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Early life maternal separation stress augmentation of limbic epileptogenesis: The role of corticosterone and HPA axis programming

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Summary Early life stress causes long-lasting effects on the limbic system that may be relevant to the development of mesial temporal lobe epilepsy (MTLE) and its associated psychopathology. Recent studies in rats suggest that maternal separation (MS), a model of early life stress, confers enduring vulnerability to amygdala kindling limbic epileptogenesis. However, the mechanisms underlying this remain unknown. Here, we tested whether hypothalamic-pituitary-adrenal (HPA) axis hyper-reactivity induced by MS — specifically the excessive secretion of corticosterone following a seizure — was involved in this vulnerability. In adult female rats subjected to MS from postnatal days 2—14, seizure-induced corticosterone responses were significantly augmented and prolonged for at least two hours post-seizure, compared to control early-handled (EH) rats. This was accompanied by reduced seizure threshold ($p < 0.05$) and increased vulnerability to the kindling-induced progression of seizure duration ($p < 0.05$) in MS rats. Pre-seizure treatment with the corticosterone synthesis inhibitor, metyrapone (MET) (50 mg/kg sc) effectively blocked seizure-induced corticosterone responses. When delivered throughout kindling, MET treatment also reversed the MS-induced reduction in seizure threshold and the lengthened seizure duration back to levels of EH rats. These observations suggest that adverse early life environments induce a vulnerability to kindling epileptogenesis mediated by HPA axis hyper-reactivity, which could have relevance for the pathogenesis of MTLE.

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1. Introduction

The pathogenesis of mesial temporal lobe epilepsy (MTLE), the most prevalent form of drug-resistant focal epilepsy in adults (Engel, 2001), is currently viewed as a multistage process which could initiate early in life, even though seizures often do not commence until adolescence or adulthood (Scharfman and Pedley, 2006; Scharfman, 2007). Amongst the range of early life factors implicated in MTLE causation, which include birth trauma, febrile seizures or infection (Scharfman, 2007), environmental stressors may be important contributors. Early life stress, a strong risk factor implicated in several psychiatric disorders (Gunnar and Quevedo, 2007), may serve as a common causal or contributory factor for MTLE and the psychopathologies that are often comorbid with the epilepsy (Hermann et al., 2008; Kanner, 2009, 2012). In the last decade, a consistent body of experimental research has provided evidence to support this theory, documenting an enduring increase in vulnerability to epileptogenesis following early postnatal stress (Huang et al., 2002; Lai et al., 2006, 2009; Salzberg et al., 2007; Gilby et al., 2009; Jones et al., 2009; Cabral et al., 2011; Kumar et al., 2011). Research into the biological mechanisms underlying this relationship, however, remains sparse (Koe et al., 2009; Kumar et al., 2011).

Amongst several candidate mechanisms, a strong possibility is the propensity for early life adversity to alter hypothalamic-pituitary-adrenal (HPA) axis function in adulthood. From the abundant literature generated from a range of species showing that early life exposures influence the programming of the HPA axis, the majority of studies conclude that stressors in early life result in exaggerated HPA axis responses to stress (see Ladd et al., 2000; Sanchez et al., 2001; Levine, 2005; Heim et al., 2008; Rao et al., 2008; Lupien et al., 2009). However, this is not a uniform finding, and different influences, including later experiences (e.g., Ladd et al., 2005; Goldman-Mellor et al., 2012), the type of stressor (e.g., Richardson et al., 2006) and genetic make-up (e.g., Tyrka et al., 2009) can differentially impact the resultant function of the HPA axis. In our hands (Kumar et al., 2011), early life maternal separation (MS) stress in Wistar rats leads to hyperresponsivity of the HPA axis in adulthood when compared to early handled (EH) controls. Adult stressors, such as restraint or seizures, therefore result in greater corticosterone release, and this may represent a mechanism underlying the augmented limbic epileptogenesis evoked by early postnatal stress. Evidence for pro-seizure and pro-epileptogenic effects of glucocorticoids is extensive in animal models (Joels, 2009), supporting the idea that excessive glucocorticoids can exacerbate MTLE development and progression. For example, administration of exogenous corticosterone aggravates kindling epileptogenesis in rats (Karst et al., 1999; Taher et al., 2005; Kumar et al., 2007), an effect that was ameliorated with corticosteroid receptor antagonists (Kumar et al., 2007). Conversely, removal of endogenous corticosterone by adrenalectomy reduced seizure susceptibility and severity, which were restored with glucocorticoid replacement (Cottrell et al., 1984; Lee et al., 1989). Also, restraint stress which elicits a corticosterone response accelerates kindling epileptogenesis (Jones et al., 2013).

Recently, we reported that the acceleration of amygdala kindling in rats previously exposed to MS was associated with larger corticosterone responses immediately following a kindled seizure (Kumar et al., 2011), promoting HPA axis hyperactivity as a potential key mechanism for the enhanced vulnerability to MTLE. Here, we aimed to characterise the time course of corticosterone elevation post-seizure, and to determine whether blocking seizure-induced corticosterone release was able to attenuate the MS-induced effects on kindling epileptogenesis. Specifically, we hypothesised that female rats previously exposed to early life MS would exhibit HPA hyper-reactivity manifested by an augmented and prolonged corticosterone elevation following a seizure, and that inhibition of the corticosterone response using the corticosterone synthesis inhibitor metyrapone (MET) (Temple and Liddle, 1970) would reverse the pro-epileptogenic effects of MS. We focussed on females in this study since our previous work showed that MS increased vulnerability to kindling selectively in females (Salzberg et al., 2007). In addition, female, but not male, MS rats displayed hyperactive HPA axis function during kindling (Kumar et al., 2011), and HPA axis response to stress has been shown to be more prominent in females (McCormick et al., 2002; Bale, 2006; Slotten et al., 2006). Furthermore, there is evidence that the rate of cryptogenic temporal lobe epilepsy (for which the most common cause is MTLE) is more common in women than in men (Christensen et al., 2005).

2. Materials and methods

2.1. Experimental animals

Inbred Wistar rats were mated in the Department of Zoology, University of Melbourne which was maintained at 20 °C on a 12 h light/dark cycle (lights on at 0600 h). Pregnant rats were checked for litters daily and the day of birth was assigned postnatal day 0 (P0). Each mother was used for breeding only once. Litters were weaned on P21, and female pups were group-housed (2—3 per cage) until electrode implantation surgery. Male pups were used for other experiments not described here. All procedures were approved by the University of Melbourne Animal Ethics Committee and performed in accordance with the guidelines published by the Australian National Health and Medical Research Council (NHMRC).

2.2. Maternal separation and early handling

For the entire pre-weaning period (i.e., P0—P21), litters were group-housed with the dams and sires, with the exception of the times of maternal separation. On P2, litters consisting of 8—12 pups were assigned to one of two separation protocols, which were carried out from P2 to P14, inclusive. MS consisted of daily separation of litters from their dams and sires for 3 h (0800—1100 h), while early handling (EH) involved daily brief separations of 15 min (0800—0815 h). First, dams and sires were removed from the home cage and placed in a quiet, separate room. Pups were then removed from the nest one at a time and placed together in a separate plastic box on a heating pad (30 °C) to maintain normal body temperature. At the end of the separation period, pups were returned to the home