minimize influence of evoked tests on guarding.

Withdrawal Response to Mechanical Stimulation
Unrestrained rats were placed on mesh (grid 12 × 12 mm) and allowed to acclimate. Withdrawal responses to mechanical stimuli were determined using calibrated von Frey filaments applied from underneath the cage through openings in the plastic mesh to an area adjacent to the wound. Each filament was applied once, and increasing forces were applied until a withdrawal response was achieved or the 522 mN filament was reached. Each test was repeated after a 5-min test-free interval, for a total of three tests per rat. The lowest force from the three tests producing a response was considered the withdrawal threshold.

Withdrawal Latency to Heat
Rats were placed individually on a glass floor (3 mm) covered with clear plastic cage and allowed to acclimate. Withdrawal
latencies to radiant heat were assessed by applying a focused radiant heat source underneath a glass floor on the middle of incision. Before the experiments began, the intensity of the heat was adjusted to produce withdrawal latency in normal rats of 12–15 s. The latency time to evoke withdrawal was determined with a cut-off value of 20 s. Each rat was tested at least three times, at an interval of 10 min. The average of three trials was used to obtain paw withdrawal latency.

**Real-time Quantitative RT-PCR**

Total RNA was extracted with TRIzol® (Invitrogen, Carlsbad, CA) followed by DNase treatment with Turbo Free DNase® (Ambion), using the manufacturer’s recommended protocols. The samples underwent column purification using the RNeasy mini kit® (Qiagen, Valencia, CA). Total RNA underwent spectrophotometric analysis using a Nanodrop 1000™ and samples with A260/A280 ratio greater than 1.7 were used. Random samples underwent further analysis with an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) to assess RNA quality via confirmation of sharp peaks of 18S and 28S ribosomal RNA without “shoulders” or degradation. Reverse transcription of 500 ng of total RNA was carried out using First Strand Kit (SABiosciences, Frederick, MD), according to manufacturer’s directions. Complementary DNA was combined with a RT-PCR master mix containing SYBR® green (Life Technologies, Grand Island, NY) and ROX™ (Affymetrix, Santa Clara, CA) (loading control) and plated with primers for 84 different neurotrophins, neurotrophin receptors, and related growth factor genes (SABiosciences). Controls wells were used to assess reverse transcription efficiency, presence of genomic DNA, and positive RT-PCR controls. Those that failed control checks were removed from evaluation. Housekeeping genes included (1) hypoxanthine guanine phosphoribosyl transferase, (2) ribosomal protein L13A, (3) lactate dehydrogenase A, (4) β-actin, and (5) ribosomal protein, large, P1. All five housekeeping genes were included in the calculation for relative gene expression, because none changed with incision. Two-step RT-PCR was performed on a 7900 Sequence Detection system (Applied Biosystems, Foster City, CA). Thermal cycling was initiated with an initial incubation at 95°C for 10 min, followed by 40 cycles with each cycle consisting of 95°C for 15 s and 60°C for 1 min. Dissociation curves were run for each gene and the presence of only one peak was confirmed for each gene of interest.

**Behavior Data Analysis**

All behavioral data were analyzed using Prism 5.0 software (GraphPad Software, Inc., San Diego, CA). Withdrawal threshold to mechanical stimuli are noncontinuous and therefore were analyzed with nonparametric tests. The data were expressed as median and interquartile range. Differences were determined by Friedman’s ANOVA followed by Dunn’s *post hoc* test for comparing paw withdrawal threshold after incision with baseline. For guarding pain scores and withdrawal latencies to heat, data were expressed as mean ± SEM. One-way ANOVA followed by Dunnett’s *post hoc* test was used for comparisons between baseline and incision.

