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Regular Article

Suppression of Adult Neurogenesis Increases the Acute Effects of Kainic Acid

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ABSTRACT

Adult neurogenesis, the generation of new neurons in the adult brain, occurs in the hippocampal dentate gyrus (DG) and the olfactory bulb (OB) of all mammals, but the functions of these new neurons are not entirely clear. Originally, adult-born neurons were considered to have excitatory effects on the DG network, but recent studies suggest a net inhibitory effect. Therefore, we hypothesized that selective removal of newborn neurons would lead to increased susceptibility to the effects of a convulsant. This hypothesis was tested by evaluating the response to the chemoconvulsant kainic acid (KA) in mice with reduced adult neurogenesis, produced either by focal X-irradiation of the DG, or by pharmacogenetic deletion of dividing radial glial precursors. In the first 4 hrs after KA administration, when mice have the most robust seizures, mice with reduced adult neurogenesis had more severe convulsive seizures, exhibited either as a decreased latency to the first convulsive seizure, greater number of convulsive seizures, or longer convulsive seizures. Nonconvulsive seizures did not appear to change or they decreased. Four-21 hrs after KA injection, mice with reduced adult neurogenesis showed more interictal spikes (IIS) and delayed seizures than controls. Effects were greater when the anticonvulsant ethosuximide was injected 30 min prior to KA administration; ethosuximide allows forebrain seizure activity to be more easily examined in mice by suppressing seizures dominated by the brainstem. These data support the hypothesis that reduction of adult-born neurons increases the susceptibility of the brain to effects of KA.

Introduction

Adult-born neurons are generated in the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) throughout the lifetime of mammals (Seki et al., 2011; Belzung and Wigmore, 2013). In the DG, newborn neurons become granule cells (GCs) - the primary cell type. Although they constitute a relatively small fraction of the total GC population (Cameron and McKay, 2001), removal of adult-born neurons compromises normal functions of the DG such as pattern separation (Clelland et al., 2009; Sahay et al., 2011a).

Recordings from immature GCs have shown that they exhibit increased excitability and long-term potentiation (LTP) compared to GCs born in early development (Schmidt-Hieber et al., 2004; Espósito et al., 2005; Markwardt and Overstreet-Wadiche, 2008; Ge et al., 2007), which has led to the idea that young adult-born GCs increase excitability of the adult DG network (Marin-Burgin et al., 2012; Song et al., 2012). However, adult-born neurons may also play a role in DG function by a net inhibitory effect on the DG-CA3 network. In support of this idea, mature GCs innervate diverse GABAergic interneurons; this has led some to propose that a net inhibitory effect in area CA3 is normal (Acsády et al., 1998). When extracellular recordings were made in the GC layer of the DG in mice in which adult DG neurogenesis had been selectively deleted, GCs discharged in bursts that were greater than those in controls (Lacefield et al., 2012). Another study revealed that following tasks that required specific cognitive demands, animals with reduced adult neurogenesis exhibited greater expression of the immediate early gene Arc in the GC layer (indicative of greater GC activity) compared to controls (Burghardt et al., 2012). Using voltage-sensitive dye imaging, a recent study showed that a reduction in adult neurogenesis in the hippocampus increased excitability in the GC

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layer, and that increasing adult hippocampal neurogenesis had the opposite effect (Ikrar et al., 2013).

If adult-born neurons have a net inhibitory effect, they could contribute to one of the proposed functions of the DG - to act as a 'gate' to protect the hippocampus from seizures arising in neocortex (Heinemann et al., 1992; Lothman et al., 1992; Hsu, 2007). Therefore, we asked whether reducing adult-born neurons in a normal animal would influence seizures. We used C57Bl6/J mice because they have high rates of adult neurogenesis (Kempermann et al., 1997), and examined the response of mice to the convulsant kainic acid (KA). Because acute seizures are an important clinical issue, and C57Bl6/J mice are resistant to epilepsy (McKhann et al., 2003; Schawecker, 2011) we focused only on the hours immediately following KA administration (i.e., 24 hours). Two methods were used to reduce adult neurogenesis: focal, low-dose X-irradiation (Santarelli et al., 2003), and mice with herpes simplex virus thymidine kinase (hsv-TK) in glial fibrillary acidic protein (GFAP) - expressing cells (GFAP-TK mice; Sofroniew et al., 1999; Schloesser et al., 2009).

Pilot studies showed that seizures elicited by KA injection in control mice often involved running and bouncing, indicative of brainstem activation (Gale, 1992), which can mask forebrain seizures in rodents (Gale, 1992; Eells et al., 2004). Pretreatment with the anticonvulsant ethosuximide inhibits seizure activity in the brainstem (Mares et al., 1994), which is critical for the generation of the severe convulsive behaviors such as running and bouncing (Browning et al., 1981; Fromm, 1985). Therefore, we used ethosuximide pretreatment to optimize conditions for a study where forebrain networks were of interest – i.e., the location of adult-born neurons. We found that a decrease in adult born neurons in the hippocampus increases convulsive seizures, with no effect or a decrease in nonconvulsive seizures. Our results support the hypothesis that reduction of adult-born neurons increases susceptibility to effects of KA.

Materials and methods

General information

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. Mice were housed in standard mouse cages, with a 12 hr: 12 hr light/dark cycle, and food (Purina 5001; W.F. Fisher, Somerville, NJ) and water *ad libitum*.

