Third Conference
on
PROGRESS IN EPILEPSY
AND ANTIEPILEPTIC DRUGS

Lublin, November 17, 2009

Organizers: Lublin Scientific Society
Section of Pharmacology, Committee of Physiology, Polish Academy of Sciences
Polish Pharmacological Society
Department of Pathophysiology, Medical University of Lublin

Sponsored by:
UCB Pharma
Sanofi-Aventis
Ministry of Science and Higher Education
The communications presented at the Conference are printed without alterations from the manuscripts submitted by the authors, who bear the full responsibility for their form and content.
The glutamatergic tripartite synapse: the role of astrocytes in epilepsy

Jan Albrecht

Department of Neurotoxicology, M. Morsakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5, PL 02-106 Warszawa, Poland

For decades, a synaptic ending in the central nervous system has been considered to be dipartite and limited to the nerve cell, consisting of the presynaptic part where a neurotransmitter is released and the postsynaptic part where the neurotransmitter reacts with receptors. Discoveries of the last two decades of the 20th century have added a new partner to the synapse which allowed to define it as tripartite; it turned out that astrocytic processes enwrapping the pre- and postsynaptic area of a glutamatergic ending are capable of receiving signals from a neuron and transmitting them into both the presynaptic and postsynaptic receptors of the nerve ending. Astrocytes possess a spectrum of neurotransmitter receptors including metabotropic glutamate receptors (mGluR) [Pearce et al., Neurosci Lett, 1986], the activation of which mobilizes calcium waves, making the electrically inert cells “excited” [Cornell-Bell et al., Science, 1990]. In a seminal study performed on a neuronal-glial coculture Parpura et al. [Nature, 1994] demonstrated that astrocytic calcium wave is transferred to adjacent neuron in a process involving glutamate release and activation of neuronal glutamate receptors.

Astrocyto-neuron calcium signal transfer was also recorded in an electrically stimulated hippocampal slice [Porter and McCarthy, J Neurosci, 1996], and was shown to be specifically mediated by mGluR [Porter and McCarthy, 2004; Perea and Araque, Science, 2007]. Stimulated astrocytes recruit calcium both from internal stores (mitochondria, endoplasmic reticulum) and from the extracellular space [reviewed by Reyes and Parpura, Neurochem Int, 2009]. Glutamate to be released from astrocytes is packed to structures which strikingly resemble synaptic vesicles, and the mechanism by which glutamate is packed, the vesicles docked to the cell membrane and then glutamate is ejected, involves a machinery almost identical to that operating in the nerve endings [Montana et al., J Neurosci, 2004; Glia, 2006]. Astrocytic processes are spatially adjusted to transmit signals in the most economical way: they are segregated into microdomains, each astrocyte docking many dendrites and forming an autonomous structure defined as “synaptic island” [Halassa et al., Trends Mol Med, 2007]. Most recently it has been shown that the “quasisynaptic” mechanism underlying glutamate release from astrocytes also holds for the glia-derived NMDA receptor agonist, D-serine [Martineau et al., Glia, 2008].