**Gene Expression Data Analysis**

All gene expression data were analyzed with the ABI SDS RQ Manager 1.2 software (Applied Biosystems). Each value is the average of tissue from four different animals. The formula used to calculate the relative gene expression level was the ΔΔCt method described in detail by Livak and Schmittgen. In this analysis, we increased the stringency for calling significant changes in gene expression by increasing the threshold value, δ(Δ). Using Δ = 1.2 allows for the most highly ranked genes to be further analyzed, and this value of δ has been previously shown to have the highest correlation with definitive measures of expression. The false-discovery rate was defined as the percentage of falsely significant genes compared with the genes called significant. For reported genes, we maintained strict thresholds (Δ = 1.2) aiming for a false-discovery rate close to zero. Data for all tissues and times after incision are contained within the Supplemental Digital Content (see Supplemental Digital Content 1, tables 1–3, http://links.lww.com/ALN/A852). The supplemental content also has the additional significance analysis of microarray analyses with R values (R = fold change) set to 1.5 for those readers interested in the most robustly changed genes (see Supplemental Digital Content 2, tables 1–3, http://links.lww.com/ALN/A853, which contain the false discovery rates and lists of significant genes).

**Results**

**Pain Behaviors**

Similar to previous studies, the mean guarding pain score was increased after surgery through the first 2 postoperative days. The scores were 0.5 ± 0.5, 18.8 ± 2.6, 22.5 ± 1.0, 18.3 ± 2.0, and 17.2 ± 3.1 at baseline, POD 1 h, POD 4 h, POD 1, and POD 2, respectively (*P < 0.001, n = 6, fig. 1A). The
median withdrawal threshold decreased from 522 mN to 13 mN at 1 h postincision ($P < 0.01$, $n = 6$, fig. 1B), and recovered by day 10. The average heat withdrawal latencies were $16.8 \pm 1.4$, $3.1 \pm 1.0$, $2.5 \pm 0.4$, $3.1 \pm 0.4$, $4.7 \pm 1.2$, and $14.3 \pm 1.7$ s at baseline, POD 1 h, POD 4 h, POD 1, and POD 2, respectively ($P < 0.05$, $n = 6$, fig. 1C). Pain-related responses were greatest within 24 h after incision and all were not different than baseline by POD 10.

**Gene Expression Trends**

Compared with the sham group, plantar incision regulated 51 of 84 neurotrophin and cytokine profiles in the skin on POD 1. Of these, 16 were increased by 1.5-fold or more, nine were down-regulated, and 68 were unchanged (fig. 2). In muscle, one was upregulated, 11 were down-regulated, and 72 were unchanged. In DRG, none were changed. On POD 2, muscle was more reactive than skin; 10 factors were upregulated, zero were down-regulated, and 74 were unchanged in skin, whereas in muscle, 41 were upregulated, four were down-regulated, and 39 were unchanged. There were no changes in DRG. The magnitude of regulation was 2- to 90-fold in the skin, and 2- to 4,400-fold in the muscle. Altogether, more genes were up-regulated in the skin compared with muscle; muscle regulation was mostly down-regulation, except on POD 2, when there was marked up-regulation of many genes. The overall relative magnitude of effect was greatest in the muscle, modest in the skin, and absent in the DRG after plantar incision.

**RT-PCR Analysis of Pronociceptive Factors**

The GDNF family of neurotrophins, which includes GDNF and artemin, are each expressed in skin, muscle, and DRG (fig. 3). In general, artemin expression was increased in both skin and muscle after incision. In skin, artemin messenger RNA (mRNA) was increased 4 h after incision (3.3 $\pm$ 1.3-fold, $^*P < 0.05$, fig. 3A). In incised muscle, artemin was increased by 4 h (1.8 $\pm$ 0.3 at 4 h, $^*P < 0.05$, fig. 3B). GDNF expression in incised muscle was markedly increased on postoperative day 2 (97.9 $\pm$ 31.3, $^*P < 0.05$, fig. 3D).

The NGF family of neurotrophins, NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-5 (NT-5), have been evaluated in several persistent pain models. These neurotrophins decreased in skin after incision (figs. 4 and 5), except NGF, which was increased, a finding supported by previous studies. In skin, the increase in NGF was apparent even 10 days after incision (3.47 $\pm$ 1.44 in skin, $^*P < 0.05$, fig. 4A). BDNF decreased after incision in the skin. The decrease in skin was apparent from 4 h through 48 h after incision (fig. 4B). In muscle, BDNF was initially down-regulated (20.0 $\pm$ 0.3-fold, $^*P < 0.05$) followed by marked up-regulation on POD 2 (351.5 $\pm$ 82.1, $^*P < 0.05$, fig. 4D). NT-3 and NT-5 were decreased in the skin at 24 h after incision (figs. 5A and B). In muscle, NT-3 and NT-5 were increased at 48 h (figs. 5C and D).

