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Seizure-induced disinhibition of the HPA axis increases seizure susceptibility

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Summary Stress is the most commonly reported precipitating factor for seizures. The proconvulsant actions of stress hormones are thought to mediate the effects of stress on seizure susceptibility. Interestingly, epileptic patients have increased basal levels of stress hormones, including corticotropin-releasing hormone (CRH) and corticosterone, which are further increased following seizures. Given the proconvulsant actions of stress hormones, we proposed that seizure-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis may contribute to future seizure susceptibility. Consistent with this hypothesis, our data demonstrate that pharmacological induction of seizures in mice with kainic acid or pilocarpine increases circulating levels of the stress hormone, corticosterone, and exogenous corticosterone administration is sufficient to increase seizure susceptibility. However, the mechanism(s) whereby seizures activate the HPA axis remain unknown. Here we demonstrate that seizure-induced activation of the HPA axis involves compromised GABAergic control of CRH neurons, which govern HPA axis function. Following seizure activity, there is a collapse of the chloride gradient due to changes in NKCC1 and KCC2 expression, resulting in reduced amplitude of sIPSPs and even depolarizing effects of GABA on CRH neurons. Seizure-induced activation of the HPA axis results in future seizure susceptibility which can be blocked by treatment with an NKCC1 inhibitor, bumetanide, or blocking the CRH signaling with Antalarmin. These data suggest that compromised GABAergic control of CRH neurons following an initial seizure event may cause hyperexcitability of the HPA axis and increase future seizure susceptibility.

Abbreviations: CRH, corticotropin-releasing hormone; CORT, corticosterone; HPA, hypothalamic-pituitary-adrenal; $GABA_AR$, $GABA_A$ receptor; KCC2, K^+/Cl^- co-transporter 2; KA, kainic acid; NKCC1, Na-K-Cl co-transporter 1.

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Introduction

Robust anecdotal evidence from the clinic suggests that there is a link between stress and epilepsy (for review see Maguire and Salpekar, 2013). The majority of patients with epilepsy self-report that stress exacerbates and/or triggers their seizures (Nakken et al., 2005; Sperling et al., 2008; Neugebauer et al., 1994; Frucht et al., 2000; Haut et al., 2003, 2007) (for review see Lai and Trimble, 1997; Maguire and Salpekar, 2013). Interestingly, some studies have demonstrated anticonvulsant effects of stress hormones, such as deoxycorticosterone (Reddy and Rogawski, 2002), which has been attributed to the production of stress-derived neurosteroids (Reddy and Rogawski, 2002). In addition, adrenocorticotropic hormone (ACTH) is an effective treatment for infantile spasms (Snead et al., 1983; Arya et al., 2012; Jaseja and Jaseja, 2013). These studies demonstrate the complex relationship between steroid hormones and epilepsy (for review see Sawyer and Escayg, 2010), which likely depends upon the age of the subject, the type of steroid hormone, and duration of exposure. However, there is substantial evidence demonstrating the pro-convulsant actions of stress and stress hormones, including corticotropin-releasing hormone (CRH) and corticosterone (for review see Joels, 2009). Consistent with the role of stress in epilepsy, cortisol is elevated in patients with epilepsy and is further increased following seizures (Culebras et al., 1987; Tunca et al., 2000; Abbott et al., 1980; Pritchard et al., 1985). Furthermore, cortisol levels are positively correlated with seizure frequency in patients with epilepsy (Culebras et al., 1987; Galimberti et al., 2005). Given the pro-convulsant actions of stress hormones, we hypothesized those seizure-induced elevations in stress hormone levels may foster a proconvulsant environment and contribute to further seizure susceptibility.

