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Reduction in the Mechanonociceptive Response by Intrathecal Administration of Glycine and Related Compounds

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We have previously reported that enhanced glycine release is produced by epidural spinal cord stimulation, a clinical method for treating neuropathic pain. Our current hypothesis is that glycine administered intrathecally reduces neuropathic pain as measured by the Randall-Selitto method. Neuropathic rats created by unilateral partial ligation of the sciatic nerve were treated with intrathecal infusion of glycine, strychnine, MK-801, or 5,7-DKA at 0.1 μmol, or artificial CSF for 2 hours at a rate of 10 μl/min. Force required to produce the pain response was significantly increased after glycine administration and reduced using strychnine, a specific glycine receptor (Gly 1) antagonist. Strychnine blocked the response to glycine when infused together. Administration of the non-specific NMDA receptor MK-801 antagonist and 5,7-DKA, a specific glycine-NMDA receptor (Gly 2) antagonist, however, failed to block the response to glycine. Our results provide evidence for the use of glycine and related compounds to treat neuropathic pain.

KEY WORDS: Glycine; neuropathic pain.

INTRODUCTION

Preliminary studies from our laboratory using microdialysis techniques in rodents revealed a significant elevation in the concentration of amino acids within the segmental extracellular space after neurological injury (1,2). These studies indicated that glycine, in particular, is released into the segmental extracellular space in response to stimulation of neural structures that are known to activate large numbers of segmental interneuron pools, chiefly the motor cortex and spinal cord (3,4). These data were obtained while using stimulation paradigms similar to those used in humans for control of neuropathic pain such as deep brain or spinal cord stimulation (5–8).

In addition, peripheral nerve stimulation techniques used in our rodent model, and mimic pain control techniques used in man, produced segmental glycine elevations (1,9). Large diameter peripheral nerve fibers were activated utilizing techniques incorporated in the clinical use of transcutaneous electrical nerve stimulation (TENS) or invasive peripheral nerve stimulation (3,7,9). Based on this pilot data, we hypothesize that neuropathic pain produced by mechanonociceptive stimulation is modified by intrathecal administration of glycine and related compounds. This phase of study was undertaken using a well established model of neuropathic pain created by Bennet and Xie in 1988 (10).

EXPERIMENTAL PROCEDURE

The surgical procedures were performed in an animal surgery room dedicated to this study. Sixty-four Sprague-Dawley male rats between 350–400 grams underwent antibiotic administration, enrofloxcin (Baytril, 0.5 mg/kg IP) 15 minutes before surgery began. The rats were anesthetized with sodium pentobarbital (Nembutal, 40mg/kg

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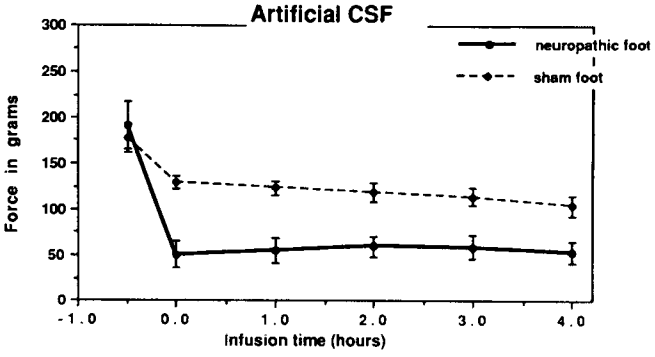

Fig. 1. A graphic illustration of the response to mechanonociceptive stimulation during intrathecal infusion of artificial CSF. Pre-infusion testing of both the neuropathic and sham limbs was 30 minutes prior, 1 hour and 2 hours during, and 1 hour and 2 hours after intrathecal infusion.

IP). Anesthesia depth was monitored by respiration rate and the lid reflex. Body temperature was monitored with a rectal probe and a heating pad and light was used as needed to maintain body temperature at 38°C. Normal saline was administered periodically for hydration.

