Mechanisms of epileptogenesis and potential treatment targets

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Prevention of epileptogenesis after brain trauma is an unmet medical challenge. Recent molecular profiling studies have provided an insight into molecular changes that contribute to formation of ictogenic neuronal networks, including genes regulating synaptic or neuronal plasticity, cell death, proliferation, and inflammatory or immune responses. These mechanisms have been targeted to prevent epileptogenesis in animal models. Favourable effects have been obtained using immunosuppressants, antibodies blocking adhesion of leucocytes to endothelial cells, gene therapy driving expression of neurotrophic factors, pharmacological neurostimulation, or even with conventional antiepileptic drugs by administering them before the appearance of genetic epilepsy. Further studies are needed to clarify the optimum time window and aetiological specificity of treatments. Questions related to adverse events also need further consideration. Encouragingly, the recent experimental studies emphasise that the complicated process of epileptogenesis can be favourably modified, and that antiepileptogenesis as a treatment indication might not be an impossible mission.

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Introduction

Epilepsy is one of the world's oldest recognised disorders, first described by Hippocrates in the 5th century BC.[1] At present, around 50 million people worldwide have active epilepsy with continuing seizures that need treatment, and 30% of patients are drug refractory.[2] Nearly 90% of epilepsy cases are in low-income countries, and in India, for example, the total cost for an estimated 5 million cases of epilepsy has been shown to be equivalent to 0·5% of the gross national product.[2] Europe has been estimated to have 6 million patients with active epilepsy, and the annual European health costs associated with epilepsy are over €20 billion.[3] In addition to the cost, the social burden associated with the disease and the two-to-three-times increased risk of death mean that there is an urgent need to find ways to prevent the disease in individuals at risk.

Currently, the most efficient ways to prevent epileptogenesis are genetic counselling or prevention of primary epileptogenic injury, for example, by wearing a helmet while riding a bike. In 2011, the prevention of epilepsy in patients at risk after acquired injury remains an unmet medical need worldwide. However, there have been recent developments in the modelling of epileptogenesis after genetic or acquired conditions in mice and rats, which increase the clinical relevance of these models. By use of these animal models, large-scale molecular profiling studies have provided clues to the mechanisms that can contribute to formation of seizure-generating (ictogenic) neuronal circuits. Finally, several laboratories have made attempts to target these mechanisms in clinically relevant experimental study designs, and some of these have shown favourable antiepileptogenic effects. We review and discuss these studies to identify unsolved problems needing attention before the current proof-of-principle studies are taken to preclinical antiepileptogenesis trials or even to the clinic.

Definitions

The term epileptogenesis is most often associated with the development of symptomatic (acquired) epilepsy that presents with an identifiable structural lesion in the brain.[4] Some studies suggest that epileptogenesis also occurs in genetic epilepsies, in which it is regulated, for example, by developmental programming of gene expression leading to abnormal circuitry during maturation.[5]

Currently, the terms epileptogenesis or latency period are used synonymously as operational terms to refer to a period that begins after the occurrence of insult (eg, traumatic brain injury [TBI] or stroke), or even during the insult (prolonged febrile seizure, status epilepticus [SE], or encephalitis), and ends at the time of the appearance of the first spontaneous seizure. Epileptogenesis refers to a dynamic process that progressively alters neuronal excitability, establishes critical interconnections, and perhaps requires intricate structural changes before the first spontaneous seizure occurs.[6] These changes can include neurodegeneration, neurogenesis, gliosis, axonal damage or sprouting, dendritic plasticity, blood–brain barrier (BBB) damage, recruitment of inflammatory cells into brain tissue, reorganisation of the extracellular matrix, and reorganisation of the molecular architecture of individual neuronal cells.[7]

Importantly, recent experimental and patient data suggest that molecular and cellular changes triggered by an epileptogenic insult can continue to progress after the epilepsy diagnosis, even though they might qualitatively and quantitatively differ at various phases of the epileptic process.[8,9] These neurobiological data raise the question of whether the term "epileptogenesis" should be extended to also include disease progression.[10] Thus, not only the prevention or delay of epilepsy but also seizure modification (less frequent or shorter seizures, milder seizure type, change from drug-resistant to drug-responsive) and even cure would be considered to be clinically relevant endpoints for antiepiletogenesis studies. Consequently, the window

for any search for treatment targets and for the initiation of antiepileptogenic treatments would extend beyond the latency phase to also cover the epilepsy phase (figure). Moreover, because epilepsy can link to several comorbidities such as memory or emotional impairment, comorbidity modification is one aspect that could be monitored in antiepileptogenesis studies.

In line with emerging neurobiological data, we use the term epileptogenesis to cover both the latency phase and the epilepsy phase, and we discuss the implications for target identification and treatment.

Identification of molecular mechanisms

If we consider epileptogenesis to be the result of circuitry reorganisation that can occur either at the synaptic or network level, a critical question is: what molecular pathways are involved in epileptogenic plasticity and how can we identify them? Because they are likely to be multiple and diverse, what reasoning should be used to select the candidate mechanism to be tested in vivo in proof-of-principle experiments?

