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Further Reading


Neuroprotective Strategies in Epilepsy

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Introduction

From the earliest attempts to develop treatments for epilepsy, the primary goal of epilepsy therapeutics has been seizure control. Since seizures are not only pathognomonic for epilepsy, but also represent a significant problem for patients, this strategy has been both rational and fruitful. However, in spite of the development of a wide array of anticonvulsant drugs, some patients have poorly controlled seizures while many more report breakthrough seizures. Efforts to seek better anticonvulsant treatments are ongoing, and these efforts have been accompanied by a concurrent effort to protect the brain from the damaging effects of seizures.

Using animal models, epilepsy researchers have discovered that seizures lead to permanent changes in the brain circuitry. Some of these changes, such as mossy fiber sprouting, could potentially be associated with seizure progression. In addition, uncontrolled seizures may cause a loss of brain cells. Humans with chronic temporal lobe epilepsy (TLE) show neuronal loss, sclerosis, and atrophy, especially in the hippocampus. In animal models, the occurrence of severe seizures leads to cell loss in various regions of the brain, especially the limbic system. As illustrated in Figure 1, the hippocampus of rodents has proved to be exquisitely sensitive to the excitotoxic cell death induced by seizures, and this cell loss in the animal model reflects the damage profile seen in human TLE.

Behavioral studies in animals have also shown that seizure-related cell loss is associated with functional impairments, such as memory decline and emotional deficits, similar to findings in humans where epilepsy has been associated with functional impairments ranging from memory loss to alterations in mood. Protecting neurons in the human epileptic brain may protect against functional impairments.

As a consequence of the realization that seizures beget neuronal loss and behavioral impairment, epilepsy researchers are increasingly interested in pursuing neuroprotective strategies in epilepsy. Protecting neurons from death or functional compromise after seizures would, in the short term, prevent some of the far-reaching consequences of breakthrough seizures. In the longer term, the optimal treatment for epilepsy would simultaneously prevent seizures and protect neurons from the damaging consequences of seizure activity.

Background

Neurotrophic Factors and Neuroprotection

The past two decades have seen a surge in interest in the potential of protein growth factors to protect cells throughout the body. From the perspective of an epilepsy researcher, those protein factors capable of protecting cells in the nervous system, termed neurotrophic factors,
represent promising therapeutic candidates. These proteins – which protect neurons from insults ranging from oxygen deprivation and serum starvation to excitotoxic damage – have moved from the Petri dish into animal models. A number of these factors have been tested in animal models of seizure disorders, with varying degrees of success.

**A Tale of Two Factors**

Our laboratory has a long-standing interest in the potential of growth factors to protect neurons, and has focused on the potential of two growth factors – brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) – as treatments for epilepsy.

**BDNF**

BDNF is a member of the neurotrophin family of growth factors. Receptors for the neurotrophins include the p75 neurotrophin receptor, which binds to neurotrophins with low affinity, and the Trk (tropomyosin-related kinase) family of receptors, which bind preferentially to certain neurotrophins with high affinity. Of the three Trk receptors, only TrkB binds BDNF. While both BDNF and its relative, nerve growth factor (NGF), are upregulated after seizures, BDNF and TrkB are more widely distributed in limbic structures, making BDNF the more promising candidate for seizure neuroprotection. Before being tested as a protective factor in the context of epilepsy, BDNF proved to be protective in a variety of other situations, ranging from developing cells in vitro through in vivo models of various disorders. BDNF was first tested for neuroprotective potential after seizures using the kainic acid model of status epilepticus. In this model, animals are administered kainic acid intraperitoneally and develop status epilepticus (SE) within about an hour. Starting 24 h after SE, significant neuronal loss can be detected in the CA1 and CA3 pyramidal neurons as well as in the hilar neurons of the hippocampus. When exogenous recombinant BDNF was delivered in this model, it failed to significantly protect against neuronal death in CA1, and actually significantly worsened damage in CA3. Since BDNF had previously been considered a well-established neurotrophic factor, this result was unanticipated.

