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Finding a better drug for epilepsy: Antiepileptogenesis targets

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Summary

For several decades, both in vitro and in vivo models of seizures and epilepsy have been employed to unravel the molecular and cellular mechanisms underlying the occurrence of spontaneous recurrent seizures (SRS)—the defining hallmark of the epileptic brain. However, despite great advances in our understanding of seizure genesis, investigators have yet to develop reliable biomarkers and surrogate markers of the epileptogenic process. Sadly, the pathogenic mechanisms that produce the epileptic condition, especially after precipitating events such as head trauma, inflammation, or prolonged febrile convulsions, are poorly understood. A major challenge has been the inherent complexity and heterogeneity of known epileptic syndromes and the differential genetic susceptibilities exhibited by patients at risk. Therefore, it is unlikely that there is only one fundamental pathophysiologic mechanism shared by all the epilepsies. Identification of antiepileptogenesis targets has been an overarching goal over the last decade, as current anticonvulsant medications appear to influence only the acute process of ictogenesis. Clearly, there is an urgent need to develop novel therapeutic interventions that are disease modifying—therapies that either completely or partially prevent the emergence of SRS. An important secondary goal is

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to develop new treatments that can also lessen the burden of epilepsy comorbidities (e.g., cognitive impairment, mood disorders) by preventing or reducing the deleterious changes during the epileptogenic process. This review summarizes novel antiepileptogenesis targets that were critically discussed at the XIth Workshop on the Neurobiology of Epilepsy (WONOEP XI) meeting in Grottaferrata, Italy. Further, emerging neurometabolic links among several target mechanisms and highlights of the panel discussion are presented.

Keywords

Epileptogenesis; Neuroprotection; Antiepileptic drug; Ion channel; Epigenetics; Neurotrophic factors; Metabolism; Biomarker; Ketogenic diet

To date, pharmacologic strategies to mitigate or prevent epileptogenesis in humans—most notably after head injury—have proven to be ineffective (Holtkamp & Meierkord, 2007; Löscher & Brandt, 2010), despite vast laboratory evidence that many anticonvulsant medications possess neuroprotective properties (Pitkänen, 2002; White, 2002). This failure is often believed to be a consequence of several factors, including species differences, inappropriate timing of intervention, focus on nonessential molecular targets, differential genetic susceptibility, and perhaps long-term neurotoxicity of drugs (Pitkänen & Lukasiuk, 2011). The same issues apply to the stroke field but perhaps even more so, as dozens of prospective randomized clinical trials—rationally based on specific molecular targets validated in animal models—have failed to show significant effects in humans (Engel, 2001; Löscher & Brandt, 2010). Another important consideration is that the molecular targets that have traditionally been studied may be more relevant to ictogenesis (i.e., induction of an acute seizure) than to epileptogenesis, the latter which reflects a variable process resulting in an enduring state of spontaneous recurrent seizures. Such limitations highlight the need to identify novel molecular targets for antiepileptogenesis.

The unpredictability of seizure activity, and indeed an increasingly recognized progressive disease state that defines many forms of epilepsy, poses numerous challenges toward our understanding of pathophysiology, efforts in designing and implementing laboratory investigations, and conducting controlled clinical studies. It is important to recognize that there are highly variable outcomes after epileptogenic brain insults, and little is known about the factors that govern such differential pathways. Moreover, the modifying influences of environment, attempts at treatment, and comorbid medical problems (intrinsic or acquired) serve to complicate an already confounding set of processes. Against this frustrating backdrop, there are growing international efforts to develop reliable biomarkers and surrogate disease markers of epileptogenesis, which may help clinicians identify patients at high risk following a brain insult (whether they be febrile seizures, status epilepticus [SE], head trauma, or brain inflammation). Such markers will also undoubtedly prove immensely useful in assessing patients with underlying genetic etiologies and in those individuals where the underlying cause(s) of spontaneous unprovoked seizures remain a mystery. However, the overall utility of biomarkers and surrogate markers rests in large measure on how these reflect the true pathophysiology of the epileptogenic process itself. And it should be duly noted that like the heterogeneous nature of the epilepsies, a singular mechanism or

molecular pathway may not be applicable to epileptogenesis in general. Investigators should bear in mind this critical notion when attempting to advance experimental therapeutics designed to arrest or modify the epileptogenic disease process.

