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Gene Transfer of Glutamic Acid Decarboxylase Reduces Neuropathic Pain

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Abstract

We tested whether transfer of the gene coding for glutamic acid decarboxylase to dorsal root ganglion using a herpes simplex virus vector to achieve release of GABA in dorsal horn would attenuate nociception in this condition. Subcutaneous inoculation of a replication-defective herpes simplex virus vector expressing glutamic acid decarboxylase (vector QHGAD67) 7 days after selective L5 spinal nerve ligation reversed mechanical allodynia and thermal hyperalgesia; the antiallodynic effect lasted 6 weeks and was reestablished by reinoculation. QH-GAD67 inoculation also suppressed induction of c-Fos and phosphorylated extracellular signal-regulated kinase 1 and 2 in the spinal cord.

Peripheral neuropathic pain is a common and difficult to treat concomitant of polyneuropathy or structural nerve injury. Opioids are relatively ineffective, and their use is limited by side effects. Antidepressants and anticonvulsants have demonstrated efficacy in randomized controlled trials but provide only 50% relief in less than half of patients treated.¹ Among the complex mechanisms underlying neuropathic pain, partial nerve injury results in a selective loss of GABAergic inhibitory synaptic currents in spinal cord² that contribute to abnormal pain sensitivity and the phenotypic features of the neuropathic pain syndrome. GABAergic agents have not been widely used in the treatment of neuropathic pain because the therapeutic window of these agents is modest and the dose is limited by side effects.

Gene transfer represents a novel and useful means to target expression of peptides to focal sites within the nervous system. We have shown that transduction of sensory neurons of the dorsal root ganglion (DRG) by footpad inoculation with herpes simplex virus (HSV)-based vectors can be used to achieve a regional antinociceptive effect. HSV vectors coding for proenkephalin or the glial cell-derived neurotrophic factor produce an antihyperalgesic and antiallodynic effect in rodent models of inflammatory pain, neuropathic pain, and pain resulting from cancer in bone,^{3–6} and an HSV-vector expressing glutamic acid decarboxylase (GAD) provides an analgesic effect in the central neuropathic pain syndrome resulting from spinal cord injury.⁷ In this study, we examined the antinociceptive effect of GAD expressed from an HSV-based vector in the spinal nerve ligation (SNL) model of neuropathic pain in the rat.

Materials and Methods

The nonreplicating HSV vector QHGAD67, which is defective in expression of the HSV immediate early genes *ICP4*, *ICP22*, *ICP27*, and *ICP47* and contains the human *GAD67* gene under the control of the human cytomegalovirus immediate early promoter in the *U_L41* locus, has been described previously.⁷ The control vector QOZHG is defective in the same HSV genes but contains the green fluorescent protein and *Escherichia coli lacZ* reporter genes.⁷

Male Sprague–Dawley rats weighing 225 to 250gm underwent selective L5 SNL, as described previously,⁵ with the approval of the University Committee on Use and Care of Animals. One week after SNL, 30μl of vector (either QH-GAD67 or QOZHG, 4×10^8 plaque-forming units per milliliter) was injected subcutaneously in the plantar surface of the left hind paw, ipsilateral to the ligation. Mechanical allodynia induced by SNL was determined by assessing the response of paw withdrawal to von Frey hairs of graded tensile strength as described previously,^{5,8} with a tactile stimulus producing a 50% likelihood of withdrawal determined using the up-down method.⁹ Thermal hyperalgesia was determined using a Hargreaves apparatus,¹⁰ recording the time to withdrawal from a radiant thermal stimulus positioned directly under the hind paw.

Results

After L5 SNL, rats displayed a significant decrease in the magnitude of the mechanical stimulus necessary to evoke a brisk withdrawal response to von Frey hair stimulation (Fig 1A) and a significant reduction in latency to withdraw from a heat stimulus (thermal hyperalgesia; see Fig 1B). Rats inoculated with QH-GAD67 showed a statistically significant increase in mechanical threshold beginning 1 week after inoculation. The antiallodynic effect of QHGAD67-mediated GABA expression was sustained and continuous, lasting 5 to 6 weeks and peaking at 2 weeks after inoculation (see Fig 1A). The peak value of mechanical threshold, 8.6gm, was close to the preoperative value. By 7 weeks after inoculation, the antiallodynic effect of vector transduction disappeared, and the mechanical threshold of QHGAD67-injected rats was identical to that of control rats. Reinoculation into the same paw with the same dose of QHGAD67 reestablished the antiallodynic effect (see Fig 1A).