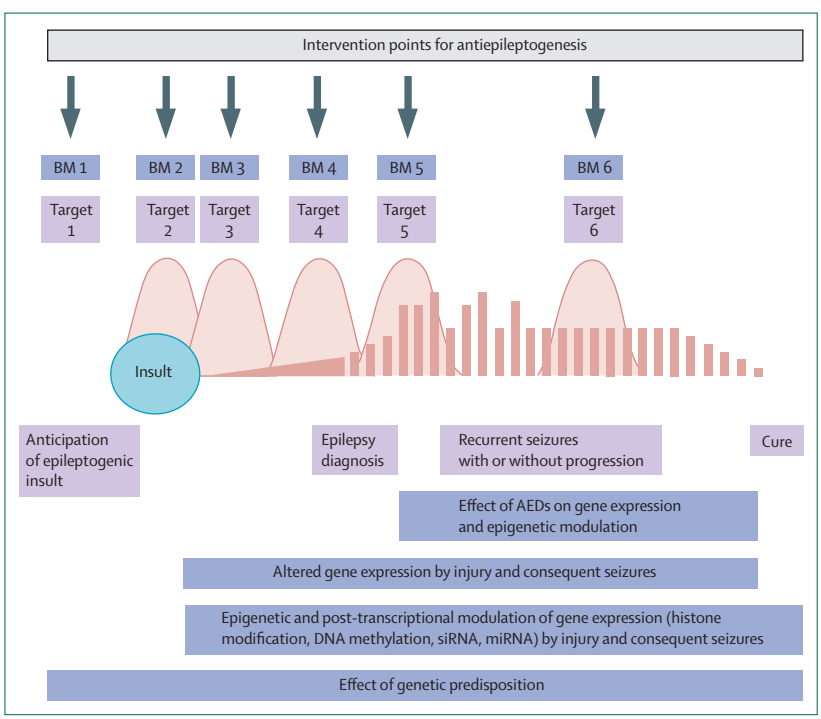

Figure: **Mechanisms and intervention points during epileptogenesis**
Epileptogenesis includes both the latency period between the insult and occurrence of seizures, and the progression of epilepsy. In an optimum case, treatment results in cure associated with the reversal of epileptogenic pathological changes. The natural course of epileptogenesis and the screening of mechanisms of epileptogenesis can be influenced by genetic predisposition, epigenetic mechanisms, and the use of AEDs. Data available (table 1) suggest that injury-induced gene expression depends on the time of sampling. Additionally, different patterns of gene expression can be observed, including genes constantly regulated following insult and those regulated only in specific time windows, which results in dynamic changes in the transcriptome over time. These data suggest that the target for antiepileptogenesis can vary over time. Moreover, polytherapy might be favoured over monotherapy. Similarly, the expression of a biomarker might vary at the time of investigation. Arrows indicate the potential timepoints for therapeutic interventions. Favourable effects have been found when treatments have been given either at early or later phases of the latency period, and even at the time of established epilepsy (table 1). Pretreatment could be a clinically relevant intervention point, for example, before surgical interventions that carry a risk of brain ischaemia or haemorrhage. Status epilepticus or encephalitis are conditions in which antiepileptogenic treatment can be started during the insult (ie, as co-administration with AEDs; insult-modifying treatment). BM=biomarker. AEDs=antiepileptic drugs. siRNA=small interfering RNA. miRNA=microRNA.

Transcriptomics

The introduction of methods to analyse gene expression at the whole transcriptome level in the mid-1990s raised expectations for the prompt discovery of molecular mechanisms of epileptogenesis, which would allow researchers to single out targets for antiepileptogenic therapies (table 1).[11–21] This hope has not yet been fulfilled. Only a few studies have been designed to specifically study the latency period or time period after the occurrence of the first seizures (table 1). Furthermore, the analysis of transcriptomic data to identify common epileptogenic mechanisms in different preparations is a challenge. This relates to use of different array platforms, normalisation algorithms, or cutoff points for selecting regulated genes, use of different animal species and strains,[22] analysis of different brain structures,[12,15,22] use of variable insults to trigger epileptogenesis, selection of timepoints for tissue sampling after the insult, and characterisation of epilepsy phenotype at the time of sampling.[15,17] Consequently, when we compared the lists of genes regulated during epileptogenesis, only 46 (7·4%) of 624 regulated genes were found to have abnormal regulation in more than one study. Such genes with a known function are summarised in table 2. 17 (37%) of these 46 genes were regulated in both SE and TBI models, indicating similarity in molecular events during epileptogenesis between different conditions.

Only a few reports have studied changes in the transcriptome throughout epileptogenesis from early after the insult to the chronic phase.[11,12,15,17,18,23] Individual genes show different expression profiles. Some genes are regulated throughout the latent phase and also after epilepsy diagnosis, whereas others are only transiently regulated. We can also observe waves of orchestrated gene expression, because clusters of genes show similar patterns of expression changes over time. These observations might be relevant for new therapeutic strategies. First, the timing could be a crucial factor for a successful intervention if abnormalities had to be targeted at the time of occurrence. Second, because some types of molecular dysfunction that are present in the latent period persist into the chronic phase, it might be reasonable to extend antiepileptogenic interventions beyond the time of epilepsy diagnosis. In the latter scenario, a notable factor is that many antiepileptic drugs (AEDs; eg, levetiracetam, phenytoin, lamotrigine, valproate), which would be administered in parallel with antiepileptogenic treatments, can also modify gene expression.[24,25] Finally, because some regulated genes can contribute to post-insult recovery that occurs in parallel with epileptogenesis (eg, after TBI), it is important not to sacrifice their beneficial effects while preventing epileptogenesis.

The next question is whether bioinformatics tools have helped the analysis. Although over the past few years the accessibility, quality, and user-friendliness of data mining tools have improved, allowing more in-depth and sophisticated interpretation of microarray data, the basic knowledge about the proteins encoded by genes affected by epileptogenesis is often lacking. It is not surprising that microarray data have triggered further studies on the identified proteins or pathways. Only after gaining additional data on their function in the normal and diseased brain (including analysis of human tissue from epilepsy surgery) can their involvement in epileptogenesis or epilepsy be tested.[26–36] Unfortunately, this is a very laborious path, and relatively few of the leads obtained from arrays have been systematically followed. Examples include studies on the role of cystatin C (*CST3*),[26,31,36] urokinase-type plasminogen activator (*PLAU*),[32,33] secreted phosphoprotein 1 (*SPP1*; formerly osteopontin),[37] tweety homolog 1 (*TTYH1*),[30] sodium channel type 7 subunit A (*SCN7A*),[34] transforming growth factor β (*TGFB*) signalling,[27] prostaglandin G/H synthase 2 (*PTGS2*; formerly cyclo-oxygenase 2),[38,39] ferritin (*FTH* or *FTL*),[40] complement activation,[28] and proteolysis[35] in epileptogenesis. None of the genes identified has yet led

For **DAVID Bioinformatic Resources version 6.7** see http://david.abcc.ncifcrf.gov

