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Divergent effects of levetiracetam and tiagabine against spontaneous seizures in adult rats following neonatal hypoxia

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ABSTRACT

Animal models are valuable tools for screening novel therapies for patients who suffer from epilepsy. However, a wide array of models are necessary to cover the diversity of human epilepsies. In humans, neonatal hypoxia (or hypoxia-ischemia) is one of the most common causes of epilepsy early in life. Hypoxia-induced seizures (HS) during the neonatal period can also lead to spontaneous seizures in adulthood. This phenomenon, i.e., early-life hypoxia leading to adult epilepsy − is also seen in experimental models, including rats. However, it is not known which anti-seizure medications are most effective at managing adult epilepsy resulting from neonatal HS. Here, we examined the efficacy of three anti-seizure medications against spontaneous seizures in adult rats with a history of neonatal HS: (1) phenobarbital (PHB), the oldest epilepsy medicine still in use today; (2) levetiracetam (LEV); and (3) tiagabine (TGB). Both LEV and TGB are relatively new anticonvulsant drugs that are ineffective in traditional seizure models, but strikingly effective in other models. We found that PHB and LEV decreased seizures in adult rats with a history of HS, whereas TGB exacerbated seizures. These divergent drug effects indicate that the HS model may be useful for differentiating the clinical efficacy of putative epilepsy therapies.

1. Introduction

A major goal of preclinical epilepsy research is to identify novel therapies. Existing animal models of epilepsy have proved exceptionally useful for assessing the utility of novel therapies, however, up to a third of patients with epilepsy have seizures that are unresponsive to current therapies (Kwan and Brodie, 2000). Thus, there is a clear need for new therapeutic strategies. As historically key components of the Epilepsy Therapy Screening Program workflow (https://panache.ninds.nih.gov/), the maximal electroshock seizure (MES) and acute pentylenetetrazole (PTZ) seizure tests played critical roles in the discovery and development of many therapies which are now in use in the clinic. However, there is concern that excessive dependence on these models will by definition only identify drugs that work by similar mechanisms to current therapies (Löscher, 2016,2011), resulting in incremental improvements for patients already responsive to known anti-seizure medications, but no improvement for patients who are unresponsive to exiting treatments.

The identification of fundamentally different anti-seizure medications has previously required fundamentally different seizure models. For example, both levetiracetam (LEV) and tiagabine (TGB) are ineffective in the MES model, but were identified based on their efficacy in the amygdala kindling model of seizures (Dalby and Nielsen, 1997a, 1997b; Klitgaard et al., 1998; Löscher and Hönack, 1993). The difference in drug efficacy in these preclinical models is likely related to fundamental differences in the seizures generated in each model: the MES model engages brainstem networks mediating tonic seizures while the amygdala kindling model engages forebrain seizure networks more typical of complex partial seizures. Both drugs display superior efficacy in partial, as compared to tonic seizure models, despite divergent mechanisms of action. LEV modulates presynaptic neurotransmitter release (Lynch et al., 2004; Vogl et al., 2012) while TGB inhibits GABA reuptake and has been suggested to suppress seizures through increases in ambient (perisynaptic) GABA levels (Suzdak and Jansen, 1995).

While both drugs shared a promising and unconventional profile in preclinical screens, LEV and TGB differ dramatically in their human

Abbreviations: HS, hypoxiainduced seizurel; ISI, interseizure interval; LEV, levetiracetam; PHB, phenobarbital; TGB, tiagabine

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clinical utility. In several assessments of anti-seizure drug prescription rates, levetiracetam is consistently among the most commonly prescribed anti-seizure drug; indeed in some studies it is the *most common* (Malerba et al., 2010). Meanwhile, tiagabine is one of the least utilized (Bauer and Cooper-Mahkorn, 2008) and can in some cases exacerbate seizures (Ettinger et al., 1999; Knake et al., 1999). Therefore, there remains a need for seizure or epilepsy models that can a) identify drugs that may be ineffective in traditional MES/PTZ or amygdala kindling models, b) demonstrate predictive value for clinical usefulness based on existing drugs' success and failures.

The model of neonatal hypoxia-induced seizures (HS), initially used to study hypoxic/ischemic encephalopathy, has recently been appreciated as an etiologically relevant model of spontaneous seizures in the adult (Jensen et al., 1991; Rakhade et al., 2011). Hypoxia and hypoxia-ischemia are two of the most common causes of neonatal seizures in humans, and have been associated with the development of epilepsy later in life (Bergamasco et al., 1984). However, few studies have examined the effects of existing anticonvulsants on seizures in adult seizures arising from a history of neonatal HS. Thus, this model is poorly understood, both in relation to the multitude of other existing seizure models, and as a potential screening tool for the development of new therapies.

Seizures in adult rats with a history of HS are subtle, consisting solely of electrographic manifestations accompanied by behavioral arrest and facial automatisms. Epidural elecetroencephalographic (EEG) recordings have revealed a characteristic pattern of seizure onset, ictal tonic activity, and seizure termination with small amplitude spikes and post-ictal slow waves (Rakhade et al., 2011). Seizures in this model arise in forebrain networks, raising the possibility that they may be sensitive to atypical drugs such as LEV and TGB. As rats age from P60 − P180, seizure frequency increases from ~1/hour to ~5/hour and average duration increases from ~4 s to ~13s. Despite subtle behavioral manifestation, HS causes the same hippocampal mossy fiber sprouting (Lippman-Bell et al., 2013; Rakhade et al., 2011; Talos et al., 2013) seen in many epilepsy models (Babb et al., 1991; Holmes et al., 1999).