An iodine skin preparation was applied to both hind limbs and occipital regions following hair removal. Sterile instruments, trays, drapes, gloves were used. A simple cut-down to the sciatic nerve in the upper hind limb was performed. Chromic ligatures (4-O), four in number were loosely secured around the entire nerve diameter and separated 1 mm apart. The ligatures were tightened around one sciatic nerve only to a point just reducing the diameter as visualized by an operative microscope. These wounds were closed and an incision was made over the atlanto-occipital membrane. An intrathecal catheter (PE-10) was placed into the cisterna magna and threaded to the lumbosacral region. A connector was secured to the catheter which was secured to the skull base with dental acrylic and the wound was closed. Daily flushing of the catheter using strict aseptic technique with artificial CSF (<0.1 cc) was done until the chronic phase of study.

Along with daily flushing of the intrathecal catheter, the animal comfort was maintained using butorphanol tartrate (Torbutrol 0.1 mg/kg IP) every twelve hours for five days. In addition, enrofloxcin (Baytril 0.1 mg/kg IP) was also administered. The animal was maintained for 12 days prior to neuropathic pain testing. In the interim, any animal exhibiting undue stress was immediately sacrificed. On the twelfth day, the animal was then tested for sensitivity to mechanical stimuli.

Although a variety of methods have been developed to quantify pain, experimentally, pain is generally measured using mechanical stimulation or thermal stimulation, each activating a relatively select group of nociceptors. The time to limb movement or vocalization is generally used to determine pain sensation in laboratory animals. Mechanical stimulation of pain receptors via calibrated pressure and thermal stimulation by a hot plate or a focused light source are the most accepted means of pain measurement, particularly in the laboratory setting.

Mechanical stimulation of nociceptors is provided by the Randall-Sellito technique. A pneumatic pressure device increases the weight in grams applied to the footpad of the neuropathic and sham limb in a calibrated fashion. The sensitivity of the animal is based on the amount of pressure applied by the device that results in limb withdrawal, or the pain response. Both hind limbs from each animal were tested and measured as grams force to cause withdrawal.

For the study of neuropathic pain, glycine, strychnine, MK-801 or 5,7-DKA (dichlorokynurenic acid) were infused at concentrations of 0.1 μmol in artificial CSF at 10 μl/min in separate animal groups (n = 7 to 9 animals per group). Glycine was also infused together with either strychnine, MK-801, or 5,7-DKA in additional separate animal groups, and all groups were compared to animals receiving only artificial CSF. After pre-infusion sensitivity testing was completed, the drugs were administered for 2 hours through the intrathecal catheter. Testing was done 30, 60, and 120 minutes after the onset of infusion. Additional sensitivity testing was done using the same paradigm 60 minutes after completion of the infusion.

At the conclusion of the study, the animals were perfused via cardiac puncture with 4% paraformaldehyde after being anesthetized with 2% halothane and oxygen by mask. The spinal cords were removed for standard neuroimmunohistochemical staining. Significant differences between treatment groups compared to animals treated with artificial CSF, as calculated by ANOVA for repeated measures, were defined as $p < 0.05$.

RESULTS

The force in grams required for limb withdrawal was significantly greater in 12 days after injury, as illustrated in Fig. 1. Although this finding was pronounced in the neuropathic limb, increased sensitivity to painful mechanical stimulation was measured in the non injured limb as well. During intrathecal infusion of artificial CSF, measurements in both the neuropathic and sham limb were consistent throughout the duration of each experiment.

Our results also show that the force necessary to produce a pain response, or withdrawal of the neuropathic limb, was increased from 70 gr to approximately 120 gr after intrathecal administration of glycine, as illustrated in Fig. 2. Again, similar observations were made in the non injured limb. In contrast, strychnine significantly lowered the threshold for pain from approximately 70 gr to 40 gr, as illustrated in Fig. 3. A similar response was observed in the sham limb. However, when glycine and strychnine were administered together, the effect of glycine on pain is blocked, as illustrated in Fig. 4. The latter observation mirrored the response to artificial CSF infusion.

