Cell and Gene Therapies for Refractory Epilepsy

Detlev Boison*

RS Dow Neurobiology Laboratories, Legacy Research, Portland, OR 97232, USA

Abstract: Despite recent advances in the development of antiepileptic drugs, refractory epilepsy remains a major clinical problem affecting up to 35% of patients with partial epilepsy. Currently, there are few therapies that affect the underlying disease process. Therefore, novel therapeutic concepts are urgently needed. The recent development of experimental cell and gene therapies may offer several advantages compared to conventional systemic pharmacotherapy: (i) Specificity to underlying pathogenetic mechanisms by rational design; (ii) specificity to epileptogenic networks by focal delivery; and (iii) avoidance of side effects. A number of naturally occurring brain substances, such as GABA, adenosine, and the neuropeptides galanin and neuropeptide Y, may function as endogenous anticonvulsants and, in addition, may interact with the process of epileptogenesis. Unfortunately, the systemic application of these compounds is compromised by limited bioavailability, poor penetration of the blood-brain barrier, or the widespread systemic distribution of their respective receptors. Therefore, in recent years a new field of cell and gene-based neuropharmacology has emerged, aimed at either delivering endogenous anticonvulsant compounds by focal intracerebral transplantation of bioengineered cells (*ex vivo* gene therapy), or by inducing epileptogenic brain areas to produce these compounds *in situ* (*in vivo* gene therapy). In this review, recent efforts to develop GABA-, adenosine-, galanin-, and neuropeptide Y- based cell and gene therapies are discussed. The neurochemical rationales for using these compounds are discussed, the advantages of focal applications are highlighted and preclinical cell transplantation and gene therapy studies are critically evaluated. Although many promising data have been generated recently, potential problems, such as long-term therapeutic efficacy, long-term safety, and efficacy in clinically relevant animal models, need to be addressed before clinical applications can be contemplated.

Key Words: Epilepsy, epileptogenesis, cell therapy, gene therapy, GABA, adenosine, galanin, neuropeptide Y.

INTRODUCTION

Epilepsy is a common seizure disorder affecting around 60 million people worldwide, making it one of the most prevalent neurological disorders worldwide [68]. It is thought to be due to an imbalance between glutamate mediated excitatory and γ -aminobutyric acid (GABA) -ergic inhibitory networks, changes in ionotropic receptor function and composition, altered calcium-mediated second messenger activity, or altered endogenous anticonvulsant and neuroprotectant activities (Table 1). Thus, dysfunctional components of these building blocks of neuronal networks combine to trigger excessive discharges of neurons, which cause epileptic seizures and subsequent brain damage [72]. Apart from dysfunction of neuronal networks the role of astrocytes in epileptogenesis is becoming more and more recognized, thus adding an additional level of complexity to the underlying disease processes [17, 159]. Conventional pharmacotherapy of epilepsy has largely been postsynaptic focusing on ion channels involved in neurotransmission and the modulation of neurotransmitter systems (Table 1). However, despite optimal treatment with currently available antiepileptic drugs (AEDs), the development of tolerance [99] and pronounced side effects remain significant problems and seizures persist in about 35 percent of patients with complex partial epilepsy [115]. For these intractable cases, surgical resection offers a final option, but only if a discrete focus of seizure genesis can be identified and only if the surgical intervention does not interfere with essential brain functions. Even though epilepsy surgery has palliative effects in many patients, a less invasive therapy would constitute a major therapeutic advance for the treatment of refractory epilepsy. Apart from seizure control the prevention of epileptogenesis remains an important therapeutic goal, such as after posttraumatic epilepsy, a condition, which is highly prevalent among pediatric brain injury victims [151].

Recently, new insights into endogenous mechanisms of the brain to modulate and control neuronal excitability and epileptogenesis have opened new prospects for the development not only of novel anticonvulsant but also of antiepileptogenic therapies. Thus, apart from the well-known anticonvulsant effects of GABA, neuromodulators, such as adenosine, galanin, or neuropeptide Y, are

Table 1. Causes for Epilepsy and Options for Therapeutic Intervention

Causes for Epilepsy	Therapeutic Intervention*
Changes in ion channels: K^+ , Na^+ , Ca^{++}	Modulation of ion channels: carbamazepine, phenytoin, valproate, ethosuximide, lamotrigine, levetiracetam; Ineffective in pharmacoresistant epilepsy
Decreased inhibitory neurotransmission	Increase of inhibitory neurotransmission: barbiturates, benzodiazepines, felbamate, vigabatrin, tiagabine, gabapentin; Ineffective in pharmacoresistant epilepsy
Excessive excitatory neurotransmission	Decrease of excitatory neurotransmission: felbamate, topiramate; Ineffective in pharmacoresistant epilepsy
Deficiency in endogenous neurotransmitters and neuromodulators	Restoration/augmentation of neuromodulatory systems: GABA, adenosine, galanin, neuropeptide Y; Focal application by cell and gene therapies; Effective in pharmacoresistant epilepsy?

* some of the antiepileptic drugs listed here have more than one mode of action

thought to have potent anticonvulsive and antiepileptogenic effects. However, systemic application of these compounds is compromised by limited bioavailability, poor penetration of the blood-brain barrier, and/or the widespread systemic distribution of their respective receptors. This review focuses on novel strategies to circumvent these problems with cell and gene therapies to make therapeutic use of endogenous anticonvulsant principles (Fig. 1).

CELL THERAPIES FOR EPILEPSY - RATIONALE

Temporal lobe epilepsy, one of the most common forms of focal, or partial, epilepsy is also one of the most difficult forms of epilepsy to treat since seizure activity often progresses from focal to secondarily generalized, – and frequently pharmacoresistant – seizures. Thus, therapeutic alternatives are urgently needed and several focal treatment approaches for refractory epilepsy have been tested. These experiments demonstrated that focal drug delivery is generally well tolerated and devoid of major side effects [119]. Focal drug delivery can be achieved by devices such as synthetic slow-release polymers, pump systems, which can be coupled to

*Address correspondence to this author at Robert Stone Dow Neurobiology Laboratories, Legacy Research, 1225 NE 2nd Ave, Portland, OR 97232, USA; Tel: (503) 413-1754; Fax: (503) 413-5465; E-mail: dboison@downeurobiology.org

Fig. (1). Summary of cell- and gene- based neuropharmacological approaches for the treatment of pharmacoresistant focal epilepsy. The endogenous anticonvulsive compounds GABA, adenosine, galanin and neuropeptide Y can directly be delivered to an epileptogenic region making use of cell and gene therapies. Cell transplantation can affect the epileptic brain by cell replacement, network interactions or factor delivery. Potential platforms for cell transplantation are heterologous fetal cell grafts, encapsulated cells, embryonic stem cells, or – potentially autologous – adult stem cell grafts. Cell-based brain implants can be engineered to release antiepileptic agents (*ex vivo* gene therapy), or the expression of antiepileptic agents can be directed to an epileptogenic region using gene delivery by viral vectors (*in vivo* gene therapy).

integrated seizure prediction systems [153], or by cellular implants. Strategies that were followed focused either on the cell-mediated paracrine release of antiepileptic compounds, the replacement of lost neurons, the functional integration of cellular implants into preexisting neuronal networks, or various combinations thereof. One method of packaging and implanting cell-loaded devices into the CNS of recipients is by encapsulating cell suspensions in a polymer membrane prior to implantation [38]. Cells/tissue packaged within an encapsulating membrane obviate the need for immunosuppressive therapies in transplant recipients. In addition, the device output can be quantified prior to implantation, and following the removal of the implant. It has been demonstrated that encapsulated cells can survive for at least a year in graft recipients [169]. The ability to retrieve the devices with the presently used tubular configurations also confers an additional margin of safety over non-encapsulated cell implants. Encapsulated cell grafting is currently being developed for a wide range of applications including chronic pain control [170] and has already proceeded into clinical trials almost 10 years ago [1, 2]. However, the long-term survival of encapsulated cell grafts – a requirement for epilepsy patients, who are expected to live for decades after treatment – is still a major challenge requiring the design of improved biomaterials and matching cells.

In contrast, the direct transplantation and functional integration of therapeutic cells into the brain may offer the perspective for long-term survival of the graft. However, for the design of direct cell therapies inflammatory and immunological responses of the brain have to be considered: While lipopolysaccharide-induced brain inflammation strongly impaired basal hippocampal neurogenesis in rats [36], more recent data suggest that long-term impairment of dentate neurogenesis, as reported previously after kainic acid-induced status epilepticus, is not a general feature of chronic epilepsy [16]. Thus, a substantial proportion of mature granule cells found six months after status epilepticus were formed during the first two weeks after the insult despite chronic inflammation [16]. These findings imply that inflammatory responses of the epileptic hippocampus are not likely to compromise the efficacy of

cellular implants. On the other hand the brain is not as “immunologically privileged” as previously thought and immunological interactions have to be considered, when designing cell transplantation studies [7]. Despite these potential obstacles, clinical cell transplantation trials have been performed in Parkinson’s disease, Huntington’s disease, demyelinating diseases, stroke, and epilepsy, mostly with the aim to achieve cell replacement [7]. In most clinical trials, encompassing more than 1900 patients, the source material for cell transplantations was largely derived from human fetuses of allogeneic origin [124].

Cell transplantation for epilepsy can most easily be achieved by grafting of fetal tissue or cells. In one example, grafting of fetal hippocampal tissue with the aim to repair hippocampal networks in the intrahippocampal kainic acid model of TLE led to partial reversal of characteristic histopathological changes of hippocampal sclerosis, however epileptic seizures were not further investigated [131, 149]. These studies clearly demonstrated beneficial network effects of the transplanted cells and the capability for long-term survival. Although cell transplantation approaches using fetal tissue or cells have yielded important basic results, stem cells have in recent years reached most attention and publicity as an alternative cell source for regenerative medicine. Stem cells are capable to self-renew but at the same time can give rise to progenitor cells, which produce differentiated progeny [114]. Thus, stem cells can become an unlimited resource for the generation of specific neurons, and cell preparations can be quality-controlled and standardized prior to transplantation. Depending on their origin, embryonic and adult stem cells have to be distinguished.

Embryonic stem cells from both mouse and human sources are derived from the inner cell mass of a preimplantation embryo (blastocysts), are totipotent and have the capacity to give rise to all types of differentiated progeny from all three germ-layers. They can differentiate into tissue-specific progenitor cells, which remain pluripotent during adulthood. The directed differentiation of ES cells provides new perspectives for the generation of clinically relevant cell types, including neurons [120], and glia [21]. Following transplantation into the rodent CNS, ES cell-derived neural precursors integrated into the host tissue and yielded functional improvement [11, 79]. Since ES cells are amenable to a wide spectrum of genetic modifications, the ES cell technology holds great promise not only for cell replacement therapies, but also for cell-based drug delivery.