The pronociceptive interleukins (IL), IL-6 and IL-1$\beta$, were markedly increased in skin after plantar incision; the range of the peak increase was from 10- to 200-fold (fig. 6). In skin, IL-6 peaked immediately after incision (241.3 $\pm$ 41.7-fold, fig. 6A) and remained increased through the first 2 days. IL-1$\beta$ also remained increased in skin through POD 2,
but the peak occurred later, at 4 h (87.0 ± 35.4-fold, fig. 6B).
In incised plantar muscle, the gene expression of IL-6 and IL-1β were also increased; the magnitude of increases was similar to skin but the peak expression tended to occur later (figs. 6D and E).

The mRNAs for neurotrophin receptors were present in skin and muscle. Receptors for the GDNF family were generally down-regulated in skin (table 1). For muscle, GFRα1 was down-regulated for 2 days after incision, whereas GFRα2 and GFRα3 were up-regulated at 48 h. The receptors for the NGF family of neurotrophins also changed after incision. TrkA was down-regulated in skin and muscle for the first 24 h, whereas p75 was increased in muscle (table 1). In the DRG, few genes changed after plantar incision, yet none were significant using significance analysis of microarrays set at $\Delta = 1.2$ (see Supplemental Digital Content 2, table 3, http://links.lww.com/ALN/A853). Several genes were found to be significantly regulated in all three tissues at some point during the 10-day study period (table 2).

**Discussion**

Pronociceptive growth factors such as artemin, GDNF, and NGF are potential therapeutic targets for the treatment of postoperative pain. NGF monoclonal antibodies have been used in human clinical trials to treat various chronic pain conditions with moderate success, but have not been reported for clinical acute pain conditions such as postoperative pain.21,22 We found that expression of pronociceptive growth factors and immune-related genes were increased in incisions but, in general, up-regulated to a greater extent and for a longer period of time in muscle compared with skin. Other neurotrophins, like NT-3, NT-5, and BDNF, could contribute to nociception after surgery as well, but the effect of these factors on sensory afferents, peripheral or central sensitization, and plasticity has not been as extensively studied. Importantly, these pronociceptive growth factors were concomitantly up-regulated in incisions where they could interact to contribute to nociception. Combinations of pro-

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**Fig. 2.** Three-dimensional summary plate layouts depicting fold gene regulation for all genes studied. Data are expressed as fold difference of incision/sham. Values greater than 1.00 represent up-regulation after incision compared with sham. Values less than 1.00 represent down-regulation after incision compared with sham surgery. Each bar represents the average fold regulation from eight animals (four incision and four sham). DRG = dorsal root ganglion; POD = postoperative day.
nociceptive factors have been measured in experimental human pain models as well as after surgical incision. The combination of NGF and artemin increases the duration of heat hyperalgesia compared with either alone. The observed increase in the expression of several neurotrophins within 48 h may contribute to sensitization and plasticity early after surgery, when pain behaviors and clinical postoperative pain are greatest.

Expression of Glial-derived Neurotrophic Factor Family Ligands

Artemin is thought to be pronociceptive. It belongs to the GDNF family of neurotrophic factors, which promote differentiation and survival of motor, sympathetic, and large and small sensory neurons during development. Artemin expression is increased after inflammation in skin and visceral tissues and sensitization of nociceptors occurs after acute artemin administration or with congenital overexpression. Pain-related behaviors are also transiently increased after local artemin administration.

After incision, artemin expression was immediately increased in skin. Muscle artemin expression was increased through 24 h and could contribute to deep tissue pain; however, artemin and muscle nociception have not been studied. In addition to pain, artemin could support restoration of sensory function after incision, which includes tissue and nerve injury, or recovery of sympathetic innervation in blood vessels. These other roles for artemin could contribute to repair in incised tissues, a response that may be most pronounced in muscle tissue.

