Current Literature in Basic Science

When Newborn Neurons Stray

Continuous Cytosine-β-D-arabinofuranoside Infusion Reduces Ectopic Granule Cells in Adult Rat Hippocampus with Attenuation of Spontaneous Recurrent Seizures Following Pilocarpine-induced Status Epilepticus

Jung KH, Chu K, Kim M, Jeong SW, Song YM, Lee ST, Kim JY, Lee SK, Roh JK

Eur J Neurosci 2004;19(12):3219–3226

Brief or prolonged seizures induce various patterns of plasticity. Axonal or dendritic remodeling and development of ectopic granule cells have been described in the hilus and molecular layer of the adult rodent hippocampus. Hippocampal cell proliferation also occurs after seizures. However, whether the seizure-induced cell proliferation plays a pathologic or reparative role in the epileptic brain is unknown. In this study, we attempted to suppress the seizure-induced cell proliferation with the antimitotic agent cytosine-β-D-arabinofuranoside (Ara-C) and to examine the development of spontaneous recurrent seizures (SRSs). Experimental status epilepticus was induced with pilocarpine, and Ara-C or vehicle alone was infused continuously with an osmotic minipump. SRSs were video-monitored. Bromodeoxyuridine (BrdU) immunohistochemistry was used for the spatial and temporal analysis of hippocampal cell proliferation, and double labeling with NeuN, calbindin, and glial fibrillary acidic protein (GFAP) antibodies was performed for the differentiation of BrdU-positive cells. Timm staining also was performed for evaluation of mossy-fiber sprouting (MFS). With continuous Ara-C infusion, the likelihood of developing SRSs was decreased, and during the latent period, the development of ectopic granule cells in the hilus and new glia in the CA1 area was reduced when compared with that in the vehicle-infused group, whereas MFS was not altered. The results suggest that the hippocampal cell proliferation plays a proepileptogenic role rather than a compensatory role, and that the epileptogenic process may be associated with the generation of new glia in the CA1 area or new neurons in the dentate gyrus, particularly the ectopically located hilar granule cells, or both.

Increased Neurogenesis and the Ectopic Granule Cells after Intrahippocampal BDNF Infusion in Adult Rats

Scharfman H, Goodman J, Macleod A, Phani S, Antonelli C, Croll S

Exp Neurol 2005;192(2):348–356

Evidence suggests that brain-derived neurotrophic factor (BDNF) influences the birth of granule cells in the dentate gyrus, which is one of the few areas of the brain that demonstrates neurogenesis throughout life. However, studies to date have not examined this issue directly. To do so, we compared the effects of BDNF, phosphate-buffered saline (PBS), or bovine serum albumin (BSA) on neurogenesis after infusion into the hippocampus of the normal adult rat, by using osmotic pumps that were implanted unilaterally in the dorsal hilus. BDNF, PBS, and BSA were infused for 2 weeks. The mitotic marker BrdU was administered twice daily during the 2-week infusion period. At least 1 month after infusion ended, brains were processed immunocytochemically by using antibodies to BrdU, a neuronal nuclear protein (NeuN), or calbindin D28K (CaBP), which labels mature granule cells. Stereology was used to quantify BrdU-labeled cells in the dorsal hippocampus that were double-labeled with NeuN or CaBP. A statistically significant increase in $BrdU^+/NeuN^+$ double-labeled cells was noted in the

Epilepsy Currents, Vol. 5, No. 6 (November/December) 2005 pp. 231–233
Blackwell Publishing, Inc.

granule cell layer after BDNF infusion, relative to that in controls. The values for $BrdU^+/NeuN^+$ cells were similar to those for $BrdU^+/CaBP^+$ cells, indicating that most new neurons were likely to be granule cells. In addition, $BrdU^+/NeuN^+$-labeled cells developed in the hilar region after BDNF infusion; these have previously been identified only after severe continuous seizures (status epilepticus) and associated pathologic changes. Remarkably, neurogenesis also was increased contralaterally, but BDNF did not appear to spread to the opposite hemisphere. Thus, infusion of BDNF to a local area can have widespread effects on hippocampal neurogenesis.