Excessive glutamatergic transmission is a key pathogenic factor in epilepsy: epileptic seizures are closely correlated with a rapid increase of extracellular glutamate, a pool ready for interaction with glutamate receptors [During and Spencer, 1993]. Synthesis and degradation of glutamate involves a cooperative action of astrocytes and neurons in the so-called “glutamate-glutamine cycle”. Glutamine is synthesized in astrocytes from glutamate and ammonia by glutamine synthetase (GS), and then transferred to neurons where it is metabolized to glutamate and ammonia by phosphate-activated glutaminase (PAG). Glutamate so formed feeds a major proportion of the neurotransmitter pool of glutamate. There is evidence that excessive accumulation of glutamate in epilepsy is due to a slow down of glutamate recycling to glutamine [Petroff et al., Epilepsia, 2002]. In epileptic hippocampus, this slow down appears to be due to both decreased GS [Eid et al., Lancet, 2004], and increased PAG activity [Eid et al., Acta Neuropathol, 2007]. Decreased GS activity in rat cerebral cortex and hippocampus of rat with PTZ-induced epilepsy is associated with increased tyrosine nitrosylation on the enzyme, a straight consequence of increased oxidative/nitrosative stress [Bidmon et al., Epilepsia, 2008]. Apart from contributing to the dysfunction of the glutamate-glutamine cycle, reactive astrocytes residing in epileptic foci appear to be directly involved in the increase of glutamatergic activity. Paroxysmal depolarization shifts (PDSs) in hippocampal slices evoked by 4-aminopyridine are not blocked by TTX indicating that they do not require neuronal firing, but rely on increased glutamate release from astrocytes [Tian et al., Nat Med, 2005]. Interestingly, major antiepileptic drugs (valproate, gabapentin, phenytoin) ameliorate ATP-evoked calcium signal in astrocytes of the rat sensory cortex in situ [Ding et al., 2007]. Durable spontaneous calcium oscillations in mice with pilocarpine-induced status epilepticus have been shown to be associated with activation of mGluR on...
Astrocytes [Ding et al., J Neurosci, 2007]. Astrocytic dysfunction in epilepsy is associated with profound structural changes: in rat, epileptic discharges following mechanical brain damage or in the kainate model are correlated with the loss of microdomain organization [Oberheim et al., J Neurosci, 2008]. Hence, astrocytes are not able anymore to dock the dendrites properly. Taken together, the state of the art points to astrocytes as a potential target of therapy in epilepsy.

Supported by the Ministry of Science and Education of Poland, grant no S 005/P-N/2007/01.

Lacosamide action on sodium channels

Marcin Balcerzak
UCB Pharma/VEDIM Sp. z o.o., Medical Affairs Department, Warszawa, Poland

Lacosamide ((R)-2-acetamido-N-benzyl-3-methoxypropionamide) is a D-serine derivative approved as an antiepileptic drug (AED) in adjunctive therapy of partial-onset seizures. Lacosamide acts on voltage-dependent sodium channels responsible for action potential initiation and propagation. Sodium channels can exist in different conformations depending on cell membrane potential and accounting for specific functional states. Membrane depolarization causes sodium channels to transition from a resting to an open state, allowing sodium ion influx according to the electrochemical gradient and generating an action potential. Potassium conductance is activated at the peak membrane potential and at the same time sodium channels shift into the fast-inactivated state (occurring within milliseconds). During fast inactivation, a cytoplasmic region of the channel occludes the pore by binding to a docking site, thus preventing sodium ions from entering the cell. This allows for a physiological mechanism to avoid action potential back-propagation and generates impulses with certain amplitude and frequency. Carbamazepine (CBZ), phenytoin (PHE) and lamotrigine (LTG) bind preferentially to the fast-inactivated state [Kuo, Mol Pharmacol, 1998] and exert their anticonvulsant effect by increasing the number of channels in the inactivated state and by delaying the recovery and transition to the resting state. In sustained repetitive firing experiments performed on pyramidal neurons using whole-cell patch-clamp technique, lacosamide inhibits the firing rate of action potentials after seconds in contrast to fast-inactivation modifying antiepileptic agents (CBZ, LTG, PHE), which inhibited firing within about 100 ms after burst initiation [Errington et al., Mol Pharmacol, 2008]. These results suggest that the mechanism of action of lacosamide is different from other anticonvulsive sodium channel blockers.

In addition to fast inactivation, another mechanism of sodium channel modulation discovered in 1978 [Rudy et al., J Physiol, 1978] likely involves channel structural rearrangement that occurs under conditions of slight depolarization and prolonged repetitive firing. This process called “slow inactivation” develops over a timescale of seconds to minutes. In experiments performed on cloned sodium channels or channels endogenously expressed in cells, lacosamide was able to shift the voltage dependence of slow inactivation to more hyperpolarized potentials (increasing the number of channels in the slow inactivated state) without affecting the fast inactivated state [Errington et al., Mol Pharmacol, 2008; Sheets et al., J Pharmacol Exp Ther, 2008; Wolff et al., Epilepsia 2009, supp. 10]. In vitro experiments have similarly indicated that lacosamide at clinical concentrations modulates sodium channels in a slow inactivated state, reducing channel availability with no effect on fast inactivation of sodium channels.