GDNF sensitizes nociceptors in a variety of pain models, but few studies have been able to evaluate the role of GDNF in pathophysiologic models by interfering with its action(s). Many pain studies have examined responses to intrathecally administered GDNF; however, GDNF was not up-regulated in the DRG, and so spinal effects of GDNF are not likely germane to incisional nociception. GDNF may have a significant role in neuropathic pain models, including partial sciatic nerve ligation or spinal nerve ligation, brachial plexus avulsion, or diabetes. After incision, GDNF mRNA increased in muscle, suggesting tissue-specific regulatory pathways for expression after incision.
Expression of NGF Family Ligands and Receptors

NGF is pronociceptive. In human volunteers, NGF produced myalgias and localized pain at the injection site and was not therapeutic in clinical trials for diabetic neuropathy. In animal models, NGF contributes to sensitization after inflammation, tissue damage, and nerve injury. NGF is increased in skin and muscle following incision. Pain behaviors after incision can be reduced by NGF sequestration demonstrating a pronociceptive role. The current study demonstrated sustained NGF mRNA up-regulation in both skin and muscle consistent with our previous protein measurements. This greater duration of increased mRNA expression for NGF in the present study may be a result of using five control, housekeeping genes.

The neurotrophin receptors include TrkA, TrkB, and p75 (also known as the low-affinity NGF receptor). All the neurotrophins can bind to p75 and at least one of the Trk receptors. The TrkA receptor is the high-affinity receptor for NGF, and signals nociceptive properties of NGF; p75 also is pronociceptive. We found that TrkA and p75 mRNA were down-regulated in skin. These changes may be because of reciprocal regulation such as ligand-receptor coupling down-regulating the receptor. The receptors for NGF were up-regulated in muscle at 48 h at a time when ligand and pain behaviors caused by muscle incision were also up-regulated. The differences in muscle expression of NGF and its cognate receptors may contribute to greater activation of nociceptive sensory neurons by incision that includes muscle. These receptors

Table 1. Fold Change in Gene Expression for Neurotrophin Receptors in Skin and Muscle after Hindpaw Incision

