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LATERAL THALAMIC CONTROL OF NOCICEPTIVE RESPONSE AFTER WHISKER PAD INJECTION OF VARICELLA ZOSTER VIRUS

PHILLIP R. KRAMER,[a]* CRYSTAL STINSON,[a] MIKHAIL UMORIN,[a] MOHONG DENG,[b] MAHESH RAO,[a] LARRY L. BELLINGER,[a] MICHAEL B. YEE[c] AND PAUL R. KINCHINGTON[c]

[a] *Texas A&M University College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, United States*

[b] *Department of Oral and Maxillofacial Surgery, The State Key Laboratory Breeding Base of Basic Science of Stomatology & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University, Luoyu Road 237, Wuhan 430079, China*

[c] *Department of Ophthalmology and of Microbiology and Molecular Genetics, University of Pittsburgh, 203 Lothrop Street, Pittsburgh, PA 15213, United States*

Abstract—Pain is a common complication of herpes zoster (HZ) infection which results from reactivation of a latent varicella zoster virus (VZV). A third of HZ patients' progress to a chronic pain state known as post herpetic neuralgia (PHN), and about a quarter of these patients' have orofacial pain. The mechanisms controlling the pain responses are not understood. Studies suggest central pathways involving the thalamus could control pain related to HZ, and studies in our lab suggest (VGAT) in the lateral thalamus influences orofacial pain. We hypothesized that thalamic VGAT functions, in part, to reduce pain, particularly orofacial pain, associated with VZV. To address this hypothesis VZV was injected into the whisker pad. Affective and motivational aspects of pain were measured using the Place Escape/Avoidance Paradigm. Thalamic neuronal activity was modulated after injecting an adeno-associated virus (AAV) expressing an engineered acetylcholine Gi-protein-coupled receptor. This receptor inhibits neuronal firing when bound by clozapine-n-oxide (CNO). VGAT expression was attenuated in the thalamus by injecting an AAV construct that expressed a VGAT silencing shRNA. VZV-induced nociception was significantly decreased after administering CNO in male rats. Nociception significantly increased concomitant with increased thalamic c-fos expression after attenuating thalamic VGAT expression. These data establish that the lateral thalamus (posterior, ventral posteromedial, ventral posterolateral and/or reticular thalamic nucleus) controls VZV-induced nociception in the orofacial region, and that GABA in this region appears to reduce the response to VZV-induced nociception possibly by gating facial pain input.

Keywords: VGAT, GABA, pain, shingles, orofacial, thalamus.

INTRODUCTION

Infection with the Herpesvirus Varicella zoster virus (VZV) causes chickenpox, a self-limiting childhood disease that is followed by a latent state. Reactivation of VZV results in the clinically distinct disease herpes zoster (HZ), commonly termed ''shingles" (Johnson et al., 2010). The trigeminal ganglia has the highest viral load (Mahalingam et al., 1990) and cadaver studies suggest about 80% of the population harbors VZV in the trigeminal ganglia (Pevenstein et al., 1999). Moreover, a significant fraction of HZ cases (up to 56%) have facial involvement (Ragozzino et al., 1982; Pavan-Langston, 1995). A form of HZ known as herpes zoster ophthalmicus (HZO) may develop in ocular tissues resulting in ocular pain and loss of visual acuity; HZO occurs in about 50,000 individuals annually (Pavan-Langston, 2000). Zoster-associated pain occurs during the acute phase, while chronic pain lasting longer than 90 days develops in about 20–30% of HZ sufferers (Mitchell et al., 2003; Dubinsky et al., 2004; Colon, 2009; Fashner and Bell, 2011; Kinchington and Goins, 2011). This chronic condition is termed post-herpetic neuralgia (PHN). In approximately 30% of PHN patients, pain can last for more than one year (Kawai et al., 2014). HZ incidence increases with age, with most HZ sufferers being 60 years of age or older. Given that the population over 60 years of age is predicted to double in the next 50 years (Kawai et al., 2014), HZ and the associated pain are increasingly important problems in the future. Although vaccines for both varicella and HZ are available, the HZ vaccine prevents only about half of HZ and reduces ''clinical illness" (including PHN) by about two-thirds (Harpaz et al., 2008). Thus, even in a fully vaccinated population (which is far from being achieved) zoster associated pain, HZ and PHN will remain common.

A rat model of PHN was first detailed by Fleetwood Walker's group, where footpad inoculation of VZV induced long-term hyperalgesia and allodynia mirroring the chronic allodynia seen in PHN patients (Fleetwood-Walker et al., 1999; Dalziel et al., 2004). In this model the rat exhibits pain behaviors after a primary infection

*Corresponding author. Address: Department of Biomedical Sciences, Texas A&M University College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, United States. Fax: +1-214-874-4538.
E-mail addresses: pkramer@tamhsc.edu (P. R. Kramer), kinchingtonp@upmc.edu (P. R. Kinchington).

Abbreviations: CNO, clozapine-n-oxide; HZ, herpes zoster; PEAP, Place Escape/Avoidance Paradigm; PHN, post herpetic neuralgia; VGAT, vesicular GABA transporter; VZV, varicella zoster virus.

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rather than reactivation, currently no animal model of reactivation is known. The response profile of VZV-induced pain was similar to that seen in humans; for example, antivirals, opioids or NSAIDS were not highly effective treatments (Garry et al., 2005; Hasnie et al., 2007). Even with current treatment there remains a need for more effective therapy, since up to half of PHN patients gain little to no relief from any current therapy (Beal et al., 2012; Derry et al., 2013). Mechanisms for VZV-induced pain are not yet resolved, but VZV may not need to replicate completely to induce a response (Surman et al., 1990; Hempenstall et al., 2005; Guedon et al., 2015). VZV gene expression is required for pain (Guedon et al., 2014) and the VZV transcriptional regulator IE62 has been postulated to alter host gene expression programs leading to pain induction (Kinchington and Goins, 2011).