Lacosamide enhances slow inactivation of neuronal sodium channels and is proposed to exert its antiepileptic effect primarily via this mechanism in pathological situations: (1) when neurons are slightly depolarized having reduced activation threshold or (2) when neurons repeatedly depolarize participating in seizure activity.
Epilepsy and allergy

Barbara Błaszczyk1,2

1 Department of Neurology, Neuropsychiatric Hospital, Grunwaldzka 47, PL 25-736 Kielce, Poland
2 Faculty of Health Sciences, Higher School of Economics and Law, Jagiellońska 109 A, PL 25-734 Kielce, Poland

Epilepsy is a common neurological disorder affecting about 1% of the population.

The World Health Organization (WHO) estimates that there are some 50 million people with epilepsy worldwide. Each year, there are some 2 million new cases. About 70% of patients receiving antiepileptic drugs (AEDs) have a good control of epilepsy, but in some of them idiosyncratic drug reactions are observed. Idiosyncratic drug reactions are unexpected and unpredictable adverse reactions, fundamentally different from dose-related side effects of drugs. A variety of idiosyncratic reactions may be seen, such as aplastic anemia, acute liver failure, and rash. Genetic factors may be important in many of these events. Drug-induced rashes are the most common type of idiosyncratic reaction due to use of AEDs.

The risk for adverse cutaneous reactions to medications has been reported to be 2–3% in hospitalized patients [Griebel, Epilepsia, 1998]. AEDs have long been recognized as being among the most common medications associated with severe cutaneous adverse reactions (SCARs), with relative risks reported to be 15, 11, and 13 for phenobarbital (PB), carbamazepine (CBZ), and phenytoin (PHT), respectively [Shear and Spielberg, Clin Invest, 1988].

Fortunately, many cutaneous reactions to AEDs are not severe. They are most commonly exanthematous or morbilliform and fade within a few days without consequence.

It may be difficult to determine initially whether a rash indicates the potential for a more serious reaction. However, association of the rash with fever, lymphadenopathy, mucosal involvement, facial edema, purpura, blisters, or urticaria usually mandates cessation of the AED. SCARs have been classified as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), or SJS-TEN overlap syndrome, depending on the extent of epidermal detachment: <10% in SJS, 10–30% in SJS TEN overlap, and ~30% in TEN [Roujeau, J Invest Dermatol, 1994]. The AED hypersensitivity syndrome (AHS), which is believed by some investigators to represent a separate entity, is associated with an erythematous morbilliform eruption which can develop into an exfoliative dermatitis. These entities share some common features, such as fever and hepatitis, but other features differ, such as degree of mucosal involvement, which is less remarkable in AED hypersensitivity syndrome (AHS) than in SJS-TEN. The time course of development of eruptions is also different; being delayed in AHS. Prognosis is also variable, with mortality rates ranging from 4% in SJS to 10% in AHS and to 30% in TEN. Although the rash in AHS may initially appear benign, the syndrome complex has been defined as including not only the rash and fever but also systemic toxicity. Multiple organs in addition to the skin can be involved, including the liver, kidneys, and lungs. Lymphadenopathy and blood dyscrasias are common [Chang and Shear, Semin Neurol, 1992; Griebel, Epilepsia, 1998].

AHS is an adverse drug reaction associated with the aromatic antiepileptic drugs (AEDs) phenytoin (PHT), carbamazepine (CBZ), phenobarbital (PB), and primidone (PRM). It is usually defined by the triad of fever, skin rash, and symptomatic or asymptomatic internal organ involvement. Even now, many practitioners still appear to be unfamiliar with AHS. Correct diagnosis of AHS may be difficult because of the wide variety of possible clinical and laboratory abnormalities and manifestations [Vittorio and Muglia, Arch Intern Med, 1995] and because the syndrome may mimic infectious, neoplastic, or collagen vascular disorders [Licata and Louis, Comprehens Ther, 1996]. Further complicating the situation may be the lack of a generally accepted nomenclature. Several terms, such as “Dilantin hypersensitivity reaction”, “phenytoin/Dilantin syndrome”, “Kawasaki-like syndrome”, “hypersensitivity to aromatic anti-convulsant agents”, or “mononucleosis-like syndrome”, have been used. Because misdiagnosis or delayed diagnosis has resulted in death, for a correct diagnosis it is crucial to define a standardized nomenclature and definition of AHS [Schlienger and Shear, Epilepsia, 1998]. The incidence of the syndrome is unclear because its variable presentation, diverse clinical features, and laboratory abnormalities have led to inaccurate reporting [Vittorio and Muglia, Arch Intern Med, 1995]. In a recent record linkage study, the risk for developing an AHS within 60 days of the first or second prescription in new users of PHT
or CBZ was estimated to be 2.3–4.5 per 10,000 and 14.1 per 10,000, respectively. Whether the incidence of LTG-induced AHS is comparable to that of older aromatic AEDs is not known [Schlienger and Shear, Epilepsia, 1998]. After the occurrence of AHS associated with PHT, CBZ or PB, it is important to reassess the necessity for use of an AED. If seizure control is needed, then alternative drug therapy should be chosen. It must be remembered that older aromatic AEDs exhibit high degree of cross-reactivity and that PRM is metabolized, in part, to PB. It remains unclear whether LTG might be a safe alternative in these patients, because LTG is usually considered as a safe alternative in these patients. However, at least one case report of AHS probably related to VPA therapy has been published.