**VEGF**

In contrast to BDNF, VEGF was first described as a vascular factor. It was only a decade later that its potential as a neurotrophic factor began to be appreciated. Perhaps most relevant for seizure researchers, VEGF was shown to protect against excitotoxic damage in hippocampal cultures. However, because VEGF’s primary signal-transducing receptors, VEGFR1 (Flt-1) and VEGFR2 (Flk-1, KDR), were not constitutively expressed on adult neurons, it was unclear whether VEGF would work as a protective factor in adult epilepsy. The discovery that VEGF receptors were upregulated after brain insult – e.g., after stroke or after status epilepticus – made this possibility more likely. Using pilocarpine-induced status epilepticus in rats (a chemoconvulsant model resembling the kainic acid model), we discovered that VEGF significantly protected hippocampal neurons from damage (see Figure 2).

**Physiological Effects of Neurotrophic Factors**

The interpretation of the protective effects of various neurotrophic factors has been complicated by the initially unexpected finding that many of these factors have acute physiological effects on neurons. Traditionally, these protein factors were thought to mediate only longer-term effects on cell survival, function, and morphology. As physiologists became more interested in studying growth factors, the initially heretical idea that these protein modulators could cause relatively rapid alterations in cell firing became a well-established fact. Because excitability is an important contributor to excitotoxicity, enhancement or suppression of neuron excitability has the potential to complicate both the interpretation of and the efficacy of neuroprotective effects of growth factors, including BDNF and VEGF.
Based on its established protective effects, BDNF was predicted to protect neurons from seizure-induced cell death. It was therefore surprising when BDNF increased neuronal loss in CA3, as indicated earlier. However, BDNF had also been shown to enhance synaptic transmission in hippocampal slices and to induce hyperexcitability – resembling epileptiform activity – in entorhinal/hippocampal slices. In vivo, mice transgenically overexpressing BDNF seized more readily while those deficient in BDNF were more resistant to seizure activation. Further, administration of BDNF protein to normal rats induced seizures in the absence of other convulsant stimuli. Some laboratories, however, have reported beneficial effects of BDNF against epileptogenesis, perhaps as a consequence of TrkB downregulation during certain administration paradigms. Given these results, BDNF’s ability to increase excitability, potentially leading to increased excitotoxicity, could outweigh its ability to directly protect neurons (see Figure 3). It is for this reason that it is crucial for all potential neuroprotective agents to be evaluated for effects on neuronal excitability.

In contrast to the proexcitability effects of BDNF, the neurotrophic factor VEGF appears to ‘quiet’ neurons, making them less excitable. Like BDNF, this factor showed promising neuroprotective effects in other situations before being tested for efficacy against status epilepticus-induced neuronal loss. But unlike BDNF, VEGF showed significant efficacy in protecting hippocampal neurons from death after status epilepticus. While it is tempting to attribute this effect solely to VEGF’s demonstrated protective effects, the interpretation of the mechanism of its efficacy is complicated by its physiological effects (see Figure 3). Specifically, since VEGF decreases the excitability of neurons in hippocampal slices, its protective effects could be mediated through reduced excitability rather than direct neuroprotection. The frustrations involved in interpreting the nature of VEGF’s protective effects are, however, more than compensated for by the exciting possibility that VEGF may represent a therapeutic approach which both protects neurons from excitotoxic damage and reduces the likelihood that the patient will have seizures.

Methods

While there are many animal models of epilepsy and epilepsy-induced neuronal loss available, our laboratory has chosen to use the pilocarpine and kainic acid models of acute SE. Both models induce severe SE in the majority of animals injected, and both produce replicable damage to the brain, most striking in the pyramidal cells of the hippocampal CA1 and CA3 subfields, as well as hilar neurons. Damage continues to progress for several days...
to a week after SE, which allows us the flexibility to study the brain either several days after SE to determine the full extent of damage, or 24 h after SE to study the process of cell death.

In either model, we introduce growth factors directly into the brain via implanted cannulae, because these large growth factors do not cross the blood–brain barrier. Growth factors can be administered acutely via injection through the cannula, chronically via infusion into the cannula by means of a pump, or genetically by introducing a virus delivering DNA or RNA for the growth factor into brain cells. This latter approach results in longer-term delivery of physiologically relevant levels of growth factors since the brain cells will then make increased levels of growth factor protein using the extra genetic material. In our laboratory, we deliver growth factors into the hippocampus, both because the cell loss after SE is striking in that structure, and also because hippocampal damage has been associated with well-defined behavioral impairments.