The thalamus has a role in orofacial pain responses (Bezdudnaya and Keller, 2008). A facial HZ patient with PHN was found to have reduced thalamic activity on a PET scan (Iadarola et al., 1995). In humans, MRI studies have shown that reduced GABA activity in the thalamus was associated with pain involving the trigeminal pathway (Henderson et al., 2013). Lesion of the lateral thalamic region, including the reticular thalamic nucleus, can heighten pain responses (Saade et al., 1999). Interestingly, all neurons in the reticular thalamic region express GABA with only a miniscule population of GABA-positive cells in the adjacent ventroposterior nuclei (Barbaresi et al., 1986). Neurons in the ventroposterior thalamic nucleus send collaterals to the adjacent reticular thalamic nucleus and GABA-positive neurons in the reticular thalamic nucleus send axons back to the ventroposterior thalamic nucleus "gating" pain signals passing through the thalamus to the cortex (Vahle-Hinz et al., 1994; Lam and Sherman, 2011). The trigeminal nuclei project to the posterior thalamic nucleus and GABA neurons in the zona incerta affect thalamic activity (Masri et al., 2009; Chang et al., 2012). To extend these studies our lab has shown that vesicular GABA transporter (VGAT) expression was elevated in the lateral thalamic region, including the reticular thalamic nucleus, when the nociceptive response was decreased (Umorin et al., 2016). Although it has been hypothesized that central pain pathways have a role in HZ pain the genes and pathways involved have not been established. In this study we hypothesized that VGAT expression in the lateral thalamic region will affect HZ associated pain in the orofacial region of the rat. Most VGAT is expressed in the reticular thalamic nucleus of the lateral thalamic region (Lein et al., 2007). Accordingly, we evaluated nociception by measuring an escape and/or avoidance behavior after injecting the whisker pad with VZV and determined the response after modulating lateral thalamic neuronal activity and VGAT expression.

EXPERIMENTAL PROCEDURES

This study was approved by the Baylor College of Dentistry Institutional Animal Care and Use Committee. Male (300–350 g) Sprague–Dawley rats from Envigo (Indianapolis, IN, USA) were kept on a 14:10 light/dark cycle. The rats were given food and water *ad libitum*. After a 4-day acclimation period experiments were carried out in accordance with the NIH regulations on animal use. Two experiments using two different batches of rats were completed, in Experiment #1 twenty-two male rats were injected with 100 μl of a high concentration of VZV, the parent Oka strain (pOka), at >1000 plaque forming units (pfu)/μl (provided by Dr. Kinchington). In Experiment #2 thirty-four male rats were injected with a lower concentration of VZV from a different preparation (650 pfu/μl).

Surgery

Adult male Sprague–Dawley rats were anesthetized with 2% isoflurane and an air flow of 2 liter per minute. Aseptic stereotaxic (David Kopf Instruments, Tujunga, CA, Model 1460-61) injection of virus was performed with the needle tip (Hamilton #7002KH Neuros syringe, Reno, NV, USA) at coordinates 3.6 mm posterior of Bregma, 3.0 mm from midline at a depth of 6.0 mm. A Stoelting stereotaxic syringe pump system was used to infuse 0.250 μl of $2–8 \times 10^{12}$ pfu/ml AAV8 or 1×10^{13} pfu/ml AAV5 or AAV9 at a rate of 20 nanoliters per minute. In the vehicle (no virus) group 0.250 μl of vehicle (350 nM NaCl, 5% D-Sorbitol in PBS) was infused. After infusion the needle was left in place for 5 min and then removed. In Experiment #1 twenty-two male rats were infused bilaterally with AAV8 expressing a neuronal silencing construct Syn–hM4D(Gi)-mcherry (Gene Therapy Center Vector Core, University of North Carolina at Chapel Hill) or vehicle. Expression of the receptor was driven by the neuronal synapsin-1 promoter (Syn), which drove expression in most neurons. The hM4D(Gi) gene was an engineered acetylcholine Gi-protein-coupled receptor that inhibits neuronal firing when bound by clozapine-n-oxide (CNO) (Rogan and Roth, 2011). Upon CNO binding the receptor stimulates calcium release, ERK1/2 activation, inhibits forskolin-induced cAMP formation and potentially GIRK activation, thereby causing hyperpolarization and inhibition of basal action potential firing (Rogan and Roth, 2011). To affect change in neuronal activity, the modified acetylcholine Gi protein-coupled receptor was expressed primarily in the posterior, ventral posteromedial and ventral posterolateral thalamic nuclei of Sprague–Dawley rats (Fig. 1A), and activated by binding CNO (Alexander et al., 2009). In Experiment #2 the right thalamus of thirty-four male rats was injected with AAV9 containing either a verified VGAT shRNA construct (5′-CACCGCAT CATCGTGTTCAGCTACACTCGAGTGTAGCTGAACAC GATGATGCTTTTT-3′) driven by the U6 promoter with a mCherry tag or AAV5 containing a scrambled shRNA construct (5′-AGGATCCAGTACTGCTTACGATACGGTT CAAGAGACCGTATCGTAAGCAGTACTTTTTTT-3′) driven by the U6 promoter containing a GFP tag (Vector Biolabs, Philadelphia, PA, USA). Sites of injection were verified by identification of cells positive for the immunofluorescent tag. Animals were given a 5 mg/kg dose of nalbuphine I.M. (Hospira, Lake Forest, IL, USA) after surgery and allowed to recover 7 days.