Infusion of MK-801 alone did not have significant influence on the pain response, as illustrated in Fig. 5. Infusion of 5,7-DKA, however, resulted in a small but significant increase in limb sensitivity in the sham limb only, as illustrated in Fig. 6. When administered with glycine, MK-801 did not prevent the reduction in the pain response to either limb, as illustrated in Fig. 7. Likewise, 5,7-DKA when administered with glycine failed to completely block the reduction in pain sensi-

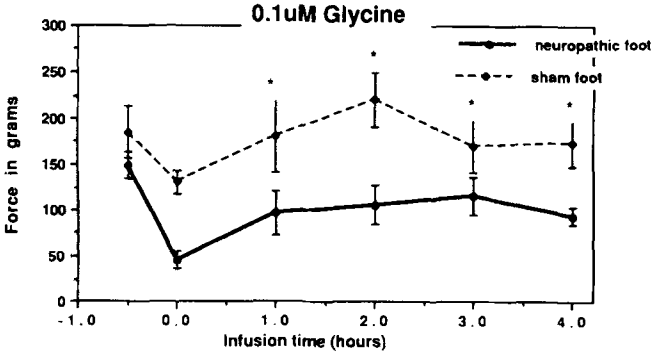

Fig. 2. A graphic illustration of the response to mechanonociceptive stimulation during intrathecal infusion of glycine. Pre-infusion testing of both the neuropathic and sham limbs was 30 minutes prior, 1 hour and 2 hours during, and 1 hour and 2 hours after intrathecal infusion. Significant differences in the values for glycine versus CSF are highlighted with an asterix (*). Both neuropathic and sham limb responses were significantly influenced by glycine administration at 1 hour and 2 hours during infusion. The effects of glycine remained significant, although less pronounced, 1 hour and 2 hours after infusion only for the neuropathic limb.

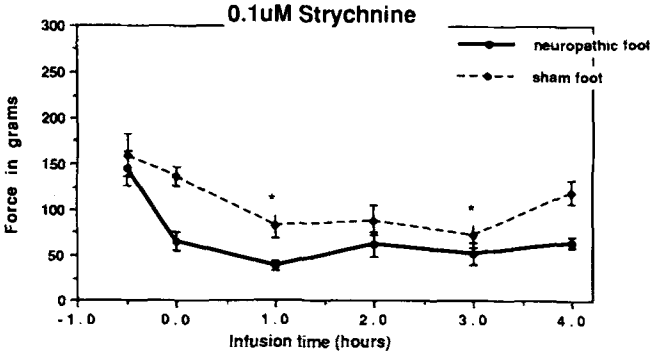

Fig. 3. A graphic illustration of the response to mechanonociceptive stimulation during intrathecal infusion of strychnine. Pre-infusion testing of both the neuropathic and sham limbs was 30 minutes prior, 1 hour and 2 hours during, and 1 hour and 2 hours after intrathecal infusion. Significant differences in the values for glycine versus CSF are highlighted with an asterix (*). The neuropathic limb response (at 1 hour of infusion) and the sham limb response (at 1 hour during and 1 hour after infusion) were significantly influenced by strychnine administration.

tivity for the neuropathic limb. A small but significant maintenance of the effects of glycine on the neuropathic limb is illustrated in Fig. 8.

DISCUSSION

Neuropathic pain, following damage to peripheral or central nervous system structures, has been described since the early American Civil War experience (11).

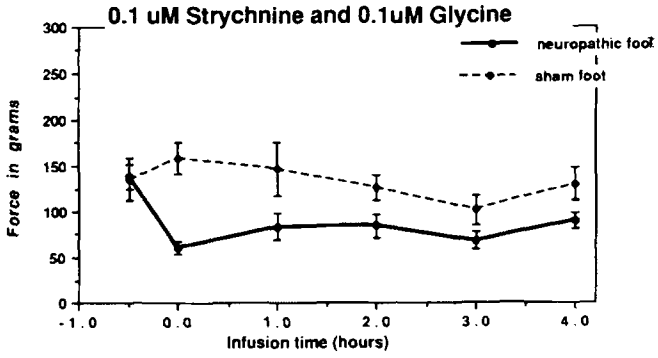

Fig. 4. A graphic illustration of the response to mechanonociceptive stimulation during intrathecal infusion of glycine and strychnine together. Pre-infusion testing of both the neuropathic and sham limbs was 30 minutes prior, 1 hour and 2 hours during, and 1 hour and 2 hours after intrathecal infusion. No significant differences were seen with glycine and strychnine together compared to CSF infusion, in either the neuropathic or sham limb.