Adult stem cells have been isolated from several easily accessible tissue sources of adult mammals [84], including bone marrow [8, 78, 113], skeletal muscle [63, 75] and skin [47, 48, 160]. All of these stem cells preferentially generate differentiated cells of the same lineage as the tissue of origin. However, transplant studies indicate that adult stem cells can also generate cells of a different embryonic lineage *in vivo*. For example, neural stem cells were shown to generate blood [12] and skeletal muscle cells [57]. Similarly, transplanted bone marrow stem cells contributed to skeletal muscle [49, 63], liver [127], and generated cells producing neuronal markers in the brain [19, 78, 112, 177]. Adult rodent and human skin was shown to contain a new type of stem cell termed SKP with the potential to differentiate into neurons [47, 160, 161], both *in vitro* and *in vivo* [48]. Under neurogenic conditions, SKPs differentiated into β III tubulin positive, but GFAP negative cells, both *in vitro* and *in vivo*. SKP derived intrahippocampal implants survived for several weeks, both in normal as well as in kainic acid treated hippocampus [48]. These findings have therapeutic implications, since neural stem cells can promote functional recovery upon transplantation into the injured nervous system [24, 25, 77, 130]. Thus, adult stem cells provide an easily accessible source for autologous grafting of stem cell derived (neuronal) progeny.

It needs to be emphasized, however, that different neuropathological conditions usually affect more than one particular type of neuron and thus the phenotype of the descendants of transplanted

stem or progenitor cells are critical for the success of replacement therapies. For the successful development of novel cell therapies several issues have to be considered: (i) Transplantation of pluripotent cells may lead to the formation of tumors with tissue components resembling normal derivatives of all three germ layers (teratoma). Therefore, methods are needed to pre-differentiate stem cells *in vitro* and thus to direct them into specific differentiation pathways [21, 120]. (ii) Differentiation of pluripotent cells frequently results in the formation of neurons with more than one phenotype as well as in the formation of glial cells [134, 152]. (iii) The ontogeny of neuronal development can be directed or predetermined by exogenous factors, such as co-culture of differentiating cells with feeder cells, or the addition of growth and transcription factors [167]. (iv) Temporal coordination of neurotransmitter receptor expression is essential to maintain neuronal differentiation, as has been demonstrated in developmental pathways of embryonal carcinoma cells. Thus, the dysfunction of a single receptor species can change the fate of neuronal differentiation [162].

Taking these issues into consideration, stem cell-derived neurons have successfully been transplanted into rat models of chronic TLE [25, 143]. In the latter case ES cell derived neurons grafted into the hippocampus of epileptic rats developed a dense network within the host tissue and revealed intrinsic and synaptic properties characteristic of neurons [143]. These studies generally demonstrate that stem cell derived brain implants are versatile and promising tools for regenerative medicine with the potential for long-term survival and functional integration into pre-existing neuronal networks. These properties make stem cell derived neuronal brain implants particularly interesting candidates for antiepileptic cell therapies.

GENE THERAPIES - RATIONALE

In contrast to cell therapy approaches aimed at replacement, repair, and network interactions, the ability of gene transfer to provide sustained levels of a therapeutically active compound in a localized manner to the CNS [73], suggest that gene therapies are particularly suited for the treatment of chronic seizure disorders, for which an intractable focus of electrical activity can be identified. In contrast to seizure suppression, i.e. symptom suppression-treatment, gene therapy holds the potential to address the underlying disease mechanisms and therefore holds the potential for cure. Recently, the interaction of genetic and acquired factors has been implicated in the pathogenesis of some forms of epilepsy [9]. For example, a particular form of myoclonic epilepsy (Unverricht-Lundborg disease) results from a defective gene coding for the protease cystatin B [126]. Consequently, reconstitution of active cystatin B by gene therapy would be a rational approach to cure this form of epilepsy. Another example, in which a particular gene defect causes seizures, is deficiency of aspartoacylase (ASPA) in the spontaneously epileptic rat. Thus, delivery of the ASPA gene by viral vectors to the brain ameliorated tonic convulsions and other behavioral defects in two independent studies [111, 148]. However, a similar study using adeno associated virus-mediated overexpression of ASPA reported rescue of the seizure phenotype, but no effects on hypomyelination and motor defects, suggesting that this gene therapy approach reduced neuronal hyperexcitation, but not oligodendrocyte dysfunction [80].

Unfortunately, the most prevalent forms of epilepsy do not result from a single identifiable gene defect [9]. Nevertheless, gene therapy could be used to induce the local release of anticonvulsant or antiepileptogenic compounds with the aim to restore the balance between inhibition and excitation in the brain, to reinforce ion channels, or to support the function of endogenous neuromodulators. Another rational strategy could be to prevent seizure induced neuronal cell loss as has been pioneered in Robert Sapolsky's lab by herpes simplex virus induced overexpression of a glucose transporter gene [89]. While prevention of neuronal cell death might

prevent the progression of epileptogenesis, it has to be noted that, on the other hand, sparing neurons from death does not necessarily spare those neurons from dysfunction [33, 110]. Based on lessons learned from gene therapy of brain tumors [39], neurodegenerative disorders such as Parkinson's or Alzheimer's diseases [3, 88], or stroke [29, 62, 144, 174], several potential vector systems have been established, which can be used to deliver DNA to brain cells (Table 2). Herpes simplex virus (HSV) is neurotrophic and therefore a natural candidate for the delivery of genes into brain cells [86]. Although HSV-1 can persist life-long in neural tissue, during much of this time it persists in a latent form shutting down most of its genes [85]. HSV vectors have successfully been used to express a variety of neuroprotective compounds in stroke and neurotoxicity models [144]. In a recent approach the growth-compromised herpes simplex virus type 2 (HSV-2) vector DeltaRR carrying the HSV-2 antiapoptotic gene ICP10PK was administered intranasally in rats and mice. ICP10PK was expressed in the hippocampus of treated animals for at least 42 days and prevented kainic acid-induced seizures, neuronal loss, and inflammation [87]. Adenovirus vectors have the capability to infect both dividing and non-dividing cells [28]. However, adenovirus-mediated gene transfer is associated with risks of inflammation, immune responses, and demyelination [32, 43]. Adenovirus vectors have successfully been used to deliver recombinant DNA to the hippocampus in an animal model of head trauma [81]. Adeno-associated virus (AAV) is a nonpathogenic parvovirus, which causes human infection only in the presence of a helper virus, such as adenovirus, but can generate long-term gene expression *in vivo*, without the risks associated with adenovirus [22]. In particular, AAV transduction occurs with a high efficacy in neurons, where it is possible to obtain stable long-term gene expression with little associated toxicity, thus making this type of virus highly suited for CNS gene therapies [106]. The expression pattern and stability of gene expression of AAV vectors is dependent on promoter choice and the serotype of AAV vector used. The use of specific AAV chimeras to avoid immune responses in the human population, which are commonly directed against the AAV2 serotype [106], can further refine targeting strategies. Due to the versatility of AAV vectors, they have found widespread application in experimental models of Parkinson's disease, Huntington's disease, Canavan's disease, ALS, lysosomal storage diseases, and epilepsy (see below) [106]. Lentiviruses have two key advantages over other gene delivery systems. First, they can infect non-cycling and post-mitotic cells [116, 117]. Second, transgenes expressed from lentiviruses are not silenced during development and seem to be expressed for the longest durations when compared with other vector systems [117, 128]. Recently, vectors derived from human foamy virus (HFV), with their nonpathogenic nature and wide tissue tropism, have successfully been used as retroviral gene transfer vehicles to transduce cultured hippocampal neurons [95]. Although the viral vectors described above provide a highly efficient system for delivery and expression of foreign genes in the CNS, the inherent properties of the vectors used and the selection of suitable promoters can determine the ultimate outcome of any therapeutic intervention. Thus, in order to develop an effective therapy, it is not only necessary to select the right target gene, but also to consider the modality of gene transfer and expression. Finally, gene therapy approaches can be combined with cell therapy in a way that therapeutic cells (e.g. stem cells, or patient derived cells) are transduced *in vitro*, before being transplanted into the brain (i.e. *ex vivo* gene therapy). In the following chapters more specific approaches for cell and gene therapies are reviewed, which make use of specific therapeutic rationales to target epilepsy.

GABA - THERAPEUTIC RATIONALE

Numerous experimental and clinical observations suggest that epilepsy may result from an imbalance in excitatory and inhibitory neurotransmitter systems resulting in decreased net inhibition.

Table 2. Viral Gene Therapy Vectors for Epilepsy

Vector	Targets	Advantages	Disadvantages	Selected Examples
Herpes simplex virus (HSV)	Neurons Possibility for nasal administration	Life-long persistence	Gene inactivation during latency	Delivery of the antiapoptotic gene ICP10PK: prevention of KA-induced seizures, neuronal loss and inflammation [55]
Adenovirus	Dividing and non-dividing cells	No permanent alteration of host genome through episomal configuration	Risks of: - inflammation - immune responses - demyelination	Intracerebroventricular gene transfer of aspartoacylase ameliorated tonic convulsions in spontaneously epileptic rats [48]
Adeno-associated virus (AAV)	High efficacy in neurons	Non-pathogenic Lack of CNS toxicity Long-term gene expression	Size-limitations for therapeutic gene inserts	Therapeutic delivery of galanin and neuropeptide Y in several models of epilepsy (see text for details) [129-131, 133, 162]
Lentivirus	High efficacy in multiple cell types, preference for neurons	Lack of CNS toxicity Infection of non-cycling and post-mitotic cells Lack of gene silencing during development	Permanent integration/alteration of host genome	Lentiviral expression of GAD ameliorated seizures in genetically epilepsy prone rats [86]
Human foamy virus (HFV)	Wide tissue tropism	Non-pathogenic	Permanent integration/alteration of host genome	No <i>in vivo</i> studies to date; <i>In vitro</i> : efficient expression of GAD [65]

Thus, conventional antiepileptic drugs suppress seizures by increasing GABA_A receptor activity (e.g. barbiturates, benzodiazepines), or by inhibition of GABA breakdown (vigabatrin) or reuptake (tiagabine) [98]. Although potentially effective, GABA-based conventional pharmacotherapy may be compromised by the development of tolerance, pharmacoresistance, or unspecific side effects. Alternatively, seizure suppression should become feasible by transplanting cells that release inhibitory neurotransmitters into seizure modulating brain nuclei [10]. Experimental evidence suggests that the piriform cortex (PC; primary olfactory cortex) plays a critical role in the development and maintenance of limbic motor seizures [96, 109]. In this system, an imbalance between GABAergic inhibition and excitatory (glutamatergic) transmission is thought to promote epileptic seizure activity [66]. Due to its unique intrinsic circuitry and its numerous connections to and from other limbic brain areas, the PC is a prime candidate for the development and propagation of seizure activity as a consequence of slight imbalances in neurotransmission [96]. Thus, an ipsilateral reduction of GABAergic neurons was found in the central PC in response to kindling of the basolateral amygdala [91]. Consequently, vigabatrin, a blocker of the GABA degrading enzyme GABA transaminase, was shown to be antiepileptogenic and anticonvulsant when microinjected bilaterally into the central PC during or after amygdala kindling [146]. Likewise, focal application of the GABA_A receptor agonist muscimol into the central PC had anticonvulsant effects in kindled rats [147]. In conclusion, focal augmentation of GABA in the limbic system is an obvious strategy for seizure control.

