Intradermal recombinant human nerve growth factor induces pressure alldynia and lowered heat-pain threshold in humans

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Article abstract—Nerve growth factor (NGF) plays a biologic role in the development and maintenance of sympathetic and small sensory neurons. Because it facilitates nerve fiber regeneration, lowers heat-pain threshold (hyperalgesia), and prevents or improves nerve dysfunction in experimental neuropathy, it is being considered as a putative treatment for certain human polyneuropathies. In 16 healthy subjects, we tested whether intradermal injection of minute doses of recombinant human NGF (1 or 3 μg) compared with saline induces hyperalgesia or alters cutaneous sensation (at the site of injection) as measured by symptom scores, clinical examination, or quantitative sensory testing with Computer Assisted Sensory Examination (CASE IV). Most subjects had, as their only symptom, localized tenderness of the NGF-injected site and only when the site was bumped or compressed. Slight discomfort developed in volar wrist structures (with flexion of fingers) or tenderness of deep structures to palpation over the bicipital groove or supraclavicular region. The Neuropathy Symptoms and Change questionnaire indicated that pressure allodynia was significantly localized to the NGF-injected side from 3 hours to 21 days after injections. Light stroking of the skin did not induce tactile allodynia. Compression of injected sites induced pressure allodynia that occurred more frequently and significantly on the NGF-injected side after 3 hours and was maintained for several weeks. No abnormality of vibratory or cooling detection threshold developed from NGF injection. By contrast, heat-pain threshold (HP 0.5, \( p = 0.003 \)) and an intermediate level of heat-pain (HP 5.0, \( p < 0.001 \)) were significantly lowered 1, 3, and 7 days (and in some cases at 3 hours and 14 and 21 days) after NGF injection. The time course of pressure allodynia and heat-pain hyperalgesia is too rapid to be explained by uptake of NGF by nociception terminals, retrograde transport, and upregulation of pain modulators. Local tissue mechanisms appear to be implicated. It remains to be tested whether recombinant human NGF prevents, stabilizes, or ameliorates small fiber human neuropathies.

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Much has been learned about the role of nerve growth factor (NGF) as a survival and trophic factor for sympathetic and certain classes of sensory neurons.\(^1\) Administration of NGF also prevents or ameliorates sympathetic or peptidergic sensory nerve fiber dysfunction in various experimental polyneuropathies. Apfel et al.\(^2\) found that NGF coadministered with paclitaxel (Taxol) prevented development of polyneuropathy, as judged by normalization of the heat-pain tail flick test and substance P content of spinal ganglia—both increased when paclitaxel was given without NGF. The same authors\(^3\) developed a model of cisplatin neuropathy (recognized by behavioral, biochemical, and electrophysiologic alterations) in mice. These alterations could be prevented or delayed by the coadministration of recombinant human NGF (rhNGF). In a third study, Apfel et al.\(^4\) studied the effect of experimental diabetes on the heat-pain tail flick test and level of substance P and calcitonin-related peptide in spinal ganglia. NGF ameliorated the functional deficits that had developed in experimental diabetes.

To assess whether rhNGF might have a beneficial effect on some human neuropathies, Petty et al.\(^5\) initiated Phase I studies in healthy human subjects. They gave doses from 0.03 to 1 μg/kg intravenously or subcutaneously without causing major adverse reactions. Myalgias occurred at doses above 0.1 μg/kg. Persons who received subcutaneous injections experienced hyperalgesia at the injection site that persisted for a variable time up to 7 weeks and was dose dependent. This hyperalgesia was described as tenderness to touch or to heat at the injection site.

The present study tested whether intradermal (ID) injection of minute doses of rhNGF induces hy-
peralgesia and alterations of thresholds of other modalities of sensation by using quantitative sensory testing with CASE IV (Computer Assisted Sensory Examination, which uses standardized stimuli and algorithms of testing and for which normal limits are known specific for test, site, and certain physical variables). We were also interested in the anatomic distribution and the time course of the sensory alterations to infer mechanisms of action of NGF. The time course of hyperalgesia might be used to distinguish between local mechanisms and central mechanisms (e.g., upregulation of pain peptides from retrogradely transported NGF).