	Status epilepticus (chemically induced)				Status epilepticus (induced by electrical stimulation)			Traumatic brain injury	
	Okamoto et al[11]	Becker et al[12]	Elliott et al[13]	Lauren et al[14]*	Gorter et al[15]	Hendriksen et al[16]	Lukasiuk et al[17]*	Kobori et al[18]*	Crawford et al[19]*
Species	Rat	Rat	Rat	Juvenile rats	Rat	Rat	Rat	Mouse	Mice overexpressing APOE4
Induction method/drug	Pilocarpine	Pilocarpine	Pilocarpine	Kainic acid	Angular bundle stimulation	Angular bundle stimulation	Amygdala stimulation	Controlled cortical impact	Controlled cortical impact
Video-EEG monitoring	Yes	No	No	No	Yes	Yes	Yes	No	No
Gene expression platform†	CodeLink	Affymetrix	Affymetrix	Illumina	Affymetrix	SAGE	Research Genetics	Incyte Genomics	Affymetrix
Day of tissue sampling	7	14	14	7	7	8	14	14	28
Brain structure	Hippocampus	Dentate gyrus or CA1	Dentate gyrus	CA1	CA3 or entorhinal cortex	Hippocampus	Hippocampus or temporal lobe	Cerebral cortex	Hippocampus or cortex
Regulated genes (n)	328	50 in dentate gyrus, 400 in CA1	129	1592	1400 in CA3, 2240 in entorhinal cortex	79	13 in hippocampus, 24 in temporal lobe	10	281 in hippocampus, 152 in cortex
Immune response	Yes	No	Yes	No	Yes	No	No	Yes	Yes
Inflammatory response	Yes	No	No	Yes	Yes	No	No	No	Yes
Response to wounding	No	No	Yes	No	Yes	No	No	No	Yes
Regulation of cell death/ cell damage	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No
Signal transduction	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lipid metabolism	No	No	No	No	Yes	No	No	No	No
Protein transport and processing	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Regulation of transcription	No	No	No	Yes	Yes	Yes	No	Yes	No
Regulation of translation	No	No	No	Yes	Yes	Yes	No	No	Yes
Cell growth, proliferation, and differentiation	Yes	No	Yes	No	Yes	Yes	No	No	Yes
Cell motility	Yes	No	No	No	No	No	No	No	Yes
Energy metabolism	No	No	No	No	No	No	Yes	No	No
Synaptic transmission and plasticity	No	Yes	Yes	Yes	Yes	No	No	No	Yes
Structural plasticity	No	Yes	Yes	No	No	Yes	No	No	No
Neurotransmitter synthesis and secretion	No	Yes	No	No	Yes	No	No	No	Yes
Ion transport	No	No	No	No	Yes	No	No	No	Yes
Protein phosphorylation	No	No	No	No	Yes	No	No	No	No
Regulation of cytoskeleton	No	No	Yes	Yes	No	No	No	No	Yes

Functional gene classes are shown as provided by authors of original publications. EEG=electroencephalogram. CA1=cornu ammonis 1. CA3=cornu ammonis 3. SAGE=Serial Analysis of Gene Expression. DAVID=Database for Annotation, Visualization and Integrated Discovery. *Indicates studies on which we did additional analyses: gene identifiers or accession numbers were converted to official gene symbols and analysed by use of the Biological Function FAT annotation chart option of DAVID Bioinformatic Resources version 6.7.[20,21] †Note that platforms containing different gene sets were used in various studies: CodeLink (Applied Microarrays, Tempe, AZ); Affymetrix (Santa Clara, CA); Illumina (San Diego, CA); Research Genetics (Huntsville, AL); Incyte Genomics (Palo Alto, CA).

Table 1: **Gene functions most frequently regulated during epileptogenesis induced by status epilepticus or traumatic brain injury**

to rigorous testing of antiepileptogenic approaches in preclinical studies.

Serendipity

Interpretation of transcriptome alterations at the level of functional gene groups or signalling pathways seems more rewarding than focusing on individual genes when attempting to pinpoint epileptogenic mechanisms. This approach has highlighted gene groups with relatively unspecific functions, such as those regulating signal transduction or transcription, which can underlie any molecular process. Importantly, more specific functional gene groups that contribute to the generation of specific network alterations already linked to epileptogenesis have also been detected. These include inflammation, immune response, reaction to wounding, synaptic transmission and plasticity, ion transport, channel and receptor function, and neurotransmitter metabolism. To search for more specific targets, one can match the transcriptome data with search terms in literature databases.

Cell proliferation and plasticity

As one tries to match the "omics" data with the literature database and extract specific targets from the articles published in that category, there is evidence that within the "epileptogenesis and plasticity" category, neurotrophins show remarkable changes during epileptogenesis in different animal models, especially brain-derived neurotrophic factor (BDNF) and neurotrophic tyrosine kinase receptor type 2 (NTRK2). Their concentrations are altered in experimental and/or human epileptic tissue, and genetically modified NTRK2 regulates excitability in vivo in mice, whereas some studies suggest that a BDNF polymorphism might play a part in human epilepsy.[41] Recently, Paradiso and colleagues[42] tested the hypothesis that limiting tissue damage and enhancing repair by neurotrophins alleviates epileptogenesis. These investigators triggered SE with pilocarpine and 4 days after SE, rats received a unilateral hippocampal injection of a vector expressing fibroblast growth factor 2 (FGF-2) and BDNF. On the basis of 20-day video electroencephalogram (EEG) monitoring, there was no evidence that the treatment lowered the proportion of rats that developed epilepsy. However, a clear seizure-modifying effect was seen and FGF-2 and BDNF duotherapy reduced both the frequency and severity of spontaneous seizures. This was associated with a normalised pattern of neurogenesis as well as preserved dendritic inhibition of granule cells by surviving hilar somatostatin neurons.

Erythropoietin also has neurotrophic effects, in addition to its role in antiapoptotic, antioxidant, and anti-inflammatory signalling. Thus, even though erythropoietin itself has not been revealed as a target by molecular profiling on the basis of the data available to date, its functions cover several differentially regulated

	Genes (n)	Official gene symbol*
Cell–cell signalling	12	*C1QA*, *GABRD*†, *NPY*, *GRIA2*, *SLC6A1*, *SYT4*, *NPTX2*†, *APOE*, *GRIN2C*, *CAMK2G*, *GABRA5*, *GABARAP*
Ion transport	12	*KCNC2*, *GABRD*†, *NPY*, *GRIA2*, *SCN3B*, *GRIN2C*, *CAMK2G*, *SCN2A*, *GABRA5*, *CAMK2B*†, *CACNG2*, *KCNK1*
Synaptic transmission	10	*GABRD*†, *NPY*, *GRIA2*, *SLC6A1*, *SYT4*, *NPTX2*†, *APOE*, *GRIN2C*, *GABRA5*, *GABARAP*
Regulation of cell proliferation	9	*PTPN6*†, *PPP1R9B*, *PTGS2*, *APOE*, *GRN*†, *CLU*, *CD81*, *IL6R*†, *SPARC*†
Response to wounding	8	*C1QA*, *PTPN6*†, *C1QB*, *GRIN2C*, *CLU*, *IL6R*†, *CTSB*, *C1QC*
Immune response	8	*C1QA*, *C1QB*, *CLU*, *IL6R*†, *CTSS*, *C1QC*, *CD74*, *B2M*
Behaviour	7	*PTGS2*, *NPY*, *SLC6A1*, *S100B*†, *GABRA5*, *IL6R*†, *CALB1*
Regulation of apoptosis	7	*PTGS2*, *APOE*, *CLU*, *DNAJC5*†, *IL6R*†, *CTSB*, *CD74*
Leucocyte-mediated immunity	6	*C1QA*, *C1QB*, *CLU*, *IL6R*†, *C1QC*, *CD74*
Regulation of synaptic transmission	6	*PTGS2*, *GRIA2*, *SLC6A1*, *APOE*, *GRIN2C*, *CALB1*
Adaptive immune response	5	*C1QA*, *C1QB*, *CLU*, *C1QC*, *CD74*
Learning or memory	5	*PTGS2*, *SLC6A1*, *S100B*†, *GABRA5*, *CALB1*
Inflammatory response	5	*C1QA*, *C1QB*, *CLU*, *IL6R*†, *C1QC*
Cell proliferation	5	*RPS27*, *NPY*, *S100B*†, *CD81*, *CD74*
Regulation of phosphorylation	5	*PPP1R9B*, *APOE*, *CD81*, *IL6R*†, *CD74*
Complement activation	4	*C1QA*, *C1QB*, *CLU*, *C1QC*
Regulation of synaptic plasticity	4	*PTGS2*, *APOE*, *GRIN2C*, *CALB1*
Response to steroid hormone stimulus	4	*C1QB*, *PTGS2*, *SLC6A1*, *IL6R*†
Lipid transport	3	*NPC2*†, *APOE*, *CLU*
Response to oxidative stress	3	*PTGS2*, *APOE*, *CLU*