In our laboratory we use two approaches to quantifying the neuroprotective effect of growth factors – measuring neuronal density and evaluating behavioral outcome. Neuronal density is determined using stereological software which allows for unbiased estimates of cell density. Behavioral outcome is determined using the Morris water maze for learning and memory ability and cell density. Behavioral outcome is determined using the Morris water maze for learning and memory ability and cell density. Behavioral impairments can be induced in animals, for instance, simply by causing abnormal electrical spiking in the intact hippocampal circuitry. In addition, abnormal brain chemistry has been found to be correlated with cognitive deficits in human epileptic patients, independent of gross anatomical lesions. Given the importance of functional preservation, it seems appropriate to eventually expand our definition of ‘neuroprotection’ to cover not just prevention of neuronal loss, but also protection of normal neuronal functioning. Indeed, researchers increasingly include preservation of normal neuronal physiology and circuitry in their definition of neuroprotection.

Directions for Future Research

While work in the area of neuroprotection in epilepsy is progressing, challenges remain. In addition to our work with BDNF and VEGF, promising data have been obtained by others with protein factors such as glial-derived neurotrophic factor (GDNF) and fibroblast growth factor-alpha (FGFα). Perhaps the biggest impediment to using any of these growth factors in human epilepsy is that the factors are too large to cross the blood–brain barrier. Therefore, chronic administration of an indwelling cannula into the brain, an approach which would not only be invasive, but would expose human epileptics to the risk of infection.

In an attempt to minimize the invasive nature of growth factor treatment, more research is currently being conducted using acute administration of viral vectors containing DNA or RNA (i.e., gene therapy) for growth factors. While this approach is invasive, it requires only one surgery, after which the brain can be left to heal. Preliminary data from our laboratory has suggested that gene therapy introducing extra copies of the VEGF gene is at least as effective as protein infusions in preventing neuronal death after status epilepticus. Gene therapy using other protective agents (e.g., GDNF) has already shown therapeutic potential in animal models of epilepsy. Once gene therapy is optimized in animal models, it may become possible to move this treatment strategy into humans. However, this approach is only likely to be undertaken in severe cases because it requires brain surgery. Treatment with growth factors for less severe cases may need to await the development of small molecules targeting growth factor receptors and signaling pathways.

Results

Functional Preservation

While some work has been done looking at the potential of growth factors to protect against the neuronal loss caused by severe seizures, less work has been carried out to determine if this protection translates into behavioral rescue. Should neuroprotective factors rescue neurons from death, but fail to preserve their ability to function normally, these treatments would ultimately be of little clinical value.

Memory impairment stands out as the most carefully validated impairment in epileptic patients, especially those showing hippocampal sclerosis. While memory and emotional disturbances have been reported repeatedly in animal models of epilepsy, we have found that neuronal rescue and functional rescue after status epilepticus are not always linearly related. With VEGF, for example, we have documented approximately 50% rescue of neurons after status epilepticus. Two weeks after SE, VEGF-treated animals show full rescue from impairments in anxiety, but only partial rescue from learning and memory impairments in the Morris water maze. This ‘discrepancy’ points to the likelihood that the relation between neuronal and behavioral rescue will be complex, and that the ultimate functional outcome of neuronal rescue may depend on the type and location of rescued neurons, on the nature of the behavior measured, and on the functional status of the hippocampal circuitry that remains intact. Behavioral impairments can be induced in animals, for instance, simply by causing abnormal electrical spiking in the intact hippocampal circuitry. In addition, abnormal brain chemistry has been found to be correlated with cognitive deficits in human epileptic patients, independent of gross anatomical lesions. Given the importance of functional preservation, it seems appropriate to eventually expand our definition of ‘neuroprotection’ to cover not just prevention of neuronal loss, but also protection of normal neuronal functioning. Indeed, researchers increasingly include preservation of normal neuronal physiology and circuitry in their definition of neuroprotection.

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In order to minimize the need for invasive therapies, our work is increasingly focusing on identifying the receptors and signaling pathways involved in growth factor-induced neuroprotection. Once these pathways are established, small molecule reagents could be sought which would emulate the beneficial effects of large protein factors without the need for brain surgery. The development of a neuroprotective small molecule would pave the way for a systemically administered drug (such as a pill) which could combat the brain damage and resultant behavioral impairments suffered by some epileptics.

