The Glt1 glutamate receptor mediates the establishment and perpetuation of chronic visceral pain in an animal model of stress-induced bladder hyperalgesia

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ACKERMAN AL, JELLISON FC, LEE UJ, BRADESI S, RODRIGUEZ LV. The Glt1 glutamate receptor mediates the establishment and perpetuation of chronic visceral pain in an animal model of stress-induced bladder hyperalgesia. Am J Physiol Renal Physiol 310: F628–F636, 2016. First published December 23, 2015; doi:10.1152/ajprenal.00297.2015.—Psychological stress exacerbates interstitial cystitis/bladder pain syndrome (IC/BPS), a lower urinary tract pain disorder characterized by increased urinary frequency and bladder pain. Glutamate (Glu) is the primary excitatory neurotransmitter modulating nociceptive networks. Glt1, an astrocytic transporter responsible for Glu clearance, is critical in pain signaling termination. We sought to examine the role of Glt1 in stress-induced bladder hyperalgesia and urinary frequency. In a model of stress-induced bladder hyperalgesia with high construct validity to human IC/BPS, female Wistar-Kyoto (WKY) rats were subjected to 10-day water avoidance stress (WAS). Referred hyperalgesia and tactile allodynia were assessed after WAS with von Frey filaments. After behavioral testing, we assessed Glt1 expression in the spinal cord by immunoblotting. We also examined the influence of dihydrokainate (DHK) and ceftriaxone (CTX), which downregulate and upregulate Glt1, respectively, on pain development. Rats exposed to WAS demonstrated increased voiding frequency, increased colonic motility, anxiety-like behaviors, and enhanced visceral hyperalgesia and tactile allodynia. This behavioral phenotype correlated with decreases in spinal Glt1 expression. Exogenous Glt1 downregulation by DHK resulted in hyperalgesia similar to that following WAS. Exogenous Glt1 upregulation via intraperitoneal CTX injection inhibited the development of and reversed preexisting pain and voiding dysfunction induced by WAS. Repeated psychological stress results in voiding dysfunction and hyperalgesia that correlate with altered central nervous system glutamate processing. Manipulation of Glu handling altered the allodynia developing after psychological stress, implicating Glu neurotransmission in the pathophysiology of bladder hyperalgesia in the WAS model of IC/BPS.

bladder pain syndrome; interstitial cystitis; hyperalgesia; glutamate processing; ceftriaxone

VISCERAL PAIN DISORDERS ARE significant clinical problems, affecting as much as 25% of the population (29). Interstitial cystitis/bladder pain syndrome (IC/BPS) is one such condition, characterized by refractory discomfort referable to the lower urinary tract associated with urinary urgency/frequency and defecatory dysfunction. The perception of bladder fullness at low volumes is exquisitely painful, a phenomenon known as hyperalgesia or allodynia, pain in response to mildly noxious or innocuous stimuli, respectively.

Current evidence suggests IC/BPS may be a manifestation of afferent neurological dysfunction (6). Abnormal processing of sensory information related to the genitourinary tract results in bladder pain and voiding dysfunction via sensitization of visceral organ primary afferents, altered excitability of second-order pain-transmitting neurons, and dysregulation of descending pathways modulating bladder function and nociception, leading to central augmentation (2).

It is now well accepted that stress facilitates the central augmentation involved in these abnormal nociceptive responses. A majority of patients with IC/BPS report symptom exacerbation following psychological and experimental stresses (28). While the exact interplay with neuronal dysfunc
tion is unclear, stress in genetically susceptible individuals is associated with failure of local and descending inhibitory systems, magnifying pronociceptive visceral responses to typically nonnoxious stimuli (18).