Genes that were regulated in at least two studies presented in table 1[11-19] were assigned to functional classes using the Biological Function FAT option of DAVID Bioinformatic Resources 6.7.[20,21] DAVID=Database for Annotation, Visualization and Integrated Discovery. *See HUGO Gene Nomenclature Committee website for full gene names. †Genes regulated by both status epilepticus and traumatic brain injury.

Table 2: **Genes belonging to different functional gene classes**

For the **HUGO Gene Nomenclature Committee website** see http://www.genenames.org/

gene classes revealed by transcriptomics.[43] Chu and co-workers[44] induced SE in rats with lithium-pilocarpine and administered erythropoietin starting immediately after SE cessation for 7 days. The proportion of rats that developed epilepsy in the treatment group was no different to that in the vehicle group. However, the seizure frequency and duration as assessed by video monitoring were reduced in the erythropoietin group compared with the vehicle group. This was associated with reductions in BBB damage, neurodegeneration, microglial activation, development of ectopic granule cells in the hilus, and gliosis.

Inflammation and immune response

For most of the other functional categories, it is difficult to extract a single specific target. For example, in the category of inflammation and immune response, various compounds that inhibit different inflammatory pathways have been used (table 3).[38,42,44–61] Lukasiuk and Sliwa[50] investigated the effect of tacrolimus on SE-induced epileptogenesis. Tacrolimus is an immunosuppressant that binds to intracellular immunophilins. The tacrolimus–immunophilin complex inhibits the activity of calcineurin, resulting in the inability of T cells to respond to activation by antigen-presenting cells. Consequently, no functional cytokine response occurs.[62] Tacrolimus was started 24 h after SE and continued for 2 weeks. On the basis of 4-week continuous video-EEG monitoring, no positive effects were observed on the animals that developed epilepsy, latency to the first seizure, seizure frequency, or seizure type.

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used in preclinical antiepileptogenesis trials on the basis of their ability to inhibit PTGS2. PTGS2 inhibition reduces activation of prostanoid pathways, resulting in reduced microglial activation, leucocyte infiltration, suppressed cytokine release and oxidative stress, and reduced neurodegeneration.[62] The first NSAID tested in epileptogenesis models was celecoxib. Jung and colleagues[51] induced SE with lithium-pilocarpine in adult rats and started celecoxib 1 day after SE, and then continued the treatment for 42 days. On the basis of video monitoring of seizures, the treatment did not reduce the proportion of rats that developed epilepsy. However, celecoxib treatment decreased the seizure frequency and duration. In addition, celecoxib reduced hippocampal neurodegeneration and microglial activation, and inhibited both the generation of ectopic granule cells in the hilus and new glia in CA1.

Parecoxib, another NSAID, belongs to the second generation of selective PTGS2 inhibitors. Polascheck and co-workers[52] administered parecoxib for 18 days after pilocarpine-induced SE. Several weeks after SE, rats underwent video-EEG monitoring to detect the occurrence of spontaneous seizures. No reductions in the occurrence of epilepsy or frequency or duration of seizures were observed. However, parecoxib slightly reduced the behavioural severity of seizures compared with vehicle alone.

The third NSAID that has been tested is SC58236, a selective inhibitor of PTGS2.[38] SE was triggered by electrical stimulation of the angular bundle and allowed to continue for 4 h. SC58236 treatment was then started and continued for 7 days. Animals underwent continuous video-EEG monitoring for up to 35 days after SE. SC58236 treatment did not delay the latency to the occurrence of spontaneous seizures or the proportion of rats that developed epilepsy. It did not affect seizure duration and had no effect on the severity of neurodegeneration, mossy-fibre sprouting, or microglial activation.

Inflammatory cell adhesion

Fabene and colleagues[53] showed that integrin α4/β1 and P-selectin glycoprotein ligand 1 are the mediators of leucocyte adhesion to endothelial cells in cerebral blood vessels after pilocarpine-induced SE. This was proposed to result in increased leucocyte extravasation, cerebral inflammatory response, leakage of the BBB, impaired K^+ buffering, and epileptogenesis. They hypothesised that preventing leucocyte adhesion by using an integrin-α4-specific monoclonal antibody (α4 MAb) after SE would prevent epileptogenesis. To address this question, they induced SE in C57BL/6 mice and administered α4 MAb starting at 1 h after SE. Treatment was continued every other day for 20 days. On the basis of video-EEG monitoring for 5–20 days after SE, the latency to the appearance of spontaneous seizures was similar in the α4 MAb and vehicle groups. Also, the duration of seizures was not altered by treatment. However, the seizure frequency as assessed during α4 MAb therapy was reduced from about 0·8 to 0·2 seizures per day. Importantly, mice treated with α4 MAb had less severe BBB damage at the acute phase (18–24 h after SE) and reduced chronic neurodegeneration (30 days after SE). In addition, their exploratory behaviour was better preserved than in the vehicle group. Thus, unlike other treatments targeted to alleviate inflammatory response, α4 MAb treatment had both seizure-modifying and comorbidity-modifying effects on epileptogenesis.