SNL induced a decrease in the thermal latency from 10.7 to 6.5 seconds, which lasted 3 weeks before gradually recovery. Rats inoculated with QHGAD67 showed a statistically significant increase in thermal latency in the ipsilateral paw beginning 1 week after inoculation (see Fig 1B), an effect that was sustained and continuous, lasting 3 to 4 weeks (see Fig 1B). Sham-operated animals had no change in mechanical threshold or thermal latency (data not shown).

Expression of c-Fos and phosphorylated extracellular signal–regulated kinase 1 and 2 (p-ERK1/2) induced by gentle touch is one indirect biological marker of nociceptive processes.¹¹ Three weeks after SNL, gentle touch was applied once every 4 seconds for 10 minutes, with the flat surface of the experimenter's thumb to the rat's paw, and the number of immunoreactive cells (anti-c-Fos or anti-p-ERK1/2 antibodies; Santa Cruz Biotechnology, Santa Cruz, CA) detected avidinbiotin horseradish peroxidase followed by nickel-enhanced diaminobenzidine (Vector Laboratories, Burlingame, CA).¹² The number of Fos-LI–positive neurons was substantially increased ipsilateral to SNL compared with sham-operated control rats (Fig 2), and inoculation of vector QHGAD67 significantly reduced the number Fos-LI–positive neurons in laminae I–VI (see Fig 2). p-ERK1/2 expression in laminae I and II was also increased in rats after gentle touch stimulation with SNL, and that increase was blocked in animals inoculated with QHGAD67 (Fig 3).

Discussion

These results demonstrate that subcutaneous inoculation of an HSV vector expressing GAD to transduce DRG *in vivo* attenuated the behavioral manifestations of mechanical allodynia and thermal hyperalgesia in a model of peripheral neuropathic pain; the effect on behavior was confirmed by histological measures showing a block in the induction of expression of c-Fos and p-ERK1/2 in the ipsilateral spinal dorsal horn. We have demonstrated previously that transduction of primary sensory neurons with QHGAD67 results in the release of GABA from those cells *in vitro* and from sensory nerve terminals in spinal cord *in vivo*.⁷ Subcutaneous inoculation of the vector results in transduction of both large and small neurons in the DRG, as determined by immunocytochemical detection of GAD expression.⁷ GABA release from transduced cells is constitutive and is mediated by the GABA transporter type 1 (GAT-1). In the spinal cord hemisection model of central neuropathic pain, transduction of DRG by subcutaneous inoculation of QHGAD67 in both hind paws reduces below-level mechanical allodynia and thermal hyperalgesia.⁷ This study is the first application of this vector to peripheral neuropathic pain.

In the spinal cord, GABA is found primarily in interneurons of the superficial dorsal horn predominantly in laminae II and III that form axoaxonic synapses on primary afferent terminals and axodendritic synapses on projection neurons. Tonic GABAergic inhibition plays an important role in normal sensory processing, as indicated by increased behavior responsiveness¹³ and electrophysiological activity in spinal nociceptive neurons¹⁴ after spinal administration of the GABA_A antagonist bicuculline to normal animals and by the development of mechanical allodynia after spinal administration of the GABA_B receptor antagonist CGP35348.¹⁵

The mechanical allodynia that results from selective SNL can be reversed by the GABA_B agonist baclofen¹⁶ or the GABA_A agonist muscimol,¹⁷ and both mechanical allodynia and thermal hyperalgesia after spinal nerve injury are reversed by the GABA_A agonist 4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol (isoguvacine) and baclofen.¹⁸ These findings are consistent with observations of loss of GABA activity in dorsal horn after chronic constriction injury of sciatic nerve,¹⁹ a decrease in GABA_A receptor-mediated inhibitory postsynaptic potentials, reduction of GAD65, and evidence of apoptosis in dorsal horn in both the chronic constriction and spared nerve injury models of neuropathic pain.² Transgene-mediated GABA released in the dorsal horn acts at both GABA_A and GABA_B receptors. As we have demonstrated previously, the antinociceptive effect produced by QHGAD67-mediated GABA release can be partially blocked by the GABA_A antagonist bicuculline or by the GABA_B antagonist phaclofen.⁷

