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Enhanced Postsynaptic $GABA_B$ Receptor Signaling in Adult Spinal Projection Neurons after Neonatal Injury

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Abstract—Clinical research and basic science research have revealed persistent effects of early-life injury on nociceptive processing and resulting pain sensitivity. While recent work has identified clear deficits in fast $GABA_A$- and glycine receptor-mediated inhibition in the adult spinal dorsal horn after neonatal tissue damage, the effects of early injury on slow, metabotropic inhibition within spinal pain circuits are poorly understood. Here we provide evidence that neonatal surgical incision significantly enhances postsynaptic $GABA_B$ receptor signaling within the mature superficial dorsal horn (SDH) in a cell type-dependent manner. *In vitro* patch-clamp recordings were obtained from identified lamina I projection neurons and GABAergic interneurons in the SDH of adult female mice following hindpaw incision at postnatal day (P)3. Early tissue damage increased the density of the outward current evoked by baclofen, a selective $GABA_B$ receptor agonist, in projection neurons but not inhibitory interneurons. This could reflect enhanced postsynaptic expression of downstream G protein-coupled inward-rectifying potassium channels (GIRKs), as the response to the GIRK agonist ML297 was greater in projection neurons from neonatally incised mice compared to naive littermate controls. Meanwhile, presynaptic $GABA_B$ receptor-mediated reduction of spontaneous neurotransmitter release onto both neuronal populations was unaffected by early-life injury. Collectively, our findings suggest that ascending nociceptive transmission to the adult brain is under stronger control by spinal metabotropic inhibition in the aftermath of neonatal tissue damage.

Key words: dorsal horn, pain, metabotropic, inhibition, synapse.

INTRODUCTION

Clinical studies report that infants in the neonatal intensive care unit (NICU) are subjected to an average of 14 invasive procedures per day in the first two weeks of life (Simons et al., 2003). Medically necessary procedures, such as heel lance, reliably evoke nociceptive responses (Cornelissen et al., 2013) and dramatically alter pain thresholds in neonates (Fitzgerald et al., 1989). While invasive procedures during the preterm period have been linked to reduced pain reactivity to modest stimuli (Oberlander et al., 2000), time spent in the NICU is correlated with increased activation of pain-related brain areas in response to moderately painful stimuli during adolescence (Hohmeister et al., 2010). Adolescents previously admitted to the NICU also exhibited greater perceptual sensitization and less habituation during repeated noxious stimulation (Hohmeister et al., 2010). Similarly, a single hindpaw injury in neonatal rats causes acute hyperalgesia followed by a generalized hypoalgesia, and yet exacerbates pain severity following reinjury (Ren et al., 2004; Walker et al., 2009). Unfortunately, the cellular and molecular mechanisms underlying these prolonged changes in pain processing after early-life tissue damage remain poorly understood.

The spinal cord serves as a conduit between the periphery and the brain for sensory and motor functions. In the SDH, lamina I projection neurons receive primary afferent input and convey signals to supraspinal pain centers, while local inhibitory interneurons normally suppress nociceptive transmission and reduce the output of the SDH network (Todd, 2010). Given the ongoing reorganization of pain circuits in the CNS during the early postnatal period, aberrant sensory input during this sensitive period of development can exert dramatic and

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Abbreviations: SDH, superficial dorsal horn; P, postnatal day; GIRK, G protein-coupled inward-rectifying potassium channel; NICU, neonatal intensive care unit; $GABA_BR$, $GABA_B$ receptor; eGFP, enhanced green fluorescent protein; GAD67, glutamic acid decarboxylase 67; PB, parabrachial; aCSF, artificial cerebrospinal fluid; TTX, tetrodotoxin; mPSCs, miniature postsynaptic currents; mEPSCs, miniature excitatory postsynaptic currents; mIPSCs, miniature inhibitory postsynaptic currents; AUC, area under the curve; KCTD, potassium channel tetramerization domain-containing protein.

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long-lasting effects on circuit function (Fitzgerald, 2005). For example, GABAergic signaling is highly plastic in the neonatal spinal cord, with significant regulation occurring throughout early postnatal and adolescent life (Keller et al., 2001, 2004), raising the possibility that tissue damage during this critical period could interfere with the normal maturation of spinal inhibitory circuits. Indeed, early-life surgical incision persistently dampens fast inhibitory neurotransmission in the adult mouse SDH (Li et al., 2013, 2015). However, although persistent deficits in $GABA_A$ receptor-mediated signaling occur after neonatal incision, little is known about the relationship between early-life injury and metabotropic inhibitory signaling in the mature dorsal horn.