Alterations in glial cell function may be fundamental in this enhancement of neuronal sensitivity and nociceptor activity through a complex interplay of cellular activation and production of soluble mediators. Several studies have identified altered neurotransmitter and cognate receptor expression within peripheral neurons, dorsal root ganglia, and the spinal cord in animal models of hyperalgesia. Glutamate is the key excitatory neurotransmitter within the central nervous system (CNS) participating in nociception (7). Regulation of glutamate levels in the synaptic cleft is mediated by transporters that reuptake extracellular glutamate; Glt1, expressed primarily on astrocytes, is responsible for 90% of glutamate clearance in the spinal cord (23). Glt1 is downregulated in multiple models of neuropathic pain (27) (17) as well as in a chemically induced cystitis model of IC/BPS (30), making this a promising therapeu
tic target in chronic bladder pain conditions. We sought to examine the role of glutamate processing in a physiological animal model of IC/BPS with high construct and face validity, induced by chronic exposure to stress through repetitive water-avoidance testing. These rodents exhibit enhanced nociceptive signaling in response to urinary bladder distention (22), generalized somatic hyperalgesia, colonic overactivity (4), and voiding dysfunction characterized by frequent small voids, increased mast cell activation in the bladder, and bladder hyperalgesia that is durable beyond the inciting stress (12).
MATERIALS AND METHODS

Animals. Female Wistar-Kyoto (WKY) rats 11–12 wk old (200–300 g) were purchased from Charles River Laboratories (Wilmington, MA). This strain is genetically predisposed to elevated levels of anxiety (16). Animals were maintained on a normal light-dark cycle; food and water were available ad libitum. Animals were allowed to accommodate for 2 wk before experimentation. Animals were housed in pairs in standard cages. All protocols were approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles (ARC 2011-143). All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

Chronic water avoidance stress protocol. Rats were placed on a cylindrical glass pedestal (8 × 8 × 11.5 cm³) in the center of a plexiglas cage (24 × 45 × 15 cm³). The container was filled with 25°C water to 1 cm below the top of the pedestal. Animals were exposed to water avoidance stress (WAS), a well-characterized psychological stressor, for 1 h/day for 10 days between 8:00 AM and 12:00 PM to minimize circadian effects (26). Control rats were handled for 1 min at the beginning and end of the experimental period (similar to rats exposed to WAS), but instead of exposing animals to water avoidance, they were placed in clean cages in isolation for 1 h. WAS-exposed rats were housed in pairs in the same facility as controls. No fewer than four rats per testing condition were used in any experiment. All rats were weighed immediately before the stress period was started. Control rats were watered to a normal light-dark cycle; flow of cerebrospinal fluid from this puncture site ensured appropriate placement of the incision. After flushing the intrathecal catheter (ReCathCo) with sterile saline, it was introduced into the puncture site and advanced slowly to the lumbar level. The catheter was immediately removed if any resistance or twitching from the animal was encountered. The catheter was secured in place to the muscle and allowed to exit the skin at the incision site. The catheter was then heat sealed to prevent cerebral spinal fluid (CSF) leakage. One hour before behavioral testing, DHK (20 μg/10 μl; EMD Millipore, Billerica, MA) or sterile saline (vehicle) was injected through the catheter, and the catheter was resealed to prevent leakage of CSF.

Fig. 1. Downregulation of an astrocytic transporter responsible for glutamate clearance (Glt1) during water avoidance stress (WAS). A: protein samples isolated from sacral spinal cord homogenates from Wistar-Kyoto (WKY) rats subjected to the specified days of WAS were analyzed by immunoblotting with antibodies specific for Glt1 (top). The relative protein levels were quantitated by densitometry and normalized to actin levels for consistency (bottom), demonstrating the downregulation of Glt1 levels in the spinal cord with increasing duration of psychological stress. Spinal samples were also examined after intraperitoneal administration of 200 mg ceftazidime (CTX), which exhibited enhanced Glt1 expression. 3T3, lysates from NIH/3T3 cells, which do not express Glt1, served as a negative control. *P = 0.016, **P = 0.007. B: percent response to stimulation of the bladder (visceral hyperalgesia) and the hindpaw (tactile allodynia) with an 8-g von Frey filament at the time points specified demonstrate increasing pain responses with greater duration of WAS. A minimum of 4 animals/treatment group were examined (n = 8 for t = 0, 10). NS, not significant. *P < 0.0001.
animals per treatment group were examined. 
Pendure after CTX treatment was discontinued (WAS/postCTX Washout). Four the increases in voiding frequency seen after WAS, but this effect did not WAS period was completed (WAS/vehicle) had no effect. When administered after the normal saline (WAS/CTX) decreased voiding frequency to baseline levels, while naive (Pre-WAS) or control animals. Intraperitoneal injection of CTX during, WAS increased the average number of voids per session compared with control animals after the WAS period was completed (WAS/postCTX). CTX was still able to reverse the increases in voiding frequency seen after WAS, but this effect did not endure after CTX treatment was discontinued (WAS/postCTX Washout). Four animals per treatment group were examined. \*P = 0.03. **P = 0.003. ***P = 0.001.