SJS and TEN (Lyell syndrome) are severe albeit rare adverse drug reactions to several AED such as PHT, CBZ, PB, and VPA [Schlienger et al., Epilepsia, 1998; Gilman and Duchowny, Epilepsia, 1998].

Therefore, it is important for the practitioner who prescribes AEDs to understand the spectrum of cutaneous drug reactions, to recognize the symptoms when they occur, and to be able to manage the patient clinically. Because a hereditary component is involved in the development of AHS, first-degree relatives of patients who experienced AHS should be informed about the increased risk for AHS in response to aromatic AEDs. In most situations, it is suggested that a patient who has had AHS should avoid PHT, PB, CBZ, and PRM. One is comfortable about recommending the other AEDs [Schlienger and Shear, Epilepsia, 1998].

Neuroprotection and epileptogenesis

Stanis³aw J. Czuczwar¹,²

¹Department of Pathophysiology, Medical University, Jacekwskiego 8, PL 20-690 Lublin, Poland
²Department of Physiopathology, Institute of Agricultural Medicine, Jacekwskiego 2, PL 20-690 Lublin, Poland

Some antiepileptic drugs, particularly diazepam, valproate, lamotrigine, and topiramate have been documented to provide neuroprotection in animal models of status epilepticus [Trojnar et al., Pol J Pharmacol, 2002; Stepien et al., Pharmacol Rev, 2005]. With the discovery of neuroprotection a possibility was considered whether the neuroprotective effects of antiepileptic drugs would result in an inhibition of epileptogenesis.

If this assumption were correct than some antiepileptic drugs could be ascribed clear-cut curative activities in epilepsy.

To verify the hypothesis that neuroprotection by antiepileptic drugs in the brain is positively correlated with the reduced epileptogenesis a number of experiments were carried out with the use of experimental status epilepticus in rats and subsequent administration of antiepileptic drugs. Next, the occurrence of spontaneous seizures and cognitive performance were evaluated.