<table>
<thead>
<tr>
<th>Gene</th>
<th>1 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>10 Days</th>
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<tbody>
<tr>
<td>Skin</td>
<td></td>
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<tr>
<td>GFRα1</td>
<td>1.94 ± 0.30 (0.330)</td>
<td>2.38 ± 0.18 (0.096)</td>
<td>4.98 ± 0.05 (0.001)</td>
<td>2.18 ± 1.59 (0.325)</td>
<td>3.03 ± 0.79 (0.005)*</td>
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<tr>
<td>GFRα2</td>
<td>1.05 ± 0.42 (0.896)</td>
<td>1.06 ± 0.16 (0.728)</td>
<td>1.18 ± 0.14 (0.224)</td>
<td>2.06 ± 0.59 (0.445)*</td>
<td>1.42 ± 0.69 (0.499)</td>
</tr>
<tr>
<td>GFRα3</td>
<td>1.06 ± 0.56 (0.897)</td>
<td>2.23 ± 0.11 (0.020)</td>
<td>5.86 ± 0.08 (0.009)*</td>
<td>4.19 ± 0.16 (0.083)</td>
<td>1.17 ± 0.28 (0.527)</td>
</tr>
<tr>
<td>Trk A</td>
<td>1.56 ± 2.28 (0.723)</td>
<td>1.97 ± 0.16 (0.077)</td>
<td>15.50 ± 0.05 (0.015)†</td>
<td>3.69 ± 0.14 (0.081)</td>
<td>3.20 ± 0.25 (0.189)</td>
</tr>
<tr>
<td>Trk B</td>
<td>1.07 ± 1.10 (0.938)</td>
<td>1.70 ± 0.26 (0.279)</td>
<td>3.22 ± 0.13 (0.036)†</td>
<td>3.48 ± 0.25 (0.206)</td>
<td>1.36 ± 0.56 (0.700)</td>
</tr>
<tr>
<td>p75</td>
<td>1.38 ± 0.34 (0.514)</td>
<td>2.54 ± 1.10 (0.075)</td>
<td>1.70 ± 0.27 (0.297)</td>
<td>2.38 ± 1.60 (0.244)</td>
<td>3.24 ± 0.88 (0.005)*</td>
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<table>
<thead>
<tr>
<th>Muscle</th>
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<tbody>
<tr>
<td>GFRα1</td>
<td>1.03 ± 0.34 (0.938)</td>
<td>1.89 ± 0.13 (0.039)†</td>
<td>5.88 ± 0.08 (0.009)†</td>
<td>10.34 ± 0.07 (0.041)†</td>
<td>2.79 ± 1.33 (0.075)</td>
</tr>
<tr>
<td>GFRα2</td>
<td>1.47 ± 0.17 (0.204)</td>
<td>1.00 ± 0.17 (0.991)</td>
<td>1.14 ± 0.34 (0.744)</td>
<td>42.52 ± 11.29 (0.003)*</td>
<td>1.23 ± 0.50 (0.625)</td>
</tr>
<tr>
<td>GFRα3</td>
<td>1.58 ± 1.25 (0.621)</td>
<td>1.42 ± 0.45 (0.603)</td>
<td>3.26 ± 0.35 (0.342)</td>
<td>50.81 ± 25.47 (0.002)*</td>
<td>1.26 ± 0.37 (0.638)</td>
</tr>
<tr>
<td>Trk A</td>
<td>3.11 ± 0.33 (0.312)</td>
<td>3.15 ± 0.11 (0.014)†</td>
<td>17.88 ± 0.07 (0.061)</td>
<td>448.04 ± 142.05 (0.00007)*</td>
<td>3.60 ± 0.11 (0.016)</td>
</tr>
<tr>
<td>Trk B</td>
<td>1.64 ± 0.50 (0.599)</td>
<td>1.77 ± 0.23 (0.205)</td>
<td>2.09 ± 0.33 (0.329)</td>
<td>1.83 ± 0.92 (0.358)</td>
<td>1.26 ± 0.54 (0.617)</td>
</tr>
<tr>
<td>p75</td>
<td>1.21 ± 0.34 (0.690)</td>
<td>3.62 ± 1.84 (0.044)*</td>
<td>1.16 ± 0.27 (0.656)</td>
<td>159.26 ± 16.53 (0.00002)*</td>
<td>2.83 ± 1.38 (0.077)</td>
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Data are expressed as mean fold change (incision/sham) ± SD. Values in parenthesis are the P value.
* Significantly up-regulated. † Significantly down-regulated.

Fig. 6. Time course of gene expression for interleukin mRNA in skin (A–C) and muscle (D–F) following plantar incision. Data are expressed as mean fold change (incision/sham) ± SD. *P < 0.05 versus sham, n = 4 per group. IL = interleukin.
may also contribute to muscle repair,\(^{49}\) apoptosis of sensory neurons,\(^{50}\) or regeneration of injured axons.\(^{47}\)

BDNF, NT-3, and NT-5 influence nociceptor function. Most studies on the nociceptive actions of BDNF have largely been in the central nervous system, but the gene and protein are constitutively expressed in DRG neurons. BDNF expression can be up-regulated by NGF.\(^{51}\) BDNF is down-regulated in small DRG neurons after axotomy, but up-regulated in large-diameter DRG neurons.\(^{52}\) BDNF can sensitize c-fibers to noxious heat in vitro.\(^{53}\) The therapeutic efficacy of delivering BDNF to DRG in painful neuropathy has shown promising results in animals.\(^{54}\) In the current study, we find that incision decreased BDNF expression over a time course where pain behaviors were the greatest, and future work could involve replacing BDNF in incised tissues as a potential analgesic treatment.

