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Partial TrkB receptor activation suppresses cortical epileptogenesis through actions on parvalbumin interneurons

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ABSTRACT

Post-traumatic epilepsy is one of the most common and difficult to treat forms of acquired epilepsy worldwide. Currently, there is no effective way to prevent post-traumatic epileptogenesis. It is known that abnormalities of interneurons, particularly parvalbumin-containing interneurons, play a critical role in epileptogenesis following traumatic brain injury. Thus, enhancing the function of existing parvalbumin interneurons might provide a logical therapeutic approach to prevention of post-traumatic epilepsy. The known positive effects of brain-derived neurotrophic factor on interneuronal growth and function through activation of its receptor tropomyosin receptor kinase B, and its decrease after traumatic brain injury, led us to hypothesize that enhancing trophic support might improve parvalbumin interneuronal function and decrease epileptogenesis. To test this hypothesis, we used the partial neocortical isolation ('undercut', UC) model of posttraumatic epileptogenesis in mature rats that were treated for 2 weeks, beginning on the day of injury, with LM22A-4, a newly designed partial agonist at the tropomyosin receptor kinase B. Effects of treatment were assessed with Western blots to measure pAKT/AKT; immunocytochemistry and whole cell patch clamp recordings to examine functional and structural properties of GABAergic interneurons; field potential recordings of epileptiform discharges in vitro; and video-EEG recordings of PTZ-induced seizures in vivo. Results showed that LM22A-4 treatment 1) increased pyramidal cell perisomatic immunoreactivity for VGAT, GAD65 and parvalbumin; 2) increased the density of close appositions of VGAT/gephyrin immunoreactive puncta (putative inhibitory synapses) on pyramidal cell somata; 3) increased the frequency of mIPSCs in pyramidal cells; and 4) decreased the incidence of spontaneous and evoked epileptiform discharges in vitro. 5) Treatment of rats with PTX BD4–3, another partial TrkB receptor agonist, reduced the incidence of bicuculline-induced ictal episodes in vitro and PTZ induced electrographic and behavioral ictal episodes in vivo. 6) Inactivation of TrkB receptors in undercut $TrkB^{F616A}$ mice with 1NMPP1 abolished both LM22A-4-induced effects on mIPSCs and on increased perisomatic VGAT-IR. Results indicate that chronic activation of the tropomyosin receptor kinase B by a partial agonist after cortical injury can enhance structural and functional measures of GABAergic inhibition and suppress posttraumatic epileptogenesis. Although the full agonist effects of brain-derived neurotrophic factor and tropomyosin receptor kinase B activation in epilepsy models have been controversial, the present results indicate that such trophic activation by a partial agonist may potentially serve as an effective therapeutic option for prophylactic treatment of posttraumatic epileptogenesis, and treatment of other neurological and psychiatric disorders whose pathogenesis involves impaired parvalbumin interneuronal function.

1. Introduction

Cortical injury is a major cause of sensory, motor and cognitive deficits in both civilian and military populations, accounting for ~20% of all symptomatic epilepsies (Hauser et al., 1991; Annegers et al., 1998)., This emphasizes the need for understanding the underlying pathophysiological processes and for development of prophylactic strategies (Garga and Lowenstein, 2006). Many structural and functional changes are present following traumatic brain injury (TBI) (Prince et al., 2009; Giblin and Blumenfeld, 2010; Li et al., 2011; Vezzani et al., 2013; Aronica et al., 2017; Lippmann et al., 2017) and, of these, decreased GABAergic inhibition due to alterations in inhibitory

Abbreviations: BDNF, brain derived neurotrophic factor; Pyr, pyramidal; IPSCs, inhibitory postsynaptic currents; eIPSCs, evoked inhibitory postsynaptic currents; VGAT, vesicular GABA transporter; GAD, glutamic acid decarboxylase; IR, immunoreactivity; TrkB-R, tropomyosin receptor kinase B; LM, LM22A-4; 1NMPP1, 1, 1-(1,1-dimethylethyl)-3-(1-naphthalenylmethyl)-1H–pyrazolo[3,4-*d*]pyrimidin-4-amine; BD, PTX BD 4–3; UC, undercut; PPR, paired pulse ratio; FS, fast-spiking; PV, parvalbumin

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interneurons is prominent (Ribak and Reiffenstein, 1982; De Lanerolle et al., 1989; DeFelipe, 1999; Cossart et al., 2001; Li and Prince, 2002; Kumar and Buckmaster, 2006; Zhang and Buckmaster, 2009; Faria and Prince, 2010; Faria et al., 2012; Ma and Prince, 2012).