The results demonstrate that BDNF administration to the dentate gyrus leads to increased neurogenesis of granule cells. They also show that ectopic granule cells develop after BDNF infusion, which suggests that ectopic migration is not necessarily confined to pathologic conditions. These results are discussed in light of the evidence that BDNF increases neuronal activity in hippocampus. Thus, the mechanisms underlying neurogenesis after BDNF infusion could be due to altered activity as well as to direct effects of BDNF itself, and this is relevant to studies of other growth factors because many of them have effects on neuronal excitability that are often not considered.

COMMENTARY

Neurogenesis persists throughout life in the mammalian dentate gyrus (1,2). The physiologic function of dentate granule cell (DGC) neurogenesis in adulthood remains obscure, although recent evidence implicates a role in learning and memory (3). Many factors, ranging from neurotransmitters to environmental enrichment and exercise, influence adult DGC neurogenesis (4). Various forms of brain injury, including prolonged seizures, stimulate neurogenesis in the dentate gyrus and the other persistent germinative region, the forebrain subventricular zone (4,5). The mechanisms by which injury increases adult neurogenesis are unknown.

Seminal observations by Houser 15 years ago suggest a link between some pathophysiologic features of human temporal lobe epilepsy (TLE) and aberrant neurogenesis (6). The author described prominent abnormalities of the DGC layer in resected hippocampi from patients with pharmacoresistant TLE. The abnormalities include dispersion of the DGC layer and the appearance of ectopic DGC clusters in the dentate hilus and molecular layer. The investigator suggested that insults during early development affect the migration of newly generated DGCs. Because DGC "development" is an ongoing process, however, the potential for insults to alter neurogenesis is not restricted to early life.

Many of the pathologic lesions of human TLE are recapitulated in adult rodent epilepsy models. The induction of status epilepticus by systemic administration of the chemoconvulsant pilocarpine, for example, causes hippocampal cell loss, mossy-fiber sprouting, and astroglial proliferation similar to that found in human TLE. Pilocarpine-induced status epilepticus also leads to DGC layer dispersion and the appearance of ectopic hilar and molecular layer DGCs (5,7,8). Newborn neurons in the adult rat contribute to the ectopic DGCs (5,7). How status epilepticus induces the ectopic formation of DGCs and the functional relevance of the ectopic cells are unknown. Two recent reports begin to shed light on these questions.

Jung et al. studied the effects of inhibiting neurogenesis, including hilar ectopic DGC formation, on spontaneous recurrent seizures in the adult rat pilocarpine TLE model. To block neurogenesis, the investigators infused the antimitotic agent cytosine-β-D-arabinofuranoside (Ara-C) intracerebroventricularly for 14 days beginning 1 day before status epilepticus induction. Proliferating cells were labeled by daily bromodeoxyuridine (BrdU) administration for the first 2 weeks after status epilepticus. Vehicle-infused or Ara-C–infused pilocarpine-treated rats were examined for spontaneous, recurrent seizures by video monitoring for about 12 hours per day during the light cycle (the time of highest seizure frequency) on days 28 to 34 after status epilepticus. Neuronal loss and mossy-fiber sprouting were evaluated by Nissl and Timm stains, respectively.

The authors found that Ara-C infusion inhibited seizure-induced cell proliferation in the subventricular zone and dentate hilus. Compared with pilocarpine-treated rats infused with vehicle, Ara-C infusion after status epilepticus markedly decreased the numbers of adult-generated neurons in the DGC layer and hilus as well as the numbers of proliferative astrocytes in areas CA1 and CA3 of the hippocampus proper. Dorsal hippocampal cell loss and mossy-fiber sprouting were similar between vehicle-infused and Ara-C–infused groups, except for slightly increased hilar cell loss with Ara-C. Pilocarpine-induced status epilepticus led to later spontaneous recurrent seizures in eight of nine vehicle-treated versus six of nine Ara-C–treated rats. Video monitoring detected significant reductions in seizure frequency (70% decrease) and duration (34% decrease) with antimitotic treatment but no change in seizure severity.

