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Phenytoin- and carbamazepine-resistant spontaneous bursting in rat entorhinal cortex is blocked by retigabine *in vitro*

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Summary Hyperexcitability in the medial entorhinal cortex-hippocampal (mEC-HC) circuit in the initial weeks after prolonged seizure activity may contribute to the epileptogenic process in animal models of temporal lobe epilepsy (TLE). The present study examined combined mEC-HC slices (400 μm) using field potential recordings 1—2 weeks following the multiple administration, low-dose kainic acid (KA) model of TLE [Hellier, J.L., Patrylo, P.R., Buckmaster, P.S., Dudek, F.E., 1998. Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy. Epilepsy Res. 31, 73—84]. Field potential recordings in slices from KA-treated rats demonstrated hallmarks of hyperexcitability in the mEC and in the CA1 and CA3 cell body regions of the HC. Spontaneous burst (SB) activity was observed under baseline recording conditions in the mEC of several slices from KA-treated rats, but not in the slices from saline-treated control rats. Elevating ACSF $[K^+]_o$ (6 mM) in the presence of picrotoxin (50 μM) increased SB rates in all slices tested. However, there was a significantly shorter latency to onset of bursting and prolonged evoked response durations in layer II of the mEC of slices from KA-treated rats versus those from controls. Neither carbamazepine (CBZ) nor phenytoin (PHT) abolished SB activity in slices from KA-treated rats; whereas, SB activity in slices from control rats was dose-dependently reduced at 100 μM CBZ. In contrast, the novel anticonvulsant retigabine (RGB) dramatically reduced SB frequency in both control and KA-treated groups. The hyperexcitability observed in combined mEC-HC brain slices from KA-treated rats suggests that the mEC, as well as the HC, may contribute to the epileptogenic process after KA-induced seizure activity. This model may provide an efficient, flexible *in vitro* paradigm for differentiating novel AEDs in a model of pharmacoresistant bursting.

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Introduction

The multiple, low-dose KA administration model of temporal lobe epilepsy

Following prolonged KA-induced seizure activity, characteristic loss of neurons in the temporal lobe are observed, most notably in layer III pyramidal cells of the mEC, in the hilar region of the dentate gyrus, and in the CA3 and CA1 pyramidal cell layers of the HC (Du et al., 1995b; Bear et al., 1996; Buckmaster and Dudek, 1997b). These patterns of neuronal degeneration are similar to the type of loss reported in patients with chronic TLE and hippocampal sclerosis (Engel et al., 1989; Sutula et al., 1989; Babb et al., 1992; S.S. Spencer and D.D. Spencer, 1994). Animal models of TLE, such as the kainic acid (KA) model, have greatly expanded our knowledge and understanding of the pathophysiology involved and therapeutic options for its management.

Systemic KA has classically been administered to rats as a single, bolus dose of 10—20 mg/kg (i.p.). Hellier et al. (1998) modified this dosing regimen, utilizing multiple, low-dose (5 mg/(kg per hour)) injections of KA titrated to each individual animal's onset of stage 4/5 seizure activity to improve upon the limitations of the traditional model (Hellier et al., 1998). This multiple administration, low-dose paradigm was shown to reliably produce chronically epileptic rats and resulted in little, if any, mortality (Hellier and Dudek, 1999; Smith and Dudek, 2002). While these initial studies utilizing the low-dose KA model described the functional changes in the dentate gyrus of KA-treated rats, they did not address morphological and physiological changes that occur in parahippocampal regions, like the mEC. Histopathological changes in the mEC following prolonged seizure activity have been suggested to play a key role in epileptogenic process and may contribute to pharmacoresistant bursting reported in the superficial mEC (Di Chiara et al., 1993; Du et al., 1993, 1995a; D.D. Spencer and S.S. Spencer, 1994; Bragin et al., 2000, 2002).