The mechanisms underlying interneuronal alterations in models of epileptogenesis following injury are largely unknown, and therapy to improve the function of surviving interneurons in injured brain is not available. Previous in vitro slice experiments in the partial neocortical isolation ("undercut" or "UC") model of posttraumatic epileptogenesis showed that fast-spiking (FS) parvalbumin (PV)-containing inhibitory interneurons were structurally and functionally abnormal in the area of injury and deafferentation (reviewed in (Prince et al., 2012). Results showed impaired inhibitory synaptic transmission from fast-spiking (FS) parvalbumin (PV)-containing interneurons onto excitatory neurons, as well as to other FS cells (Ma and Prince, 2012). The abnormalities of inhibitory synaptic transmission were compatible with dysfunction in presynaptic inhibitory terminals, as evidenced by increased failure rates; decreased amplitude of unitary (u) IPSCs (Ma and Prince, 2012); decreased mIPSC frequency in Pyr cells (Li and Prince, 2002); decreased probability of release (Pr) for monosynaptic IPSCs (Faria and Prince, 2010) and a reduction in presynaptic N current Ca^{++} channel function (Faria et al., 2012). Although there was no significant reduction in density of PV interneurons in the UC cortex (Gu et al., 2017), our results (Prince et al., 2009; Prince et al., 2016; Gu et al., 2017) showed a significant decrease in perisomatic immunoreactivity (IR) for PV, GAD65/67 and VGAT around layer V Pyr cells in UC rat sensorimotor cortex, due predominantly to alterations in terminals of fast-spiking (FS) PV-containing interneurons that target Pyr cell somata (Chaudhry et al., 1998). These structural changes suggested a reduction in inhibitory synapses on Pyr cells after cortical injury, which was confirmed by electron microscopy showing decreased density of symmetrical synapses on layer V Pyr cell somata (Gu et al., 2017).

Many the above-mentioned alterations are also reported in inhibitory neurons of deafferented barrel cortex in immature mice (Jiao et al., 2006, 2011) and in interneurons of mutant mice lacking activity-dependent BDNF expression (Hong et al., 2008; Lin et al., 2008; Sakata et al., 2009; Jiao et al., 2011). BDNF/TrkB signaling has well-known effects on interneuronal development, maintenance, plasticity and inhibitory transmission. Interneuronal developmental maturation depends on trophic support through activation of TrkB receptors by BDNF (Marty et al., 1996; Marty et al., 1997; McAllister et al., 1999). The complex effects of TrkB-R activation by BDNF include enhancement of interneuronal structure and inhibitory function (Croll et al., 1994; Rutherford et al., 1997; Turrigiano, 1999; Baldelli et al., 2002; Aguado et al., 2003; Baldelli et al., 2005). BDNF treatment in vivo can restore presumed inhibitory terminals that had been "stripped" from axotomized spinal cord motoneurons (Novikov et al., 2000), and reverse degenerative changes in axotomized corticospinal neurons (Giehl and Tetzlaff, 1996; Brock et al., 2010), that are a principal source of BDNF in neocortex. In UC cortex, BDNF is decreased in pyramidal (Pyr) cells (Prince et al., 2009; Gu et al., 2017), presumably in response to axotomy and the deafferentation resulting from the partial cortical isolation. Loss of BDNF in single Pyr cells reduces their GABAergic innervation and their mIPSC frequency, presumably due to decrease in paracrine effects of released BDNF on presynaptic inhibitory terminals (Kohara et al., 2007). These results and the availability of a small molecule, LM22A-4, ("LM" below) that selectively activates TrkB receptors and has favorable effects in brain disease models (Massa et al., 2010; Han et al., 2012; Schmid et al., 2012; Simmons et al., 2013; Kron et al., 2014; Yu and Wang, 2015; Li et al., 2017), led us to hypothesize that injury-induced epileptogenic structural and functional abnormalities in neocortical PV interneurons might be reduced or reversed by partial activation of TrkB receptors with LM.