GABA – THERAPEUTIC APPROACHES

Based on the rationale described above, several attempts were performed to transplant GABA releasing cells into discrete regions within the limbic system. Intravenous transplantation of neural stem cells into chronically epileptic rats differentiated into GABAergic interneurons within the damaged hippocampus thus decreasing neuronal excitability and suppressing recurrent epileptic seizures [24, 25]. Another strategy made use of neurotransmitter releasing cells with the aim to modulate network excitability. Thus, fetal striatal tissue containing GABAergic neurons reduced afterdischarge thresholds and seizures in amygdala kindled rats, when grafted into the substantia nigra [97]. However, seizure suppression was only transient. As further improvement in cell-mediated drug-delivery to the brain, cells can be engineered to release endogenous anticonvulsive substances. Thus, Thompson *et al.* engineered mouse

neurons to release GABA under the control of tetracycline dependent GAD₆₅ expression. Prior to kindling these cells were transplanted into the substantia nigra [156] or piriform cortex [58] of rats, but the transplantation had only weak effects on afterdischarge threshold and kindling rate. In a more recent study the same cells were injected into the substantia nigra in a status epilepticus model with spontaneous seizures [157]. In GABA-releasing graft recipients a robust anti-seizure effect was observed 7 to 10 days after transplantation. In another approach the same group transplanted the cells into the dentate gyrus of rats prior to kindling and documented an enhancement of seizure thresholds, a reduction of afterdischarges and a retardation in acquiring stage 5 kindled seizures [158]. Apart from the expression of the GABA-producing enzyme GAD in engineered cells for the prospective use in cell therapies as described above, GAD is highly suited for expression in viral gene therapy vectors. Thus, primary hippocampal neurons transduced with a GAD-expressing HFV-vector showed a significant increase in isoform-specific expression of GAD, synthesis of GABA and stimulation-evoked GABA release [95] and lentiviral expression of GAD ameliorated seizures in genetically epilepsy prone rats [40].

A recent approach made use of the differential expression of GABA_A receptor subunits in the hippocampus of patients and animals with temporal lobe epilepsy (TLE) [133]. Thus, in the adult rat pilocarpine model of TLE GABA_A receptor $\alpha 1$ subunits were reduced, while $\alpha 4$ subunits were increased in the dentate gyrus [132]. To address the question whether this altered subunit composition is a critical determinant of spontaneous seizure development Raol *et al.* introduced an $\alpha 1$ gene driven by an $\alpha 4$ promoter into an AAV-2 vector [132]. This condition-dependent promoter construct was upregulated after pilocarpine-induced status epilepticus, resulted in increased expression of the $\alpha 1$ subunit in the dentate gyrus, a three-fold increase in mean seizure-free time after SE and a 60% decrease in the number of rats developing epilepsy in the first 4 weeks after SE [132]. This work nicely exemplifies that the rational design of a gene therapy approach has the potential to prevent epileptogenesis. In summary, the examples presented here suggest that focal modulation of GABAergic neuromodulation by cell or gene therapies has the potential to suppress seizures and to prevent epileptogenesis. However, since GABA elevating drugs are available, which fail to be effective in pharmacoresistant epilepsy, it remains to be determined whether GABA enhancing cell or gene therapies would be effective in pharmacoresistant epilepsy.

ADENOSINE - THERAPEUTIC RATIONALE

Purines have long been recognized to modulate neurotransmission [23]. In particular, the purine ribonucleoside adenosine is a prototypic endogenous inhibitory neuromodulator, its predominant inhibitory action mediated primarily *via* adenosine A₁ receptors [53, 54], which belong to the family of G-protein coupled adenosine receptors (A₁, A_{2A}, A_{2B}, A₃) [52]. Thus, adenosine has extensively been characterized as an endogenous anticonvulsant of the brain [30, 34, 90] with potent capabilities for seizure control [14]. Apart from regulating seizure activity, adenosine exerts powerful neuro-protective effects [27, 31, 50, 141] and is involved in the control of neuropathic pain [108].

Adenosine formation is largely the result of a metabolic cascade initiated from the breakdown of ATP [168]. Thus, adenosine has the unique capability to adapt metabolic activity to energy supplies, and – consequently – has been termed a retaliatory metabolite [118]. Adenosine levels can be reduced by conversion to inosine by adenosine deaminase or by re-phosphorylation to AMP by adenosine kinase (ADK) [15]. Due to its low K_M for adenosine and extensive experimental evidence, astroglial [154] ADK is believed to be the key enzyme for the regulation of ambient adenosine [15]. Since intra- and extracellular pools of adenosine are in dynamic exchange by equilibrative [4] and concentrative [61] nucleoside transporters, extracellular concentrations of adenosine are largely regulated by the activity of intracellular ADK [15].

In healthy adult brain, endogenous adenosine concentrations are normally kept in the range of 25 – 250 nM [35] by a steady state expression of ADK, which is mostly restricted to astrocytes [60]. Thus, physiological adenosine concentrations are kept in the range of the affinity of the A₁ receptor for adenosine (around 70 nM) [35]. Consequently, small increases in ambient adenosine can augment inhibitory A₁ receptor mediated functions and adenosine receptor antagonists such as caffeine, have stimulatory effects on brain function [51]. In human patients as well as in animal models of epilepsy the density of A₁ receptors changes as a consequence of seizure activity and, depending on the convulsants used and depending on the duration or recurrence-frequency of seizures, both upregulation as well as downregulation of A₁ receptors have been described [121]. In general, it appears that acute seizure activity is associated with upregulation of A₁ receptors, whereas chronic seizure activity is usually accompanied by downregulation of A₁ receptors [59]. Furthermore, the loss of adenosine-mediated anticonvulsant mechanisms may cause status epilepticus [175]. Likewise, in the hippocampus of amygdala-kindled epileptic rats deficits of the adenosine system have recently been described and found to be due to a combined decrease in the density of A₁ receptors and to metabolic changes that led to lower basal levels of adenosine [135]. Indeed, overexpression of ADK in epileptic hippocampus has been associated with astrogliosis and seizures in a mouse model of kainic acid induced status epilepticus [60]. Likewise, transgenic overexpression of ADK led to spontaneous seizure activity and to increased sensitivity to ischemia [45, 129]. Similarly, mice lacking the A₁ receptor display increased seizure activity and cell loss after brain injury [46, 82], suggesting that ADK is an upstream regulator of the A₁ receptor mediated response. Thus, deficits in adenosinergic neuromodulation clearly contribute to epileptogenesis, seizure activity, and neuronal vulnerability. Consequently, therapeutic strategies, which augment the adenosine system after astrogliosis-induced upregulation of ADK, constitute a rational treatment approach.

Thus, A₁ receptor agonists and ADK inhibitors have been developed, which provide potent anticonvulsant and antinociceptive activity [76], and which are effective in a mouse model pharmacoresistant epilepsy [103, 108]. Among these agents ADK inhibitors show an improved therapeutic window compared to A₁ receptor agonists [76]. This may be due to the fact that systemic admini-

stration of ADK inhibitors can elicit a site- and event-specific enhancement of endogenous adenosine levels *in vivo* [20, 122]. However, the systemic application of ADK inhibitors might not be a therapeutic option due to liver toxicity of ADK deficiency [13] and the incidence of brain hemorrhage in rats and dogs after application of the ADK inhibitor GP-3966 [42].

ADENOSINE – THERAPEUTIC APPROACHES

In order to avoid systemic side effects focal cell-based augmentation of adenosine appears to be more promising. Thus, adenosine-based cell therapy approaches for partial epilepsy have been developed based on engineering BHK fibroblasts and C₂C₁₂ myoblasts to lack ADK. The engineered cells were demonstrated to release adenosine and were subsequently encapsulated into semipermeable polymer membranes to selectively study the paracrine effects of adenosine and to avoid immune rejection. Adenosine releasing intraventricular brain implants provided protection from convulsive seizures and from epileptiform electric afterdischarges in rats kindled in the hippocampus, a model of TLE [65, 74]. Thus, the focal delivery of adenosine, as opposed to the systemic application of an adenosine A₁ receptor agonist, did not cause sedation [65]. The anticonvulsant effect lasted up to 8 weeks and corresponded to the life expectancy of the encapsulated cells. The effect was adenosine-mediated since seizure suppression was transiently reversed after application of an A₁ receptor selective antagonist. Thus, paracrine focal adenosine delivery by cell grafts is a promising strategy to control seizures without accompanying sedative side effects.

Based on the anticonvulsant properties of focally released adenosine and on the long-term survival potential of stem cell derived brain implants, adenosine releasing stem cells may constitute a novel tool for the treatment of epilepsy. In contrast to encapsulated cell grafts, which provide therapeutic effects exclusively by paracrine action, direct stem cell derived brain implants may combine paracrine effects with network interactions [143]. With the aim to develop a stem cell-based delivery system for adenosine, both alleles of ADK were disrupted by homologous recombination in ES cells [44]. To avoid teratoma formation after transplantation, a protocol has been developed to differentiate ES cells into pure populations of glial precursor cells [21]. Accordingly, *Adk*^{-/-} ES cells were subjected to a glial differentiation protocol and, as a result, gave rise to proliferating glial precursors, which were further differentiated into mature adenosine releasing glia cells. Thus, a lack of ADK does not compromise the glial differentiation potential of ES cells [44]. Since adenosine can readily be released from *Adk*^{-/-} cells into the surrounding culture medium *in vitro* or into host tissue *in vivo*, it is unlikely to accumulate within the cells. *Adk*^{-/-} ES cell derived neural and glial progenitor cells potentially suppressed ischemic brain damage, when transplanted into mouse brain one week prior to middle cerebral artery occlusion, thus demonstrating the *in vivo* functionality of the cells [129].

An attempt was made to investigate the potential of differentiated *Adk*^{-/-} ES cell progeny for seizure suppression by paracrine adenosine release. To isolate paracrine effects of stem cell-derived implants from effects caused by network integration, ES cell-derived embryoid bodies and glial precursor cells were encapsulated and grafted into the lateral brain ventricle of kindled rats. While seizure activity in kindled rats with wild-type implants remained unaltered, rats with adenosine-releasing *Adk*^{-/-} ES cell-derived implants displayed transient protection from convulsive seizures and a profound reduction of afterdischarge activity in EEG-recordings [64]. Long-term seizure suppression was precluded by limited viability of the encapsulated cells. Thus, proof has been established that *Adk*^{-/-} ES cell-derived brain implants can suppress seizure activity by a paracrine mode of action. More recently, the same cells, after induction of neuronal differentiation and direct intrahippocampal injection, were shown (*i*) to integrate and to sur-

vive for at least 26 days within the hippocampus of rats, (ii) to express a marker indicating neuronal differentiation (NeuN), and (iii) to suppress kindling epileptogenesis [92]. Suppression of kindling epileptogenesis by ADK-deficient ES cell-derived intrahippocampal implants was more effective than suppression of kindling epileptogenesis by paracrine adenosine release from fibroblast-based intrahippocampal implants, indicating that ES cell-derived intrahippocampal implants may have the added advantage of providing beneficial trophic or network-mediated effects [92].

GALANIN – THERAPEUTIC RATIONALE

Another group of potential targets for cell and gene therapies includes a number of neuroactive peptides with anticonvulsant activity *in vivo*. Among those galanin and neuropeptide Y (see below) are the most thoroughly studied. The neuropeptide galanin is highly inducible and shows distinct up- or down-regulation after pathological disruption of the nervous system. Thus, galanin expression is increased after peripheral nerve injury, while seizure activity depletes galanin in the hippocampus. Galanin acts predominantly as an inhibitory, hyperpolarizing neuromodulator. Two types of galanin receptors [galanin receptor type 1 (GalR1) and galanin receptor type 2 (GalR2)] contribute to the inhibition of epileptic activity [102]. The anticonvulsant effects of galanin are well established and have best been described in models of limbic epilepsy [83, 102-105, 107, 145]. Mechanistically, the suppression of seizures by galanin is due to the opening of G protein-activated or ATP-sensitive potassium channels and ultimately pre-synaptic inhibition of glutamatergic transmission [101].