To examine the effect of DHK treatment on CTX-mediated pain modulation, intrathecal catheters were placed and attached to pre-equilibrated osmotic pumps (DURECT, Cupertino, CA) containing DHK as specified in the manufacturer’s protocol to provide a release rate of 0.5 μl/h over 14 days (24 μg/day). Osmotic pumps were implanted in a subcutaneous pocket below the scapulae before skin closure. Animals were housed individually after surgical procedures and allowed to recover for 3 days before testing. CTX or vehicle was then administered intraperitoneally immediately before each stress period. Behavioral testing was performed as specified above after completion of the 10-day WAS protocol.

Voiding frequency. Baseline micturition parameters in all animals were obtained using a metabolic cage (Techniplast United States, Exton, PA) for 2-h sessions before and after exposure to WAS or control conditions. Animals had free access to food and water. Voiding frequency, intervals, and volumes, as well as fecal pellet excretion and water intake, were recorded.

Immunoblotting. After euthanasia, spinal cords were hydroextruded withiced saline, and the lumbar L6—S2 spinal segments were dissected and sonicated in T-PEPER buffer (Pierce Biotechnology, Rockford, IL). After determination of total protein concentrations by standard BCA assay (Pierce), 20 μg protein were loaded onto an 8% SDS-PAGE gel and transferred to nitrocellulose membranes. Lysates from the NIH/3T3 cell line, which does not express Glt1, served as a negative control. After 1-h blocking in albumin, the membranes underwent overnight primary antibody (rabbit anti-Glt1 antibody, 1:1,000; rabbit anti-actin antibody, 1:1,000; Santa Cruz Biotechnology, Dallas, TX) and 1-h secondary antibody (horseradish peroxidase-conju-gated rabbit IgG, 1:3,000; Santa Cruz Biotechnology) incubation, followed by X-ray film exposure with Enhanced Chemiluminescent Substrate (Pierce). Band intensity was quantitated by densitometry; relative protein levels were determined by normalizing experimental intensities to those determined for actin in each sample.

Statistical analysis. Quantitative data are expressed as means ± SE. To compare single paired means, we used the paired Student’s t-test. For tactile allodynia, linear regression analysis was used to evaluate the effects of force on pain levels. For bladder-specific visceral hyperalgesia testing, a nonlinear mixed effects model repeated measures (MMRM) multivariate analysis with random intercepts was used to account for multiple measurements. This model utilized a quadratic effect of force on withdrawal percentage, interacted with both WAS and treatment status, to allow for calibrated force stimulus (von Frey filament weight) to vary by both treatment condition and the presence or absence of preceding WAS. For multiple treatment conditions, one-way ANOVA followed by the Fisher least significant difference test was utilized. A P value <0.05 was considered to be a statistically significant difference between the experimental groups.

RESULTS

Altered neurotransmitter processing after chronic stress. After subjecting rats to chronic stress with daily WAS for 10 days, we examined the levels of Glt1 in lysates from the sacral bulb of the spinal cord. Quantitative analysis of these immunoblots revealed that Glt1 transporter levels are decreased in the spinal cord after chronic stress with water avoidance compared with control animals (Fig. 1A).

The time course of Glt1 downregulation and its association with pain symptoms in these animals were then assessed. Animals were subjected to 0, 1, 3, 5, 8, or 10 days of WAS, then tested for tactile alldynia and visceral hyperalgesia im-

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**Fig. 2. Voiding dysfunction after WAS.** Following WAS, the average voided volume decreased significantly (A; \*P = 0.002), concomitant with increases in the number of voids (B; \*P = 0.03) compared with controls. C: we examined the ability of CTX to reverse WAS-associated voiding dysfunction. As seen in B, WAS increased the average number of voids per session compared with naive (Pre-WAS) or control animals. Intraperitoneal injection of CTX during WAS (WAS+CTX) decreased voiding frequency to baseline levels, while normal saline (WAS+vehicle) had no effect. When administered after the WAS period was completed (WAS+postCTX), CTX was still able to reverse the increases in voiding frequency seen after WAS, but this effect did not endure after CTX treatment was discontinued (WAS/postCTX Washout). Four animals per treatment group were examined. \*P = 0.03. **P = 0.003. ***P = 0.001.