GABAergic therapy for neuropathic pain has had limited success. Gene transfer offers the possibility of targeted alteration in the neurotransmitter phenotype to produce the local release of selected neurotransmitters. Among the many viral and nonviral vectors that may be used for gene transfer, HSV, because of its natural neurotropism and high-affinity retrograde transport in sensory neurons, is uniquely suited for modifying neurotransmitter release in dorsal horn after subcutaneous inoculation.²⁰ We have found previously that HSV-mediated transfer of proenkephalin produces a sustained antiallodynic effect in the SNL model of neuropathic pain,⁵ and that similar results can be achieved with a vector expressing glial cell-derived neurotrophic factor.⁶ The magnitude of the antiallodynic effect achieved with the GAD67-expressing vector is substantially greater than that produced by either the proenkephalin- or glial cell-derived neurotrophic factor-expressing vectors. We propose that this differential effect reflects the role of reduced spinal GABAergic tone in the pathogenesis of neuropathic pain and the selective ability of QHGAD67 to correct that deficit locally. The results suggest that this novel approach may prove useful in the treatment of neuropathic pain.

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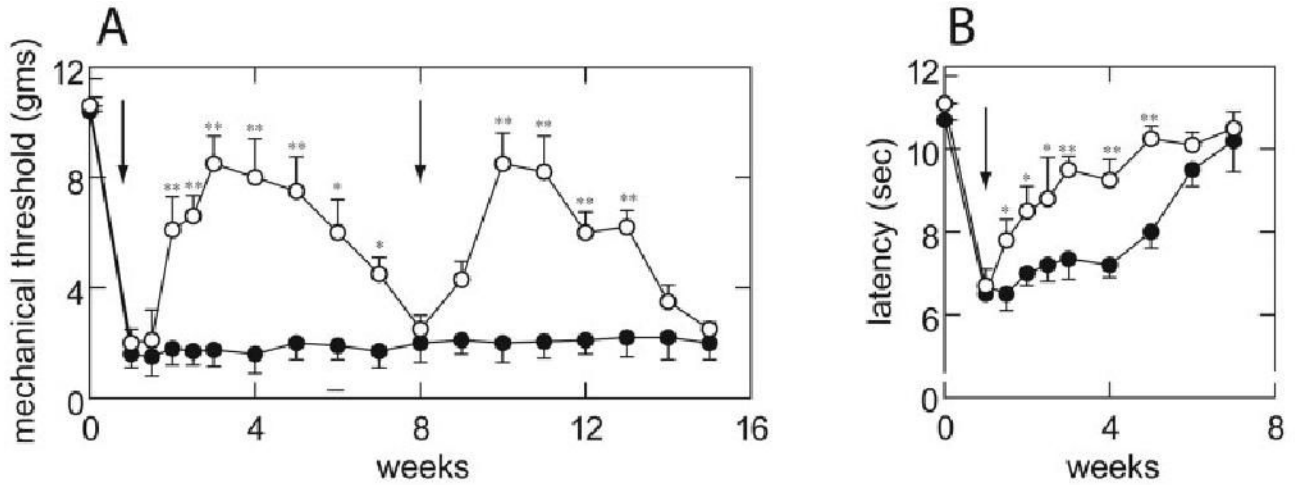
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**Fig 1.**

*Antinociceptive effect of QOGAD67 in neuropathic pain. (A) L5 spinal nerve ligation (SNL) caused a significant decrease in the threshold to tactile stimulation, which persisted for more than 4 months. Subcutaneous inoculation of QHGAD67 (arrow) produced an antiallodynic effect reflected in an increase in the mechanical threshold. Reinoculation of QHGAD67 7 weeks after the initial inoculation (arrow) reestablished the antiallodynic effect. Results are expressed as mean \pm standard error of the mean. (open circles) QHGAD67; (closed circles) QOZHG; * $p < 0.05$; ** $p < 0.01$; $n = 8$ animals per group. (B) L5 SNL also caused a significant thermal hyperalgesia, which persisted for 6 weeks. Inoculation with QHGAD67 (arrow), but not QOZHG, reversed the thermal hyperalgesia induced by spinal nerve injury. * $p < 0.05$; ** $p < 0.01$ versus QOZHG-inoculated; $n = 8$ animals per group. The statistical significances of the differences were determined by analysis of variance (StatView 5.2; SAS Institute, Cary, NC) corrected for the number of post hoc comparisons using Scheffé's F test.*