GALANIN – THERAPEUTIC APPROACHES

The only approaches so far making therapeutic use of galanin for the treatment of epilepsy have focused on the AAV-mediated expression and secretion of galanin. Initially, the inclusion of the fibronectin secretory signal sequence (FIB) in an AAV vector caused significant gene product secretion *in vitro*. More importantly, the combination of this secretory signal with the coding sequence for the active galanin peptide (AAV-FIB) significantly attenuated *in vivo* focal seizure sensitivity, even with different promoters, and prevented kainic acid-induced hilar cell death [67]. In a different approach to study the effect of vector-mediated galanin over-expression on seizures, rats were administered kainic acid intrahippocampally 2.5 months following infusion of a galanin expressing AAV vector (AAV-GAL) or corresponding empty control vector (AAV-Empty). In this study AAV-GAL-injected rats showed a decreased number of seizure episodes and a decrease in total seizure time compared to AAV-Empty rats, despite similar latencies to the development of the first EEG seizure and similar levels of neuronal damage in the CA3 region in both groups [93]. These data demonstrated that recombinant AAV mediates strong and stable over-expression of galanin when injected into the rat hippocampus resulting in a significant anticonvulsive effect. In the most recent study, bilateral infusion of an AAV-FIB galanin expression vector into the rat piriform cortex significantly attenuated kainic acid-induced seizures [107]. In these experiments 11 out of 12 rats exhibited no limbic seizures. In parallel to seizure suppression, this gene therapy vector prevented electrographic seizure activity. In contrast, bilateral infusion of a control vector did not alter the behavioral or electrographic seizure activity [107]. To assess whether prior seizure exposure could influence the activity of the vector, an additional group of rats received daily electrical stimulation of the piriform cortex until three consecutive stage 5 seizures were elicited. After completion of kindling the AAV-FIB galanin vector or a respective control vector were infused adjacent to the stimulating electrode. One week after the infusion of the galanin expression vector the animals exhibited a significant increase in the stimulation current necessary to evoke limbic seizure activity, while the control vector had no effect [107].

These experiments suggest that seizure suppression mediated by galanin expression in the hippocampus by viral gene therapy vectors may lead to novel therapeutic strategies for the treatment and management of intractable seizures with focal onset. Although technically feasible and scientifically justified, cell therapies for the local secretion of galanin have not yet been accomplished.

NEUROPEPTIDE Y – THERAPEUTIC RATIONALE

Since its discovery more than two decades ago [155], neuropeptide Y (NPY) has been characterized as a modulator of a diverse range of physiological functions including energy homeostasis, cardiovascular and neuroendocrine functions, anxiety, and cognition [71, 125]. Apart from these functions NPY has more recently been identified to modulate neuronal excitability, seizures, and neuroprotection [150, 164, 166]. Both, in experimental seizure models, as well as in patients with intractable temporal lobe epilepsy, prominent changes in NPY and its receptors have been identified in brain regions, which play a role in the initiation and propagation of epileptiform activity, such as the hippocampus [55, 56, 166]. Furthermore, a potent anticonvulsive role of NPY has been demonstrated by the suppression of epileptiform activity by intracerebral infusion of NPY, its analogues, or receptor agonists in various experimental models of epilepsy [164, 172, 173].

The potential therapeutic effects of NPY are mediated by binding to G-protein coupled receptors, of which six subtypes have been identified (Y1 – Y6) [69, 70, 137, 138]. Among those the Y1 and Y2 receptors were found to be the dominant receptor subtypes of the hippocampus [136]. The use of various NPY- and Y-receptor subtype-specific knockout and transgenic animals yielded important results in the study of NPY function and the role of individual receptors. As direct evidence for the anticonvulsant role of NPY, NPY-deficient mice develop spontaneous seizure activity and have lowered thresholds for chemical seizure induction [41, 123]. Conversely, transgenic overexpression of NPY in CA1 of rats resulted in increased protection against seizures [165]. Recent studies suggest an important role of the Y5 receptor in mediating inhibitory effects of NPY in the hippocampus [5, 6, 100], however, the two major hippocampal NPY receptors of the hippocampus, Y1 and Y2, appear to mediate opposite effects with the Y2 receptor playing a critical role in the anticonvulsive properties of NPY [37, 94] and the Y1 receptor mediating a proconvulsive action of NPY [94]. These differential receptor-dependant actions of NPY on seizure modulation need to be considered for therapy development.

NEUROPEPTIDE Y – THERAPEUTIC APPROACHES

The first demonstration that NPY possesses anticonvulsant activity *in vivo* was achieved in 1997 by intraventricular application of the peptide [171]. In this study NPY led to powerful inhibition of motor as well as electroencephalographic seizures induced by kainic acid [171]. Subsequently, a seven-day chronic infusion of NPY into the hippocampus delayed the progression of hippocampal kindling in the rat, whereas anti-NPY immunoglobulin aggravated seizure development [139]. These results suggest that NPY might also have anti-epileptogenic properties. However, the first NPY-mediated gene therapy approach was only realized recently by Richichi *et al.* [142], who demonstrated a range of antiseizure effects by AAV-mediated expression of prepro-neuropeptide Y. In this study hippocampal gene expression of prepro-neuropeptide Y prevented the appearance of status epilepticus after intracerebroventricular administration of kainic acid. Furthermore, intrahippocampal expression of this gene therapy vector increased electrical seizure thresholds in the hippocampus and retarded the rate of kindling epileptogenesis. This study also addressed the use of different AAV serotypes. While neuronal transduction with the AAV2 serotype was limited, the authors were able to demonstrate an increased transduction efficacy in a wider range of neurons, and thus an increased expression of prepro-neuropeptide Y, when they used a

hybrid AAV1/2 serotype. Thus, this study clearly established the antiepileptic potential of AAV-mediated prepro-neuropeptide Y expression *in vivo* over a period of months without any evidence of receptor desensitization. However, it remains to be determined whether receptor desensitization might constitute a problem for the prolonged therapeutic time frame necessary for future clinical applications.

Subsequently, the same rAAV1/2-NPY vector used for the rat studies described above was also used in a mouse model of kainic acid induced seizures [94]. The focal overexpression of NPY in the hippocampus effectively reduced motor seizures in wild-type mice by at least two-fold, despite the severity of the seizure model used. However, in contrast to the rat study [142], NPY overexpression in mice did not delay the onset of seizures [94]. These differences could be due to the severity of the seizure model used, to the read-out of seizure activity (motor seizures versus EEG), or species dependent differences in the efficacy of NPY. Using the same vector in both Y1 and Y2 receptor knockout mice Lin *et al.* were able to demonstrate that the anticonvulsant effect of NPY was mediated by Y2 receptors, while Y1 receptors mediate proconvulsive effects. Consequently, a therapeutic strategy targeting NPY-dependent neuromodulation should be receptor selective. Receptor selectivity in favor of seizure suppression becomes at least partially possible by the observation that in human epileptic hippocampus the proconvulsive Y1 receptor appears to be downregulated, while upregulation of the “beneficial” Y2 receptor was found [55]. Eventually, the combination of NPY overexpression with Y1 receptor blockade, e.g. by RNAi, may improve the therapeutic efficacy against limbic seizures.

PERSPECTIVES AND LIMITATIONS

The examples presented here demonstrate that therapeutic augmentation of endogenous anticonvulsant regulatory systems of the brain (GABA, adenosine, galanin, neuropeptide Y) holds great promise for the development of rational therapies for epilepsy aimed not only at seizure suppression and neuroprotection, but also at the prevention of epileptogenesis. While augmentation of the small-molecule based GABAergic and adenosinergic neuromodulation can effectively be achieved by intracerebral transplantation of cells engineered to release GABA or adenosine, viral vector-based gene therapy approaches making use of these compounds may be further down the road. Conversely, the delivery of peptide-based antiepileptic neuromodulators, such as galanin or neuropeptide Y may be more effective if delivered directly in viral vector-based gene therapy approaches. However, it remains to be determined whether cells engineered to secrete galanin or neuropeptide Y might be equally effective in providing antiepileptic efficacy.

Intractable focal seizures are particularly suited for novel cell and gene based therapies for several reasons (Table 3): (i) By focal augmentation of endogenous anticonvulsive mechanisms, effective therapy becomes possible without widespread side-effects; (ii) augmentation of endogenous antiepileptic mechanisms may be effective in the suppression of pharmacoresistant seizures; (iii) direct application of these antiepileptic compounds either by transplanted cells or by direct delivery *via* gene therapy vectors circumvents the therapeutic limitations of the blood brain barrier.

Although promising in rodent models of epilepsy, cell and gene therapies for epilepsy have to meet the following challenges before clinical trials can be envisaged (Table 3):

- (i) Prove of long-term efficacy: In contrast to many other neurological disorders in which patients are either aged and/or have a limited life expectancy (e.g. Parkinson's, Huntington's, Alzheimer's disease, certain brain tumors), patients with epilepsy are often young and have the perspective to live for many decades. Thus, cell or gene therapies for epilepsy should ideally be effective for decades, requiring life-long

Table 3. Opportunities and Hurdles for Clinical Implementation of Cell and Gene Therapies for Refractory Epilepsy

Opportunities	Hurdles
<ul style="list-style-type: none"> • Potential to avoid side effects by focal application • Promise to combine neuroprotective, antiepileptic and antiepileptogenic effects by rational use of endogenous modulators of epileptic activity • Potential to be effective in pharmacoresistant epilepsy 	<ul style="list-style-type: none"> • Proof of long-term therapeutic efficacy • Proof of efficacy in clinically relevant animal models of epilepsy • Demonstration of long-term safety • Demonstration of efficacy in resected human epileptic hippocampi

survival of implanted cells or life-long therapeutic gene expression. To this end specific treatments are currently tested to enhance the survival of grafted cells [176] and lentiviral gene expression vectors hold the promise for stable, differentiation-independent, long-term gene expression [26].

- (ii) Proof of efficacy in clinically relevant animal models: Most of the published cell and gene therapy approaches in animal models yielded promising results when the time between the initial result and the cell transplantation or vector application was short; when specific deficits were induced prior to therapeutic intervention and the therapy was tailored to compensate this deficit; or when treatment was initiated prior to the induction of an epileptic phenotype, i.e. in naïve brain. Therefore, there is a strong need to proof therapeutic efficacy of cell and gene therapies in clinically relevant animal models, which represent true epileptogenesis and the development of chronic spontaneous, and – ideally – pharmacoresistant seizures. Some of the kainic acid models of epileptogenesis meet these criteria and mimic human temporal lobe epilepsy [18, 140]. Thus, it would be important to demonstrate that therapeutic interventions discussed in this review are able to prevent spontaneous seizures and – preferably – epileptogenesis in clinically relevant animal models. A further caveat is the relatively small size of a rodent brain and extrapolation of results to the human brain may be difficult to achieve without the use of a large animal model (e.g. in monkeys or swine), mimicking the dimensions of the human brain.
- (iii) Demonstration of long-term safety: The studies discussed in this review were all limited to weeks or months. Although focal treatment approaches are in general less destructive than resective surgery, in regard to the normal life expectancy of a patient with epilepsy, the assessment of the benefit-risk ratio requires careful scrutiny and long-term experiments, ideally in the range of years, to exclude potential risks, such as tumor formation or cognitive impairment. Such long-term experiments should be coupled with behavioral paradigms (e.g. Morris water maze) to assess cognitive functions of treated animals.
- (iv) Demonstration of efficacy in resected human epileptic hippocampus: Before clinical trials are contemplated it would be desirable to test the therapeutic efficacy of therapeutic cells or gene therapy vectors in slice cultures prepared from surgically resected human epileptic hippocampi. This approach is feasible, since epileptic activity can be recorded from hippocampal slice cultures. Thus, stem cells can integrate into epileptic hippocampus [48, 143] and gene expression vectors can be used to infect hippocampal slices [163]. The demonstration of *in vitro* efficacy of cell and gene therapies on slices prepared from human epileptic hippocampi would constitute a major preclinical advance.