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**Fig. 3. Dihydrokainate (DHK) administration induces visceral and tactile alldynia similar to WAS.** A: percent withdrawal responses to suprapubic stimulation with von Frey filaments increased significantly after 10 days of WAS (vis-ceral hyperalgesia). B: thresholds for withdrawal after hindpaw presentation (tactile alldynia) were significantly lower after WAS. A single intrathecal dose of DHK generated a pain phenotype indistinguishable from animals after WAS. Four animals per treatment group were examined. \*P <0.001.
Intrathecal DHK induces similar pain responses as chronic stress. To recreate the downregulation of Glt1 in the absence of psychological stress, we utilized DHK, a selective Glt1 inhibitor that does not cross the blood-brain barrier. After placement of indwelling intrathecal catheters, naive nonstressed animals were given a single intrathecal dose of vehicle (sterile saline) or DHK (20 μg) 1 h before assessment of tactile allodynia and visceral hyperalgesia. Glt1 inhibition via a single DHK administration was sufficient to generate a clinical phenotype of lowered pain thresholds in behavioral testing indistinguishable from that seen after WAS (Fig. 3, P < 0.001). Interestingly, neither a single dose nor continuous 14-day infusion of intrathecal DHK was able to induce the increases in voiding frequency and decreases in voided volume seen after chronic WAS (Fig. 4).

CTX prevents development of WAS-induced allodynia. CTX, a β-lactam antibiotic that increases Glt1 expression and activity in the brain and spinal cord, has good penetration into the CNS and a duration of action of several days. Intraperitoneal injection has been shown to attenuate mechanical allodynia in rats in different models of neuropathic pain (20) (8). To determine whether reversal of Glt1 downregulation could abrogate the induction of visceral pain by chronic stress, we administered CTX or vehicle (saline) throughout the 10-day WAS protocol. We first confirmed that daily intraperitoneal injection of 200 mg/kg CTX was able to upregulate Glt1 in the sacral spinal cord to levels averaging 171 ± 12.7% (P = 0.007) of that seen at baseline, even after the 10-day WAS period (Fig. 1A). In behavioral testing, this CTX-mediated increase in Glt1 levels prevented the induction of visceral hyperalgesia and tactile allodynia seen after WAS (Fig. 5, A and B; P < 0.001). Pain responses in CTX-treated animals were not significantly different from those seen in control animals that did not experience psychological stress (P = 0.64). CTX also prevented the increased voiding frequency seen in these animals when administered throughout the inciting WAS period (Fig. 2C, P = 0.003). CTX treatment of naive animals before any handling had no significant effect on pain responses (P = 0.262) or voiding parameters (P = 0.990) (Fig. 6).
CTX decreases pain responses via upregulation of Glt1. Cephalothin (CLT) is a β-lactam antibiotic with anti-inflammatory and antimicrobial effects similar to CTX that does not alter spinal Glt1 levels (13). We confirmed that CLT administration did not change spinal levels of Glt1 before or after 10 days of WAS (Fig. 7, P = 0.89). To confirm that the effect of CTX on pain responses resulted from its effect on Glt1 levels, and not from its antimicrobial or anti-inflammatory activities, we tested the ability of CLT to abrogate WAS-induced pain. While CTX significantly reversed the induction of pain by WAS (P < 0.001), animals treated with CLT at the same dose and administration schedule as CTX exhibited similar tactile allodynia and referred hyperalgesia as vehicle-treated animals (Fig. 8, P = 0.78), confirming that the effect of CTX on pain after chronic stress was independent of its action as an antibiotic.

These results suggest that CTX mediated its effect on pain by upregulating Glt1 levels in the spinal cord, an effect that should be reversible by direct Glt1 inhibition with DHK. We examined the ability of a continuous intrathecal administration of DHK throughout WAS to abrogate the CTX-mediated reversal of chronic pain development. An osmotic pump was attached to an implanted intrathecal catheter subcutaneously to deliver DHK continuously over a 14-day period. Three days after implantation, animals were subjected to WAS with daily CTX administration, which normally would prevent the induction of increased pain responses. Administration of DHK completely reversed the effects of CTX on pain after WAS, confirming the role of Glt1 downregulation in pain development (Fig. 9, P < 0.001).