**Methods.**

**Study design.** The study was approved by our Institutional Review Board, and subjects gave written informed consent. Eight healthy subjects had a general medical examination and were given 1 μg of rhNGF ID into the skin of a randomly chosen right or left mid-volar forearm by the nonmasked physician (M.K.B.). An equal volume of saline was injected into the homologous region of the other arm. A square, corresponding in size to the testing thermode, was drawn with a gentian violet indelible pencil on the skin with the ID injection site at its center. The skin marks were refreshed every 2 to 3 days as needed, so testing could be performed at the same sites. After we had shown that 1 μg doses were tolerated, eight additional healthy subjects received 3 μg of rhNGF ID. Subjects were examined medically at baseline and after completion of the rhNGF trial by personnel (M.K.B. and K.A.L.) who knew the randomization schedule, performed the injections, and took responsibility for care of the patients. The examination included a comprehensive battery of hematologic and biochemical tests of blood and urine. Antibodies to NGF were measured before and 18 days after the NGF injection. Symptoms and findings were monitored by masked observers (P.J.D. and D.A.G.). The evaluations were done, in duplicate (at the beginning of the study) and at 3 hours, 1 and 3 days, and weekly until symptoms or abnormal findings had disappeared.

**Neuropathic symptoms.** The Neuropathy Symptoms and Change (NSC), a 38-item true and false questionnaire of symptoms encountered in peripheral neuropathy, was completed by one of the observers at each evaluation. The test was separately administered for the right and left arms. The questions take the general form, “Do you experience (the symptom inquired after) to an abnormal degree?” If the answer is “yes,” the subject is asked whether it is mild (1), moderate (2), or severe (3). Irrespective of the answer to the primary question, the subject is then asked whether it is the same, better, or worse than at the preceding evaluation (a previous time set by the examiner). For the first evaluation, comparisons were made to 1 week earlier. For later evaluations, comparisons were to the preceding evaluation. If the answer is better or worse, the degree of change (mild [+1 or −1], moderate [+2 or −2], or severe [+3 or −3]) is recorded. Scores include number of symptoms—sum of severity responses and sum of the change responses.

**Tactile allosteryia.** The injected sites were stroked with long-fiber cotton wool (cotton wool test). The subject was asked whether the stimulus was felt and whether it elicited any discomfort or pain. In the puff of air test, short (<0.5 second) bursts of compressed air were directed at the injection sites from a distance of approximately 20 cm. The lumen diameter of the plastic straw directing the air was 1 mm. The subject was asked whether the stimulus was felt and whether it was uncomfortable or painful. If the stimulus was uncomfortable or painful, it was to be graded by choosing a number from 1 (the least pain) to 10 (the most severe pain).

**Pressure allodynia.** The pulp of the examiner’s thumb or index finger depressed the skin of the injected site for approximately 1 second. Strong pressure was exerted to indent the skin to a depth of 0.5 to 1 cm. In normal subjects, this does not elicit discomfort or pain. If tenderness or pain was elicited, surrounding tissue of forearm, wrist, hands, elbows, upper arms, shoulders, and neck and trunk was also tested for pressure allodynia. The distribution of tenderness was recorded on a drawing of the human body.

**Vibratory detection threshold assessment.** Vibratory detection threshold was assessed at each of the injection sites at each evaluation using CASE IV designed and fabricated at our medical center and described previously. The 4, 2, and 1 stepping algorithm was used to estimate threshold. Values were expressed as the natural log (ln) of microns of displacement.

**Cooling detection threshold.** Cooling detection threshold was assessed at each of the two injection sites at each evaluation using CASE IV (see preceding section). The 4, 2, and 1 stepping algorithm was used.

**Estimating heat-pain threshold.** The CASE IV system was used to estimate heat-pain threshold (HP 5.0) and an intermediate heat-pain response (HP 5.0) by using a standard nonrepeating ascending with null stimuli algorithm.

**Analysis of data.** Standard statistics were used to assess for differences at various times after rhNGF (or saline) injection and at baseline between rhNGF- and saline-injected sides.

**Results.**

**Patient characteristics and systemic symptoms.** Thirteen men and three women volunteered for these studies (average age, 41.6 ± 13.5 [SD] years; range, 18 to 66 years). Typically, subjects did not experience any systemic symptoms. No hematologic, urinary, or biochemical abnormalities developed.

Sera were analyzed for antibodies to NGF on the day of NGF injection and 18 days later. None of the 32 sera samples had detectable antibodies to NGF.