Bolanos et al. [Neurology, 1998] have evaluated phenobarbital and valproate, given for 40 days after kainate-induced status epilepticus. Phenobarbital was administered ip in a dose of 70 mg/kg for 30 days, then 35 mg/kg for 5, and 17.5 mg/kg for the last 5 days. Valproate – 600 mg/kg ip twice daily for 30 days, 300 mg/kg twice daily for 5 days, and 300 mg/kg once a day for 5 days. Phenobarbital-treated rats, similarly to control animals, exhibited recurrent seizures (evaluated once a week within the period of the drug administration), neurodegeneration in the hippocampus and dentate hilus, and impaired performance in the water maze test (measured one week after stopping the antiepileptic drug). In contrast, valproate-treated animals were not spontaneously seizing, their hippocampal lesions were reduced and the water maze test evaluating visuospatial learning was performed without any deficit. Also, gabapentin was tested in a similar experimental paradigm – first 200 mg/kg, ip (30 days), then 100 mg/kg (5 days) and eventually,
50 mg/kg (5 days). The outcome indicates that gabapentin reduced the incidence of spontaneous convulsions and the extent of neuronal degeneration along with the better learning in the water maze test [Cilio et al., Neuropharmacology, 2001]. Halonen et al. [Epilepsy Res, 2001] induced status epilepticus in rats via electric stimulation of the perforant pathway in rats. Carbamazepine administration (30 mg/kg ip, twice daily) started 3 days prior to status epilepticus and continued for two weeks after the status. Lamotrigine (12.5 mg/kg, twice a day) was either initiated 3 days before or started one hour following the status and was also extended for two weeks. Spontaneous seizures were not evaluated, however, the pretreatment with carbamazepine was ineffective as regards neuronal damage in the hilus and did not prevent status epilepticus-produced deficit in the water maze performance. Lamotrigine was found moderately neuroprotective but, similarly to carbamazepine, did not improve spatial memory deficit. In the kainate model of temporal lobe epilepsy in rats, the N-methyl-D-aspartate receptor antagonist, dizocilpine (MK-801), distinctly protected against neurodegeneration in the hippocampus and piriform cortex but did not stop the occurrence of spontaneous recurrent seizures [Brandt et al., Neuropharmacology, 2001]. Brandt et al. [Neuropharmacology, 2006] also used a model of status epilepticus resulting from prolonged electrical stimulation of the rat basal amygdala which was terminated by diazepam after 4 h. Then, ip injections of valproate followed, 400 mg/kg in a bolus and next, 3 times daily of 200 mg/kg for 4 weeks. Again, there were potent neuroprotective effects of valproate in the hippocampal formation with an inclusion of the dentate hilus but spontaneous seizures were not prevented. Anyway, functional outcome was significantly improved. Diazepam was given only once in a dose of 20 mg/kg, 2 or 3 h after the onset of status epilepticus in rats produced by electrical stimulation of the amygdala [Pitkanen et al., Epilepsy Res, 2005]. Whilst 94% of the animals in the control (unprotected) group developed spontaneous convulsions, diazepam (2 h) reduced this percentage to 42 and diazepam (3 h) – to 71. Also, the spontaneous seizures in the diazepam (particularly 2 h group) were less frequent and the severity of the hippocampal neurodegeneration was considerably reduced. Rigoulot et al. [J Pharmacol Exp Ther, 2004] administered diazepam (two injections of 2.5 mg/kg, im on the day of status epileptics induced by lithium and pilocarpine in rats) or topiramate (two injections of 10, 30 or 60 mg/kg on the day of the status and then twice daily for 6 days). Although the topiramate-treated groups exhibited significant neuroprotection in the hippocampus, all rats surviving status epilepticus developed spontaneous seizures. According to Andre et al. [Epilepsia, 2007], the enthorhinal and piriform cortices are associated with the early phase of epileptogenesis whilst the hippocampal hilus seems to be involved in the initiation and/or maintenance of seizures. This conclusion is based on the fact that pregabalin (50 mg/kg ip followed by 10 mg/kg) given chronically after the induction of lithium-pilocarpine status epilepticus in rats exerted neuroprotective effects in both enthorhinal and piriform cortices which was correlated with a delay in epileptogenesis. Neuroprotection limited to the hippocampal fields CA_1 and/or CA_3 (chronic topiramate 10–60 mg/kg or vigabatrin 250 mg/kg) was without any effect upon the latency to spontaneous seizure activity. There are also data available that the free radical scavenger, N-tert-butyl-α-phenylnitrone, reduced neuronal injury in immature rats after lithium-pilocarpine status epilepticus which was correlated with better cognitive functions but on the other hand, enhanced epileptogenesis was observed [Kubova et al., Pharmacol Rep, 2007]. The available results on the association between neuroprotection and epileptogenesis are not unequivocal. Some authors represent opinions that neuroprotection may be positively correlated with cognitive functions without any major effect on epileptogenesis [Brandt et al., 2006] whilst many of them suggest no correlation between neuroprotection against status epilepticus and acquired spontaneous seizure activity [Brandt et al., 2003; Rigoulot et al., 2004]. Some authors did report a relationship between neuroprotection and a subsequent reduction in spontaneous seizures [Bolanos et al., 1998; Cilio et al., 2001; Pitkanen et al., 2005]. However, antiepileptic drugs were administered in high doses for a very long period of time [Bolanos et al., 1998; Cilio et al., 2001]. The only exception is diazepam which was given only once but in very high dose [Pitkanen et al., 2005]. Interestingly, diazepam at a much lower daily dose of 5 mg/kg did not prevent the development of spontaneous seizure activity in rats surviving status epilepticus.
Majority of patients with progressive facial hemiatrophy (Parry-Romberg syndrome) are affected at developmental age. Progressive destruction concerns the subcutaneous tissue, cartilage and bone structures. Various neurological (seizures, migraine-type headache, trigeminalgia) and ophtalmological manifestations were observed in this syndrome and sometimes occurred prior to the facial hemiatrophy [Yano et al., Pediatr Neurol, 2000; Cory et al., AJNR, 1997]. Other neurological symptoms reported were pyramidal tract impairment, dysphonia, diplopia, progressive atrophy of the orbit and ocular motor nerve dysfunction.