Another advantage of identifying the particular receptors involved in the protective effects of factors such as VEGF is that most growth factors bind at multiple receptors which mediate multiple effects within the brain (e.g., VEGF signals through both VEGFR1 and VEGFR2 which have been proposed to modulate inflammation and vascular plasticity as well as protection). Selective targeting of the receptor which mediates neuroprotection would reduce side effects that could result from binding at those receptors not involved in the protective effects. Our laboratory is currently using reagents which preferentially block one VEGF receptor at a time in an attempt to discover which receptor mediates VEGF’s protective effects in epilepsy.

While the search for therapeutic reagents emulating protein growth factors is ongoing, other laboratories are targeting the pathways which lead to neuronal death. All cells, for instance, share common biochemical cascades which mediate apoptosis, or programmed cell death. Protein growth factors are known to act via these pathways, with phosphorylation of their receptors leading to activation of the mitogen-activated protein kinase (MEK), extracellular signal-regulated protein kinase (Erk1/2), and/or phosphatidylinositol-3-kinase (PI3K)-Akt survival pathways (see Figure 4). Indeed, much of VEGF’s neuroprotective effect in other systems appears to be mediated via the Akt pathway, but it also has the potential to mediate survival via the Erk pathway. Both of these pathways, as well as other trophic signaling pathways, lead to effects on components of cell death and survival pathways such as caspases, BAD, Bcl-2, Bax, Bim, and Bcl-w – all of which could impact on neuronal survival during seizures (Figure 4). For instance, animals deficient in Bcl-w and animals treated with a caspase-3 inhibitor both show enhanced cell protection after status epilepticus, validating the idea that neurons could be protected from SE-induced neuronal death by blocking the biochemical pathways leading to cell death.

While it is challenging to identify small molecule reagents that cross the blood–brain barrier, are soluble in water-based solutions, and selectively activate prosurvival signaling pathways, aggressive research in this area should lead us ever-closer to viable therapeutics. Inhibitors of programmed cell death would have to be used with care in young children with epilepsy, as programmed cell death is an important component of normal development. In older children and adults, however, this ‘downstream’ strategy for neuroprotection may one day be possible.

**Figure 4**  Signaling cell survival. Most protein growth factors transduce signals through biochemical pathways which can inhibit cell death. Two common prosurvival pathways are the PI3K/Akt pathway and the MEK/Erk pathway, both of which can be induced by transphosphorylation of dimerized receptor tyrosine kinases. This simplified cartoon illustrates some of the key players in these pathways, including the procell death mediators Bim, Bad, Bax, Bid, Bak, and cleaved caspases in red and the prosurvival mediators Bcl-2, Bcl-w, Bcl-x, in green. These substances could, themselves, become potential targets for neuroprotective therapeutics.
Whatever the eventual neuroprotective approach taken in humans, be it growth factor-related or not, preserving the brain of the epileptic patient is a promising strategy for maintaining normal functioning. The success of neuroprotective approaches could lead to a substantial enhancement in the quality of life of epileptic patients.

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See also: Epileptogenesis; Epileptogenesis and Plasticity; Inflammation: Neurodegeneration, Neuroprotection and Inflammation in the Brain; Neurotrophic Factors: Activity-Regulated BDNF Expression: Contributions to Synaptic Plasticity and Neuroprotection; Role of BDNF in Animal Models of Epilepsy; The Influence of Neurotrophins on Excitability in Hippocampus and its Potential Relevance to Epilepsy.

Further Reading


Rudge JS, Mather PE, Paskowski EM, et al. (1998) Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective. Experimental Neurology 149: 396–410.


Role of BDNF in Animal Models of Epilepsy

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Introduction

Elucidating the cellular and molecular mechanisms of epileptogenesis could lead to novel therapeutic approaches aimed at the prevention or management of the disease. The discovery that limbic seizures increase nerve growth factor (NGF) mRNA levels led to the idea that seizure-induced expression of neurotrophic factors might contribute to the lasting structural and functional changes underlying epileptogenesis. Similarly, increases in neurotrophin expression that follow other insults (e.g., ischemia and traumatic brain injury) could also contribute to epileptogenesis. In the past decade, there has been an exciting confluence of in vitro and in vivo findings that strongly implicate the neurotrophin, brain-derived neurotrophic factor (BDNF), in particular, limbic circuits involved in the cascade of electrophysiological and behavioral changes underlying the development of the epileptic state.