To test this hypothesis, we performed Western blots to measure effects of LM on pAKT/AKT; immunocytochemistry and whole cell patch clamp recordings to examine functional and structural properties of GABAergic interneurons; and field potential recordings of epileptiform discharges in vitro. To confirm that the effects of LM actions are mediated through TrkB receptors, 1NMPP1 was used in vivo to inactivate these receptors in $TrkB^{F616A}$ mice (Chen et al., 2005). Also, video-EEG recordings were obtained to test the effects of another TrkB partial agonist, PTX BD4–3, on PTZ-induced seizures in vivo. Our results show that chronic activation of TrkB receptors after cortical injury decreases structural and functional abnormalities in GABAergic inhibition and has prophylactic antiepileptogenic effects.

2. Materials and methods

All experiments were carried out according to National Institutes of Health Guide for the Care and Use of Laboratory Animals and protocols approved by the Stanford Institutional Animal Care and Use Committee.

2.1. Partial cortical isolations

Male Sprague-Dawley rats aged P21 (55–60 g, P0 = date of birth) and mice aged P21 were used for UC surgery. Partial isolations of sensorimotor cortex were made as previously described (Hoffman et al., 1994; Graber and Prince, 2006; Gu et al., 2017) (See Supplementary material for details). After surgery, animals were given carprofen (5 mg/kg sc). Lesioned animals recovered from surgery uneventfully and were re-anesthetized for slice experiments 2 weeks later. Two weeks after removal of the bone window, a thin covering of bone had regrown over the skull opening. In some experiments, partial cortical isolations were made in transgenic $TrkB^{F616A}$ mice (Chen et al., 2005), kindly provided by Michael Stryker at U.C.S.F. These mice carry a mutated TrkB receptor in which alanine is substituted for phenylalanine at residue 616 within kinase subdomain V, making TrkB kinase activity sensitive to inhibition by a small-molecule derivative of the protein kinase inhibitor protein phosphatase 1, 1NMPP1.

2.2. Protocols for LM, 1NMPP1 and PTX BD4–3 (BD) administration

Animals were randomly selected for treatment with LM obtained from Ricercar LLC, as described in Massa et al. (2010), or saline for 2 weeks, beginning ~10 min after completion of UC surgery. Dosage was 50 mg/kg i.p. once a day, together with 5 mg/kg intranasal, a protocol previously shown to achieve a brain concentration similar to, or exceeding that at which LM is biologically active and able to affect TrkB function (Massa et al., 2010). Immunocytochemical experiments and electrophysiological recordings were performed 2–7 days after the last LM dose. For experiments to assess the effects of TrkB activation on seizures in vivo, PTX BD4–3 (BD) (50 mg/kg, once a day, ip x 7d) was used. BD is a new partial TrkB receptor agonist kindly provided by Professor Frank Longo. It has better blood brain barrier penetration and a longer half-life than LM22A-4 (F. Longo, personal communication). In experiments on $TrkB^{F616A}$(mice, 1NMPP1 was dissolved in DMSO (100 mM) and stored at −20 °C. Before use, 100 μl 1NMPP1 was diluted in 2 ml saline and 2.5% Tween-20 solution and then added to 400 ml of distilled drinking water (final concentration 25 μM) (Chen et al., 2005; Wang et al., 2009). 1NMPP1 administration was begun on the day of the UC surgery, as soon as the mice recovered from anesthesia and began to drink, and continued for 2 weeks, along with LM treatment.

2.3. Neocortical slice preparation and field potential recordings

Neocortical slice preparation was as previously described (Graber and Prince, 2006) (see Supplementary Material). After incubation, slices were transferred to an interface chamber where the partially isolated cortical area was easily identified under a dissecting microscope and evoked field potentials recorded in layer V with an ACSF-filled glass pipette. Focal extracellular 100 μs single square-wave pulses were delivered at 0.1 Hz through a concentric bipolar electrode (FHC,