Epigenomics

There is some evidence that gene expression triggered by epileptogenic brain insults occurs in temporally coordinated waves. This has been proposed to be orchestrated by regulation of transcription by specific transcription factors. One such transcription factor is inducible cyclic AMP early repressor (ICER), which has been suggested to play a part in epileptogenesis because it suppresses kindling (repeated subthreshold stimulation culminating in the occurrence of generalised seizures).[63,64] Another candidate mechanism for the clustering of post-injury gene expression relates to the epigenetic regulation of transcription by alterations in DNA methylation or histone modifications (table 4).[65–78]

	Treatment	Model	Mechanism of action	Time of administration of treatment	Antiepileptogenesis						
					Prevention		Seizure modification				Cure
					Decrease in proportion of animals that develop epilepsy	Delay in onset	Decrease in frequency	Decrease in duration	Milder seizure type	Prevention of progressive increase in seizure frequency	Increase in proportion of animals that become seizure free
Zeng et al[45]	Rapamycin	Tsc1GFAP CKO mice	mTOR inhibition	Postnatal day 14 (~2 weeks before onset of seizures)	Yes	Yes	Yes	Yes	Yes	Yes	..
Zhou et al[46]	Rapamycin	Pten CKO mice	mTOR inhibition	Age 4–6 weeks (presymptomatic phase)	No	..	No	Yes	Yes	Yes	..
Ljungberg et al[47]	Rapamycin	Pten CKO mice	mTOR inhibition	Age 4–5 weeks	..	..	..	Yes	..	..	..
Zeng et al[48]	Rapamycin	Kainic-acid-induced SE in rats	mTOR inhibition	24 h post-SE for 6 days, then every other day for 6 weeks	..	..	Yes	..	..	Yes	..
Huang et al[49]	Rapamycin	Pilocarpine-induced SE in rats	mTOR inhibition	>10 weeks after SE when spontaneous seizures occur	..	..	Yes	Yes	Yes	Yes	No
Lukasiuk et al[50]	Tacrolimus	Electrical stimulation-induced SE in rats	Inhibition of T-cell response by binding to immunophilin	24 h post-SE for 2 weeks	No	No	No	..	No	..	..
Jung et al[51]	Celecoxib	Lithium-pilocarpine-induced SE in rats	COX-2 inhibition	1 day post-SE for 14 days	No	..	Yes	Yes	..	..	..
Polascheck et al[52]	Parecoxib	Pilocarpine-induced SE in rats	COX-2 inhibition	Immediately after interruption of SE (>90 min), continued for 17 days (twice daily)	No	..	No	No	Yes	..	..
Holtman et al[38]	SC58236	Electrical stimulation-induced SE in rats	COX-2 inhibition	4 h post-SE (orally) for 7 days	No	No	No	No	..	..	..
Fabene et al[53]	Integrin-α4-specific MAb	Pilocarpine-induced SE in mice	Targeting integrin α4 action	1 h after beginning of SE, every other day for 20 days	..	No	Yes	No	..	..	..
Chu et al[44]	Erythropoietin	Lithium-pilocarpine-induced SE in rats	Erythropoietin receptor binding	1 h after beginning of SE	No	..	Yes	Yes	..	..	..
Paradiso et al[42]	FGF-2 and BDNF gene therapy	Pilocarpine-induced SE in rats	FGF and NTRK2 receptor binding	4 days, post-SE	..	..	Yes	..	Yes	..	..
Yan et al[54]	Levetiracetam	Spontaneously epileptic rats	Binding to synaptic vesicle protein SV2A	Age 5–9 weeks (presymptomatic phase)	..	..	Yes	Yes	..	..	..
Blumenfeld et al[55]	Ethosuximide	WAG/Rij rats with spontaneous absence seizures	Inhibition of T-type Ca^{2+} channel	Postnatal day 21 and for up to 5 months of age	..	..	Yes	No	..	..	..
Pitkänen et al[56]	Atipamezole	Electrical stimulation-induced SE in rats	α2-adrenergic antagonist	1 week post-SE for 9 weeks via osmotic minipumps	No	..	Yes	No	No	..	..
Echegoyen et al[57]	Rimonabant	Lateral FPI-induced TBI in rats	CB1 cannabinoid receptor antagonist	2 min post-TBI	Prevention of reduction in seizure threshold	..	..	..	..	..	..

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	Treatment	Model	Mechanism of action	Time of administration of treatment	Antiepileptogenesis						
					Prevention		Seizure modification				Cure
					Decrease in proportion of animals that develop epilepsy	Delay in onset	Decrease in frequency	Decrease in duration	Milder seizure type	Prevention of progressive increase in seizure frequency	Increase in proportion of animals that become seizure free
(Continued from previous page)											
Dudek et al[58]	SR141716A	Kainic-acid-induced SE in rats	CB1 cannabinoid receptor antagonist	During first electrographic seizure	No	..	No	..	..	..	..
Chen et al[59]	SR141716A	Hyperthermia in postnatal day 16–18 rats	CB1 cannabinoid receptor antagonist	2 min after start of seizure induction	Prevention of reduction in seizure threshold	..	..	..	..	..	..
Chrzaszcz et al[60]	Minozac	Closed skull TBI (CD-1 mouse)	Reduction of proinflammatory cytokine production by activated glia	3 h and 6 h post-TBI	Reduced seizure susceptibility to ECS-induced seizure at 7 days, post-TBI	..	..	..	..	..	..
Brandt et al[61]	Bumetadine	Lithium-pilocarpine-induced SE in adult rats	NKCC1 inhibition	90 min after initiation of SE for 2 weeks	No	..	No	No	No	..	..

CKO=conditional knockout. mTOR=mammalian target of rapamycin (serine-threonine protein kinase). SE=status epilepticus. COX-2=cyclo-oxygenase 2. MAb=monoclonal antibody. FGF=fibroblast growth factor. BDNF=brain-derived neurotrophic factor. NTRK2=neurotrophic tyrosine kinase receptor 2. FPI=fluid-percussion injury. TBI=traumatic brain injury. ECS=electroconvulsive shock. NKCC1=sodium-potassium-chloride co-transporter. ..=no data/not applicable.

Table 3: **Studies of the effects of various treatments on epileptogenesis induced by status epilepticus or traumatic brain injury**

Interest in the role of histone acetylation as a possible therapeutic target for epileptogenesis was increased by the discovery that valproate, a widely used AED, is a histone deacetylase (HDAC) inhibitor.[79] In particular, HDAC inhibition explains why valproate blocks seizure-induced neurogenesis, which is one of the changes in the neuronal network triggered by various epileptogenic stimuli (ie, SE, TBI) as well as by single brief seizures.[80] Valproate also regulates the expression of several genes that regulate synaptic transmission.[81] Whether epigenetic mechanisms contribute to the antiepileptic effect of valproate is debatable because other HDAC inhibitors do not suppress seizures.[72,73] Another question is whether valproate would prevent acquired epileptogenesis after SE. So far, there is no evidence that valproate started during the latency period or after the initiation of spontaneous seizures would have any effect on the epileptogenic process if its effect on the severity of the epileptogenic insult itself (ie, SE) is excluded.[10,82] Another AED with known epigenetic properties is phenobarbital, although these effects have been described only in extraneuronal tissue.[83] As for valproate, there is no convincing evidence that phenobarbital would block epileptogenic circuitry reorganisation without affecting the insult itself. However, it is too early to draw any conclusion about the antiepileptogenic potential of epigenetic modulation, because only SE models with a very severe initial epileptogenic insult have been tested, and the study designs have not been tailored to address the epigenetic modulation.