At present, there is a wide range of animal models of epileptogenesis, but many of them are likely not clinically meaningful to a large extent when viewed from the standpoint of precipitating causes. Other than traumatic brain injury, insults such as electrical brain stimulation (e.g., kindling) or exposure to chemoconvulsants (e.g., pilocarpine) or excitotoxins (e.g., kainic acid) generally do not occur in humans who develop epilepsy. As such, the question can and should be raised as to the relevance of molecular and cellular changes observed in such models. Specifically, the spectrum of alterations reported during the presumed epileptogenesis process in animal models include neuronal injury and cell death, axonal and dendritic plasticity, presynaptic and postsynaptic modifications, neurogenesis, neuroinflammation, glial cell activation, vascular damage and angiogenesis, disruption of extracellular matrix integrity, as well as structural (i.e., subunit) and functional changes in ion channels properties (Pitkänen et al., 2007).

Summarized below is a composite review of presentations made at the 11th Workshop on the Neurobiology of Epilepsy (WONOEP XI) on the topic of antiepileptogenesis targets, which was organized by the Neurobiology Commission of the International League Against Epilepsy (ILAE) in Grottaferrata, Italy (August 23–27, 2011). Both older and newer molecular targets and approaches were discussed, and all were framed with novel therapeutic perspectives. These include α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, thrombospondin receptors, epigenetic regulation, granular “hub” cells, neurotrophic factors, erythropoietin (EPO)–derived peptide mimetics, ketogenic diet, and bumetanide derivatives as potential treatment approaches as well as diffusion tensor (magnetic resonance) imaging (DTI) to monitor treatment effects.

We propose that true antiepileptogenesis targets should be validated by their ability to either completely prevent the emergence of spontaneous recurrent seizures, delay their onset (i.e., partial prevention), or by modifying seizure frequency, duration, and/or severity. Broadly speaking, such changes would fall under the rubric of disease or syndrome modification, implying that one or more therapeutic interventions based on such targets have some critical effects on the epileptogenic process(es). Following brief overviews of the diverse array of potential targets, an attempt at weaving the seemingly unrelated topics into a common “neurometabolic” fabric is made.

Mechanisms of Epileptogenesis

Epigenetics

Although many mechanisms of epileptogenesis have been postulated, one emerging topic discussed during WONOEP XI was epigenetics. Epigenetics summarizes alterations to the chromatin template (i.e., DNA methylation, histone tail modifications, and incorporation of histone variants, activity of noncoding RNAs, and chromatin remodeling) that collectively establish and propagate different patterns of gene expression from the same genome. Aberrant epigenetic chromatin modifications have been increasingly recognized in many

neurologic disorders including autism, bipolar disorder, schizophrenia, brain tumors, and neurodegeneration (Mehler, 2008; Urdinguio et al., 2009; Qureshi & Mehler, 2010). Recent evidence suggests neuronal membrane excitation is linked to epigenetic chromatin modifications (Dulac, 2010; Peleg et al., 2010), thereby adapting gene expression levels to functional network activity. Seizures are regarded as paroxysms of hypersynchronous epileptogenic network excitation and likely compromise the epigenetic regulation machinery (Huang et al., 2002; Tsankova et al., 2004; Sng et al., 2006; McClelland et al., 2011; Zhu et al., 2012).

Recently, it has been proposed that aberrant promoter methylation is an important pathogenic mechanism underlying epileptogenesis. Specifically, increased levels of DNA promoter methylation was shown in surgically-resected specimens from patients with human temporal lobe epilepsy (Kobow et al., 2009; Kobow & Blumcke, 2011). Preliminary investigations in rodents appear to validate these findings (K. Kobow, unpublished data), but are as yet not generalizable as only single genes have been investigated thus far.

Although aberrant epigenetic gene regulation in epileptogenesis is an attractive hypothesis that could explain the synergistic misregulation of multiple genes in major proepileptogenic pathways—including neuroinflammation, synaptic reorganization, and development of pharmacoresistance (Kobow & Blumcke, 2011)—the pathogenic link between epigenetic chromatin modifications on neuronal excitability and epileptogenesis awaits experimental confirmation. Future studies will be needed to (1) confirm seizure-associated global alterations in DNA methylation and other epigenetic phenomena, for example, impact of poly-comb and trithorax proteins, histone modifications, chromatin remodelling, and microRNA; (2) analyze the effects on genes or noncoding genomic regions (regulation of microRNA expression); and (3) demonstrate a causal relationship between functional gene regulation by epigenetic chromatin modification and seizure genesis. A greater understanding of these mechanisms may open new therapeutic windows for difficult-to-treat epilepsies. In addition, epigenetically active pharmacologic compounds may be recognized as novel anticonvulsant (and potentially, antiepileptogenic treatments), which may rapidly advance to clinical application.