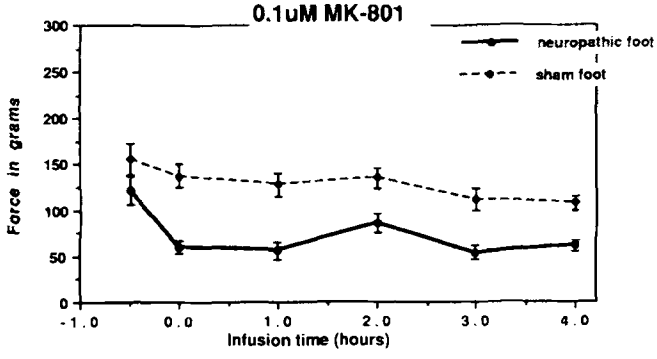

Fig. 5. A graphic illustration of the response to mechanonociceptive stimulation during intrathecal infusion of MK-801. Pre-infusion testing of both the neuropathic and sham limbs was 30 minutes prior, 1 hour and 2 hours during, and 1 hour and 2 hours after intrathecal infusion. No significant differences were seen compared to CSF infusion in either the neuropathic or sham limb.

Since that time, many theories have been proposed to implicate peripheral and central nervous system mechanisms in the production of neuropathic pain (12). After the "Gate Theory" of pain was proposed by Melzack and Wall, several investigations have shown that interneuron pools mediate nociceptive information from afferent sources (12–15). Influence from these interneurons is likely due to many complex neurochemical events, including amino acid neurotransmitters with excitatory characteristics such as aspartate and glutamate, and inhibitory features such as glycine and taurine (13–17).

The role of glycine as an inhibitory neurotransmitter was initially proposed by Aprison in 1965, and since that time it has been established as the chief postsynaptic inhibitory neurotransmitter in the spinal cord (18,19).

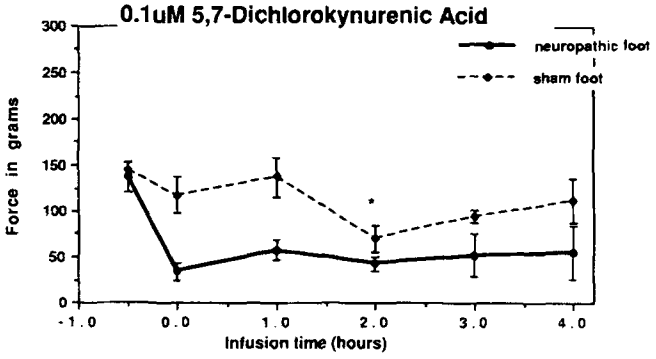

Fig. 6. A graphic illustration of the response to mechanonociceptive stimulation during intrathecal infusion of 5,7-DKA (dichlorokynurenic acid). Pre-infusion testing of both the neuropathic and sham limbs was 30 minutes prior, 1 hour and 2 hours during, and 1 hour and 2 hours after intrathecal infusion. Significant differences in the values for 5,7-DKA versus CSF are highlighted with an asterix (*). A small but significant difference was seen only in the sham limb (at 2 hours of infusion) when compared to CSF infusion.

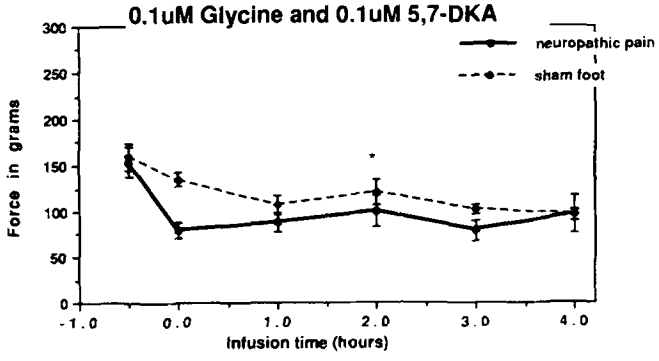

Fig. 8. A graphic illustration of the response to mechanonociceptive stimulation during intrathecal infusion of glycine and 5,7-DKA together. Pre-infusion testing of both the neuropathic and sham limbs was 30 minutes prior, 1 hour and 2 hours during, and 1 hour and 2 hours after intrathecal infusion. A significant difference was seen only in the neuropathic limb (at 2 hours of infusion) when compared to CSF infusion.