Given the etiological validity of this model, we hypothesized that it might serve as a useful tool for drug screening. Accordingly, we subjected Long Evans rats to hypoxia as neonates and monitored their seizures in adulthood. We examined the efficacy of phenobarbital (positive control), levetiracetam and tiagabine against these spontaneous seizures during adulthood. The findings we present here are discussed in the context of the HS model as a screening tool for anti-seizure drugs.

2. Materials and methods

2.1. Animals

Experimental procedures were approved by the Georgetown University Animal Care and Use Committee and conducted using male Long Evans rats (n = 11) (Charles River). Animals were housed in a temperature and humidity controlled room on a 12-h light/dark cycle (Lights on 0700–1900). Food (Lab Diet #5001) and water were available *ad libitum*. Animals arrived at the facility at P8 and were subjected to graded global hypoxia (described below) at P10. Following hypoxia, animals were returned to their dam until weaning at P21. At P21, animals were weaned and pair-housed until surgery at P65-75 for EEG assessment. All experimental manipulations occurred during the light phase of the light cycle.

2.2. Graded global hypoxia

We selected this model as it has been demonstrated to produce spontaneous seizures in adult animals, but little is known about its profile of sensitivity to anti-seizure medications. Graded global hypoxia was induced as previously described (Jensen et al., 1991; Rakhade et al., 2011; Sun et al., 2016). Briefly, P10 Long-Evans rat pups were placed on a heating pad in a large airtight chamber regulated by an OxyCycler Oxygen Profile Controller (Model A84XOV; BioSpherix, Redfield, NY, USA). Nitrogen was introduced into the chamber, displacing oxygen until the desired oxygen levels (7% for 8 min, 5% for 6 min, 4% for 1 min) were reached. Transitions between oxygen concentrations took 15–30 s. All animals exhibited acute seizures, characterized by head bobbing, wet dog shakes, and tonic-clonic behavior.

2.3. EEG electrode placement surgery

At P65-P75, rats were anesthetized with 2.8 mg/kg equithesin (a mixture of chloral hydrate, magnesium sulfate and pentobarbital sodium) (Sigma, St Louis, MO), delivered by intraperitoneal injection in prepration for electrode implantation, which occurred as previously described (Soper et al., 2016). Epidural screw electrodes were implanted into the skull such that the bottom of each screw was in contact with the dura. Each animal was implanted with a total of 6 electrodes, with screws placed bilaterally over the parietal and frontal lobes, and the cerebellum (for ground and reference). EEG wires were routed into a plastic pedestal (PlasticsOne, Roanoke, VA) and held in place with dental acrylic. Animals were then returned to their home cages for 2–3 months of recovery to allow for normalization of seizure threshold (Forcelli et al., 2013).

2.4. Drug treatments

During drug treatments, all animals were P130-P150. Levetiracetam (LEV; Keppra, UCB Pharma), tiagabine (TGB; Sigma-Aldrich), or phenobarbital (PHB; Sigma-Aldrich) were dissolved or diluted from stock in 0.9% saline and warmed before intraperitoneal injection. All solutions were prepared such that 1 ml/kg of body weight was injected. To assess the effect of drug treatment within animals, an ordered treatment schedule was used (Saline, LEV 13 mg/kg, LEV 35 mg/kg, LEV 54 mg/kg, TGB 0.5 mg/kg, TGB 1.0 mg/kg, TGB 2.0 mg/kg, PHB 50 mg/kg), but where animals started in the ordered schedule was pseudorandomized. Animals had an average of 2.5 days between drug treatment sessions to allow for drug washout. Electrographic seizure activity was analyzed for each of the up to eight sessions.

2.5. EEG monitoring

Animals were placed in plexiglass monitoring cages filled with 0.5 inches of bedding for at least 10 min before drug/saline injection. Recordings began within five min of drug administration and lasted for 1.5 h. EEGs were obtained by coupling the plastic EEG pedestal to a preamplifier/amplifier (Pinnacle Technologies, Lawrence, KS). Data were recorded using LabChart 7 and 8 (AD Instruments, Colorado Springs, CO) with a 60 Hz low-pass filter. A subset of animals were subject to concurrent video monitoring. In no case did we observe seizures with manifestations beyond cessation of ongoing behavior and facial twitches.

2.6. EEG analysis

As described above, 1.5 h electrographic records were reviewed for each drug or saline session. Electrographic seizures (or epileptiform events) were identified manually post hoc by an observer (RLD) blind to the treatment status of the animals; a subset of records were also reviewed by PAF. Criterion for scoring a seizure included: (1) the presence at least five spikes that were > 2x baseline signal; (2) a spike frequency of ~10–50 Hz; and (3) the presence of the activity on at least two electrode channels. These criteria were consistent with previous reports analyzing HS adult seizures (Rakhade et al., 2011). Epileptiform activity was "generalized" across the cortical leads (left and right