Biomarkers of Epileptogenesis

Diffusion tensor MRI

Currently, there are no reliable biomarkers or surrogate markers that can predict or track the development and severity of epilepsy in patients at risk after a brain insult. And suffice it to say that without such markers for the human epileptogenic process, clinicians will remain in the dark about the nature and evolution of the epileptogenic process. In addition, they will not be able to properly assess the impact of their interventions. For many obvious reasons, magnetic resonance imaging (MRI) has emerged as perhaps the most versatile clinical and research tool, providing relatively high-resolution images of the brain over long periods of time in a noninvasive manner. MRI is widely used in the detection of structural, functional, and metabolic alterations in the intact brain.

DTI is one contemporary imaging tool that provides a high tissue contrast based on microstructural characteristics of water diffusion (Le Bihan, 2003). Alterations of the tissue cytoarchitecture in pathologic conditions cause transient or permanent changes in water diffusion detectable by DTI (Beaulieu, 2002; Horsfield & Jones, 2002). Although DTI contrast is most often associated with highly organized white matter, it also provides useful contrast for organized gray matter structures (Mori & Zhang, 2006).

Although definitive information regarding MRI changes during the period of epileptogenesis is not yet forthcoming in humans, parallel studies in animal models have provided useful insights (Laitinen et al., 2010; Sierra et al., 2011). Tract-based spatial statistics (TBSS) analysis was applied in the analysis of DTI data acquired from rat brain in a kainic acid–induced SE model of temporal lobe epilepsy (TLE) and after traumatic brain injury (TBI). Together with targeted histology (including Nissl, Timm, and myelin staining), DTI changes in the epileptic brain were associated with alterations in myelinated axons, neurodegeneration, and/or calcification of the tissue.

Specifically, diffusion ellipsoids were created to help visualize more refined changes in diffusion tensor, likely to be associated with orientation and density of the cellular structures hindering water diffusion. In SE and TBI models, altered shapes of the diffusion ellipsoids were seen mainly in the CA3 hippocampus and dentate gyrus, whereas changes in orientation were more pronounced in CA3 and lacunosum-moleculare of CA1. As predicted from histology, DTI changes after SE were more robust and widespread than after TBI. These investigators proposed that diffusion ellipsoids generated from different hippocampal subfields or areas can serve as plasticity sensors and provide important information about the dynamics of ongoing changes after brain injury—and if indeed validated, this may become the one of the first reliable neuroimaging biomarker or surrogate marker of epileptogenesis in humans.

Targeting Epileptogenesis

Neurotrophic factors

One innovative strategy for the control of seizures may be augmentation of neurotrophic factors (NTFs) such as brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 2 (FGF-2), which have been increasingly shown to exert neuroprotective effects. Given the favorable effects of NTFs on neuronal survival following a brain insult, it has been suggested that limiting tissue damage and enhancing repair by administration of NTFs may alleviate epileptogenesis—particularly for the prevention and treatment of TLE. However, it is unclear which specific NTFs should be administered, at what doses, for what duration of treatment, and through which route(s) of administration, in order to promote repair of neuronal damage and to avoid possible proepileptic effects (Simonato et al., 2006).

In this light, the potential utility of BDNF and its principal receptor target TrkB—tropomyosin-receptor-kinase B, a member of the tyrosine kinase family—remains a matter of intense debate (Acharya et al., 2008). Paradiso et al. (2009, 2011) used a herpes-based vector to locally supplement FGF-2 and BDNF in the hippocampus 3 days after pilocarpine-induced SE, that is, when epileptogenic damage was already in place (Bovolenta et al.,