A case history of 12-year-old boy with progressive facial hemiatrophy, syringomyelia and epileptic seizures preceded by headaches is presented herein. Two years ago this boy with Parry-Romberg syndrome was admitted because of extremely severe headache attacks associated with vomiting, which appeared few days before hospitalization. Physical examination on admission revealed hyperpigmentation of suboccular region and cheek, left enophthalmus and smaller eyeball. Abnormalities of skeletal systems involved short neck, limited movement in the humeral joint and pathological gait pattern (with forward bending of the head and trunk). Additionally, thorough skin examination revealed cafe-au-lait spots. Optic disc examination was normal. Family history of neurological disorders including migraine and epilepsy was negative.

Thorough neuroimaging diagnostics was performed. MR of head revealed numerous abnormalities concerning soft tissues and bone structures of the left affected side of the head. MR of the cervical part of spinal cord revealed Arnold Chiari malformation type I and syringomyelia. Reduction of middle cerebral arteries peripheral branches, pathological course of vertebral arteries and asymmetry of posterior communicating arteries were found in MRA. HMRS revealed lesion of left part of cerebellum suggested ischemia or necrobiosis. Due to facial deformation the boy was referred to plastic surgeon and invasive treatment was suggested after cessation of bone growth.

During hospitalization, severe headaches associated with sweating and reddening of the face, hot flashes and with hyperventilation, were observed several times. However, three of such headache attacks were followed soon by partial complex seizures. The EEG pattern revealed localized discharges of sharp waves and single sharp and slow wave complex, in right back temporal and parieto-occipital region with tendency to synchronization during hyperventilation and photostimulation. Antiepileptic treatment with carbamazepine was instituted (13 mg/kg/24 h) with initial good effect, subsidence of headache attacks and epileptic seizures for one month. During next two months, the boy was admitted to local hospital four times due to recurring headache attacks and epileptic seizures, usually complex partial and three times secondary generalized tonic seizures. All of epileptic seizures were preceded with headache attacks accompanied by autonomic symptoms and hyperventilation and usually occurred in the morning. An increase of carbamazepine dose was instituted (18 mg/kg/24 h). On the following day the boy was admitted to our department and similarly headache attacks sometimes followed by epileptic seizures (usually complex partial, once generalized tonic seizure) were observed. Carbamazepine was replaced by oxcarbazepine (27 mg/kg /24 h). After treatment modification, headaches were observed several times but less intense and subsided without treatment usually during interactions with other patients.

Nevertheless one week later the boy was admitted to our department again due to recurrent headaches and optic discs blurred margins. Control ophthalmological examination performed two weeks later was normal. Visual evoked potentials were recorded and revealed elongation of P100 latency over the left hemisphere. Values of brainstem auditory and somatosensory evoked potentials were within normal limits. Oxcarbazepine dose was increased to 32 mg/kg/24 h. Since last hospitalization no headache attacks as well as epileptic seizures has been observed.

The patient reported in this paper presented headache attacks accompanied by reddening, sweating of the face and hot flashes associated with complex partial seizures and sometimes followed by generalized tonic seizures. Autonomic symptoms observed in this
case can be considered as vegetative part of epileptic attack or as isolated equivalent of epilepsy. The symptoms were bilateral however the EEG revealed localized discharges in the right hemisphere, contralateral to the hemifacial atrophy. Clinical migraine-like headaches coexist in this case with syringomyelia, mild Arnold Chiari malformation, middle cerebral and vertebral arteries abnormalities and their influence on headache features is hard to define [Headache Classification Committee of The International Headache Society, Cephalalgia, 2004]. Occurrence of migraine in patients with Parry-Romberg syndrome was reported in the literature as well as different intracranial vessels abnormalities, but localized on side of the facial hemiatrophy [Pichiecchio et al., Neurology, 2002].

