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Prostaglandin E_2 and BDNF levels in rat hippocampus are negatively correlated with status epilepticus severity: No impact on survival of seizure-generated neurons

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Partial and generalized status epilepticus (pSE and gSE) trigger the same level of progenitor cell proliferation in adult dentate gyrus, but survival of new neurons is poor after gSE. Here, we show markedly elevated levels of prostaglandin E_2 (PGE_2) and brain-derived neurotrophic factor (BDNF) in rat hippocampal formation at 7 days following pSE but not gSE. Administration of the cyclooxygenase (COX) inhibitor flurbiprofen for 1 week, starting at day 8 post-SE, abated PGE_2 and decreased BDNF levels, but did not affect survival of new neurons 4 weeks later. Thus, high PGE_2 and BDNF levels induced by pSE are probably not of major importance for survival of new neurons during the first days after formation. We propose that they modulate other aspects of synaptic and cellular plasticity, and thereby may influence epileptogenesis.

Keywords: Prostaglandin E_2 (PGE_2); Cyclooxygenase-2 (COX-2); Brain-derived neurotrophic factor (BDNF); EP2; EP3; Isoprostanes; Flurbiprofen; Status epilepticus; Neurogenesis

Introduction

The restorative potential of the adult brain following injury and its plasticity to environmental and behavioral cues arise partly from the ability of its own neural stem cells to react to physio-pathological changes in their "niche" with a complex neurogenic response (Gage, 2002). The process of neurogenesis comprises at least four distinct steps: proliferation, survival, migration, and differentiation, with each step having a specific regulatory machinery. In the adult dentate gyrus (DG), multipotent progenitors located in the subgranular zone (SGZ) continuously generate neuroblasts, which migrate into the granule cell layer (GCL), adopt the morphological characteristics of granule cells, and extend axonal projections to their appropriate target, the CA3 region (Lie et al., 2004). The new neurons develop into functional granule cells (van Praag et al., 2002) but have also been reported to differentiate into inhibitory interneurons (Liu et al., 2003). The formation of new neurons in the adult SGZ is modulated by different physiological stimuli, and circumstantial evidence suggests a link between level of hippocampal neurogenesis and cognitive function (Lie et al., 2004). Insults to the adult brain, such as epileptic seizures and cerebral ischemia, trigger increased formation of neurons in the SGZ (Bengzon et al., 1997; Parent et al., 1997; Jin et al., 2001; Arvidsson et al., 2001; Liu et al., 1998). Whether the new DG neurons generated after brain insults contribute to functional recovery or impairment is not known. For example, it has been proposed that the new neurons formed after epileptic seizures participate in the neural circuits which underlie the pathological excitability in chronic epilepsy (Scharfman, 2004).

Following status epilepticus (SE), SGZ cell proliferation is high during about 2 weeks after the insult but then returns to baseline (Parent et al., 1997). We have previously demonstrated that increase of cell proliferation in the SGZ following electrically induced SE, peaking at 7 days post-SE, is independent of the severity of SE (Mohapel et al., 2004). In contrast, the degree of survival of the newborn neurons is clearly influenced by the severity of the initial epileptic insult. In rats exhibiting partial SE (pSE), defined by the predominance of non-clonic convulsions, the new neurons formed at 1 week showed no significant decrease over the subsequent 4 weeks. In contrast, there was a 65% loss of new neurons during the same

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time period in rats with generalized SE (gSE), defined by the predominance of clonic convulsions (Mohapel et al., 2004). The severity of the initial injury and the associated local inflammation, sustained by microglial cells, were probably main causes of poor survival (Ekdahl et al., 2003a,b; Monje et al., 2003, Mohapel et al., 2004). Better knowledge of mechanisms involved in the marked loss of the newborn neurons, which is observed also during striatal neurogenesis after stroke (Arvidsson et al., 2002), is crucial to provide opportunities of manipulating endogenous neurogenesis and exploit its possible therapeutic potential.

Here, we have induced SE by electrical stimulation in the hippocampus, and compared pSE and gSE rats in order to identify factors in the microenvironment which are differentially regulated by SE severity and may underlie differences in neurogenesis. We particularly addressed the question whether SE severity differentially affects the synthesis of prostaglandin E_2 (PGE_2) and cyclooxygenase (COX)-2, the enzyme catalyzing the first committed step in PGE_2 synthesis. COX-2 is expressed by neurons in an activity-dependent way (Yamagata et al., 1993; Kaufmann et al., 1996), and increases dramatically after seizures (Marcheselli and Bazan, 1996; Tu and Bazan, 2003). In addition, PGE_2 has recently emerged as a putative neuroprotective factor in several paradigms of neurodegeneration, depending on the extent of induction, cellular source, and subset of specific receptors dominantly expressed in a given area (Minghetti, 2004). Finally, there is experimental evidence indicating that COX-2 and PGE_2 can influence hippocampal neurogenesis by promoting proliferation of SGZ progenitors (Uchida et al., 2002; Sasaki et al., 2003).