2010). This dual NTF treatment decreased hippocampal mossy fiber sprouting, and also reduced the frequency and severity of spontaneous seizures. Additionally, neuronal loss was mitigated in both hilus and the CA3 subfield. Despite such observations, there are well-recognized challenges in employing viral vectors to treat conditions such as TLE—for example, concerns regarding the injection of potentially pathologic wild-type virus, and the need for invasive stereotactic delivery. Hence, in search of alternative methods of administration, a new strategy based on the use of mesangioblasts (MABs) was proposed. MABs are multipotent progenitors of mesodermal tissues which can give rise to multiple differentiated mesodermal phenotypes. Specifically, MABs can be isolated from adult perivascular tissue, and can reach perivascular targets especially in damaged areas due to their high adhesin-dependent migratory capacity (Galvez et al., 2006; Sampaolesi et al., 2006). Bystander effects of MABs producing BDNF (MABs-BDNF) have been tested (Su et al., 2012). MAB-BDNF–conditioned medium increased the survival and functionality of cultured neurons and slices in a TrkB-receptor–dependent manner. Moreover, initial evidence for selective homing to the lesioned areas in a model of Alzheimer's disease was obtained. Therefore, MABs may prove useful for autologous transplants (i.e., they can be prepared from a patient's biopsy), may be peripherally administered (i.e., obviate the need for surgery), and may selectively be targeted to a specific brain area (i.e., potentially reduce side-effects).

Erythropoietin-derived peptide mimetics

EPO is a glycoprotein produced mainly in the renal cortex and acts primarily on the hematopoietic system as a cytokine to induce red blood cell production in the bone marrow. However, EPO is also expressed in several nonhematopoietic tissues, where it acts to prevent apoptosis and inflammation due to hypoxia, toxicity, or injury (Chateauvieux et al., 2011). As such, there is intense interest in studying the actions of EPO, and synthesizing nonerythropoietic peptide derivatives, which may exert beneficial effects in the central nervous system (CNS). Considering the neuroprotective, neuroregenerative, and antiinflammatory potential of EPO, it is of extreme interest to determine whether EPO-derived mimetic peptides can exert disease-modifying or antiepileptogenic effects. In a post-SE model, the nonerythropoietic EPO-peptide pHBSP (pyroglutamate helix B surface peptides) promoted hippocampal cell proliferation, neuronal differentiation, and survival of newborn neurons (Seeger et al., 2011). Moreover, this peptide reduced the activation of microglial cells, the production of proinflammatory cytokines, the microglial phagocytotic activity, and the formation of reactive oxygen species (ROS). Despite its effects on neurogenesis and neuroinflammation, neither pHBSP nor recombinant human EPO affected the number of animals exhibiting spontaneous recurrent seizures. However, pHBSP was shown to attenuate epilepsy-associated spatial learning deficits. Notwithstanding the negative effects on seizures, the fact that pHBSP can modify the long-term detrimental cellular and cognitive changes following SE is encouraging, and suggests that this strategy may eventually yield EPO-derived molecules that possess disease-modifying properties. As with the NTFs discussed earlier, future studies are necessary to delineate the optimum treatment parameters and indeed the most susceptible animal models. At present, the design of EPO-derived peptide mimetics offers intriguing possibilities for selectively preserving certain effects while excluding undesirable actions of a parent molecule based on the

selection of a minimal active sequence. Moreover, as a general rule, the rationale design of peptides allows for the development of therapeutic compounds with improved stability and brain distribution.

Cell-specific therapy

The dentate gyrus (part of the hippocampal formation) is one of the very few brain structures known to exhibit highly active adult neurogenesis (Ehninger & Kempermann, 2008), a phenomenon that has been linked to epileptogenesis (Siebzehnrubl & Blumcke, 2008). Specifically, seizures increase neurogenesis in the dentate gyrus in a robust manner, unlike other manipulations that induce this phenomenon. It is important to note that adult-born granule cells show a maturation-dependent vulnerability to SE-induced abnormal structural plasticity (Kron et al., 2010). Therefore, it has been suggested that newly born dentate granule cells might represent targets for antiepileptogenic treatments, but this strategy may pose several complications (Scharfman & McCloskey, 2009). Based on preliminary findings, the innovative idea was proposed that a key player in epileptogenesis occurring in TLE may be the group of dentate granule cells that are “caught” by the insult at the most plastic stage of their development. This hypothesis is highly attractive because it is well established that adult-born granule cells go through defined stages of development in an adult neuronal environment (Ge et al., 2008), and that a precipitating injury during a highly vulnerable period may be what is required to activate the epileptogenic process. At approximately 4 weeks of age, dentate granule cells exhibit prominent signs of neuronal plasticity, including appearance of dendritic spines, new synaptic contacts, a shift in intracellular Ca^{2+} binding proteins, highly plastic neurotransmitter receptors, and possibly basal dendrite formation. By examining the cellular and network properties of retrovirally labeled cells at different stages of development at the time of the precipitating insult, Istvan Mody’s group was able to “fingerprint” these cells in terms of their electrophysiologic and synaptic properties. Based on their preliminary findings, the 4-week-old neurons at the time of SE are very likely to evolve into the hypothetical “hub” cells of the dentate gyrus (Morgan & Soltesz, 2008). These highly interconnected granular hub cells have been postulated by computer models to be in a position to coordinate the activity of thousands of their target neurons, eventually leading to the formation of aberrant and epileptic activity. If validated, these studies could pave the way for the first cell-specific anti-epileptogenic therapy.

