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Research Paper

Galanin contributes to monoaminergic dysfunction and to dependent neurobehavioral comorbidities of epilepsy

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ABSTRACT

Status epilepticus (SE) in rats, along with chronic epilepsy, leads to the development of behavioral impairments resembling depressive disorder and/or attention deficit/hyperactivity disorder (ADHD), thus reflecting respective comorbidities in epilepsy patients. Suppressed neurotransmitter tone in the raphe nucleus (RN)-prefrontal cortex (PFC) serotonergic pathway and in the locus coeruleus (LC)-PFC noradrenergic pathway underlies depressive- and impulsive-like behavioral deficits respectively. We examined possible mechanisms leading to the monoamine dysfunction in brainstem efferents, namely modulatory effects of the neuropeptide galanin on serotonin (5-HT) and norepinephrine (NE) signaling. SE was induced in young adult male Wistar rats by LiCl and pilocarpine. Epileptic rats were categorized vis-à-vis behavioral deficits as not impaired, "depressed" and "impulsive". Depressive- and impulsive-like behaviors were examined in the forced swimming test (FST). The strength of serotonergic transmission in RN-PFC and of noradrenergic transmission in LC-PFC was analyzed using in vivo fast scan cyclic voltammetry. Galanin receptor type 1 (GalR1)/type 2 (GalR2) antagonist M40, and a preferential GalR2 antagonist M871 were administered over 3 days locally into either RN or LC by means of ALZET osmotic minipumps connected to locally implanted infusion cannulas. Intra-RN injection of M40 improved serotonergic tone and depressive-like behavior in epileptic "depressed" rats. Intra-LC injection of M40 improved noradrenergic tone and impulsive-like behavior in epileptic "impulsive" rats. The effects of M40 were only observed in impaired subjects. The treatment did not modify neurotransmission and behavior in naïve and epileptic not impaired rats; in "depressed" rats the effects were limited to serotonergic transmission and immobility, while in "impulsive" rats – to noradrenergic transmission and struggling behavior. Intra-RN administration of M871 exacerbated depressive-like behavior, but had no effects on any other of the examined parameters in any category of animals. These findings suggest that endogenous galanin, acting through GalR1 may be involved in the pathophysiology of epilepsy-associated depression and ADHD via inhibiting RN-PFC serotonergic and LC-PFC noradrenergic transmissions respectively.

1. Introduction

Depressive disorder and attention deficit/hyperactivity disorder (ADHD) are two common comorbidities of epilepsy. Prevalence of depression among epilepsy patients is 30–50% vs. 5–17% in people without epilepsy (Kanner, 2003; Kanner et al., 2012); for ADHD the numbers are 25% and 3–5% respectively (Parisi et al., 2010; Schubert, 2005).

Numbers aside, psychiatric disorders have profound detrimental effects on the quality of life of people with epilepsy, as they exacerbate the severity of the disease and hamper the effectiveness of anticonvulsant interventions (Baca et al., 2011; Kanner, 2016; Luoni et al., 2011).

In a series of studies, we showed that sub-populations of rats with post-status epilepticus (SE) chronic epilepsy consistently presented with either depressive-like, ADHD-like behavioral impairments, or with both (Kumar et al., 2016; Mazarati et al., 2008; Pineda et al., 2014, 2012). We established that depressive-like behavior stemmed from the suppressed serotonergic transmission in the raphe nucleus (RN) – prefrontal cortex (PFC) pathway. At the same time, ADHD-like impairments developed as a result of deficient noradrenergic transmission in the locus coeruleus (LC)-PFC pathway (Mazarati et al., 2008; Pineda et al., 2014).

Events that lead to the dysfunction of respective monoaminergic systems are of significant interest both from a mechanistic point of view and for their therapeutic implications. For example, the upregulation of presynaptic receptors, specifically 5-HT1A in RN (Pineda et al., 2011; Pineda et al., 2012), and α2A adrenoreceptors in LC (personal

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unpublished data) may contribute to the observed monoamine deficiencies.

Galanin is a bioactive peptide ubiquitous in the mammalian brain (Gundlach et al., 1990; Merchenthaler et al., 1993; Skofitsch and Jacobowitz, 1986), with a broad spectrum of neurophysiological and neurobehavioral actions (Lang et al., 2015; Mitsukawa et al., 2010). Galanin is a well-established potent modulator of all types of monoaminergic transmission (Hökfelt et al., 1998; Kuteeva et al., 2008; Lundstrom et al., 2005; Picciotto et al., 2010). Three galanin receptor (GalR) subtypes have been cloned, all being G-protein coupled receptors (GPCR). GalR1 is coupled to G_i protein and its activation produces membrane hyperpolarization. GalR2 is coupled to $G_{q/11}$ and thus has a depolarizing effect. GalR1 and GalR2 are likely present both in RN and in LC, while $G_{i/o}$ – coupled GalR3 (Smith et al., 1998) is not (Le Maitre et al., 2013; Lundstrom et al., 2005; Mitsukawa et al., 2010; Webling et al., 2012).

Coexistence of galanin with serotonin (5-HT) and norepinephrine (NE) suggests that the peptide may regulate monoamine-dependent behaviors. Indeed, effects of GalR ligands on depression have been well documented, whereby activation of RN GalR1 exerts pro-depressant, and of GalR2-antidepressant effects via negative and positive regulation of serotonergic transmission respectively (Kuteeva et al., 2010; Kuteeva et al., 2008; Lu et al., 2005; Mazarati et al., 2005).