Neurotrophin-3 (NT-3) has antinociceptive properties in the peripheral nervous system. NT-3 reverses inflammatory hyperalgesia after complete Freund’s adjuvant,\(^{55}\) attenuates pain behaviors after nerve crush injury,\(^{56}\) and reverses mechanical hyperalgesia after intramuscular acid injection.\(^{57}\) We demonstrate down-regulation of NT-3 in skin and muscle 24 h after incision, with a rebound increase only in muscle at 48 h after incision, patterns that may augment pain behavior after incision early and lead to recovery at later time periods. Neurotrophin-5 (NT-5) may have pronociceptive properties. Intraplantar injection of NT-5 induced thermal hyperalgesia in rats, an effect that was sustained for 24 h.\(^{53}\) Like NGF, NT-5 decreased heat threshold in c-fibers without altering mechanical responses.\(^{58}\) In our study, NT-5 was down-regulated in skin, but up-regulated in muscle, again peaking at 48 h after incision.

### Expression of Other Neurotrophin Receptors

Other receptors for the neurotrophins changed after incision (table 3). The receptor for artemin, GFR\(\alpha\)-3, was down-regulated after incision. GFR\(\alpha\)-3 increased at 48 h when the ligand mRNA was decreased, following a similar pattern to NGF and its receptors. The etiology of this biphasic regulation is unclear, but may represent negative feedback autoregulation similar to other growth factor receptors within the central nervous system.\(^{59}\)

### RT-PCR Analysis of Interleukins

Inflammatory cytokines, including IL-1\(\beta\) and IL-6, are increased in inflammatory pain models and produce peripheral and central sensitization and hyperalgesia.\(^{60,61}\) IL-1\(\beta\) has a time course where pain behaviors were the greatest, and future work could involve replacing BDNF in incised tissues as a potential analgesic treatment.

