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Valproate Prevents Dysregulation of Spinal Glutamate and Reduces the Development of Hypersensitivity in Rats After Peripheral Nerve Injury

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Abstract: The present study examined whether the histone deacetylase inhibitor valproate prevents downregulation of glutamate transporters in the primary cultured astrocytes and in the spinal cord after L5-L6 spinal nerve ligation (SNL) and whether this action of valproate on spinal glutamate transporters prevents spinal glutamate dysregulation and development of hypersensitivity after SNL. In cultured astrocytes, valproate prevented downregulation of glutamate transporter-1 (GLT-1) and glutamate-aspartate transporter in a concentration-dependent manner. Repeated oral administration of valproate reduced the development of hypersensitivity and prevented the downregulation of spinal GLT-1 and glutamate-aspartate transporter expression in rats after SNL, but did not affect mechanical nociception and expression of those transporters in normal rats. Valproate's effects on hypersensitivity and spinal GLT-1 expression in SNL rats were blocked by intrathecal administration of the selective GLT-1 blocker dihydrokainic acid or the GLT-1 selective small interfering RNA (siRNA). Extracellular glutamate concentration in the spinal cord, measured by microdialysis, was increased in animals with SNL or after GLT-1 selective siRNA treatment, and valproate prevented the SNL-induced glutamate increase. These results suggest that valproate reduces the development of chronic pain after nerve injury in part by preventing downregulation of glutamate transporters, especially GLT-1, to maintain normal extracellular glutamate concentrations in the spinal cord.

***Perspective:** This study demonstrates that valproate prevents the downregulation of glutamate transporters in the spinal cord, which contributes in part to the development of chronic pain after nerve injury. Given clinical availability and established safety profiles, perioperative use of valproate should be tested to prevent chronic pain after surgery.*

***Key words:** Valproate, glutamate transporter, chronic pain, astrocyte, spinal cord.*

The preeminent excitatory neurotransmitter glutamate underlies normal physiology and pathophysiology in the central nervous system. Extracellular concentrations of glutamate are regulated by 2 types of astroglial glutamate transporters, predominantly by glutamate transporter-1 (GLT-1) and, to a lesser extent, by glutamate-aspartate transporter (GLAST).[19,20] Impairment or downregulation of astroglial glutamate transporters results in elevation of extracellular glutamate, contributing to many neurologic diseases including amyotrophic lateral sclerosis, epilepsy, stroke, and chronic pain.[3,4,9,21]

Clinical studies have demonstrated that the antiepileptic drug valproate is effective in various painful conditions such as migraine headaches,[14] diabetic neuropathy,[16] and postherpetic neuralgia.[17] Valproate is known to act by several mechanisms to reduce pain, including inhibition of voltage-gated sodium channels, increasing γ-aminobutyric acid (GABA) levels by enhancing GABA synthesis and inhibiting GABA degradation, and direct stimulation of $GABA_A$ receptors.[6] In addition to those classic actions, valproate also inhibits histone deacetylase (HDAC).[8] Histone deacetylation by HDACs has been recognized as an important mechanism in regulation of gene transcription responsible for the induction and maintenance of chronic pain, including proinflammatory cytokine production and neuroglia plasticity in the central nervous system. Previous studies

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demonstrated that direct inhibition of HDAC by valproate activated gene transcription of glutamate transporters in cultured glial cells[1,18] and reduced hypersensitivity in mice after peripheral inflammation.[2] Similarly, we recently observed in rats after L5-L6 spinal nerve ligation (SNL) that repeated oral administration of valproate starting from 3 weeks after surgery restored downregulated expression of GLT-1 and GLAST in the spinal cord and reduced chronic hypersensitivity after this nerve injury.[13] These results suggest that increase in expression of glutamate transporters is an important mechanism of valproate action in the spinal cord to reduce established chronic pain.

The current study examined whether valproate, in addition to its actions in established pathology, also *prevents* downregulation of glutamate transporters in the primary cultured astrocytes in vitro and in the spinal cord after SNL in vivo and whether this action of valproate on glutamate transporters, especially on GLT-1, prevents hypersensitivity and elevation of extracellular glutamate concentration in the spinal cord after SNL. We also examined whether elevation of extracellular glutamate concentration by knockdown of GLT-1 with the selective small interfering RNA (siRNA) induces hypersensitivity in normal rats.