Antiepileptic cell and gene therapies may finally lead to clinical trials to demonstrate safety and efficacy in the suppression of pharmacoresistant seizures. Clinical trials could be envisaged in patients with MTL. This form of epilepsy would be selected for three reasons: (i) it is a common form of epilepsy, (ii) it has a relatively homogeneous pathophysiology, and (iii) it displays rather uniform clinical characteristics. The patients afflicted with this disease are frequently drug resistant and 70-80% of these are candidates for amygdalo-hippocampectomy. Patients who are referred for amygdalo-hippocampectomy would be selected for future cell or gene therapy. The vectors (cells or virus) would be injected stereotactically into the epileptic focus. It is understood that patients, who undergo such a treatment will require sophisticated follow-up studies to document the clinical efficacy of the treatment on the epilepsy of the patient (epileptological and EEG controls), the structural characteristics of the target area (MRI), to detect and measure functional/metabolic changes after treatment (1H-MRS), and neuropsychological evaluations. In the case that grafted patients do not show seizure suppression, the primary epileptogenic zone would be excised using a conventional surgical approach. The resected brain region including the implant/vector would then be carefully studied using advanced histological and biochemical methods to gain further insight into the behavior of reactions to the implant/vector within the human brain.

In conclusion, cell and gene therapies for refractory epilepsy hold substantial therapeutic promise but several hurdles need to be overcome before clinical trials can be planned (Table 3). Thus, the development of cell and gene therapies for pharmacoresistant focal epilepsies will continue to progress further into an area of intense scientific and therapeutic interest.

ACKNOWLEDGMENTS

The work of the author is supported by grant R01 NS047622-01A2 from the NIH, by the Epilepsy Research Foundation through the generous support of Arlene & Arnold Goldstein Family Foundation, and by the Good Samaritan Hospital Foundation.

REFERENCES

- Aebischer, P., Buchser, E., Joseph, J. M., Favre, J., de Tribolet, N., Lysaght, M., Rudnick, S., Goddard, M. (1994) Transplantation in humans of encapsulated xenogeneic cells without immunosuppression. A preliminary report. *Transplantation*, **58**, 1275-1277.
- Aebischer, P., Pochon, N. A., Heyd, B., Déglon, N., Joseph, J. M., Zurn, A. D., Baetge, E. E., Hammang, J. P., Goddard, M., Lysaght, M., Kaplan, F., Kato, A. C., Schlup, M., Hirt, L., Regli, F., Porchet, F., De Tribolet, N. (1996) Gene therapy for amyotrophic lateral sclerosis (ALS) using a polymer encapsulated xenogenic cell line engineered to secrete hCNTF. *Hum. Gene Ther.*, **7**, 851-860.
- Baekelandt, V., De Strooper, B., Nuttin, B., Debyser, Z. (2000) Gene therapeutic strategies for neurodegenerative diseases. *Curr. Opin. Mol. Ther.*, **2**, 540-554.
- Baldwin, S. A., Beal, P. R., Yao, S. Y., King, A. E., Cass, C. E., Young, J. D. (2004) The equilibrative nucleoside transporter family, SLC29. *Pflügers Arch.*, **447**, 735-743.
- Baraban, S. C. (2002) Antiepileptic actions of neuropeptide Y in the mouse hippocampus require Y5 receptors. *Epilepsia*, **43**(Suppl. 5), 9-13.
- Baraban, S. C. (2004) Neuropeptide Y and epilepsy: recent progress, prospects and controversies. *Neuropeptides*, **38**, 261-265.
- Barker, R. A., Widner, H. (2004) Immune problems in central nervous system cell therapy. *NeuroRx*, **1**, 472-481.
- Barry, F. P., Murphy, J. M. (2004) Mesenchymal stem cells: clinical applications and biological characterization. *Int. J. Biochem. Cell Biol.*, **36**, 568-584.
- Berkovic, S. F., Mulley, J. C., Scheffer, I. E., Petrou, S. (2006) Human epilepsies: interaction of genetic and acquired factors. *Trends Neurosci.*, **29**, 391-397.
- Bjorklund, A. (2000) Cell replacement strategies for neurodegenerative disorders. *Novartis Found. Symp.*, **231**, 7-15.
- Bjorklund, L. M., Sanchez-Pernaute, R., Chung, S., Andersson, T., Chen, I. Y., McNaught, K. S., Brownell, A. L., Jenkins, B. G., Wahlestedt, C., Kim, K. S., Isacson, O. (2002) Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc. Natl. Acad. Sci. USA*, **99**, 2344-2349.
- Bjornson, C. R. R., Rietze, R. L., Reynolds, B. A., Magli, M. C., Vescovi, A. L. (1999) Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells *in vivo*. *Science*, **283**, 534-537.
- Boison, D., Scheurer, L., Zumsteg, V., Rulicke, T., Litynski, P., Fowler, B., Brandner, S., Mohler, H. (2002) Neonatal hepatic steatosis by disruption of the adenosine kinase gene. *Proc. Natl. Acad. Sci. USA*, **99**, 6985-6990.
- Boison, D. (2005) Adenosine and epilepsy: from therapeutic rationale to new therapeutic strategies. *Neuroscientist*, **11**, 25-36.
- Boison, D. (2006) Adenosine kinase, epilepsy and stroke: mechanisms and therapies. *Trends Pharmacol. Sci.*, **27**, 652-658.
- Bonde, S., Ekdahl, C. T., Lindvall, O. (2006) Long-term neuronal replacement in adult rat hippocampus after status epilepticus despite chronic inflammation. *Eur. J. Neurosci.*, **23**, 965-974.
- Borges, K., McDermott, D., Irier, H., Smith, Y., Dingledine, R. (2006) Degeneration and proliferation of astrocytes in the mouse dentate gyrus after pilocarpine-induced status epilepticus. *Exp. Neurol.*, **201**, 416-427.
- Bouilleret, V., Ridoux, V., Depaulis, A., Marescaux, C., Nehlig, A., Le Gal La Salle, G. (1999) Recurrent seizures and hippocampal sclerosis following intrahippocampal kainate injection in adult mice: electroencephalography, histopathology and synaptic reorganization similar to mesial temporal lobe epilepsy. *Neuroscience*, **89**, 717-729.
- Brazelton, T. R., Rossi, F. M., Keshet, G. I., Blau, H. M. (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. *Science*, **290**, 1775-1779.
- Britton, D. R., Mikusa, J., Lee, C. H., Jarvis, M. F., Williams, M., Kowaluk, E. A. (1999) Site and event specific increase of striatal adenosine release by adenosine kinase inhibition in rats. *Neurosci. Lett.*, **266**, 93-96.
- Brüstle, O., Jones, K. N., Learish, R. D., Karram, K., Choudhary, K., Westler, O. D., Duncan, I. D., McKay, R. D. (1999) Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science*, **285**, 754-756.
- Bueler, H. (1999) Adeno-associated viral vectors for gene transfer and gene therapy. *Biol. Chem.*, **380**, 613-622.
- Burnstock, G. (1972) Purinergic nerves. *Pharmacol. Rev.*, **24**, 509-581.
- Chu, K., Kim, M., Jeong, S. W., Kim, S. U., Yoon, B. W. (2003) Human neural stem cells can migrate, differentiate, and integrate after intravenous transplantation in adult rats with transient forebrain ischemia. *Neurosci. Lett.*, **343**, 129-133.
- Chu, K., Kim, M., Jung, K. H., Jeon, D., Lee, S. T., Kim, J., Jeong, S. W., Kim, S. U., Lee, S. K., Shin, H. S., Roh, J. K. (2004) Human neural stem cell transplantation reduces spontaneous recurrent seizures following pilocarpine-induced status epilepticus in adult rats. *Brain Res.*, **1023**, 213-221.
- Clements, M. O., Godfrey, A., Crossley, J., Wilson, S. J., Takeuchi, Y., Boshoff, C. (2006) Lentiviral manipulation of gene expression in human adult and embryonic stem cells. *Tissue Eng.*, **12**, 1741-1751.
- Cunha, R. A. (2005) Neuroprotection by adenosine in the brain: from A1 receptor activation to A2A receptor blockade. *Purinergic Signal.*, **1**, 111-134.
- Davidson, B. L., Bohn, M. C. (1997) Recombinant adenovirus: a gene transfer vector for study and treatment of CNS diseases. *Exp. Neurol.*, **144**, 125-130.
- Davis, A. S., Zhao, H., Sun, G. H., Sapolsky, R. M., Steinberg, G. K. (2007) Gene therapy using SOD1 protects striatal neurons from experimental stroke. *Neurosci. Lett.*, **411**, 32-36.
- Dragunow, M. (1986) Adenosine: the brain's natural anticonvulsant? *Trends Pharmacol. Sci.*, **7**, 128.
- Dragunow, M., Faull, R. L. M. (1988) Neuroprotective effects of adenosine. *Trends Pharmacol. Sci.*, **9**, 193-194.
- Driesse, M. J., Esandi, M. C., Kros, J. M., Avezaat, C. J., Vecht, C., Zurcher, C., van der Velde, I., Valerio, D., Bout, A., Sillevius Smitt, P. A. (2000) Intracerebral administered recombinant adenovirus causes an immune response-mediated toxicity. *Gene Ther.*, **7**, 1401-1409.
- Dumas, T. C., Sapolsky, R. M. (2001) Gene therapy against neurological insults: sparing neurons versus sparing function. *Trends Neurosci.*, **24**, 695-700.
- Dunwiddie, T. V. (1999) Adenosine and suppression of seizures. *Adv. Neurol.*, **79**, 1001-1010.
- Dunwiddie, T. V., Masino, S. A. (2001) The role and regulation of adenosine in the central nervous system. *Annu. Rev. Neurosci.*, **24**, 31-55.
- Ekdahl, C. T., Claassen, J. H., Bonde, S., Kokaia, Z., Lindvall, O. (2003) Inflammation is detrimental for neurogenesis in adult brain. *Proc. Natl. Acad. Sci. USA*, **100**, 13632-13637.
- El Bahh, B., Balosso, S., Hamilton, T., Herzog, H., Beck-Sickingler, A. G., Sperk, G., Gehlert, D. R., Vezzani, A., Colmers, W. F. (2005) The anti-epileptic actions of neuropeptide Y in the hippocampus are mediated by Y and not Y receptors. *Eur. J. Neurosci.*, **22**, 1417-1430.
- Emerich, D. F., Winn, S. R. (2000) Application of polymer-encapsulated cell therapy for CNS diseases. In: Dunnett, S.B., Boulton, A.A., Baker, G.B., Eds.; Neural transplantation methods, Humana Press: Totowa, New Jersey, Vol. **36**, pp. 233-278.
- Engelhard, H. H. (2000) Gene therapy for brain tumors: the fundamentals. *Surg. Neurol.*, **54**, 3-9.
- Epps, S. A., Venable, D. E., Faingold, C. L., Wilson, S. P., Coleman, J. R. (2006) Effects of alterations of GAD by lentivirus mediated gene transfer on seizure severity in genetically epilepsy prone rats. *Exp. Neurol.*, **198**, 568-568.
- Erickson, J. C., Clegg, K. E., Palmiter, R. D. (1996) Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature*, **381**, 415-421.
- Erion, M. D., Wiesner, J. B., Rosengren, S., Ugarkar, B. G., Boyer, S. H., Tsuchiya, M. (2000) Therapeutic potential of adenosine kinase inhibitors as analgesic agents. *Drug Dev. Res.*, **50**, S14-06.
- Fathallah-Shaykh, H. M., Kafrouni, A. I., Zhao, L. J., Diaz-Arrastia, R., Garcia, J. A., Frawley, W. H., Forman, J. (2000) Demyelination but no cog-