Recent evidence suggests a functional link between PGE_2 and the neurotrophin brain-derived neurotrophic factor (BDNF), in which BDNF expression in the rat hippocampus appears to be under the control of COX-2 activity (Shaw et al., 2003). In accordance with this idea, also BDNF protein levels are markedly increased in hippocampal subregions after recurring seizures (Elmer et al., 1998). BDNF has been shown to promote differentiation and survival of neuronal progenitors in rat hippocampus and cortex (Lee et al., 2002; Barnabé-Heider and Miller, 2003), and functional BDNF signaling is required for long-term survival of newborn DG neurons in the mouse (Sairanen et al., 2005). However, depending on the type of the insult, and the level of BDNF and its mode of delivery, this neurotrophic factor may also counteract neurogenesis (Larsson et al., 2002; Gustafsson et al., 2003).

The specific objectives of the present study were threefold: first, to quantify PGE_2 and BDNF levels and determine the distribution and magnitude of COX-2 expression in hippocampal subregions at different time points after pSE and gSE; second, to analyze the expression of PGE_2 receptors on different DG cell types, especially on the newly formed neuroblasts, to assess if these cells can be directly influenced by PGE_2 in the early phases of neurogenesis; finally, to explore whether manipulation of PGE_2, and possibly BDNF synthesis by the non-selective COX inhibitor flurbiprofen, could affect the survival of the newborn neurons generated following SE.

Materials and methods

Animals and surgery

125 male Sprague–Dawley rats (Möllegaard's Breeding Center, Copenhagen, Denmark), weighing 250 g at the time of surgery, were housed separately under 12 h light/12 h dark conditions with ad libitum access to food and water. 116 rats were anesthetized with halothane and implanted unilaterally with a twisted insulated stainless-steel stimulating/recording electrode (Plastics One, Roanoke, VA) into the right ventral hippocampal CA1–CA3 region (coordinates: 4.8 mm caudal, 5.2 mm lateral to bregma, 6.3 mm ventral from dura, toothbar at −3.3 mm; Paxinos and Watson, 1997). Rats were then either subjected to electrically induced SE ($n = 76$) or used as non-stimulated controls and referred as to sham ($n = 40$). Nine rats not subjected to electrode implantation were used as intact controls and referred as to controls. Experiments followed guidelines set by the Malmö-Lund Ethical Committee for use and care of laboratory animals.

Induction of status epilepticus

Seven days after electrode implantation, SE was induced as originally described by Lothman et al. (1989). Afterdischarge (AD) threshold was determined for each rat through a 1 s 50 Hz electrical current, starting at 10 μA and increasing in 10 μA increments at 1 min intervals until an AD lasting 5 s or more was registered (Chart 3.6.3, PowerLab/MacLab; AD Systems, Hastings, UK). Thirty minutes later, rats received 1 h supra-threshold stimulation with 10 s trains of 1 ms biphasic square wave pulses, at a frequency of 50 Hz. Every 10 min, stimulations were interrupted for 1 min of electroencephalogram (EEG) recordings and AD measurements. After 1 h of stimulation, all rats exhibited continuous self-sustained ictal EEG activity. Based on the severity of behavioral convulsions, two different SE profiles were distinguished (Mohapel et al., 2004): partial SE (pSE, including grade 1–2 according to Racine's scoring system for kindled seizures; Racine, 1972) and generalized SE (gSE, including grade 3–4). Examples of typical EEG recordings from sham, pSE, and gSE rats are given in Figs. 1A–C. Behavioral convulsions and ictal EEG activity were arrested with pentobarbital (65 mg/kg, i.p.) 2 h after cessation of stimulation.

Immunocytochemistry

Thirty-five rats with partial and 13 rats with generalized SE, and 26 electrode-implanted, non-stimulated rats were used for immunocytochemistry. Rats received an overdose of sodium penthobarbital (200 mg/kg, i.p.) and were transcardially perfused with 250 ml of saline followed by 250 ml of ice-cold formaldehyde solution (4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4). Brains were removed, post-fixed overnight in the same fixative, and then placed in 20% sucrose/0.1M phosphate buffer for at least 24 h. Coronal sections (30 μm) were cut on a freezing microtome and stored in cryoprotective solution at −20°C. For COX-2/neuron-specific nuclear protein (NeuN) double-labeling, free-floating sections were first microwaved in citrate buffer (0.01M, pH 6) for 2 min for COX-2 retrieval, then rinsed in potassium phosphate-buffered saline (KPBS) before pre-incubation with 5% donkey and horse serum in 0.25% Triton X-100 for 1 h at room temperature. The sections were incubated with a goat polyclonal anti-COX-2 antibody (1:1000, M19, Santa Cruz Biotechnology Inc., Santa Cruz, CA) and a mouse anti-NeuN antibody (1:100, Chemicon, Temecula, CA) overnight at +4°C, followed by rinsing and incubation in the dark for 2 h with Cy3-conjugated donkey anti-goat IgG antibody (1:200, Jackson ImmunoResearch, West Grove, PA) and biotinylated horse anti-