The role of the mEC-HC circuit

The mEC and the HC are functionally linked to one another by reciprocal connections. Axons of layer II stellate cells in the mEC form the perforant pathway, which provides the major afferent excitatory input to dentate gyrus (DG) granule cells and CA3 pyramidal cells (Steward and Scoville, 1976; Witter et al., 1989; Witter, 1993; Naber et al., 2001). Under pathological conditions, the mEC has been implicated in both seizure onset and spread (Bragin et al., 1999, 2000, 2002, 2002a, 2002b, 2004; Shah et al., 2004). Prolonged seizures result in selective loss of layer III pyramidal cells coupled with marked hyperexcitability of stellate cells in layer II of the mEC (Du et al., 1995b, 1998; Bear et al., 1996; Behr et al., 1998; Scharfman et al., 1998; Chen and Buckmaster, 2005; Kumar and Buckmaster, 2006).

In the present study, histological and electrophysiological changes in the mEC and HC of combined slices were examined. Histological changes were examined at 24 h, 1 week, 4 weeks and 10 weeks after multiple, low-dose KA administration. In the absence of pharmacological manipulations, hyper-synchronous network activity (spontaneous discharges) in layer II of the mEC and the HC were also observed in some slices as early as 1 week after KA-induced seizure activity *in vivo*. Understanding the ontogeny of pathophysiological changes in the mEC and ventral HC following prolonged seizure activity may give insight into the epileptogenic process, potential therapeutic interventions, and improved animal models of TLE.

Pharmacoresistant activity in layer II mEC

It has been proposed that the late bursting activity observed in the superficial mEC of combined slices bathed in low magnesium media may represent an *in vitro* model for pharmacoresistant events, since it has been demonstrated to be refractory to several AEDs: including, phenytoin, carbamazepine, phenobarbital, valproic acid, and midazolam (Armand et al., 2000; Dreier et al., 1998; Zhang et al., 1995). Low-Mg^{2+}-induced epileptiform activity recorded in the entorhinal cortex of combined slices from naïve rats slowly transition from seizure-like events (SLEs) to recurring epileptiform discharges. However, the delay from pharmacosensitivity to pharmacoresistance does not make this an ideal model from a drug screening perspective. It is currently unclear if field potential recordings in combined mEC-HC slices made from KA-treated rats (rather than naïve animals) would offer any advantage as an *in vitro* model system for detecting novel anticonvulsant therapies. In the present study, we compared the ability of AEDs to block SB activity in layer II of the mEC of brain slices obtained from KA-treated and saline-treated control rats. Bursting was defined as ''pharmacoresistant'' if it was not blocked by at least two different AEDs.

Methods

Animals and housing

Adult, male Sprague—Dawley rats weighing 150—200 g (Simonsen Laboratories, Gilroy, CA) were group housed ($n = 6$) in plastic cages in a temperature- and light-controlled (12 h light:dark cycle) facility with *ad libitum* access to food (Harlan Teklad 8640 Rodent diet) and water. Animals were allowed to rest for at least 72 h prior to KA or saline administration. All experiments were conducted in accordance with the guidelines set by the National Institute of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Utah.

KA administration

Animals were treated with KA (Sigma Aldrich, St. Louis, MO; Ocean Produce International, Shelbourne, Nova Scotia) following a modified protocol described by Hellier et al. (1998). Seizure activity was scored throughout the experiment according to the Racine scale (Racine, 1972). Saline (0.9%) or KA (5 mg/kg, i.p.) was administered once every hour until animals began to exhibit behaviors consistent with focal seizure activity (stage 1—3). Typically, wet dog shakes, facial clonus and head nodding (stage 1—2 activity) were observed after the first injections of KA, with forelimb clonus (stage 3) observed soon thereafter. Once an animal had demonstrated stage 1—3 seizures, dosing was reduced to 2.5 mg/kg (i.p.) delivered every 30 min until stage 4/5 seizures (rearing and falling) were first observed. Following stage 4/5 seizure onset, injections were ceased and the number and stage of seizures observed were recorded for