- nitive, motor or behavioral deficits after adenovirus-mediated gene transfer into the brain. *Gene Ther.*, **7**, 2094-2098.
- [44] Fedele, D. E., Koch, P., Brüstle, O., Scheurer, L., Simpson, E. M., Mohler, H., Boison, D. (2004) Engineering embryonic stem cell derived glia for adenosine delivery. *Neurosci. Lett.*, **370**, 160-165.
- [45] Fedele, D. E., Gouder, N., Göttinger, M., Gabernet, L., Scheurer, L., Rulicke, T., Crestani, F., Boison, D. (2005) Astroglial gliosis in epilepsy leads to overexpression of adenosine kinase resulting in seizure aggravation. *Brain*, **128**, 2383-2395.
- [46] Fedele, D. E., Li, T., Lan, J. Q., Fredholm, B. B., Boison, D. (2006) Adenosine A₁ receptors are crucial in keeping an epileptic focus localized. *Exp. Neurol.*, **200**, 184-190.
- [47] Fernandes, K. J., McKenzie, I. A., Mill, P., Smith, K. M., Akhavan, M., Barnabe-Heider, F., Biernaskie, J., Junek, A., Kobayashi, N. R., Toma, J. G., Kaplan, D. R., Labosky, P. A., Rafuse, V., Hui, C. C., Miller, F. D. (2004) A dermal niche for multipotent adult skin-derived precursor cells. *Nat. Cell Biol.*, **6**, 1082-1093.
- [48] Fernandes, K. J. L., Kobayashi, N. R., Gallagher, C. J., Barnabe-Heider, F., Aumont, A., Kaplan, D. R., Miller, F. D. (2006) Analysis of the neurogenic potential of multipotent skin-derived precursors. *Exp. Neurol.*, **201**, 32-48.
- [49] Ferrari, G., Cusella-De Angelis, G., Coletta, M., Paolucci, E., Stornaiuolo, A., Cossu, G., Mavilio, F. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science*, **279**, 1528-1530.
- [50] Fredholm, B. B. (1997) Adenosine and neuroprotection. *Int. Rev. Neurobiol.*, **40**, 259-280.
- [51] Fredholm, B. B., Battig, K., Holmen, J., Nehlig, A., Zvartau, E. E. (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol. Rev.*, **51**, 83-133.
- [52] Fredholm, B. B., Ijzerman, A. P., Jacobson, K. A., Klotz, K. N., Linden, J. (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.*, **53**, 527-552.
- [53] Fredholm, B. B., Chen, J. F., Cunha, R. A., Svenningsson, P., Vaugeois, J. M. (2005) Adenosine and brain function. *Int. Rev. Neurobiol.*, **63**, 191-270.
- [54] Fredholm, B. B., Chen, J. F., Masino, S. A., Vaugeois, J. M. (2005) Actions of adenosine at its receptors in the CNS: Insights from knockouts and drugs. *Annu. Rev. Pharmacol. Toxicol.*, **45**, 385-412.
- [55] Furtinger, S., Pirker, S., Czech, T., Baumgartner, C., Ransmayr, G., Sperk, G. (2001) Plasticity of Y1 and Y2 receptors and neuropeptide Y fibers in patients with temporal lobe epilepsy. *J. Neurosci.*, **21**, 5804-5812.
- [56] Furtinger, S., Pirker, S., Czech, T., Baumgartner, C., Sperk, G. (2002) Altered expression of neuropeptide Y, -Y(1), and -Y(2) receptors in the hippocampus of patients with mesial temporal lobe epilepsy. *Epilepsia*, **43**(Suppl. 5), 152.
- [57] Galli, R., Borello, U., Gritti, A., Minasi, M. G., Bjornson, C., Coletta, M., Mora, M., De Angelis, M. G., Fiocco, R., Cossu, G., Vescovi, A. L. (2000) Skeletal myogenic potential of human and mouse neural stem cells. *Nat. Neurosci.*, **3**, 986-991.
- [58] Gernert, M., Thompson, K. W., Loscher, W., Tobin, A. J. (2002) Genetically engineered GABA-producing cells demonstrate anticonvulsant effects and long-term transgene expression when transplanted into the central piriform cortex of rats. *Exp. Neurol.*, **176**, 183-192.
- [59] Glass, M., Faull, R. L., Bullock, J. Y., Jansen, K., Mee, E. W., Walker, E. B., Synek, B. J., Dragunow, M. (1996) Loss of A1 adenosine receptors in human temporal lobe epilepsy. *Brain Res.*, **710**, 56-68.
- [60] Gouder, N., Scheurer, L., Fritschy, J.-M., Boison, D. (2004) Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. *J. Neurosci.*, **24**, 692-701.
- [61] Gray, J. H., Owen, R. P., Giacomini, K. M. (2004) The concentrative nucleoside transporter family, SLC28. *Pflügers Arch.*, **447**, 728-734.
- [62] Gunnett, C. A., Heistad, D. D. (2002) Virally mediated gene transfer to the vasculature. *Microcirculation*, **9**, 23-33.
- [63] Gussoni, E., Soneoka, Y., Strickland, C. D., Buzney, E. A., Khan, M. K., Flint, A. F., Kunkel, L. M., Mulligan, R. C. (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature*, **401**, 390-394.
- [64] Göttinger, M., Fedele, D. E., Koch, P., Padrun, V., Pralong, W., Brüstle, O., Boison, D. (2005) Suppression of kindled seizures by paracrine adenosine release from stem cell derived brain implants. *Epilepsia*, **46**, 1-8.
- [65] Göttinger, M., Padrun, V., Pralong, W., Boison, D. (2005) Seizure suppression and lack of adenosine A₁ receptor desensitization after focal long-term delivery of adenosine by encapsulated myoblasts. *Exp. Neurol.*, **193**, 53-64.
- [66] Haberly, L. B., Sutula, T. P. (1992) Neuronal processes that underlie expression of kindled epileptiform events in the piriform cortex *in vivo*. *J. Neurosci.*, **12**, 2211-2224.
- [67] Haberman, R. P., Samulski, R. J., McCown, T. J. (2003) Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. *Nat. Med.*, **9**, 1076-1080.
- [68] Hauser, W. A. (1992) The natural history of drug resistant epilepsy: Epidemiologic considerations. In *Surgical treatment of epilepsy*, W.H. Theodore, ed.; Elsevier: Amsterdam, pp. 25-28.
- [69] Herzog, H., Darby, K., Ball, H., Hort, Y., Beck-Sickinger, A., Shine, J. (1997) Overlapping gene structure of the human neuropeptide Y receptor subtypes Y1 and Y5 suggests coordinate transcriptional regulation. *Genomics*, **41**, 315-319.
- [70] Herzog, H. (2000) Human Y1/Y5 receptor gene cluster. Isolation and characterization. *Methods Mol. Biol.*, **153**, 13-24.
- [71] Hokfelt, T., Broberger, C., Diez, M., Xu, Z. Q., Shi, T., Kopp, J., Zhang, X., Holmberg, K., Landry, M., Koistinaho, J. (1999) Galanin and NPY, two peptides with multiple putative roles in the nervous system. *Horm. Metab. Res.*, **31**, 330-334.
- [72] Holmes, G. L., Ben-Ari, Y. (2003) Seizing hold of seizures. *Nat. Med.*, **9**, 994-996.
- [73] Hsieh, G., Sena-Esteves, M., Breakefield, X. O. (2002) Critical issues in gene therapy for neurologic disease. *Hum. Gene Ther.*, **13**, 579-604.
- [74] Huber, A., Padrun, V., Deglon, N., Aebischer, P., Mohler, H., Boison, D. (2001) Grafts of adenosine-releasing cells suppress seizures in kindling epilepsy. *Proc. Natl. Acad. Sci. USA*, **98**, 7611-7616.
- [75] Jackson, K. A., Mi, T., Goodell, M. A. (1999) Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc. Natl. Acad. Sci. USA*, **96**, 14482-14486.
- [76] Jarvis, M. F., Mikusa, J., Chu, K. L., Wismer, C. T., Honore, P., Kowaluk, E. A., McGaraughy, S. (2002) Comparison of the ability of adenosine kinase inhibitors and adenosine receptor agonists to attenuate thermal hyperalgesia and reduce motor performance in rats. *Pharmacol. Biochem. Behav.*, **73**, 573-581.
- [77] Jeong, S. W., Chu, K., Jung, K. H., Kim, S. U., Kim, M., Roh, J. K. (2003) Human neural stem cell transplantation promotes functional recovery in rats with experimental intracerebral hemorrhage. *Stroke*, **34**, 2258-2263.
- [78] Kassem, M. (2004) Mesenchymal stem cells: biological characteristics and potential clinical applications. *Cloning Stem Cells*, **6**, 369-374.
- [79] Kim, J. H., Auerbach, J. M., Rodriguez-Gomez, J. A., Velasco, I., Gavin, D., Lumelsky, N., Lee, S. H., Nguyen, J., Sanchez-Pemate, R., Bankiewicz, K., McKay, R. (2002) Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature*, **418**, 50-56.
- [80] Klugmann, M., Leichtle, C. B., Symes, C. W., Serikawa, T., Young, D., Doring, M. J. (2005) Restoration of aspartoacylase activity in CNS neurons does not ameliorate motor deficits and demyelination in a model of Canavan disease. *Mol. Ther.*, **11**, 745-753.
- [81] Kochanek, P. M., Janesko, K. L., Jenkins, L. W., Yan, H. Q., Kibbe, M. R., Rohbachaud, P., Wooditch, A. C., Clark, R. S., Dixon, C. E., Marion, D. W., Billiar, T. R. (2001) Adenovirus-mediated transfer and expression of beta-gal in injured hippocampus after traumatic brain injury in mice. *J. Neurotrauma*, **18**, 73-82.
- [82] Kochanek, P. M., Vagni, V. A., Janesko, K. L., Washington, C. B., Crumrine, P. K., Garman, R. H., Jenkins, L. W., Clark, R. S., Homanics, G. E., Dixon, C. E., Schnermann, J., Jackson, E. K. (2006) Adenosine A1 receptor knockout mice develop lethal status epilepticus after experimental traumatic brain injury. *J. Cereb. Blood Flow Metab.*, **26**, 565-575.
- [83] Kokaia, M., Holmberg, K., Nanobashvili, A., Xu, Z. Q., Kokaia, Z., Lendahl, U., Hilke, S., Theodorsson, E., Kahl, U., Bartfai, T., Lindvall, O., Hokfelt, T. (2001) Suppressed kindling epileptogenesis in mice with ectopic overexpression of galanin. *Proc. Natl. Acad. Sci. USA*, **98**, 14006-14011.
- [84] Korbli, M., Estrov, Z., Champlin, R. (2003) Adult stem cells and tissue repair. *Bone Marrow Transplant.*, **32**(Suppl. 1), S23-24.
- [85] Lachmann, R. (2003) Herpes simplex virus latency. *Expert Rev. Mol. Med.*, **2003**, 1-14.
- [86] Lachmann, R. (2004) Herpes simplex virus-based vectors. *Int. J. Exp. Pathol.*, **85**, 177-190.
- [87] Laing, J. M., Gober, M. D., Golembewski, E. K., Thompson, S. M., Gyure, K. A., Yarowsky, P. J., Aurelian, L. (2006) Intranasal administration of the growth-compromised HSV-2 vector DeltaARR prevents kainate-induced seizures and neuronal loss in rats and mice. *Mol. Ther.*, **13**, 870-881.
- [88] Latchman, D. S. (2003) Herpes simplex virus vectors for Parkinson's disease. *Int. Rev. Neurobiol.*, **55**, 223-241.
- [89] Lawrence, M. S., Ho, D. Y., Dash, R., Sapolsky, R. M. (1995) Herpes simplex virus vectors overexpressing the glucose transporter gene protect against seizure-induced neuron loss. *Proc. Natl. Acad. Sci. USA*, **92**, 7247-7251.
- [90] Lee, K. S., Schubert, P., Heinemann, U. (1984) The anticonvulsive action of adenosine: a postsynaptic, dendritic action by a possible endogenous anticonvulsant. *Brain Res.*, **321**, 160-164.
- [91] Lehmann, H., Ebert, U., Loscher, W. (1998) Amygdala-kindling induces a lasting reduction of GABA-immunoreactive neurons in a discrete area of the ipsilateral piriform cortex. *Synapse*, **29**, 299-309.
- [92] Li, T., Steinbeck, J. A., Lusardi, T., Koch, P., Lan, J. Q., Wilz, A., Segschneider, M., Simon, R. P., Brüstle, O., Boison, D. (2007) Suppression of kindling epileptogenesis by adenosine releasing stem cell-derived brain implants. *Brain*, in press.
- [93] Lin, E. J., Richichi, C., Young, D., Baer, K., Vezzani, A., Doring, M. J. (2003) Recombinant AAV-mediated expression of galanin in rat hippocampus suppresses seizure development. *Eur. J. Neurosci.*, **18**, 2087-2092.
- [94] Lin, E. J., Young, D., Baer, K., Herzog, H., Doring, M. J. (2006) Differential actions of NPY on seizure modulation via Y1 and Y2 receptors: Evidence from receptor knockout mice. *Epilepsia*, **47**, 773-780.
- [95] Liu, W., He, X., Cao, Z., Sheng, J., Liu, H., Li, Z., Li, W. (2005) Efficient therapeutic gene expression in cultured rat hippocampal neurons mediated by human foamy virus vectors: a potential for the treatment of neurological diseases. *Intervirology*, **48**, 329-335.
- [96] Loscher, W., Ebert, U. (1996) The role of the piriform cortex in kindling. *Prog. Neurobiol.*, **50**, 427-481.
- [97] Loscher, W., Ebert, U., Lehmann, H., Rosenthal, C., Nikkhah, G. (1998) Seizure suppression in kindling epilepsy by grafts of fetal GABAergic neurons in rat substantia nigra. *J. Neurosci. Res.*, **51**, 196-209.
- [98] Loscher, W., Schmidt, D. (2004) New horizons in the development of antiepileptic drugs: the search for new targets. *Epilepsy Res.*, **60**, 77-159.
- [99] Loscher, W., Schmidt, D. (2006) Experimental and clinical evidence for loss of effect (tolerance) during prolonged treatment with antiepileptic drugs. *Epilepsia*, **47**, 1253-1284.
- [100] Marsh, D. J., Baraban, S. C., Hollopeter, G., Palmiter, R. D. (1999) Role of the Y5 neuropeptide Y receptor in limbic seizures. *Proc. Natl. Acad. Sci. USA*, **96**, 13518-13523.