Methods to suppress adult neurogenesis

For focal, low-dose X-irradiation, male 6-8 week-old C57BL6/J mice (Jackson Laboratories) were anesthetized with sodium pentobarbital (6 mg/kg), placed in a stereotaxic frame, and exposed to cranial irradiation using a XRAD320 system (Precision Xray Inc., North Branford, CT) operated at 300 kV and 12 mA. Animals were protected with a lead shield that covered the entire body, except for a 4 x 14 mm treatment field above the hippocampus. X-rays were filtered using a 2 mm aluminum filter with a 36 mm source-to-skin distance. Three 2.5 Gy doses were delivered over approximately 2 min 45 sec, with 3 days between doses, and a 7.5 Gy cumulative dose.

As an alternative method to suppress adult neurogenesis, we used mice with the herpes simplex virus – thymidine kinase (hsv-TK) transgene under the control of the mouse glial fibrillary acid protein promoter (GFAP-TK+) or mice without the transgene (GFAP-TK-). Mice were backcrossed onto C57BL6/J mice (Jackson Laboratories, Bar Harbor, ME) for at least 6 generations. Starting 6 weeks of age, they were fed valganciclovir (VGCV; 165 mg/kg chow; Custom Animal Diets) 5 days/week and normal chow on other days. It is important to note that injection of GCV i.p. has adverse effects on the gastrointestinal tract but treatment with VCGV in the chow does not (Schloesser et al., 2009). Presumably this is due to a direct exposure of the intestines during i.p. injection vs. indirect exposure during oral administration. We confirmed that the intestines were normal in appearance in 3 mice that were euthanized immediately after the 6 week-long treatment with chow containing VGCV. The appearance (skin, fur) was similar to control mice. We also measured body weight before and after VCGV treatment and found that it was not significantly different (GFAP-TK-: 23.8 ± 3.4 g, n = 36; GFAP-TK+: 24.4 ± 2.3 g; n = 39; t-test, $p = 0.211$), suggesting that food intake was unaffected by the transgene.

EEG recordings of KA-induced seizures

Implantation of electrodes for EEG

Mice were anesthetized with chloral hydrate (55 mg/kg i.p.) and implanted stereotaxically with an epidural electrode (0.10"diameter screws; # 8209; Pinnacle Technologies; Lawrence, KS) over the left frontal cortex (FC; anterior-posterior coordinate relative to Bregma, AP = -0.5 mm; mediolateral coordinate relative to the midline, ML = 1.5 mm) and another electrode overlying right occipital cortex (OC; AP = -3.5 mm; ML = 2.0 mm). Stainless steel 75 μm-diameter lacquer-coated wire (#787000; A-M Systems, Sequim, WA) was twisted to make bipolar electrodes that were placed into each dorsal hippocampus (AP = -2.5 mm, ML = 2.5 mm, depth below skull surface = 2.5 mm). Electrodes were soldered to an 8-pin connector (#8400-SE4, Pinnacle) and cemented (Grip cement; Caulk Dentsply). The location of electrodes in hippocampus was confirmed in 60 of 61 mice using cresyl violet staining. Video-EEG was acquired (500 Hz; Pinnacle) and analyzed offline (Acqknowledge; Biopac Systems, Inc.).

Seizure induction

KA (16-25 mg/kg s.c.; Milestone Pharmatech) was dissolved in phosphate buffered saline (PBS); ethosuximide (150 mg/kg; i.p.) was dissolved in 20% ethanol in PBS. Between 10:30 a.m. and 2:00 p.m., animals were removed from their home cage and placed in a new cage where their pin connector was attached to a cable with a commutator (Pinnacle) to allow freedom of movement. Baseline recordings were made for >10 min, and the animal was injected s.c. between the shoulder blades with KA using a 29 gauge needle and low-volume (0.33 cc) syringe to optimize the accuracy of each dose, which we estimate (from the accuracy of this syringe) was ± 1.2 mg/kg. For this reason, doses between 20 and 25 mg/kg were not tested (see Results). KA was made as a concentrated stock solution (12 mg/ml in PBS) and was stored at 4 °C for up to 1 month. For a subset of GFAP-TK mice, the investigator was blinded to the genotype during KA administration. This was not possible for X-irradiated animals because X-irradiation led to a band of hair where irradiation occurred that lacked pigmentation.

Quantification of EEG

A seizure was defined as rapid, rhythmic (>3 Hz) deflections >2x the standard deviation of noise, lasting >3 sec (the duration of the shortest seizure in the current study was 11 sec). Seizures were rated as convulsive if they were accompanied by stage 3-5 behaviors (Racine, 1972). In three mice, the interrater reliability of convulsive vs. nonconvulsive seizures was tested for 38 seizures by two investigators and there was 100% agreement. The two investigators measured the latency to the first convulsive seizure in these animals (25.0 ± 0.9 min vs. 25.2 ± 0.9 min; t-test, $p = 0.855$).

The latency to onset of a seizure was defined as the time of KA injection to the time when the peak-to-peak amplitude of the EEG exceeded 1.25x of the baseline mean in all electrodes. To validate interrater reliability, the onset of the first seizure was examined in three mice by two investigators; there was 100% agreement. The end of a seizure was defined as the time when the EEG returned to the baseline mean or (in the case of postictal depression) a value lower than the baseline mean. Duration was defined as the time between seizure onset and the end of a seizure (Supplementary Fig. 1).

Interictal spikes (IIS) were defined as brief (<200 msec) deflections in all leads that were >2x the standard deviation of noise. Digital detection