From phenotype to genotype to target

Epilepsy is a common comorbidity in many neurological diseases caused by a wide range of genetic factors. One approach to reveal novel epileptogenic mechanisms is to understand why a mutation in a disease-causing gene is associated with an epilepsy phenotype in mice. This is particularly interesting if the mutated gene is not directly associated with the expression of ligand or voltage-gated ion channels that regulate neuronal excitability, as is seen in inherited epileptic channelopathies.

Probably the most convincing evidence to support the idea of searching for novel epileptogenic mechanisms by investigating diseases in which epilepsy is "just a comorbidity" comes from the study of tuberous sclerosis, which is caused by an inactivating mutation in either the *TSC1* or *TSC2* gene, which encode hamartin and tuberin, respectively. The generation of animals with conditional knockout of *Tsc1* in astrocytes resulted in disinhibition of the serine/threonine protein kinase mammalian target of rapamycin (mTOR) pathway, causing structural and behavioural abnormalities resembling tuberous sclerosis in human beings, including the development of spontaneous seizures. Administration of rapamycin, an mTOR inhibitor, before seizure occurrence reversed the hippocampal abnormalities (ie, pyramidal cell dispersion and astrogliosis). Moreover, epileptogenesis was suppressed. When treatment was started after the appearance of spontaneous seizures, a positive, albeit less dramatic, effect was still observed.[45] The results have

	Experimental model	Observation
DNA methylation		
Lundberg et al[65]	TBI (weight-drop model) in rats	Increased DNA methyltransferase-1 expression in reactive astrocytes at days 4 and 7
Zhang et al[66]	TBI (weight-drop model) in rats	Decreased DNA methylation in microglia/macrophages at days 1 and 2
Kobow et al[67]	Human temporal lobe epilepsy	Increased DNA methylation at reelin promotor
Histone methylation		
Gao et al[68]	TBI (CCI model) in immature rats	Decreased histone H3 methylation at 6 h, 24 h, and 72 h
Histone acetylation		
Gao et al[68]	TBI (CCI model) in immature rats	Decreased histone H3 acetylation at 6 h and 24 h
Zhang et al[69]	TBI (lateral fluid percussion injury model) in rats	Decreased histone H3 acetylation at 24 h; HDAC inhibition prevents decrease in H3 acetylation and reduces microglia inflammatory response after TBI
Dash et al[70]	TBI (CCI model) in mice	HDAC inhibition enhances learning and memory after TBI
Shein et al[71]	TBI (weight-drop model) in mice	Decreased histone H3 acetylation at 6 h and 24 h; HDAC inhibition diminishes decrease in H3 acetylation and neurodegeneration, and improves recovery
Dash et al[72]	TBI (CCI model) in rats	Valproate, but not SAHA, increases H3 and H4 acetylation, decreases neurodegeneration, and improves motor skills and cognitive functions after TBI
Hoffmann et al[73]	Intravenous pentylenetetrazole infusion	Valproate but not trichostatin A increases seizure threshold
Tsankova et al[74]	Electroconvulsive seizures in rats	Increased histone H4 acetylation at c-fos and BDNF promoters and decreased histone H4 acetylation after seizures
Huang et al[75]	Intraperitoneal pilocarpine-induced SE in rats	Decreased H4 acetylation at GluR2 promoter and increase at BDNF P2 promoter after SE
Sng et al[76]	Intraperitoneal kainate-induced SE in mice	Increased histone H4 acetylation at 0·5–6 h after SE
Rajan et al[77]	HDAC4 domain knockout mice	Mice have seizures
Histone phosphorylation		
Crosio et al[78]	Intraperitoneal pilocarpine or kainate-induced SE in mice	Increased histone H3 phosphorylation
Sng et al[76]	Intraperitoneal kainate-induced SE in mice	Increased histone H3 phosphorylation at 0·5 h after SE

TBI=traumatic brain injury. CCI=controlled cortical impact. SE=status epilepticus. HDAC=histone deacetylase. SAHA=suberoylanilide hydroxamic acid. BDNF=brain-derived neurotrophic factor. GluR2=glutamate receptor 2.

Table 4: **Studies of epigenetic modifications during epileptogenesis**

been extended to acquired epilepsy models by Zeng and colleagues,[48] who showed that administration of rapamycin at 24 h after kainate-induced SE leads to the development of milder epilepsy. Importantly, rapamycin had favourable effects even when started after established epilepsy. Huang and co-workers[49] administered rapamycin to rats that had spontaneous seizures after pilocarpine-induced SE. Rapamycin administration suppressed seizures, and the study also suggested that mossy-fibre sprouting was diminished. After cessation of rapamycin treatment, which itself has not been shown to have any anticonvulsant effect,[48,84,85] seizures were re-established. These studies show that, on the basis of the identification of the epileptogenic pathway and characterisation of its role in epilepsy-associated network reorganisation, one can indeed design treatments that modify the epileptogenic process both in genetic and acquired conditions, and even at different phases of the epileptogenic process.

Insight into novel epileptogenic mechanisms has also been revealed by investigating animal models of Alzheimer's disease and fragile-X syndrome,[86,87] in which epilepsy can be a comorbidity. The pathological proteins produced by mutated genes in these disease models do vary, and data are just emerging on their contribution to the generation of an epileptogenic network. Some studies suggest that, in murine models of Alzheimer's disease, oligomeric amyloid β might directly affect the voltage-gated or ligand-gated neuronal ion channels' modulation of neuronal excitability and axon potential firing.[88,89] Other data show that enzymes processing amyloid precursor protein could also use sodium-channel subunits as substrates, resulting in hyperexcitability.[90] Unfortunately, preclinical trials with compounds that reduce amyloid-β concentrations (ie, lithium, valproate, or γ-secretase inhibitors) have not reported the effects of chronic treatments on seizures.

In a fragile-X murine model with knockout of the *Fmr1* gene, seizure generation seemed to be related to a reduction in fragile-X mental retardation protein-mediated silencing of group I metabotropic glutamate receptor (mGluR) activation-induced dendritic mRNA. This led to the discovery that co-reduction in mGluR5 expression repaired most of the structural and functional abnormalities in *Fmr1* knockout mice, including dendritic spine density and susceptibility to audiogenic seizures.[91,92] Whether the use of an mGluR5 antagonist prevents the development of the epileptogenic network/synaptic reorganisation in patients with fragile-X syndrome remains to be explored.

The use of genetic information from patients with neurological diseases with epilepsy as a comorbidity is an exciting platform to reveal novel epileptogenic mechanisms. These data show that in addition to the occurrence of diverse network alterations, for example after SE or TBI, more localised changes in dendritic spines or the axon initial segment can also be used to locate the epileptogenic microenvironment.[87,93] Fortunately, whether the drug targets revealed by these studies have an effect beyond the specific syndrome can be tested.