In one subject, a 52-year-old man, a severe pulsating headache developed with nausea and vomiting within 30 minutes of NGF injection, but later he reported that prodromata of migraine headache, which he was subject to, had preceded the injection of the NGF. He experienced a severe migraine headache after the NGF injection, which was otherwise typical of his headaches.

In another subject, a 34-year-old physician, upper abdominal tightness and nausea and generalized moderately severe myalgias developed that persisted for 2 days. Physicians in our Community Internal Medicine Division had encountered concurrent patients with an influenza-like syndrome and myalgias, but the degree of myalgia in our subject seemed greater than in these patients. Additionally, he did not develop fever or symptoms of an upper
respiratory infection. We suspect that the NGF accentuated the symptoms of influenza, but possibly all symptoms were due to NGF.

Symptoms or examination abnormalities, to be described below, disappeared to statistically significant degrees by 28 days. A few subjects had minor findings to 8 weeks.

Neuropathic symptoms. Based on physician inquiry, 10 of 16 subjects, at one or more times, reported "tenderness," "soreness," or "hurting" with "touch" or "bumping" of the rhNGF-injected site. At one or more evaluations, 4 of 16 subjects also reported some discomfort with flexion of fingers or wrists. Two subjects also reported intermittent and mild tenderness in the medial aspect of the upper arm related to contact or pressure. One subject reported tenderness to contact in the supraclavicular region. Two subjects reported slight burning at the injection site when the forearm was immersed in hot water. No symptoms were reported for the saline-injected arm.

Figure 1 shows the percentage of injected arms with symptoms, as elicited by the NSC questionnaire. By 3 hours and for each subsequent evaluation to and including 21 days, the rhNGF-injected side was found to have significantly more frequent neuropathic symptoms (pressure tenderness) than at baseline. Symptoms were not significantly more frequent than at baseline on the saline-injected side for any postinjection time. Significantly higher frequencies of neuropathic symptoms for the rhNGF compared with the saline-injected side were found at 1, 3, 7, 14, and 21 days.

When the change in severity of symptoms (compared with the previous evaluation) was plotted, significant worsening was recognized at 3 hours and 1 day. Significant improvement occurred at days 7 and 14 (see figure 1). A statistically significant difference in number of symptoms or change was not found between 1 and 3 μg of rhNGF except for a greater change with 3 μg of NGF at 21 days.

Allodynia from cotton wool or puff of air. No tactile allodynia was induced from stroking the skin with cotton wool or from blowing air over either the rhNGF- or saline-injected skin.

Pressure allodynia. For the saline-injected side, no significant increase above baseline was found for any of the postinjection times. By contrast, for the NGF side, pressure allodynia was already significantly increased by 3 hours; was highest at 3, 7, and 14 days; and remained significantly increased at 21 days (figure 2).

The area of pressure allodynia remained at baseline for all postinjection times on the saline-injected side. The area of pressure allodynia for the rhNGF-injected side was significantly greater than for the saline-injected side at 1, 3, 7, and 14 days.

Heat-pain responses. The heat-pain threshold (HP 0.5) was slightly but significantly above baseline on the saline-injected side at 14 and 21 days. HP 5.0 was not significantly higher than baseline for any postinjection times for the saline-injected side. In contrast, striking decreases of HP 0.5 and HP 5.0 were found on the NGF-injected side. Highly significant lowering of threshold (compared with baseline and compared with the saline-injected side) was found for 1, 3, and 7 days and in some cases even for 3 hours and 14 and 21 days (figure 3).

Vibration and cooling detection threshold. The vibration and cooling detection thresholds were not significantly different in the rhNGF- and saline-injected sides for any evaluation time (not illustrated).

Discussion. We found that ID injection of small amounts of rhNGF induced pressure but not tactile, allodynia, and heat-pain hyperalgesia. Vibratory and cooling detection thresholds were not changed. With the exception of one subject in whom myalgia may have been induced for 2 days, no systemic, hematologic, or biochemical effects were found. Subjects re-
Figure 2. Pressure discomfort (allodynia) was induced in the rhNGF-injected volar forearm by strong indentation of the skin with the thumb or index finger—tissue compression insufficient to cause discomfort on the saline-injected side. (Top) There is a high percentage of patients who developed pressure allodynia on the NGF side but not on the saline-injected side. The frequency of pressure alldynia was highest at 1, 3, and 7 days but remained statistically more frequent to 21 days. (Bottom) The mean area of pressure allodynia on NGF and saline arms. The area under the NGF-injected side is significantly greater than that under the saline-injected side (p < 0.001). The symbol to the left of the plotted point is the significance value for that point compared with baseline. The symbol above the plotted point is the significance value for comparison with the saline-injected side. The symbols for statistical significance are the same as described in the legend for figure 1.