### Table 2. Genes Regulated in All Three Tissues (Skin, Muscle, and Dorsal Root Ganglion) after Plantar Incision

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>1 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>10 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemin</td>
<td>1.14 ± 0.20 (0.45)</td>
<td>1.03 ± 0.15 (0.87)</td>
<td>-1.19 ± 0.12 (0.26)</td>
<td>-1.15 ± 0.18 (0.59)</td>
<td>1.02 ± 0.20 (0.94)</td>
</tr>
<tr>
<td>BDNF</td>
<td>1.27 ± 0.28 (0.22)</td>
<td>-1.02 ± 0.15 (0.92)</td>
<td>-1.20 ± 0.14 (0.31)</td>
<td>1.39 ± 0.56 (0.44)</td>
<td>2.51 ± 1.39 (0.22)</td>
</tr>
<tr>
<td>GDNF</td>
<td>-1.05 ± 0.55 (0.91)</td>
<td>1.27 ± 0.83 (0.73)</td>
<td>-1.23 ± 0.30 (0.59)</td>
<td>2.02 ± 1.40 (0.36)</td>
<td>-1.69 ± 0.14 (0.08)</td>
</tr>
<tr>
<td>Gfra1</td>
<td>1.12 ± 0.25 (0.60)</td>
<td>1.13 ± 0.13 (0.34)</td>
<td>-1.19 ± 0.07 (0.08)</td>
<td>1.13 ± 0.24 (0.65)</td>
<td>1.47 ± 0.57 (0.43)</td>
</tr>
<tr>
<td>Gfra2</td>
<td>1.01 ± 0.20 (0.97)</td>
<td>1.02 ± 0.08 (0.86)</td>
<td>-1.20 ± 0.06 (0.04)</td>
<td>1.12 ± 0.31 (0.72)</td>
<td>1.25 ± 0.43 (0.59)</td>
</tr>
<tr>
<td>Gfra3</td>
<td>-1.10 ± 0.28 (0.71)</td>
<td>1.00 ± 0.07 (0.99)</td>
<td>-1.06 ± 0.13 (0.66)</td>
<td>-1.05 ± 0.22 (0.87)</td>
<td>1.56 ± 0.78 (0.49)</td>
</tr>
<tr>
<td>IL-10</td>
<td>4.26 ± 3.49 (0.07)</td>
<td>-2.10 ± 0.20 (0.12)</td>
<td>-1.21 ± 0.46 (0.74)</td>
<td>-1.38 ± 0.47 (0.66)</td>
<td>-2.04 ± 0.34 (0.41)</td>
</tr>
<tr>
<td>IL-1(\beta)</td>
<td>2.46 ± 1.54 (0.15)</td>
<td>-1.01 ± 0.28 (0.97)</td>
<td>1.06 ± 0.30 (0.86)</td>
<td>1.18 ± 0.42 (0.68)</td>
<td>-28.29 ± 0.05 (0.06)</td>
</tr>
<tr>
<td>IL-6</td>
<td>4.15 ± 6.04 (0.28)</td>
<td>1.48 ± 0.58 (0.36)</td>
<td>-1.36 ± 0.15 (0.18)</td>
<td>35.09 ± 41.54 (0.33)</td>
<td>3.61 ± 1.46 (0.02)</td>
</tr>
<tr>
<td>NGF</td>
<td>1.30 ± 0.29 (0.21)</td>
<td>-1.38 ± 0.22 (0.32)</td>
<td>-1.02 ± 0.44 (0.97)</td>
<td>1.04 ± 0.18 (0.85)</td>
<td>1.07 ± 0.18 (0.73)</td>
</tr>
<tr>
<td>p75</td>
<td>1.20 ± 0.33 (0.51)</td>
<td>1.35 ± 0.08 (0.00)*</td>
<td>1.21 ± 0.19 (0.27)</td>
<td>-1.05 ± 0.28 (0.89)</td>
<td>2.03 ± 1.21 (0.36)</td>
</tr>
<tr>
<td>NT-3</td>
<td>-1.34 ± 0.36 (0.31)</td>
<td>-1.28 ± 0.28 (0.52)</td>
<td>-1.67 ± 0.12 (0.04)</td>
<td>-1.15 ± 0.09 (0.20)</td>
<td>1.46 ± 0.69 (0.53)</td>
</tr>
<tr>
<td>NT-5</td>
<td>-1.54 ± 0.45 (0.15)</td>
<td>1.42 ± 0.70 (0.50)</td>
<td>1.48 ± 0.52 (0.3)</td>
<td>-1.34 ± 0.29 (0.49)</td>
<td>1.76 ± 0.92 (0.39)</td>
</tr>
<tr>
<td>trkA</td>
<td>-1.09 ± 0.63 (0.89)</td>
<td>1.17 ± 0.27 (0.54)</td>
<td>-2.57 ± 0.09 (0.01)</td>
<td>1.10 ± 0.43 (0.84)</td>
<td>-1.55 ± 0.13 (0.13)</td>
</tr>
<tr>
<td>trkB</td>
<td>1.01 ± 0.28 (0.97)</td>
<td>1.25 ± 0.14 (0.08)</td>
<td>-1.25 ± 0.10 (0.13)</td>
<td>-1.17 ± 0.16 (0.53)</td>
<td>-1.04 ± 0.19 (0.86)</td>
</tr>
</tbody>
</table>

Data are expressed as mean fold change (incision/sham) ± SD. Values in parenthesis are the P value.

* Significantly up-regulated.
† Significantly down-regulated.

BDNF = brain-derived neurotrophic factor; GDNF = glial-derived neurotrophic factor; IL = interleukin; NGF = nerve growth factor; NT = neurotrophin.
been shown to be increased in the spinal cord dorsal horn in postoperative pain models, in peri-incisional tissues, and serum of postoperative patients. IL-1β may cause hyperalgesia through the expression or release of pronociceptive compounds such as substance P, prostaglandins, and NGF. IL-1β may directly sensitize sensory neurons to heat. Although IL-1β is considered pronociceptive, the role for IL-1β in nociception after incision is not clear. Using IL-1β knockout mice, pain behavior was reduced in inflammatory and neuropathic models, but not after plantar incision. In contrast, using IL-1β receptor knockout mice and over-expression of the IL-1β receptor antagonists, mechanical hyperalgesia was reduced after incision.