CTX administration reverses previously established pain. As patients with IC/BPS present after the cycle of chronic pain is well established, we sought to determine the efficacy of Glt1 reduction in the reversal of previously established pain. We examined the therapeutic effect of CTX in animals after the establishment of visceral pain (Fig. 10). Animals were subjected to the WAS protocol, and the establishment of chronic pain was confirmed with behavioral testing (P < 0.001). Animals were then treated for 5 days with CTX or vehicle alone and retested for pain responses. Rats treated with vehicle demonstrated a persistence of the pain responses, while CTX-treated animals exhibited pain thresholds that were not significantly different from unstressed control animals (Fig. 10, P = 0.805). In addition, the increased voiding frequency seen in animals after WAS was also reversed by CTX administration (Fig. 2C, P = 0.003). To assess CTX as a potential therapeutic agent, we also tested its effect on animals with well-established pain, at time points distant from the inciting WAS stimulus. We allowed animals to recover for 60 days after WAS, periodically retesting for both tactile allodynia and visceral hyperalgesia to ensure persistence of the pain phenotype. As seen previously (12), pain responses persisted in stressed animals at this late time point (Fig. 11, P = 0.002). Administration of

![Fig. 6. CTX has no effect on pain parameters in naïve animals before WAS. Animals were injected with vehicle alone or CTX before any handling or stressors. Regardless of CTX treatment (P = 0.972), the percent withdrawal responses to suprapubic stimulation with von Frey filaments (visceral hyperalgesia; A) were minimal. The thresholds for withdrawal after hindpaw presentation (tactile allodynia; B) were greater than the stimuli tested and unchanged by CTX treatment (P = 1). The key for B is as specified in A. Four animals per treatment group were examined. *P = 0.037.](http://ajprenal.physiology.org/)

![Fig. 7. Cephalothin (CLT) does not alter Glt1 levels in the spinal cord. A: protein samples isolated from sacral spinal cord homogenates from control rats and rats subjected to WAS were analyzed by immunoblotting with antibodies specific for Glt1 (top) and actin (bottom). Glt1 protein levels were quantitated by densitometry and normalized to actin levels for consistency. Again, CTX was observed to increase Glt1 levels over baseline. Also, WAS was again seen to downregulate Glt1 levels from control animals. CLT treatment, however, had no effect on Glt1 levels in either control animals or those subjected to WAS. Four animals per treatment group were examined. 3T3, lysates from NIH/3T3 cells, which do not express Glt1, served as a negative control. *P = 0.03. **P = 0.007.](http://ajprenal.physiology.org/)
CTX for 5 days even at this late time point was sufficient to return responses in both visceral hypersensitivity and tactile allodynia testing to normal thresholds (P < 0.001).

The effect of CTX to abrogate the increased pain seen after chronic WAS, however, did not endure after discontinuation of CTX (Washout, Fig. 10). Thus, while useful therapeutically at reversing the chronic pain resulting from WAS in this model of IC/BPS, treatment with CTX was not curative and required continuous administration for persistent efficacy.

**DISCUSSION**

Previously, we demonstrated that chronic WAS, in the absence of a chemical stimulus, produces a chronic pain syndrome in susceptible animals characterized by tactile allodynia, visceral hyperalgesia, increased colonic motility, anxiety behaviors, and increased voiding frequency (12, 26), a phenotype similar to human IC/BPS. In this study, we demonstrate that these symptoms inversely correlate with alterations in spinal Glt1 expression; lower pain thresholds are associated with lower Glt1 protein levels. Exogenous inhibition of Glt1 via DHK treatment without psychological stress reproduces both bladder hyperalgesia and tactile allodynia, suggesting Glt1 inhibition is sufficient to establish chronic visceral pain. The relationship between glutamate processing and hyperalgesia is implicated in several other models of visceral pain (30), but this is the first report demonstrating a role for Glt1 in referred bladder pain and voiding dysfunction in an IC/BPS animal model generated without noxious stimuli.