- [101] Mazarati, A., Lu, X. (2005) Regulation of limbic status epilepticus by hippocampal galanin type 1 and type 2 receptors. *Neuropeptides*, **39**, 277-280.
- [102] Mazarati, A. M. (2004) Galanin and galanin receptors in epilepsy. *Neuropeptides*, **38**, 331-343.
- [103] Mazarati, A. M., Baldwin, R. A., Shinmei, S., Sankar, R. (2005) *In vivo* interaction between serotonin and galanin receptors types 1 and 2 in the dorsal raphe: implication for limbic seizures. *J. Neurochem.*, **95**, 1495-1503.
- [104] Mazarati, A. M., Lu, X., Bartfai, T. (2005) Serotonergic projection from dorsal raphe into the hippocampus: Role in seizures and regulation by galanin type 1 (GalR1) and type 2 (GalR2) receptors. *Epilepsia*, **46**, 198.
- [105] McCown, T. J. (2004) Adeno-associated virus (AAV) vector-mediated expression and secretion of galanin in the piriform cortex prevents kainic acid seizures. *Epilepsia*, **45**, 359.
- [106] McCown, T. J. (2005) Adeno-associated virus (AAV) vectors in the CNS. *Curr. Gene Ther.*, **5**, 333-338.
- [107] McCown, T. J. (2006) Adeno-associated virus-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity *in vivo*. *Mol. Ther.*, **14**, 63-68.
- [108] McGaraughy, S., Jarvis, M. F. (2006) Purinergic control of neuropathic pain. *Drug Dev. Res.*, **67**, 376-388.
- [109] McIntyre, D. C., Plant, J. R. (1989) Piriform cortex involvement in kindling. *Neurosci. Biobehav. Rev.*, **13**, 277-280.
- [110] McLaughlin, J., Rozenzweig, B., Dumas, T., Gupta, A., Ajilore, O., Hsieh, J., Ho, D., Lawrence, M., McGaugh, J. L., Sapolsky, R. (2000) Sparing of neuronal function postseizure with gene therapy. *Proc. Natl. Acad. Sci. USA*, **97**, 12804-12809.
- [111] McPhee, S. W., Francis, J., Janson, C. G., Serikawa, T., Hyland, K., Ong, E. O., Raghavan, S. S., Freese, A., Leone, P. (2005) Effects of AAV-2-mediated aspartoacylase gene transfer in the tremor rat model of Canavan disease. *Brain Res. Mol. Brain Res.*, **135**, 112-121.
- [112] Mezey, E., Chandross, K. J., Harta, G., Maki, R. A., McKercher, S. R. (2000) Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science*, **290**, 1779-1782.
- [113] Mezey, E., Key, S., Vogelsang, G., Szalayova, I., Lange, G. D., Crain, B. (2003) Transplanted bone marrow generates new neurons in human brains. *Proc. Natl. Acad. Sci. USA*, **100**, 1364-1369.
- [114] Morrison, S. J., Shah, N. M., Anderson, D. J. (1997) Regulatory mechanisms in stem cell biology. *Cell*, **88**, 287-298.
- [115] Nadkarni, S., LaJoie, J., Devinsky, O. (2005) Current treatments of epilepsy. *Neurology*, **64**, S2-11.
- [116] Naldini, L., Blomer, U., Gage, F. H., Trono, D., Verma, I. M. (1996) Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc. Natl. Acad. Sci. USA*, **93**, 11382-11388.
- [117] Naldini, L., Blomer, U., Galloway, P., Ory, D., Mulligan, R., Gage, F. H., Verma, I. M., Trono, D. (1996) *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science*, **272**, 263-267.
- [118] Newby, A. C., Worku, Y., Holmquist, C. A. (1985) Adenosine formation. Evidence for a direct biochemical link with energy metabolism. *Adv. Myocardiol.*, **6**, 273-284.
- [119] Nilsen, K. E., Cock, H. R. (2004) Focal treatment for refractory epilepsy: hope for the future? *Brain Res. Brain Res. Rev.*, **44**, 141-153.
- [120] Okabe, S., Forsberg-Nilsson, K., Spiro, A. C., Segal, M., McKay, R. D. (1996) Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells *in vitro*. *Mech. Dev.*, **59**, 89-102.
- [121] Pagonopoulou, O., Efthimiadou, A., Asimakopoulos, B., Nikolettos, N. K. (2006) Modulatory role of adenosine and its receptors in epilepsy: Possible therapeutic approaches. *Neurosci. Res.*, **56**, 14-20.
- [122] Pak, M. A., Haas, H. L., Decking, U. K. M., Schrader, J. (1994) Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices. *Neuropharmacology*, **33**, 1049-1053.
- [123] Palmiter, R. D., Erickson, J. C., Hollopeter, G., Baraban, S. C., Schwartz, M. W. (1998) Life without neuropeptide Y. *Recent Prog. Horm. Res.*, **53**, 163-199.
- [124] Pankratov, Y. V., Ivanov, A. I., Kolokoltsova, T. D., Nechayeva, Y. A., Radayeva, I. F., Korochkin, L. I., Revischin, A. V., Naumov, S. A., Khlusov, I. A., Autenshlus, A. I. (2003) Cell-based therapy of chronic degenerative diseases of the central nervous system. *Adv. Exp. Med. Biol.*, **534**, 97-105.
- [125] Pedrazzini, T., Pralong, F., Grouzmann, E. (2003) Neuropeptide Y: the universal soldier. *Cell Mol. Life Sci.*, **60**, 350-377.
- [126] Pennacchio, L. A., Lehesjoki, A. E., Stone, N. E., Willour, V. L., Virtaneva, K., Miao, J., D'Amato, E., Ramirez, L., Faham, M., Koskineniemi, M., Warrington, J. A., Norio, R., de la Chapelle, A., Cox, D. R., Myers, R. M. (1996) Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (EPM1). *Science*, **271**, 1731-1734.
- [127] Petersen, B. E., Bowen, W. C., Patrene, K. D., Mars, W. M., Sullivan, A. K., Murase, N., Boggs, S. S., Greenberger, J. S., Goff, J. P. (1999) Bone marrow as a potential source of hepatic oval cells. *Science*, **284**, 1168-1170.
- [128] Pfeifer, A., Ikawa, M., Dayn, Y., Verma, I. M. (2002) Transgenesis by lentiviral vectors: lack of gene silencing in mammalian embryonic stem cells and preimplantation embryos. *Proc. Natl. Acad. Sci. USA*, **99**, 2140-2145.
- [129] Pignataro, G., Studer, F. E., Wilz, A., Simon, R. P., Boison, D. (2006) Neuroprotection in ischemic mouse brain induced by stem cell derived brain implants. *J. Cereb. Blood Flow Metab.*, Nov 22; [Epub ahead of print].
- [130] Pluchino, S., Quattrini, A., Brambilla, E., Gritti, A., Salani, G., Dina, G., Galli, R., Del Carro, U., Amadio, S., Bergami, A., Furlan, R., Comi, G., Vescovi, A. L., Martino, G. (2003) Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature*, **422**, 688-694.
- [131] Rao, M. S., Hattiangady, B., Shetty, A. K. (2006) Fetal hippocampal CA3 cell grafts enriched with FGF-2 and BDNF exhibit robust long-term survival and integration and suppress aberrant mossy fiber sprouting in the injured middle-aged hippocampus. *Neurobiol. Dis.*, **21**, 276-290.
- [132] Raol, Y. H., Lund, I. V., Bandyopadhyay, S., Zhang, G., Roberts, D. S., Wolfe, J. H., Russek, S. J., Brooks-Kayal, A. R. (2006) Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J. Neurosci.*, **26**, 11342-11346.
- [133] Raol, Y. H., Zhang, G., Lund, I. V., Porter, B. E., Maronski, M. A., Brooks-Kayal, A. R. (2006) Increased GABA(A)-receptor alpha 1-subunit expression in hippocampal dentate gyrus after early-life status epilepticus. *Epilepsia*, **47**, 1665-1673.
- [134] Rathjen, J., Rathjen, P. D. (2001) Mouse ES cells: experimental exploitation of pluripotent differentiation potential. *Curr. Opin. Genet. Dev.*, **11**, 587-594.
- [135] Rebola, N., Coelho, J. E., Costenla, A. R., Lopes, L. V., Parada, A., Oliveira, C. R., Soares-da-Silva, P., de Mendonca, A., Cunha, R. A. (2003) Decrease of adenosine A₁ receptor density and of adenosine neuromodulation in the hippocampus of kindled rats. *Eur. J. Neurosci.*, **18**, 820-828.
- [136] Redrobe, J. P., Dumont, Y., St-Pierre, J. A., Quirion, R. (1999) Multiple receptors for neuropeptide Y in the hippocampus: putative roles in seizures and cognition. *Brain Res.*, **848**, 153-166.
- [137] Redrobe, J. P., Dumont, Y., Fournier, A., Quirion, R. (2002) The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. *Neuropsychopharmacology*, **26**, 615-624.
- [138] Redrobe, J. P., Dumont, Y., Herzog, H., Quirion, R. (2003) Neuropeptide Y (NPY) Y2 receptors mediate behaviour in two animal models of anxiety: evidence from Y2 receptor knockout mice. *Behav. Brain Res.*, **141**, 251-255.
- [139] Reibel, S., Benmaamar, R., Le, B. T., Larmet, Y., Kalra, S. P., Marescaux, C., Depaulis, A. (2003) Neuropeptide Y delays hippocampal kindling in the rat. *Hippocampus*, **13**, 557-560.
- [140] Riban, V., Bouillere, V., Pham-Le, B. T., Fritschy, J. M., Marescaux, C., Depaulis, A. (2002) Evolution of hippocampal epileptic activity during the development of hippocampal sclerosis in a mouse model of temporal lobe epilepsy. *Neuroscience*, **112**, 101-111.
- [141] Ribeiro, J. A. (2005) What can adenosine neuromodulation do for neuroprotection? *Curr. Drug Targets CNS Neurol. Disord.*, **4**, 325-329.
- [142] Richichi, C., Lin, E. J., Stefanin, D., Colella, D., Ravizza, T., Grignaschi, G., Veglianesi, P., Sperk, G., During, M. J., Vezzani, A. (2004) Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. *J. Neurosci.*, **24**, 3051-3059.
- [143] Ruschenschmidt, C., Koch, P. G., Brustle, O., Beck, H. (2005) Functional properties of ES cell-derived neurons engrafted into the hippocampus of adult normal and chronically epileptic rats. *Epilepsia*, **46**(Suppl. 5), 174-183.
- [144] Sapolsky, R. M. (2003) Neuroprotective gene therapy against acute neurological insults. *Nat. Rev. Neurosci.*, **4**, 61-69.
- [145] Schlifke, I., Kuteeva, E., Hokfelt, T., Kokaia, M. (2006) Galanin expressed in the excitatory fibers attenuates synaptic strength and generalized seizures in the piriform cortex of mice. *Exp. Neurol.*, **200**, 398-406.
- [146] Schwabe, K., Ebert, U., Loscher, W. (2004) Bilateral microinjections of vigabatrin in the central piriform cortex retard amygdala kindling in rats. *Neuroscience*, **129**, 425-429.
- [147] Schwabe, K., Ebert, U., Loscher, W. (2004) The central piriform cortex: anatomical connections and anticonvulsant effect of GABA elevation in the kindling model. *Neuroscience*, **126**, 727-741.
- [148] Seki, T., Matsubayashi, H., Amano, T., Kitada, K., Serikawa, T., Sasa, M., Sakai, N. (2004) Adenoviral gene transfer of aspartoacylase ameliorates tonic convulsions of spontaneously epileptic rats. *Neurochem. Int.*, **45**, 171-178.
- [149] Shetty, A. K., Zaman, V., Hattiangady, B. (2005) Repair of the injured adult hippocampus through graft-mediated modulation of the plasticity of the dentate gyrus in a rat model of temporal lobe epilepsy. *J. Neurosci.*, **25**, 8391-8401.
- [150] Silva, A. P., Xapelli, S., Grouzmann, E., Cavadas, C. (2005) The putative neuroprotective role of neuropeptide Y in the central nervous system. *Curr. Drug Targets CNS Neurol. Disord.*, **4**, 331-347.
- [151] Statler, K. D. (2006) Pediatric posttraumatic seizures: Epidemiology, putative mechanisms of epileptogenesis and promising investigational progress. *Dev. Neurosci.*, **28**, 354-363.
- [152] Stavridis, M. P., Smith, A. G. (2003) Neural differentiation of mouse embryonic stem cells. *Biochem. Soc. Trans.*, **31**, 45-49.
- [153] Stein, A. G., Eder, H. G., Blum, D. E., Drachev, A., Fisher, R. S. (2000) An automated drug delivery system for focal epilepsy. *Epilepsia Res.*, **39**, 103-114.
- [154] Studer, F. E., Fedele, D. E., Marowsky, A., Schwerdel, C., Wernli, K., Vogt, K., Fritschy, J.-M., Boison, D. (2006) Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. *Neuroscience*, **142**, 125-137.
- [155] Tatemoto, K. (1982) Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proc. Natl. Acad. Sci. USA*, **79**, 5485-5489.
- [156] Thompson, K., Anantharam, V., Behrstock, S., Bongarzone, E., Campagnoni, A., Tobin, A. J. (2000) Conditionally immortalized cell lines, engineered to produce and release GABA, modulate the development of behavioral seizures. *Exp. Neurol.*, **161**, 481-489.
- [157] Thompson, K. W., Suchomelova, L. M. (2004) Transplants of Cells Engineered to Produce GABA Suppress Spontaneous Seizures. *Epilepsia*, **45**, 4-12.
- [158] Thompson, K. W. (2005) Genetically engineered cells with regulatable GABA production can affect afterdischarges and behavioral seizures after transplantation into the dentate gyrus. *Neuroscience*, **133**, 1029-1037.