In the reported case epileptic seizures were always preceded with headaches. Induction of epileptic seizure by headache attack or consideration of headache attack as a part of epileptic seizure is possible. Parry-Romberg syndrome is known as a rare syndrome frequently associated with epilepsy, most often focal and particularly sensory focal. In some patients epilepsy is easily controlled but in others is drug resistant and need surgical treatment [Chbicheb et al., Rev Neurol (Paris), 2005; DeFelipe et al., Epilepsia, 2001]. On the basis of histological study of resected brain tissue of the patient with focal epilepsy vascular dysgenesis is suggested referring to neurodevelopmental theory of Parry-Romberg syndrome [Chbicheb et al., Rev Neurol (Paris), 2005]. After surgery patients were seizures free, what suggested presence of epileptic focus within the resected tissue [DeFelipe et al., Epilepsia, 2001]. Parry-Romberg syndrome is often associated with brain lesions and pathogenetic correlation even with Rasmussen disease was proposed [Shah et al., Neurology, 2003]. Contrary to these observations MR imaging of the brain in our 12-year-old patient was normal. On the other hand this patient presented syringomyelia, which in our estimation is the first report of Parry-Romberg syndrome associated with this lesion, however in 1979 year a case of Parry-Romberg with syringobulbia was reported [Tebloev and Klashnikov, Zh Nevropatol Psikhiatr Im S S Korsakova, 1979]. Localization and activation of the trigeminal nerve nucleus, trigeminal nerve and cranial para-sympathetic outflow through the trigeminal-autonomic reflex should be considered as a possible cause of the headache in such case. Our patient did not have any motor-sensory signs or other spinal dysfunction, and the neuroimaging revealed also downward displacement of cerebellar tonsils bellow foramen magnum, which may cause headache. However it seems to be that vegetative cause of the headache in this child is more probable.

The potential role of hyperactivity of the sympathetic nervous system in pathogenesis of facial hemiatrophy was stressed by other authors [Drummond et al., Cephalalgia, 2006]. Superior cervical ganglion, medial and lateral internal carotid plexus, and distal branches innervating cerebral arteries may be involved in this mechanism. It is possible that an inflammatory process triggers chronic sympathetic activity causing unilateral facial tissue destruction. Autonomic impairment may explain coexistence ipsilateral migraine-like headache, facial pain or autonomic symptoms with Parry-Romberg syndrome. Bilateral clinical symptoms of sympathetic hyperactivity observed in reported patient obligate to further investigations. This case illustrates unusual correlation of headache attacks associated with autonomic symptoms and epilepsy in Parry-Romberg syndrome, but pathogenesis of this relationship still needs explanation.
Psychiatric disorders in children and adolescents with epilepsy: selected aspects of the etiology, diagnosis and treatment

Maria M. Kaczyńska-Haładyj

Neuropsychiatric Department for Children, Neuropsychiatry Hospital, Abramowska 2, PL 20-442 Lublin, Poland