CTX administration upregulates Glt1 expression and functional glutamate uptake in the spinal cord (8, 13). CTX treatment in the WAS model inhibits the visceral pain induced by chronic psychological stress, demonstrating Glt1 downregulation is necessary for bladder hyperalgesia. This inhibition of chronic pain resulted from CTX-mediated downregulation of

Fig. 8. CTX, but not CLT, reverses induction of pain by WAS. A: as seen in Fig. 5, treatment of rats with CTX during WAS (WAS + CTX) reversed the induction of higher pain responses seen after suprapubic stimulation with von Frey filaments, while treatment with CLT, a similar antibiotic without activity against Glt1, had no effect (WAS + CLT). B: a similar inhibition of tactile allodynia was seen in hindpaw stimulation with von Frey filaments following WAS with concurrent CTX, but not CLT, treatment. The key for B is the same as in A. Four animals per treatment group were examined. *P < 0.001. **P = 0.03.

Fig. 9. Intrathecal DHK abrogates the inhibitory effect of CTX on pain induction during WAS. A: WKY rats were subjected to WAS and treated with CTX through the stress period. Previously placed intrathecal pumps delivered either vehicle or DHK continuously throughout the stress period. Animals receiving vehicle alone exhibited minimal pain to either suprapubic (A) or hindpaw (B) stimulation, due to the inhibitory effect of CTX on stress-induced pain. DHK infusion, however, abrogated the effect of CTX, resulting in elevated visceral hyperalgesia (A) and tactile allodynia (B). The key for B is the same as in A. Four animals per treatment group were examined. *P < 0.001.
pared with controls (enhanced visceral pain responses after completing the WAS protocol continued to be elevated in animals subjected to WAS (WAS d60)). This increased pain, even long after WAS.

CTX administration after cessation of the inciting stress (Treatment) reversed the induction of pain in animals subjected to WAS (WAS + pCTX), but did not affect Control animals (Control + pCTX). The effect of CTX treatment to decrease pain responses, however, did not endure after discontinuation of CTX therapy. After discontinuation of CTX, all animals who had undergone WAS (WAS and WAS + pCTX) exhibited equivalent low pain thresholds, regardless of prior CTX administration. Four animals per treatment group were examined. *P < 0.001.

Visceral hyperalgesia could be effectively treated with CTX even at this late time point, restoring pain thresholds to control levels (WAS d60 + pCTX). CTX administration 60 days after cessation of the inciting stress (WAS d60 + CTX) reversed this pain to levels similar to those seen in control animals. Four animals per treatment group were examined. *P = 0.002. **P < 0.001. ***P = 0.008.

Fig. 11. CTX inhibits previously established pain, even long after WAS. A: visceral pain responses 60 days after the initial testing period continued to be elevated in animals subjected to WAS (WAS d60) compared with control animals (Control d60). This increased visceral hyperalgesia could be effectively treated with CTX even at this late time point, restoring pain thresholds to control levels (WAS d60 + pCTX). B: hindpaw stimulation demonstrated significantly lower pain thresholds in animals even 60 days after WAS (groups as designated in A). CTX administration 60 days after cessation of the inciting stress (WAS d60 + CTX) reversed this pain to levels similar to those seen in control animals. Four animals per treatment group were examined. *P = 0.002. **P < 0.001. ***P = 0.008.

spinal Glt1, not an anti-inflammatory or antibiotic effect, as equimolar doses of CLT, a β-lactam antibiotic that does not affect spinal Glt1, had no effect on pain following chronic stress. In addition, continuous intrathecal infusion during WAS of DHK, a specific inhibitor of Glt1 activity, abrogated the effect of CTX on visceral pain. These results verify that CTX’s ability to enhance glutamate transport via Glt1 upregulation is responsible for its clinical activity in pain prevention.

Glt1 is emerging as a central mediator in multiple neurological diseases. In addition to chronic pain, glutamate modulation via Glt1 is implicated in anoxic and traumatic brain injury (19), addiction (21), neurodegenerative diseases (25), episodic neurologic illnesses such as epilepsy and migraine (1), and even psychiatric illness such as depression and schizophrenia (9). As Glt1 downregulation is both necessary and sufficient for the establishment of chronic pain in our model, Glt1 is a promising target for therapeutic intervention in syndromes of bladder hyperalgesia such as IC/BPS as well. As CTX has been used clinically for years with minimal side effects and is safe at high doses for long periods (3), selective Glt1 inhibition would likely have few deleterious effects in humans.