Glutamate receptors

Early brain development is a time of heightened activity-dependent synaptic plasticity, and as such, the timing of various CNS insults may be critical in determining long-term processes such as epileptogenesis and its associated comorbidities. It is well known that in both rodents and humans, there is a developmentally-timed increase in the expression and activity of glutamate receptors (GluRs), and that pathologic augmentation of this normal ontogeny may produce excitotoxic damage and subsequent molecular cascades that negatively impact further brain development (Silverstein & Jensen, 2007). With respect to normal cognition, hippocampal long-term potentiation (LTP) has been established as the fundamental cellular electrophysiological substrate underlying learning and memory processes (Pastalkova et al., 2006; Whitlock et al., 2006). Thus, it should not be surprising

that the mechanisms that govern hippocampal LTP might be altered during the epileptogenic process.

It has been hypothesized that seizure-induced epileptogenesis in the immature brain may result in part from activation of endogenous synaptic strengthening mechanisms involved in normal activity-dependent plasticity. Furthermore, the concept was advanced that for early life seizures, at ages that still express Ca^{2+}-permeable AMPA receptors, AMPA receptor–induced signaling events similar to LTP may participate in epileptogenic synaptic strengthening (Rakhade et al., 2008; Zhou et al., 2012). Jensen and colleagues tested the effects of seizures induced by hypoxia, kainate, and pentylenetetrazole (PTZ) during the second postnatal week in rodents, and found that brief seizures activated Ca^{2+}-dependent kinases (specifically, protein kinases A and C and calmodulin-dependent kinase II) and increased the phosphorylation of specific sites on the GluR1AMPA receptor subunit. Electrophysiologic studies revealed enhanced AMPA receptor–mediated miniature excitatory postsynaptic potentials (mEPSCs), spontaneous EPSC amplitude and frequency (Rakhade et al., 2008). Knock-in mice with mutations in GluR1 phosphorylation sites showed reduced seizure susceptibility to PTZ, and hippocampal slices from these animals failed to show the increased excitatory AMPA receptor–mediated currents in control wild-type mice. Seizures in these models were found to occlude hippocampal LTP, and postseizure blockade with the AMPA-receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfa-moyl-benzo[f]quinoxaline-2,3-dione (NBQX) prevented the seizure-induced attenuation of LTP as well as the downstream changes in key signaling pathways. Therefore, in preliminary experiments, AMPA receptor blockade following seizures was found to prevent the cellular signaling events similar to those involved in LTP, lending credence to the notion that epileptogenesis may in part involve normal mechanisms of activity-dependent synaptic potentiation. This approach may represent a potential strategy for the development of age-specific therapies aimed at preventing or attenuating epileptogenesis (Zhou et al., 2012).

Cation-chloride cotransporters

The development of neuronal hyperexcitability is thought to be a critical event in the process of epileptogenesis. Cation-chloride cotransporters play an important role in the formation of abnormal excitatory γ-aminobutyric acid (GABA)ergic neurons during early brain development as well as in pathologic states associated with epilepsy. Hence, these membrane-bound pumps may constitute potential targets for novel anticonvulsant, and possibly antiepileptogenic drugs (Kahle et al., 2008). Different proepileptogenic brain insults have been shown to downregulate the K^+-CL^- cotransporter KCC2 (which extrudes chloride) and upregulate the Na^+-K^+-$2Cl^-$ cotransporter NKCC1 (which imports chloride), resulting a net increase in intracellular $C1^-$. This reversal of the normal electrochemical gradient for chloride is the major factor governing the shift from inhibitory to excitatory GABA activity, and hence may contribute to the development of neuronal hyperexcitability.