These findings suggest a link between altered neurogenesis and epileptogenesis in the pilocarpine TLE model. A number of confounding effects, however, raise caution for interpretation of

these data. Ara-C infusion appeared to have widespread effects in a number of regions, such that the relative contributions of inhibiting dentate gyrus neurogenesis, subventricular zone neurogenesis, or astrocyte proliferation on epilepsy severity cannot easily be untangled. Moreover, antimotic-agent infusion likely has additional effects on plasticity that were not assessed in this study. In terms of outcome measurements, the use of video alone without EEG precluded the detection of smaller focal seizures, and most of the histologic analyses involved only dorsal hippocampal regions. Nonetheless, this study provides compelling evidence that the ectopic DGCs formed in experimental TLE arise from DGC precursors in the adult (5,7). These data also support prior findings that ectopic hilar, putatively newborn DGCs contribute to network excitability after status epilepticus (7,8). However, evidence that is more definitive awaits the development of experiments designed to inhibit neurogenesis specifically (perhaps by genetic manipulation) or, ideally, to prevent aberrant DGC neurogenesis, while maintaining normal neuronal development in the adult.

More recently, Scharfman and colleagues examined a specific factor that may regulate DGC neurogenesis in normal or injury states. They studied brain-derived neurotrophic factor (BDNF)—a neurotrophin family member that is highly expressed in the mature hippocampus and is upregulated by seizures. BDNF has been shown to influence adult neurogenesis in the dentate gyrus, subventricular zone, and other regions of the intact or ischemic adult rodent brain (4). The authors infused low- or higher-dose BDNF into the hilus of adult rats and examined the pattern of neurogenesis by BrdU administration and double labeling for BrdU and cell phenotype markers. They found increased DGC neurogenesis ipsilateral and, to an even greater extent, contralateral to the BDNF infusion compared with that in the control infusion groups. More hilar newborn neurons also were found in the BDNF groups. Two rats given BDNF, but none of the controls, had a single convulsive seizure identified during intermittent observation of the animals.

These important findings raise a number of questions. First, how are the BDNF effects mediated, especially contralaterally where no increased BDNF protein was detected? The authors raise the key points that BDNF effects could result from enhanced neuronal transmission or by increasing levels of neuropeptide intermediates. Another possibility is that undetected seizure activity induced by BDNF may contribute to the increased neurogenesis, as even single discrete seizures have been shown to increase DGC neurogenesis several weeks later (9). As the authors explain, rare discrete seizures, however, are unlikely to cause hilar ectopic DGC formation. Second, is BDNF necessary for the stimulation of neurogenesis seen after seizures or other brain insults? Finally, is BDNF a major mechanism leading to ectopic DGC formation after seizures? The numbers of ectopic hilar DGCs induced by BDNF infusion in this study were small compared with those found in status epilepticus models, suggesting that other factors also are involved. Nevertheless, the finding of aberrant neurogenesis in the absence of apparent injury effects is intriguing. All of these questions are fertile ground for future study.

Together, these reports suggest that the stimulation of endogenous neurogenesis by injury may have maladaptive, as well as potentially beneficial, consequences. Aberrant neurogenesis leading to altered hippocampal network formation not only has implications for epileptogenesis, but also may contribute to memory dysfunction in TLE. Experimental strategies that selectively eliminate or conditionally label newborn neurons in the adult likely will be critical to understanding their normal biologic role as well as their contribution to pathophysiology or repair after brain injury.

by Jack M. Parent, MD

References

1. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 1965;124:319–335.
2. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. Neurogenesis in the adult human hippocampus. *Nat Med* 1998;4:1313–1317.
3. Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 2001;410:372–376.
4. Lichtenwalner RJ, Parent JM. Adult neurogenesis and the ischemic forebrain. *J Cereb Blood Flow Metab* 2005 Jun 15; [Epub ahead of print]
5. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci* 1997;17:3727–3738.
6. Houser CR. Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Res* 1990;535:195–204.
7. Scharfman HE, Goodman JH, Sollas AL. Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. *J Neurosci* 2000;20:6144–6158.
8. Dashtipour K, Tran PH, Okazaki MM, Nadler JV, Ribak CE. Ultrastructural features and synaptic connections of hilar ectopic granule cells in the rat dentate gyrus are different from those of granule cells in the granule cell layer. *Brain Res* 2001;890:261–271.
9. Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc Natl Acad Sci USA* 1997;94:10432–10437.