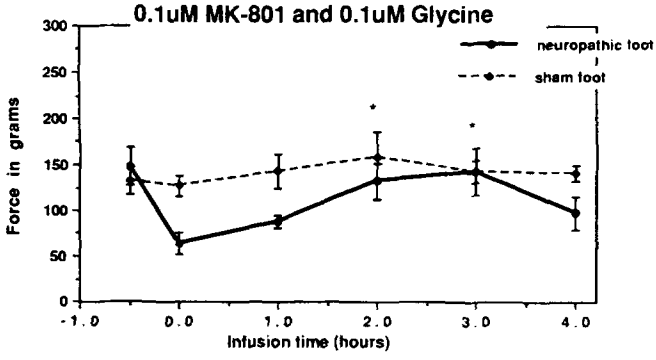

Fig. 7. A graphic illustration of the response to mechanonociceptive stimulation during intrathecal infusion of glycine and MK-801 together. Pre-infusion testing of both the neuropathic and sham limbs was 30 minutes prior, 1 hour and 2 hours during, and 1 hour and 2 hours after intrathecal infusion. Significant differences in the values for glycine and MK-801 together versus CSF are highlighted with an asterix (*). A significant difference was seen only in the neuropathic limb (at 2 hours of infusion and 1 hour afterward) when compared to CSF infusion.

Autoradiographic and immunohistochemical studies show glycine to be closely associated with medial and ventral interneuron pools (20,21). As with γ-amino butyric acid (GABA), glycine containing interneurons are located within the dorsal horn and may act in concert with GABA to modify peripheral afferent activity (15,24).

Superficial dorsal horn neurons, which respond primarily to Aδ and C fibers, are hypersensitive in neuropathic pain (25). Local interneuron pools appear to be selectively damaged under such conditions (26–28). Dorsal horn neurons that receive Aβ afferents may become sensitized without dorsal horn interneuron activity or activity from ventral interneurons that project dorsally (20–23,26–28). In addition, immunohistochemical studies show that dorsal horn neurons involved in pain perception receive numerous postsynaptic glycinergic terminals (13–16). These dorsal horn cells show reduced activity after ionophoretic application of glycinergic compounds and increased activity after application of glycine antagonists (13–16).

Glycine acts on postsynaptic neuronal membranes through two receptor subtypes, a strychnine sensitive chloride channel (Gly 1) and a much smaller population of n-methyl-D-aspartate (NMDA) sensitive channels (Gly 2) (19,29,30). Dorsal horn neurons subserving afferent inputs, particularly those involved in nociception, are readily activated by NMDA agonists (30–33). The primary effect of glycine, however, is via activation of the hyperpolarizing chloride channel (18,19). Although only recently developed, glycine-NMDA channel antagonists such as 5,7-dichlorokynurenic acid (5,7-DKA) have not been widely studied for their antinociceptive characteristics (34–36). Pain can be produced by non specific NMDA channel stimulation and reduced by antagonists such as MK-801, particularly pain triggered by thermal nociceptor stimulation (37–39).

We have shown that glycine will reduce the pain response to mechanonociceptor stimulation via the strychnine sensitive receptor, and moreover, that strychnine will amplify the pain response in this model of neuropathic pain and will block the influence of glycine when administered together. Although the impact of glycine-NMDA receptor activity and neuropathic pain is unclear, our results show that blockade of these receptors

by 5,7-DKA does influence pain. However, neither NMDA receptor associated compound blocked the influence of glycine when administered together.

The dual role of glycine in the spinal cord as it pertains to neuropathic pain has not been clearly deciphered (40). Using combinations of Gly 1 and Gly 2 agonists and antagonists, the role of this particular neurotransmitter in neuropathic pain may be better understood and is the focus of our ongoing study.

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