The diuretic drug bumetanide is a selective inhibitor of NKCC1, which can counteract the differential expression and activity of NKCC1 and KCC2 during epileptogenesis. Bumetanide exerted disease-modifying effects when administered after pilocarpine-induced SE in rats, but these effects occurred only when bumetanide was coadministered with

phenobarbital (Brandt et al., 2010). A likely explanation for this observation is the fact that bumetanide, due to its high ionization at physiologic pH, does not effectively cross the blood–brain barrier. Furthermore, the half-life of bumetanide in the rat is only about 10 min, so that effective levels are difficult to achieve and maintain in vivo (Brandt et al., 2010).

Due to these limitations, attempts are currently under way to synthesize bumetanide derivatives with enhanced brain penetration and prolonged duration of action. Fundamental to this aim is the development of lipophilic and uncharged bumetanide esters that could serve as pro-drugs. However, investigation of pharmacokinetic properties in rats revealed rapid metabolism of bumetanide esters by serum esterases resulting in limited brain penetration of the intact esters themselves. Intriguingly, there were remarkable species differences in the rates of degradation when tested in vitro using serum from mouse, rat, dog and human. In rat serum, all esters are rapidly degraded, whereas in dog and human serum all esters are stable for at least 60 min. In rodents, monooxygenases, which target the *n*-butyl sidechain of bumetanide, may also account for the rapid degradation observed. Therefore, both combined treatment of bumetanide esters with monooxygenase inhibitors (as well as application of other bumetanide derivatives) hold promise as potential antiepileptogenic treatments that principally target NKCC1.

One important caveat should be considered, however, with this class of agents. Long-term use of bumetanide-like drugs during gestation could potentially have adverse effects on the developing brain (Ben-Ari & Tyzio, 2011; Wang & Kriegstein, 2011). Yet, it is well appreciated that seizures during pregnancy also carry risks to both mother and fetus. This risk–benefit concern should be carefully weighed in the clinical setting.

Thrombospondin and BDNF

Following injury to cortical structures, aberrant new excitatory connections emerge and disinhibition of principal neurons occurs as a consequence of both structural/functional abnormalities in GABAergic interneurons. It is believed that the combination of these alterations contribute to epileptogenesis (Prince & Jacobs, 1998; Briggs & Galanopoulou, 2011). Prophylactic treatments targeting these pathophysiologic mechanisms in the chronic partial cortical isolation (i.e., “undercut” or UC) were developed in a model of posttraumatic epileptogenesis (Prince et al., 2009; Li et al., 2011). One of the novel targets studied is a class of multifunctional proteins known as thrombospondins (TSPs), which are adhesive glycoproteins released by reactive astrocytes that mediate cell-to-cell and cell-to-matrix interactions. It was recently reported that the primary target of the anticonvulsant and analgesic agent gabapentin (GBP)—the α2δ-1 subunit of voltage-gated calcium channels—is a neuronal thrombospondin receptor (Eroglu et al., 2009). GBP was found to antagonize TSP binding to the α2δ-1 subunit with high affinity and inhibited excitatory synapse formation both in vitro and in vivo (Eroglu et al., 2009). As an extension of this intriguing observation, GBP was shown to reduce injury-induced synapse formation in neocortex, and decreased both the incidence of epileptiform discharges in vitro and the frequency of excitatory synaptic currents, in acute sensorimotor cortical slices prepared from UC rat brain (Li et al., 2011). Moreover, GBP significantly reduced the density of synapses in the UC cortex and the number of injured cells as measured with Fluorojade C staining and

neurofilament immunoreactivity. Another potentially complementary approach involved providing trophic support for injured GABAergic interneurons. Prince et al. (2009) first found that BDNF mRNA and protein are significantly reduced in pyramidal cells, and TrkB-receptor protein is decreased on fast-spiking parvalbumin-containing interneurons in the UC model. Moreover, these interneurons exhibited widespread anatomic changes (decreased axonal arbors, smaller boutons, shorter and thinner dendrites, and a reduced number of perisomatic inhibitory synapses onto pyramidal neurons), which correlated with electrophysiologic measures of diminished postsynaptic inhibition. Intriguingly, treatment of UC rats for 2 weeks postinjury with a small molecule mimetic for BDNF diminished signs of interneuronal injury. Collectively, these results suggest that treatments designed to enhance trophic support of inter-neurons and limit excitatory synaptogenesis after injury may provide effective prophylaxis for posttraumatic epilepsy. Nevertheless, several important questions remain, such as whether either or both of these approaches will decrease parameters of seizure activity in vivo. Would gabapentin further interfere with functional recovery after injury? Will TrkB-receptor activation have proepileptogenic effects, via increased excitatory synaptic activity, as well as antiepileptogenic actions to “rescue” atrophic interneurons? Which action will predominate, and under which experimental conditions? Although the answers to such questions are unclear at present, there is nevertheless a strong rationale for further validating either or both approaches.