Chemistry–biology interphase target-independent discovery

Minozac (derived from inactive aminopyridazine) was discovered by using a molecular target-independent discovery paradigm.[94] The goal was to find a small molecule that suppressed the increased production of proinflammatory cytokines in glial cultures using disease-relevant endpoints rather than designing a compound targeting a specific molecular pathway. This was combined with hierarchical biological screens for oral bioavailability, toxicity, brain penetrance, and stability of candidate molecules before testing their efficacy in animal models of brain disorders. Recently, Chrzaszcz and colleagues[60] used the closed-skull midline impact model of TBI in mice and administered Minozac at 3 h or 6 h after injury. 1 week after TBI, Minozac-treated mice showed less susceptibility to electroconvulsive shock-induced seizures than did sham-operated mice (table 3). Whether Minozac treatment prevented the long-term increase in seizure susceptibility and occurrence of late seizures remains to be explored. Whether the chemistry–biology interface would provide a faster throughput approach for antiepileptogenesis drug discovery than hypothesis-driven "omics" approaches also remains to be seen.

AEDs as antiepileptogenic treatments

The first antiepileptogenesis trial in human beings was done more than 60 years ago.[95] It attempted to prevent epileptogenesis after TBI using phenytoin. Several other AEDs, including phenobarbital, carbamazepine, and valproate in monotherapy or polytherapy, as well as non-AEDs such as magnesium sulphate and glucocorticoids, have been tested since then. These studies have failed to provide evidence that the use of AEDs (or other compounds) during epileptogenesis would have favourable antiepileptogenic effects in patients.[96,97]

The analysis of data from experimental studies using AEDs as candidate antiepileptogenic agents is challenging.[98] This relates to the use of SE as an epileptogenic insult. Many studies have now shown that the shortening of SE by AEDs favourably modifies the epileptogenic process.[99,100] Therefore, unless the effect of AEDs on the duration and severity of SE is carefully controlled and quantified, it is difficult to determine whether the few positive effects on latency, seizure frequency, or seizure duration were related to the initial insult alleviation (ie, reduction in the severity and duration of SE itself by AEDs) rather than related to a true antiepileptogenic effect. Consequently, even if the most recent data on the effects of AEDs on the epileptogenic process are considered,[61,82,101] there is no evidence that the use of AEDs would be antiepileptogenic in adult rodents.

However, some recent data suggest that levetiracetam and ethosuximide could modify the epileptogenic process in immature animals with genetic predisposition to epilepsy, if the treatment is started before the expression of an epilepsy phenotype. Spontaneously epileptic rats (*zi/zi, tm/tm* double mutant) develop air-puff-induced tonic convulsions at approximately 8 weeks of age and absence seizures by about 12 weeks. Yan and colleagues[54] administered levetiracetam during weeks 5–9, before the occurrence of seizures. They found that the frequency and duration of air-puff-induced tonic seizures was reduced in the levetiracetam group compared with vehicle-treated rats. Also, the number and duration of electrographically recorded absence seizures was reduced in the levetiracetam-treated rats.

WAG/Rij rats develop absence seizures at approximately 3 months of age. Blumenfeld and co-workers[55] started the administration of ethosuximide at postnatal day 21 in these rats. Rats in which ethosuximide was discontinued at the age of 5 months had reduced seizure frequency when assessed with long-term EEG at age 5–8 months. The duration of remaining seizures was not altered compared with the vehicle group. Unfortunately, they did not mention whether epilepsy had been completely prevented in any of the rats. The investigators showed that abnormalities in the SCN1A and SCN8A sodium channels as well as in potassium/sodium hyperpolarisation-activated cyclic nucleotide-gated channel 1, as assessed by immunohistochemistry, were normalised in the ethosuximide group.

Proconvulsants

Many preclinical and clinical studies have shown that drugs designed to prevent epileptic seizures and suppress neuronal activity (ie, AEDs) do not prevent acquired epileptogenesis.[96,98] Recent data have provided surprising evidence that the administration of the proconvulsant drugs atipamezole or rimonabant could have favourable effects on antiepileptogenesis after epileptogenic brain insults, including SE and TBI.[56,57]

We induced SE with electrical stimulation of the amygdala and 1 week later started atipamezole treatment with subcutaneous osmotic minipumps for 9 weeks.[56] Atipamezole treatment had no effect on the proportion of rats that developed epilepsy. However, the seizure frequency was reduced from about 8·4 to 0·7 seizures per day. Atipamezole-treated rats also had milder hippocampal neurodegeneration and less intense mossy-fibre sprouting than did the vehicle group. This was the first study to show that SE-induced epileptogenesis can

be favourably modified by pharmacotherapy, with an experimental design in which the treatment effect on the severity of the epileptogenic insult was excluded and the assessment of efficacy was based on long-term video-EEG monitoring.

More recently, Echegoyen and colleagues[57] induced epileptogenesis by lateral fluid-percussion-induced TBI, and administered rimonabant as a single injection 2 min after injury. The threshold for kainate-induced seizures was assessed at 6 weeks after TBI. The reduction in latency to kainate-induced seizures was prevented by rimonabant. Also, the total time spent in seizures after kainate administration was reduced in the rimonabant group compared with the vehicle group. Importantly, no positive effect was found if rimonabant was administered 20 min after TBI. The same group also showed that a similarly favourable effect could be achieved in a hyperthermia model of prolonged seizures in immature rats if the treatment was initiated 2 min after the start of seizure induction.[59] Dudek and colleagues[58] extended the studies on rimonabant to an SE model in adult animals. Interestingly, if rimonabant was given after the first electrographic seizure during the kainate-induced SE (ie, 1 min after SE onset), it had no effect on the proportion of rats that developed epilepsy or seizure frequency when assessed during the first 10 weeks after SE.[58]

Even though the compounds seem to have different mechanisms of action (atipamezole is an α_2-noradrenergic antagonist and rimonabant is a cannabinoid receptor 1 antagonist), it remains to be found whether there is convergence in the molecular mechanisms or cellular location of the effects of these compounds. Furthermore, whether the effects are model specific remains to be addressed.

What should antiepileptogenic treatment look like?