The production of stress hormones is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, involving the release of CRH from the hypothalamus, which acts in the anterior pituitary to signal the release of ACTH, which then triggers the release of cortisol from the adrenal cortex in humans (corticosterone in rodents). CRH neurons are at the apex of HPA axis control and govern the production and release of stress hormones. These neurons receive input from numerous different brain regions and are regulated by multiple neurotransmitter systems (for review see Herman et al., 2003; Larsen et al., 2003; Ulrich-Lai and Herman, 2009). Ultimately, the activity of these neurons is tightly regulated by GABAergic inhibition (for review see Herman et al., 2004; Decavel and van den Pol, 1990). Our laboratory has recently uncovered the mechanisms through which CRH neurons overcome this robust GABAergic constraint to elicit the body's physiological response to stress (Sarkar et al., 2011). However, the mechanisms mediating elevations in stress hormone levels following seizures are unknown.

Alterations in $GABA_A$ receptor ($GABA_AR$) subunit expression have been shown to occur in many brain regions in both animal models of epilepsy and in patients with epilepsy (Zhang et al., 2007) (for review see Sperk et al., 2009). The majority of these studies have focused on changes in $GABA_AR$ subunit expression and GABAergic inhibition in the hippocampus following seizures. It is unknown whether similar changes occur in the paraventricular nucleus (PVN) of the hypothalamus following seizures which may alter GABAergic control of CRH neurons and contribute to elevations in the levels of stress hormones. We hypothesize that seizures induce deficits in the GABAergic control of CRH neurons, resulting in hyperexcitability of the HPA axis which may contribute to further seizure susceptibility.

Here we demonstrate that seizures impair the GABAergic regulation of CRH neurons by inducing a collapse in the chloride gradient. The chloride gradient in neurons is maintained by the K^+/Cl^- co-transporter (KCC2) in the adult brain (Rivera et al., 1999; Payne et al., 2003; Rivera et al., 2005) which is necessary for the inhibitory actions of GABA. The extrusion of chloride by KCC2 is opposed by the active transport of chloride into the cell by the Na-K-Cl co-transporter 1 (NKCC1). Our data demonstrate that there is a dephosphorylation of KCC2 residue Ser940 and a downregulation of KCC2 and an increase in NKCC1 in the PVN following seizures induced with kainic acid. Our lab previously demonstrated a role for KCC2 in mediating stress-induced activation of CRH neurons and elevations in corticosterone. Our data suggest that seizures activate the HPA axis using mechanisms similar to stress. Alterations in KCC2 expression in the PVN following seizures is associated with a reduction in the amplitude of sIPSPs and even depolarizing actions of GABA on CRH neurons, an increased firing rate of CRH neurons, elevations in circulating corticosterone, and increased seizure susceptibility. Blocking seizure-induced activation of the HPA axis with a CRH receptor antagonist, Antalarmin, or preventing the collapse in the chloride gradient with an NKCC1 antagonist, bumetanide, prevented future seizure susceptibility. These data suggest that changes in the regulation of the HPA axis following an initial seizure episode may result in HPA axis hyperexcitability and increased seizure susceptibility. Thus, insight into the mechanisms contributing to the dysregulation of the HPA axis associated with epilepsy may have significant therapeutic potential for seizure control.

Methods

Animal handling

Mice expressing green fluorescent protein (GFP) specifically in CRH neurons have previously been characterized in our laboratory (Sarkar et al., 2011). Adult male CRH-GFP mice (C57Bl/6 background) were bred and housed at the Tufts University School of Medicine, Division of Laboratory Animal Medicine. The animals (5/cage) were housed in clear plastic cages in a temperature-, and humidity-controlled environment with a 12 h light/dark cycle (light on at 7 a.m.), and were maintained on an *ad libitum* diet of lab chow and water. Animals were handled according to protocols approved by the Institutional Animal Care and Use Committee of the Tufts University School of Medicine.

Treatments

Kainic acid

Kainic acid (Sigma) was dissolved in sterile injection saline (0.9% sodium chloride) and either 10 mg/kg or 20 mg/kg was delivered by intraperitoneal (*i.p.*) injection. For acute corticosterone measurements, mice were injected with either