Along with regulating behavior, galanin signaling has been implicated in epilepsy and epileptogenesis. In the hippocampus, the activation of both GalR1 and GalR2 had anticonvulsant effects and attenuated neuronal cell death after SE (Mazarati and Lu, 2005; Mazarati et al., 2006; Mazarati et al., 2000; Mazarati et al., 1998). Antiepileptic effects of galanin were observed in the kindling model (Mazarati et al., 2006; Schlifke et al., 2006). In RN, GalR1 facilitated, while GalR2-attenuated seizures via the discussed modulation of the serotonergic RN-hippocampal pathway (Mazarati et al., 2005).

The purpose of the present study was to examine the involvement of endogenous galanin in impairments of monoamine neurotransmission, and in related behavioral deficits associated with chronic epilepsy. We report that in animals with epilepsy, pharmacological blockade of GalR1 in RN improves serotonergic transmission in RN-PFC and exerts antidepressant effect. Blockade of GalR1 in LC improves noradrenergic transmission and attenuates impulsivity.

2. Subjects, materials and methods

2.1. Experimental subjects

The experiments were performed in male Wistar rats (Charles River, Wilmington, MA), fifty days old at the beginning of the study, in accordance with the policies of the National Institutes of Health and of the UCLA Office of Protection of Research Subjects.

2.2. Experiment design

The study consisted of the following steps (Fig. 1). Induction of SE, followed 4 weeks later by animal selection and category assignments based on the spontaneous seizure frequency and the animals' performance in the forced swimming test (FST). Within each category, the subjects were then randomized for treatments. In the main experiments (solid lines, gray box), GalR blockers were administered over 3 days into either RN or LC, followed by FST and fast scan cyclic voltammetry (FSCV) of 5-HT and NE in the ascending pathways, at the time of drug infusions. In an additional experiment (dashed lines, outside the gray box), FST was repeated two weeks later (i.e. after the one-week washout), and was followed by FSCV.

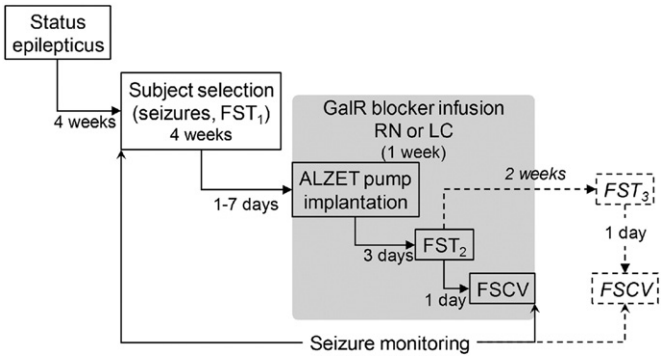

Fig. 1. Study design. Experiment design. Explanations in the Section 2.2.

2.3. Epilepsy model

SE was induced by LiCl (128 mg/kg, i.p., Sigma, St. Louis, MO) followed 24 h later by pilocarpine (40 mg/kg, s.c., Sigma). In order to alleviate the severity of SE and to decrease the frequency of subsequent spontaneous seizures, rats received i.p. injections of diazepam (10 mg/kg) and phenytoin (50 mg/kg) one and four hours after the SE onset (Kumar et al., 2016; Mazarati et al., 2008; Pineda et al., 2014, 2012). In control animals, saline was injected instead of pilocarpine. Starting from four weeks after SE and until the end of the study, the animals were continuously video-monitored for documenting spontaneous seizures (Fig. 1).

2.4. Forced swimming test (FST)

FST was used to examine depressive- and impulsive-like behaviors as described by our group (Kumar et al., 2016; Mazarati et al., 2008; Pineda et al., 2014). The first FST (FST_1) was performed 4 weeks after SE; the second (FST_2) was conducted during the administration of GalR ligands; some animals underwent the third test (FST_3) after the drug washout (Fig. 1). Time between the tests was at least 2 weeks, so as to avoid any previous forced swimming experience to affect an animal's performance (De Pablo et al., 1989; Mezadri et al., 2011). The test consisted of a single 5-minute-long session in a tank filled with water at 22°–25 °C. Behavior was video-recorded and analyzed offline in a blinded fashion. Cumulative durations of three distinct behaviors were calculated: active adaptive behavior (i.e. swimming along the walls, climbing, diving); immobility (i.e. movements were limited to maintaining the head above the surface with no escape attempts); and non-adaptive struggle (i.e. treading water away from the walls with no attempts to escape) (Mazarati et al., 2008; Pineda et al., 2014). The first two behaviors are typical for both normal animals and those with depressive-like impairments. In validated models of depression, the immobility time is increased, and this increment is regarded as both an indicator, and a measure of the inability to cope with the stress (Cryan et al., 2005). Non-adaptive struggle is negligible in normal rats, but occurs in 25–50% of animals with epilepsy (Kumar et al., 2016; Pineda et al., 2014). We established that only those animals which displayed the non-adaptive struggle during FST, presented with impulsivity in an ADHD-specific Lateralized Reaction Time Task (LRTT) (Pineda et al., 2014). The downside of LRTT and similar tasks (such as 5-choice serial reaction time task, 5-CSRT) is that they take weeks to complete (Faure et al., 2014; Jentsch, 2005; Jentsch et al., 2009) and are thus associated with substantial challenges when used in chronic epilepsy (Faure et al., 2014; Pineda et al., 2014). Based on the congruency between ADHD-specific behavior during LRTT and non-adaptive struggle during FST, we proposed that the latter could be used as a simple surrogate marker of impulsivity (Pineda et al., 2014). Such a suggestion was further confirmed by an observation that both impulsivity in LRTT and non-adaptive struggle in the FST bore a specific neurochemical