Methods

Animals

Male (5 weeks old at arrival) and pregnant female Sprague Dawley rats from Harlan Industries (Indianapolis, IN), housed under a 12-hour light-dark cycle with food and water ad libitum, were used. All experiments were approved by Animal Care and Use Committee at Wake Forest University (Winston Salem, NC).

Astrocyte Culture

Primary astrocyte cultures were prepared from the cerebral cortices of neonatal rats between postnatal days 1 and 2 as previously reported, with minor modifications.[11] Cerebral cortices were mechanically dissociated in ice-cold Hank's buffered salt solution (pH = 7.2) by fire-polished glass pipettes and centrifuged at 300 x g for 5 minutes. Cells were redissociated in ice-cold Hank's buffered salt solution and the procedure was repeated 2 times using pipettes with smaller tip diameters. Cells were seeded onto T-50 flasks and incubated in Dulbecco's modified Eagle's medium, containing 10% fetal bovine serum, 100 units/mL penicillin/streptomycin, and 2 mM L-glutamine, with or without sodium valproate (Sigma-Aldrich, St. Louis, MO), at 37°C and 5% CO_2 for up to 2 weeks.

Surgical Preparations

Spinal Nerve Ligation

L5-L6 SNL was performed as previously described.[15] Briefly, under anesthesia with 2% isoflurane in oxygen, the right L6 transverse process was removed and the right L5 and L6 spinal nerves were tightly ligated using 5-0 silk suture.

Intrathecal Catheterization

Animals were anesthetized with 2% isoflurane, and intrathecal catheterization was performed as previously described.[24] A small puncture was made in the atlanto-occipital membrane of the cistern magnum, and a polyethylene catheter (ReCathCo LLC, Allison Park, PA), 7.5 cm, was inserted so that the caudal tip reached the lumbar enlargement of the spinal cord. Animals were allowed at least 5 days to recover from the surgery.

Behavioral Testing

The researcher conducting behavioral testing (M.Y.) was blinded to the treatments.

von Frey Test

Hypersensitivity to light touch following SNL was assessed using calibrated von Frey filaments (Stoelting, Wood Dale, IL) applied to the plantar surface of the hind paw ipsilateral to surgery. Filaments were applied to the bending point for 5 seconds, and a brisk paw withdrawal was considered a positive response. Withdrawal threshold was determined using an up-down statistical method.[5]

Randall-Selitto Test

Nociceptive mechanical thresholds in normal rats were measured with the Randall-Selitto test using an analgesimeter (Ugo Basile, Comerio, Italy). The test was performed by applying pressure to the hind paw. When the animal withdrew the paw or vocalized, the pedal was immediately released and the nociceptive threshold was read on a scale. A cutoff of 250 g was used to avoid potential tissue injury.

Drugs and siRNA Treatments

Sodium valproate was dissolved with vehicle (.5% carboxymethylcellulose solution) and orally administered twice a day by a feeding tube (300 mg/5 mL/kg) from day 0 to day 21, and SNL surgery was performed at day 1. The dose of valproate was determined from our previous study,[13] and behavioral tests were performed at the morning 1 hour prior to the first administration during day 0 to day 22. For pharmacologic blockade of spinal GLT-1, dihydrokainic acid (DHK, 10 μg/10 μL/rat; Tocris Bioscience, Ellisville, MO) was dissolved in saline and intrathecally injected, followed by 10 μL saline, in valproate-treated SNL rats at day 14, and withdrawal threshold was tested 1 hour after injection. For knockdown of spinal GLT-1, an siRNA mixture for rat GLT-1 (SMARTpool #M-091209-02; Thermo Fisher Scientific Inc, Rockford, IL) or a nontargeting siRNA pool (#D-001206-14; Thermo Fisher Scientific Inc) was dissolved in double-distilled water, diluted with the transfection reagent (i-Fect; Neuromics, Edina, MN) to achieve a final concentration of .17 nmol/10 μL, and intrathecally injected for 5 days in normal or valproate-treated SNL rats from day 17 to day 21. On day 22, rats