Figure 3. Plotted intermediate heat-pain response (HP 5) and heat-pain threshold (HP 0.5) using CASE IV as described in text. (Top) Serial-measured values of HP 5 (by stimulus steps) for saline and NGF-injected arms. Whereas the HP 5 threshold rose slightly with time on the saline-injected side, it fell below the saline-injected side at 3 hours, and for all times to and including 21 days for the NGF-injected side. For the NGF-injected side, this intermediate threshold was below baseline at 1 through 14 days. (Bottom) Similar data for HP 0.5 (threshold) for saline and NGF-injected arms. Threshold was approximately constant throughout the testing period for the saline-injected side. For the NGF-injected side, it fell below baseline and the saline-injected side especially for days 1 through 7. These data provide evidence that local injection of rhNGF induces heat-pain hyperalgesia. This hyperalgesia has already begun at 3 hours, reaches a peak at 1 to 7 days, and slowly subsides thereafter. For the significance of the symbols above and to the left of the plotted points, see legend to figure 1.

ported regional discomfort of the rhNGF-injected site only when the tissue at the site of injection was bumped or compressed. A significant percentage of subjects reported tenderness to palpation at 3 hours, reaching the highest frequency by 3 days and remaining significant to 21 days.

The NSC questionnaire revealed significant worsening of pressure alldynia (compared with the previous examination time) at 3 and 24 hours and significant improvement at 7 and 14 days. In addition to providing characterization information, these results appear to indicate that the NSC is sensitive for quantifying such symptoms.

The time course and distribution of the sensory phenomena we describe provides some insight about tissue distribution and mode of action of rhNGF given ID. These hyperalgesia phenomena begin too early and perhaps peak too quickly to be explained by uptake of rhNGF by nociceptors or sympathetic postganglionic terminals, retrograde transport to spinal or autonomic ganglia somas, and upregulation of the metabolism of these neurons. This hypothesis must be considered because NGF is known to be taken up by TrkA receptors in nociceptive and sympathetic terminals and has been shown to increase such metabolites as substance P in the spinal ganglia. Assuming an average distance of ~500 mm from the site of injection to the spinal ganglia and an average transport of the peak rhNGF wave of 250 mm/day, one would not expect to find an effect for...
several days. We found an effect of NGF at 3 hours. In some of the earlier studies in which retrograde transport and upregulation of metabolism of primary sensory nerve was assumed to be the basis for hyperalgesia, it may not have been possible to recognize that the hyperalgesia occurred too early to be explained by retrograde transport and upregulation of proteins because rodents were used—the distances were too short for evaluation of the phenomena.

The distribution and time course of tenderness to regional deep pressure, slight discomfort with flexion of fingers and wrists in some cases, and tenderness over the bicipital groove and in the suprascapular region (in some cases) without tenderness of nerve trunks are in keeping with local and lymphatic spread of NGF, inflammatory cells, or locally released pain mediators. The third possibility, that NGF was distributed throughout the vascular bed and thus gained access to spinal ganglia causing early upregulation of nociceptor neurons, also seems unlikely because the rhNGF was injected ID and the contralateral side did not develop hyperalgesia.

Although the present study does not provide direct information, there are some hints of how local NGF injection might be involved in inducing hyperalgesia. Two mechanisms need to be considered: activation of primary afferent nociceptors or local induction or release of pain or inflammatory mediators, perhaps through degradation of mast, basophil, or other cells. Although obvious inflammation was not observed at the injection site, the time course and distribution of the hyperalgesia is perhaps more in keeping with the second explanation.

Our results should not be taken to indicate that NGF might not be useful in preventing, stabilizing, or improving sympathetic or peptidergic sensory neuron involvement in polyneuropathy or in other neurologic disease. However, it does indicate that hyperalgesia will need to be critically evaluated further as a marker of such improvement. It may be necessary, therefore, to show that symptoms and deficits characteristic of small fiber neuropathy can be prevented, stabilized, or improved by the use of NGF.

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References
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