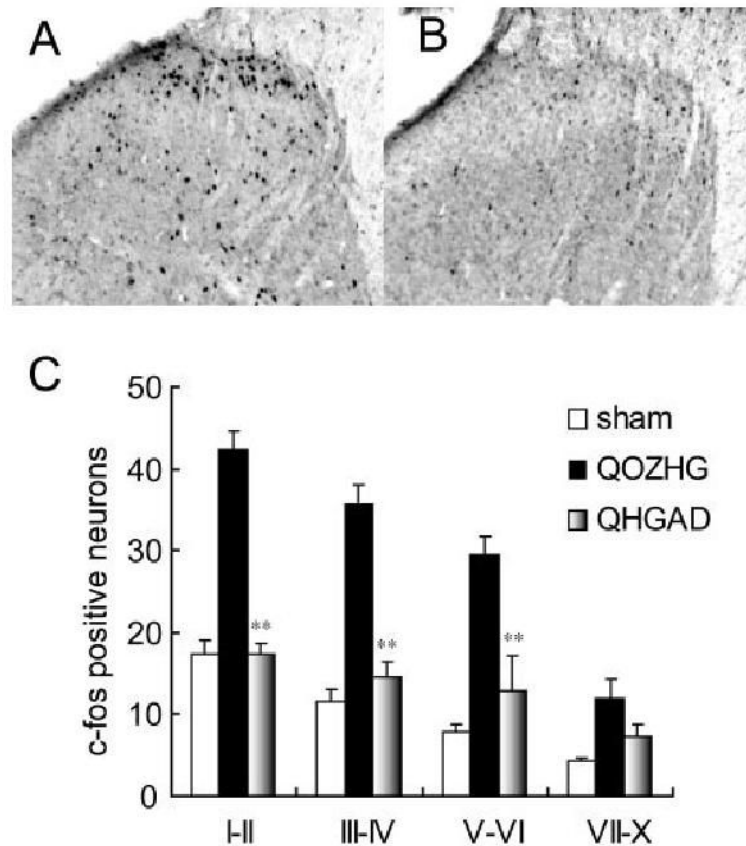


Fig 2. Effect of QHGAD67 on Fos-LI in dorsal horn. (A) Fos-LI in dorsal horn induced by 10 minutes of gentle tactile stimulation was markedly increased in rats inoculated with QOZHG 1 week after spinal nerve ligation (SNL) and tested 2 weeks later (3 weeks after SNL). (B) This increase was blocked in rats with SNL that had been inoculated with QHGAD67 1 week after SNL and tested 2 weeks later (3 weeks after SNL), and it was found in laminae I–VI of dorsal horn. Results are expressed as mean \pm standard error of the mean. ** $p < 0.01$; $n = 5$ animals per group. The difference between sham-operated and SNL animals inoculated with QOZHG was also statistically significant ($p < 0.01$).

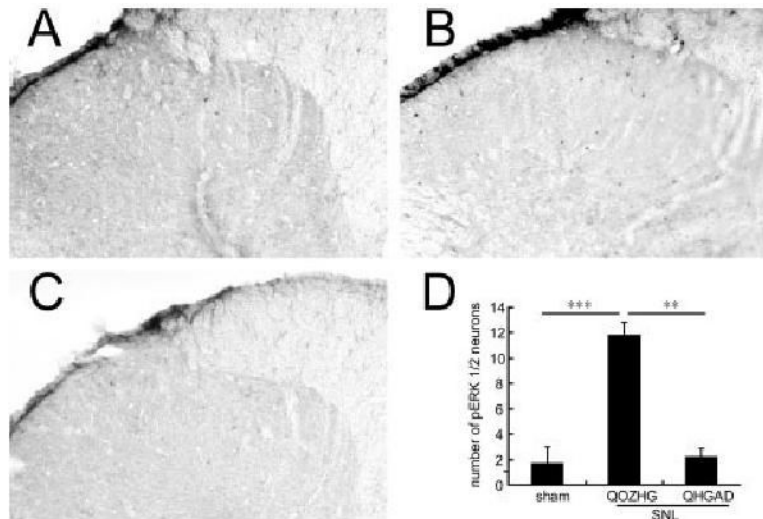


Fig 3. Effect of QHGAD67 on the phosphorylated extracellular signal–regulated kinase 1 and 2 (p-ERK1/2) expression in dorsal horn. p-ERK is not induced by 10 minutes of gentle tactile stimulation in sham-operated animals (A), but it is substantially induced after spinal nerve ligation (SNL) in animals inoculated with QOZHG (B). Touch-induced expression of p-ERK1/2 was suppressed in animals inoculated with QHGAD67 (C), confirmed by counts of p-ERK1/2–positive neurons in the dorsal horn (D). Results are expressed as mean \pm standard error of the mean. ** $p < 0.01$; *** $p < 0.001$; $n = 5$ animals per group.