IL-6, a pronociceptive early marker of the inflammatory cascade, can also enhance transient receptor vaniloid 1 activity, a key target in nociceptive heat responses. Similar to IL-1β, IL-6 is increased in inflammatory and neuropathic pain models. Increased levels of peri-incisional and plasma IL-6 are found after surgery in both humans and rodents. The increase in IL-6 expression from skin and muscle parallel observations from surgical wound dialysates and muscle injury models. The therapeutic value of interfering with IL-6 function in incisional pain is not understood.

In contrast, IL-10 is an antiinflammatory cytokine that is increased in surgical wound exudates. In other models of pain, IL-10 can reduce pain behaviors and produce pronociceptive compounds such as IL-6 and IL-1β. The significance of modifying IL-10 expression in incisional pain models is also not known.

In summary, pronociceptive cytokines IL-1β and IL-6 were up-regulated in both skin and muscle early after incision and returned to near-baseline when pain behaviors resolved. The role of these cytokines in muscle and nociception is less clear compared with that of skin where pronociception is well studied. The interplay between antinociceptive and pronociceptive cytokines in surgical wounds is not yet understood; nonnociceptive functions like wound healing, reinnervation, neovascularization, reducing infection, and hemostasis must be considered when function of these cytokines is altered.

**DRG Gene Changes in Other Pain Models**

There were few changes in gene expression in DRG after plantar incision. The time course and changes in neurotrophin mRNA expression in DRG after spinal nerve ligation are markedly different than incision. For example, BDNF increased 2 or 3 days after spinal nerve ligation, whereas NGF was increased fourfold 3 weeks after nerve injury. After sciatic nerve axotomy, BDNF mRNA increased 1.6-fold and NT-3 decreased 1.6-fold 3 days after injury; no other changes in neurotrophins in the DRG were noted. Not all studies have found mRNA changes in the neurotrophin gene families. In two models of neuropathic pain, there were no changes in NGF, BDNF, NT-3, NT-5, GDNF, artemin, or their receptors. The lack of changes in gene expression in DRG is likely related to the small amount of nerve injury after plantar incision compared with a relatively large amount of nerve injury in other pain models. The time courses of changes in DRG and nerve injury models may be one of the factors that contribute to persistent pain after nerve injury, but shorter lived pain after plantar incision.

Other models of peripheral inflammation result in short-lived changes in dorsal root ganglia neurotrophin expression. The mRNA for p75 and trkA were increased 8 and 24 h after injection of complete Freund’s adjuvant without change in NGF, BDNF, GDNF, artemin, NT-5, or trkB. In adult animals, BDNF mRNA increased sixfold in the L4/L5 DRG 1 day after hind paw complete Freund’s adjuvant administration.

**Statistical Considerations**

The expression data and subsequent analyses obtained in these experiments are reported in entirety in Supplemental Digital Content 1 and 2, [http://links.lww.com/ALN/A852](http://links.lww.com/ALN/A852) and [http://links.lww.com/ALN/A853](http://links.lww.com/ALN/A853). The P values are obtained from uncorrected t tests, which may have inflated type-I error rates. The use of a post hoc correction, such as the Bonferroni correction, is excessively conservative because the genes in any biologic sample are not truly independent. Exact P values are reported for all data so that interested readers may apply any correction. We used the significance analysis of microarrays to ascertain differentially regulated genes while allowing for some correction of multiple experiments.

**Conclusion**

Our data suggest that patterns of mRNA expression after incision are unique in magnitude and timing. Furthermore, specific factor expression in incisions is markedly different than other pain models. These factors may not only affect nociceptor activation, but also influence tissue remodeling, wound healing, reinnervation, and the immune response, depending on the tissue. The interaction among factors at different times after incision may contribute to pain after surgery and other processes in perioperative wound homeostasis. Our understanding of the interactions between the sensory, autonomic, and immune systems is incomplete, and further work is needed to determine how these mediators interact in injured tissues. Future studies will examine mechanisms for gene regulation in different tissues, the downstream signaling and subsequent impact of such changes, and a better understanding of how interference with these mediators can alter nociception after injury.

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