In the WAS animal model, CTX is also able to attenuate chronic stress-induced visceral pain in a therapeutic manner after the inciting stress. To be clinically efficacious, therapy for patients with IC/BPS must be able to reverse previously established chronic visceral pain. Even after months of established visceral pain, CTX treatment returned pain thresholds to pre-stress baseline levels, although the visceral hyperalgesia returned upon discontinuation of CTX therapy.

We hypothesize that chronic stress in anxiety-prone animals induces a cascade of events that results in the decreased pain thresholds, voiding and defecatory dysfunction, and anxiety behaviors. The rapid return of pain upon removal of CTX suggests Glt1 downregulation is maintained by other upstream cellular processes. Initial signals generated as a consequence of WAS likely result in sustained global changes in nociceptive pathways that are able to reestablish Glt1 inhibition once CTX is removed. These global changes, however, likely impact more than just glutamate metabolism. The inability of DHK to induce increased voiding frequency similar to that seen after WAS suggests that changes in glial Glt1 expression are not sufficient to generate the entire clinical phenotype seen in this model, suggesting there are Glt1-independent mechanisms that
Altered glutamate processing controls bladder hyperalgesia.

Research attempting to identify stress-induced mediators that establish chronic pain has implicated several pathways, stress-associated catecholamine production and β2-adrenergic receptor activation (18), increased mast cell activation and histamine release, or local inflammation promoting cytokine, growth factor, and chemokine release, all of which can promote changes in neural activity. Genetic alterations in glutamate transporters may also predispose to chronic pain conditions. For example, a genome-wide discovery identified an allelic variant in a regulatory gene associated with idiopathic migraine that results in Glt1 downregulation (1).

While the exact interplay between environmental stresses, intrinsic inflammation, and neuronal dysregulation in the development and maintenance of chronic pain remains unclear, a model is emerging in which inciting stress in a genetically susceptible individual results in alterations in immune and glial cell function that leads to the enhancement of neuronal sensitivity and nociceptor activity. This dysfunctional neural processing of painful stimuli may result in visceral hyperalgesia in a subpopulation of stress-susceptible individuals, which may manifest in the urinary tract as a clinical phenotype of IC/BPS. A better understanding of these underlying molecular mechanisms and the identification of specific targets critical in establishing bladder hyperalgesia will hopefully lead to the development of targeted therapies capable of providing much-wanted relief to a marginalized, debilitated population, allowing these affected individuals to improve their quality of life. As we gain additional insight into the mechanisms that govern IC/BPS, we may find that many of the results obtained in this model of bladder hyperalgesia will be applicable to other chronic functional pain syndromes.

Thus psychological stress plays a critical role in functional pain disorders like IC/BPS. In this study, we utilize a novel animal model in which chronic psychological stress induces visceral pain and urinary frequency in the absence of direct bladder irritants or inflammatory agents with high construct validity to the clinical manifestation of IC/BPS. The establishment of chronic pain correlates with alterations in glutamate neurotransmission attributable to the downregulation of glial Glt1 receptors. Exogenous upregulation of Glt1 via CTX treatment had a profound therapeutic effect; CTX both inhibited the development of and reversed established pain and voiding dysfunction. The efficacy of Glt1 manipulation over a wide range of neurological diseases points to glutamate metabolism as a critical convergence of pathologies for these varied diseases. Gaining greater insight into the molecular mechanisms controlling Glt1 expression and function is central in both understanding the pathophysiology of chronic pain and in identifying novel therapeutic approaches to these costly, treatment-refractory diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

12. Lee UJ, Ackerman AL, Wu A, Zhang R, Leung J, Bradesi S, Mayer EA, Rodriguez LV. Chronic psychological stress in high-anxiety rats in the development and reversed established pain and voiding dysfunction alone. Identification of the upstream events influenced by CTX, but independent of DHK, will be critical in understanding the effects of stress on bladder sensitivity in susceptible individuals and determining treatment modalities effective against the entire constellation of symptoms.