- [159] Tian, G. F., Azmi, H., Takano, T., Xu, Q. W., Peng, W. G., Lin, J., Oberheim, N., Lou, N. H., Wang, X. H., Zielke, H. R., Kang, J., Nedergaard, M. (2005) An astrocytic basis of epilepsy. *Nat. Med.*, **11**, 973-981.
- [160] Toma, J. G., Akhavan, M., Fernandes, K. J. L., Barnabe-Heider, F., Sadikot, A., Kaplan, D. R., Miller, F. D. (2001) Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat. Cell Biol.*, **3**, 778-784.
- [161] Toma, J. G., McKenzie, I. A., Bagli, D., Miller, F. D. (2005) Isolation and characterization of multipotent skin-derived precursors from human skin. *Stem Cells*, **23**, 727-737.
- [162] Ulrich, H., Majumder, P. (2006) Neurotransmitter receptor expression and activity during neuronal differentiation of embryonal carcinoma and stem cells: from basic research towards clinical applications. *Cell Prolif.*, **39**, 281-300.
- [163] van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D., Gage, F. H. (2002) Functional neurogenesis in the adult hippocampus. *Nature*, **415**, 1030-1034.
- [164] Vezzani, A., Sperk, G., Colmers, W. F. (1999) Neuropeptide Y: emerging evidence for a functional role in seizure modulation. *Trends Neurosci.*, **22**, 25-30.
- [165] Vezzani, A., Moneta, D., Mule, F., Ravizza, T., Gobbi, M., French-Mullen, J. (2000) Plastic changes in neuropeptide Y receptor subtypes in experimental models of limbic seizures. *Epilepsia*, **41** (Suppl. 6), S115-121.
- [166] Vezzani, A., Sperk, G. (2004) Overexpression of NPY and Y2 receptors in epileptic brain tissue: an endogenous neuroprotective mechanism in temporal lobe epilepsy? *Neuropeptides*, **38**, 245-252.
- [167] Wichterle, H., Lieberam, I., Porter, J. A., Jessell, T. M. (2002) Directed differentiation of embryonic stem cells into motor neurons. *Cell*, **110**, 385-397.
- [168] Williams, M., Jarvis, M. F. (2000) Purinergic and pyrimidinergic receptors as potential drug targets. *Biochem. Pharmacol.*, **59**, 1173-1185.
- [169] Winn, S. R., Lindner, M. D., Lee, A., Haggett, G., Francis, J. M., Emerich, D. F. (1996) Polymer-encapsulated genetically modified cells continue to secrete human nerve growth factor for over one year in rat ventricles: behavioral and anatomical consequences. *Exp. Neurol.*, **140**, 126-138.
- [170] Winn, S. R., Emerich, D. F. (2005) Managing chronic pain with encapsulated cell implants releasing catecholamines and endogenous opioids. *Front. Biosci.*, **10**, 367-378.
- [171] Woldbye, D. P., Larsen, P. J., Mikkelsen, J. D., Klemp, K., Madsen, T. M., Bolwig, T. G. (1997) Powerful inhibition of kainic acid seizures by neuropeptide Y via Y5-like receptors. *Nat. Med.*, **3**, 761-764.
- [172] Woldbye, D. P., Kokaia, M. (2004) Neuropeptide Y and seizures: effects of exogenously applied ligands. *Neuropeptides*, **38**, 253-260.
- [173] Woldbye, D. P., Nanobashvili, A., Sorensen, A. T., Husum, H., Bolwig, T. G., Sorensen, G., Ernfor, P., Kokaia, M. (2005) Differential suppression of seizures via Y2 and Y5 neuropeptide Y receptors. *Neurobiol. Dis.*, **20**, 760-772.
- [174] Yenari, M. A., Zhao, H., Giffard, R. G., Sobel, R. A., Sapolsky, R. M., Steinberg, G. K. (2003) Gene therapy and hypothermia for stroke treatment. *Ann. N. Y. Acad. Sci.*, **993**, 54-68; discussion 79-81.
- [175] Young, D., Dragunow, M. (1994) Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. *Neuroscience*, **58**, 245-261.
- [176] Zaman, V., Shetty, A. K. (2003) Pretreatment of donor cells with FGF-2 enhances survival of fetal hippocampal CA3 cell transplants in the chronically lesioned young adult hippocampus. *Exp. Neurol.*, **183**, 11-24.
- [177] Zhao, L. R., Duan, W. M., Reyes, M., Keene, C. D., Verfaillie, C. M., Low, W. C. (2002) Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Exp. Neurol.*, **174**, 11-20.

Received: December 12, 2006

Revised: March 07, 2007

Accepted: March 08, 2007