Differences across conditions and patients

As mentioned earlier, there are some similarly regulated genes in different conditions (eg, SE and TBI) during epileptogenesis. However, even considering the bias related to the use of different array platforms or other methodological issues, most analyses of epileptogenesis in rodents suggest differences in the pattern of molecular changes as well as in the time course and severity of the cellular alterations between conditions, such as electrically or chemically induced SE or TBI.[102] Even the different SE models differ substantially. Moreover, in each condition there is substantial inter-animal variability. Experimental antiepileptogenesis studies have mostly used electrically or chemically induced SE as an epileptogenic trigger (table 3). In a few reports, TBI or a genetically abnormal mTOR pathway serves as an epileptogenic trigger. Beneficial effects have been achieved by administering rapamycin, α4 MAb, or FGF-2–BDNF combination gene therapy (table 3). Only rapamycin has shown efficacy in different conditions (ie, tuberous sclerosis, cortical dysplasia, and post-SE models).

None of the studies has taken into account the qualitatively or quantitatively different mechanisms of epileptogenesis between individuals at a given time, which is difficult because we lack reliable biomarkers to pinpoint the phase of epileptogenesis in individual animals. Moreover, these studies have typically been proof-of-principle studies, which have been done in a relatively small number of animals to show the efficacy in the whole animal group, without any attempts to power the study to make subgroup analyses. Therefore, there is a possibility of false-negative results.

Monotherapy versus polytherapy

As the molecular and cellular studies have shown, acquired epileptogenesis is regulated by multiple molecular pathways. One could hypothesise that modulating several pathways at the same time or sequentially would be a more beneficial strategy than any single-bullet strategy. An antiepileptogenic effect can be shown by using relatively specific treatments, such as rapamycin for targeting the mTOR pathway or a specific monoclonal antibody to integrin α4 (table 3). However, the blockage of epileptogenesis was not complete, and thus the polytherapy hypothesis remains viable. The closest approach to polytherapy was made in the FGF-2–BDNF duotherapy study, which resulted in multiple effects, including both neurogenesis and survival of interneurons. However, the antiepileptogenic effect was partial. Finally, both α4 MAb and FGF-2–BDNF gene therapy show efficacy in the systemic pilocarpine model, even though one is given systemically (α4 MAb) and one directly to the hippocampus (gene therapy). This is of particular interest as recent studies show that both the peripheral component of inflammation (leucocyte stimulation) and the central cholinergic effect contribute to mechanisms that trigger SE after pilocarpine administration. Currently, no preclinical experiments have investigated whether a combination of different approaches is more favourable than any of the treatments alone.

When to start and how long to continue

As in patients, the progression of the epileptogenic process varies between the conditions and even between different animals with a similar epileptogenic trigger. In addition, the altered gene expression progresses in waves. Should this affect the timing of the treatment approach (figure)? Table 3 summarises the time of initiation of candidate antiepileptogenic therapies in experimental models. In all cases with a beneficial effect, the treatment was initiated within 7 days after the insult, suggesting that the therapeutic time window can be several days rather than minutes or hours, at least in SE models. In most of the studies, the time window was not specifically investigated. One exception was a study in a TBI model, in which the administration of the cannabinoid antagonist rimonabant prevented the lowering of seizure susceptibility only if it was given at 2 min, but not at

20 min, after TBI. No similar effect was found in an SE model.[58] Whether this indicates a true difference in the therapeutic time window for antiepileptogenesis between TBI and SE models remains to be studied. Furthermore, the duration of treatment has varied from a single administration to up to 9 weeks. For clinical trials, the extent of the therapeutic window is a crucial issue, as is the question of how specific is the window for each treatment, condition, and patient.

Conclusions

The molecular and cellular data on processes that underlie epileptogenesis suggest a wide spectrum of treatment targets. Therefore, is it even realistic to believe that the modulation of one target pathway would be antiepileptogenic, unless treating specific syndromes such as tuberous sclerosis? Should we focus on target selectivity versus pathophysiological process selectivity in multifactorial disorders like post-SE or post-TBI epileptogenesis? Do "omics" provide a category of biological mechanisms that can be set up as endpoints for biological screens of selective molecular chemotypes?

In addition, how can we cross over from a proof-of-principle trial to the preclinical testing of candidate antiepileptogenic treatments? Many components of the infrastructure for preclinical testing are already available. For example, we have a wide range of clinically relevant models and many laboratories have long-term video-EEG monitoring units that can provide the opportunity for more representative and reliable data acquisition. However, several challenges remain to be faced before translating the preclinical data to the clinic and some of the problems are similar to those discussed for stroke and amyotrophic lateral sclerosis.[103,104] For example, is the prevention of the lowering of seizure threshold a valid outcome measure in models, whereby only a low proportion of animals develop spontaneous seizures? Should the favourable effect be shown in more than one model to represent the different conditions? Should we aim to identify a silver-bullet therapy for large patient populations with heterogeneous epileptogenic triggers, or accept the possibility of a need for personalised treatments? Which preclinical outcome measures show the strongest indications to move to the clinic, and eventually, to labelling a compound as antiepileptogenic? Are the effects on comorbidities, such as alleviation of memory and behavioural abnormalities, an extra bonus for judging the clinical value of the treatment? What kind of adverse events can be tolerated and for how long during antiepileptogenic therapy? Finally, are the markers for treatment effects sensitive enough to highlight the full therapeutic potential of treatments and to avoid false-negative results?

Problems related to the analysis of a large amount of EEG data and lack of biomarkers indicating the stage of the epileptic process are examples of bottlenecks, which when solved will facilitate the movement from proof-of-principle studies to preclinical trials. Another challenge is the design of compounds with acceptable bioavailability to achieve stable brain concentrations, sometimes for a longer period of time. Forming preclinical consortia between the laboratories will make it realistic to do randomised and blinded preclinical trials with sufficient numbers of animals to show efficacy even within specific endophenotypes and, thus, reduce the likelihood of false-negative or false-positive data. Finally, overcoming the publication bias (ie, by reporting negative data) will save resources if repetition of unnecessary studies can be avoided.

Even though many questions remain, particularly related to translation of preclinical data to the clinic, the recent developments in modelling, target identification, and data from proof-of-principle antiepileptogenesis preclinical studies provide encouraging signals that the prevention of the complicated process of epileptogenesis is not an impossible mission, but can indeed be favourably modified.

Search strategy and selection criteria

We searched all PubMed articles published up to September, 2010, with terms "epileptogenesis" and "antiepileptogenesis". For transcriptomics in epileptogenesis, we did searches using the following terms: "microarrays and epileptogenesis", "transcriptome and epileptogenesis", "microarrays and traumatic brain injury", and "transcriptome and traumatic brain injury". Articles describing alterations in gene expression at timepoints longer than 4 days post-insult were selected. For epigenetics, a search for "epigenetic" and "epilepsy" was done. For preclinical treatment trials, only those studies in which the therapy was initiated after the epileptogenic insult were included. Articles were also identified through searches of the authors' own files. Only articles published in English were reviewed.

Contributors

Both authors contributed equally to the conception, design, literature search, and writing of this Review.

Conflicts of interest

We declare that we have no conflicts of interest.

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