Polyunsaturated fatty acids

The ketogenic diet (KD) is a high-fat and low carbohydrate diet with an established efficacy to treat medically refractory epilepsy. However, the underlying mechanisms are unfortunately not well understood. It is well known that fatty acids provide 80–90% of the total caloric intake in patients treated with the KD and trigger a metabolic switch favoring β-oxidation in mitochondria. This subsequently leads to the production of ketones, which can be used by the brain as an alternative source of energy (Acharya et al., 2008). Several experimental studies have reported that polyunsaturated fatty acids (PUFAs) alone exhibit anticonvulsant properties (Taha et al., 2010), but thus far clinical trials in epilepsy using PUFA supplements have failed to show significant effects. This discrepancy may be related to the use of different PUFAs (eicosapentaenoic acid and docosahexaenoic acid vs. linoleic acid and α-linolenic acid), varying routes of administration (oral vs. systemic), and to an experimentally well-established dose–response relationship (Auvin, 2011). Recent data strongly suggest that fatty acids contribute to the clinical efficacy of the KD via modification of cellular membrane composition in the CNS, stimulation of nuclear receptors such as PPARα/γ (peroxisome proliferator-activated receptor α or γ), and/or attenuation of neuro-inflammation. It is, however, currently difficult to know whether the quantity and/or a particular type of fatty acid are critical variables toward the anticonvulsant properties of the KD (Curatolo et al., 2011).

A commercially available form of the KD—comprising a 4:1 ratio by weight of fats to carbohydrate plus protein—elevated the PTZ threshold, but interestingly, there was no correlation between blood ketone concentrations and PTZ threshold, and there was no upregulation of PPARα and PPARγ genes, contrary to expectations. These data suggest that PUFAs may not be the essential mediators of KD action, but there remains controversy in

this research area. Ultimately, a better understanding of these (and other) mechanisms may lead to improvements in metabolism-based treatments for epilepsy. In addition, whether the KD or any other dietary formulation can exert disease-modifying effects remains unclear, but given the expanding literature on the neuroprotective effects of the KD and ketone bodies (Gasior et al., 2006; Maalouf et al., 2009), this intriguing potential is ripe for further investigation.

A Neurometabolic Synthesis

Although the mechanisms and molecular targets described above are seemingly disparate, many if not all (including those not covered in this review) may have in part an underlying basis in neurometabolism—specifically, in that the diverse array of metabolic substrates, enzymes, and biochemical pathways inherent in virtually every cell undoubtedly impacts cellular processes in a manner that can influence the actions of ion channels, neurotrophic factors, and even epigenetic regulation.

The prototypic metabolic treatment is the KD, which is clinically effective in reducing seizure activity in patients who fail to respond to conventional and even newer anticonvulsant medications. Despite the lack of a detailed understanding of how the KD works to control seizures (Masino & Rho, 2012), there is mounting evidence that the KD, ketones, and PUFAs are broadly neuroprotective (Gasior et al., 2006; Maalouf et al., 2009) through a multiplicity of effects, including, but not limited to a reduction in oxidative stress (Sullivan et al., 2004; Milder & Patel, 2011), enhancement in purinergic signaling (Masino et al., 2011), and increases in bioenergetic substrates such as ATP (Bough et al., 2006; Kim Do et al., 2010). These actions suggest that certain substrates that define such metabolism-based treatments might ameliorate a number of neurologic disease states, and indeed there is growing support for this concept (Stafstrom & Rho, 2012). With respect to epileptogenesis, the early evidence for a disease-modifying effect of the KD and its related treatments appears suggestive but not conclusive (Muller-Schwarze et al., 1999; Todorova et al., 2000; Bough et al., 2003; Stafstrom et al., 2009; Linard et al., 2010; Schwartzkroin et al., 2010; Jiang et al., 2012).