The $GABA_B$ receptor ($GABA_BR$) is a metabotropic G protein-coupled receptor that is highly expressed in laminae I–II of the SDH (Yang et al., 2001). $GABA_BR$ acts to reduce presynaptic neurotransmitter release (Chen and van den Pol, 1998; Bussieres and El Manira, 1999; Barral et al., 2000; Sakaba and Neher, 2003) and evoke postsynaptic membrane hyperpolarization via the activation of downstream G protein-coupled inward-rectifying potassium channel (GIRK) channels (Fernández-Alacid et al., 2009). Significant evidence supports the importance of the $GABA_BR$ for the modulation of spinal nociceptive processing. Intrathecal administration of $GABA_BR$ antagonists causes allodynia and thermal hyperalgesia in rodents (Malan et al., 2002). Additionally, intrathecal, but not systemic, baclofen administration mitigates hyperalgesia in adult rats after hindpaw incision (Reichl et al., 2012). This highlights the need to understand how neonatal tissue damage alters $GABA_BR$ signaling in the mature SDH. Furthermore, given the rich heterogeneity of cell types that characterizes the SDH network (Peirs and Seal, 2016), it is important to elucidate the influence of early-life injury on $GABA_BR$ function in identified neuronal populations within the spinal pain circuit.

The present results demonstrate that neonatal surgical injury enhances postsynaptic $GABA_BR$-mediated signaling in ascending projection neurons, but not inhibitory interneurons, of the adult mouse SDH. This may reflect an elevated expression of GIRK channels, as the direct activation of these channels also evoked greater outward currents in mature projection neurons after neonatal hindpaw incision. Meanwhile, early tissue damage failed to affect $GABA_BR$-mediated presynaptic inhibition of spontaneous neurotransmitter release onto either neuronal population in the adult SDH. In summary, this work suggests that noxious sensory experience during early life may strengthen the ability of spinal $GABA_BRs$ to restrict the flow of ascending nociceptive transmission to the brain.

EXPERIMENTAL PROCEDURES

Animals

In order to identify inhibitory interneurons in the spinal cord dorsal horn, homozygous transgenic mice expressing enhanced green fluorescent protein (eGFP) under control of the glutamic acid decarboxylase 67 (GAD67) gene promoter (FVB-Tg(GadGFP)4570Swn/J; Jackson Laboratory, Bar Harbor, ME) were used in all experiments. We recorded from GFP-labeled laminae I and II interneurons located within 50 μM of the myelin border. We chose to initiate our study with adult (P56–77) female mice based on previous research demonstrating sex differences after neonatal injury. Although both sexes experience hypoalgesia in adulthood after neonatal inflammation, female rats show higher mechanical thresholds and the phenotype occurs earlier (LaPrairie and Murphy, 2007). Additionally, female but not male mice show an acute elevation in spontaneous excitatory signaling in the spinal dorsal horn after neonatal incision (Li and Baccei, 2011).

Neonatal hindpaw incision

On P3, pups were anesthetized via isoflurane inhalation (5%), and the left hindpaw was incised through the plantar skin and muscle as previously described (Brennan et al., 1996). The skin was sutured (7-0; Ethicon; Cincinnati, OH, USA) and the injury completely healed within two weeks.

Projection neuron identification

The parabrachial nucleus (PB) was chosen as the site of injection since it is the target of the majority of lamina I projection neurons located on the contralateral side of the lumbar enlargement of the spinal cord (Cameron et al., 2015), and retrograde labeling would be most efficient at this site. At least three days before being euthanized, animals were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) and secured in a stereotaxic apparatus (World Precision Instruments; Sarasota, FL, USA) with non-rupture ear bars (World Precision Instruments; Sarasota, FL, USA). An incision was made in the scalp to expose a skull area that included both lambda and bregma. The coordinates to target the PB were (in mm in relation to bregma): −0.47 to 0.49 rostrocaudal, −0.12 mediolateral, and −0.40 to 0.42 dorsoventral based on an atlas of the mouse brain (Paxinos, 2013); a hole was drilled in the skull using an OmniDrill35 (World Precision Instruments; Sarasota, FL, USA). Mice received an injection (150 nL at an infusion rate of 30 nL/min) of the retrograde tracer FAST DiI oil (2.5 mg/mL; Invitrogen; Carlsbad, CA, USA) into the PB contralateral to the P3 hindpaw incision using a Hamilton syringe (62RN; 2.5 μL; 33-gauge needle). The skin was closed using Vetbond (3M; Maplewood, MN, USA) and animals were returned to the home cage upon recovery.

Electrophysiology

*Preparation of **in vitro** spinal cord slices.* Adult female GAD67-GFP mice were euthanized with sodium pentobarbital (Fatal-Plus; Vortech Pharmaceuticals; Dearborn, MI, USA) and transcardially perfused with cold, sucrose-substituted artificial cerebrospinal fluid (aCSF) (dissection solution; containing in mM: 250 sucrose, 2.5 KCl, 25 $NaHCO_3$, 1.0 NaH_2PO_4, 6 $MgCl_2$, 0.5 $CaCl_2$, and 25 glucose). All solutions were