Pertinent to this review, although it remains uncertain whether PUFAs exert direct anticonvulsant effects (given the lack of concordance between animal and human studies), it is possible that omega-3 fatty acid derivatives may be antiepileptogenic. Indeed, it was recently reported that neuroprotectin D1 (NPD1), a derivative of docosahexaenoic acid (DHA) significantly attenuated kindling progression and hippocampal hyperexcitability (Musto et al., 2011). Along a similar vein, in an attempt to mirror the glucose restriction that is a hallmark feature of the KD, Garriga-Canut et al. (2006) demonstrated that 2-deoxyglucose, an inhibitor of phosphoglucose isomerase (a key enzyme in the glycolytic pathway, which prevents the conversion of glucose-6-phosphate to fructose-6-phosphate) prevented kindling epileptogenesis in rats. Specifically, 2-DG suppressed seizure-induced increases in BDNF and TrkB, which are mediated by the transcriptional repressor neuron restrictive silencing factor (NRSF) and its nicotinamide adenine dinucleotide (NADH)–sensitive corepressor carboxy-terminal binding protein (CtBP) (Garriga-Canut et al., 2006).

At this juncture, the effects of epigenetic DNA modification on neuronal excitability and on epileptogenesis are unknown. However, preliminary investigations in Wistar rats subjected to pilocarpine-induced SE have shown that a non–calorie-restricted KD reduced seizure frequency and antagonized aberrant DNA methylation (K. Kobow, unpublished data). A distinct correlation between seizure frequency and DNA promoter methylation pattern was seen in these studies.

It has been stated above that epileptogenesis may involve aberrant recruitment of mechanisms underlying activity-dependent synaptic potentiation, in part through effects involving AMPA-receptor modulation. Of interest, there are conflicting data regarding whether a KD impacts hippocampal LTP. Koranda et al. (2011) assessed the effects of theta-burst stimulation of dentate gyrus using in vivo recordings in freely behaving normal rats, and found that KD treatment significantly diminished LTP responses. This is in contrast to the study by Thio et al. (2010) who examined paired-pulse modulation (PPM) and LTP in the medial perforant path of rats in vivo and found no significant effects with KD treatment. Moreover, these investigators found that locomotor activity and behavior in a conditioned fear test were similarly unaffected. Although the reasons for this discrepancy remain unclear, it is clear that further studies examining the metabolic effects on synaptic plasticity and integrity are warranted.

Because the cation chloride cotransporters NKCC1 and KCC2 are prominently involved in the GABA-depolarizing effect in immature neurons (see above), and given that pharmacologic antagonism of NKCC1 has been shown to render anticonvulsant effects (Dzhala et al., 2005, 2008), a logical question to ask is whether the anticonvulsant KD might alter NKCC1 or KCC2 expression. An initial study examining expression levels of both cation-chloride cotransporters using immunocytochemical methods failed to reveal a difference in the number or intensity of labeled cells in hippocampus of adolescent rats fed a KD (Gomez-Lira et al., 2011). Therefore, it appears that one could rule out such cotransporters as mechanistic targets of KD action, but it should be noted that for many such studies, these negative findings were made in normal (and not epileptic) brain.

If seizures increase neurogenesis, and preventing this might yield antiepileptogenic and anticonvulsant effects, then another straightforward question would be whether the KD alters neurogenesis. Again, there are opposing data. In normal adult rats, KD treatment for 4 weeks did not change the number of bromodeoxyuridine (BrdU) immunoreactive cells in dentate gyrus (Strandberg et al., 2008), whereas the KD was found to significantly increase the number of BrdU-positive cells (and thus enhance neurogenesis) following kainate-induced seizures in mice (Kwon et al., 2008).

In summary, although there are at present only scant data regarding the effects of metabolism-based treatments such as the KD on the panoply of mechanisms and targets that have up to now been implicated in both ictogenesis and epileptogenesis (Masino & Rho, 2012), it would not be altogether surprising to witness a steadily increasing number of publications that link specific antiepileptogenesis targets to one or more metabolic substrates, enzymes or pathways—not just whether the KD affects any of these targets. The

fundamental role of neurometabolism in cellular homeostasis and disease states is becoming increasingly appreciated, but there is much more that needs to be investigated.

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