Epilepsy is the most common pediatric neurological disorder. The multiple seizure types characterize childhood-onset epilepsy and they may evolve from one type to another during the course of illness. Children and adolescents with seizures are at increased risk for psychiatric disorders in epilepsy. The prevalence rates of psychiatric disorders have fluctuated in the wide ranges from 12% to 77% [Caplan et al., Epilepsia, 2005; Dunn et al., Dev Med Child Neurol, 2003; Ettinger et al., Epilepsia, 1998; Kanner, Epilepsy Behav, 2000; Piazziini et al., Epilepsy Behav 2001; Plioplys, Epilepsy Behav 2003; Plioplys et al., J Am Acad Child Adolesc Psychiat, 2007; Steffenburg et al., Dev Med Child Neurol, 2003; Williams et al., Epilepsy Behav 2003]. This wide range of prevalence rates of psychopathology in pediatric epilepsy reflects methodological differences across these studies. A variety of coexisting psychiatric disorders associated in children and adolescents with epilepsy, including mood disorder, anxiety, adjustment disorder, psychosis, autism spectrum disorders and attention-deficit hyperactivity disorder. The high prevalence of mental health problems in children with epilepsy has not changed over the past 30 years. The risk for comorbid psychiatric disorders in pediatric epilepsy is three to six times that of general population and significantly higher than that of children with other pediatric chronic disorders [Austin et al., Epilepsia, 1996; Austin et al., Epilepsia, 2000; Davies et al., Dev Med Child Neurol, 2003]. Children with developmental disabilities are more likely than normal children to develop epilepsy [Goulden et al., Epilepsia, 1991; Steffenburg et al., Dev Med Child Neurol, 2003]. A number of approaches have been developed to explain the multifactorial etiology of psychiatric comorbidity in epilepsy in children and adolescents and probably involves both neurobiologic and psychosocial factors [Piazziini et al., Epilepsy Behav, 2001]. The relationship among pediatric epilepsy and psychiatric disorders appears to be complex interactions between multiple etiological variables, illustrating current conceptualization of adjustment to pediatric chronic disorder. The presence of one or more coexisting psychiatric symptoms may complicate seizure control. Psychopathology arises from complex interactions between CNS, cognitive, linguistic, family variables and as the influence of antiepileptic drugs. The diagnosis of psychiatric disorders in pediatric patients is complex. At the time of the initial diagnostic evaluation, the physician who is managing a child or a adolescent with seizure disorder should make a psychiatric and psychological assessment to identify the patient’s risk for psychiatric disorder. The psychiatric disorders remain underdiagnosed and undertreated in children and young people with epilepsy [Caplan et al., Epilepsia, 2005; Dunn and Austin, Neurology, 1999; Ott et al., Epilepsia, 2003; Pellock, Epilepsy Behav, 2004]. The developmental variations of age specific psychopathology of developmental psychiatric disorders in childhood and adolescence, their specific clinical manifestations and tendency to comorbidities, multi predictors of clinical course should be considered individually in every case. Potential epilepsy-related variables include: age of onset, frequency and severity of seizures, type of seizure disorder, and type and antiepileptic drug [Lambert et al., Epilepsia, 1999; Devinsky, Epilepsia, 1995]. A question asked by many clinicians, which has still not been answered definitively, is whether a child with focal epilepsy or one with generalized epilepsy is more likely to exhibit a behavioral disturbance.

In addition, location of the seizure focus may affect predisposition to psychiatric comorbidity. Patients with seizures that are of temporal and frontal lobe origin are disproportionately affected by psychiatric comorbidity [Kanner, Epilepsia, 2003]. Potential psychosocial determinants are believed to include: increased perceived stigma, elevated number of stressful life events during the past year, poor adjustment to epilepsy, financial stress, vocational problems, external locus of control, and an earlier onset of epilepsy [Hermann et al., Br J Psychiat, 1990]. Optimal diagnosis, clinical evaluation, and choice of treatment are predicated on the proper identification of coexisting psychiatric disorders in childhood epilepsy. The goal of pharmacological therapy in pediatric epilepsy and
comorbid psychiatric disorders is to optimize management, prevent seizures and as is the identification and treatment of comorbid condition. Pediatric psychiatric disorder is treatable with both psychotherapeutic and pharmacotherapeutic approaches. Medication should be used cautiously because some of the drugs may lower seizure threshold.

SV2A protein: role in the anticonvulsant mechanism of action of levetiracetam

Rafal M. Kaminski

Levetiracetam (Keppra®) is characterized by a unique profile of activity in experimental models of epilepsy as it is devoid of protective effects in two classical, acute seizure screening models for antiepileptic drugs, the maximal electroshock (MES) and pentylentetrazol (PTZ), while it displays robust efficacy in epileptic animals with focal and generalized seizures. This anticonvulsant activity of levetiracetam in preclinical models fully translates into broad spectrum efficacy in patients with epilepsy [Klitgaard, Epilepsia, 2001; Stockis et al., Expert Rev Clin Pharmacol, 2009].

Levetiracetam is known to have a unique and specific binding target in the brain that has recently been identified as the synaptic vesicle protein 2A (SV2A) [Lynch et al., Proc Natl Acad Sci USA, 2004]. SV2A is ubiquitously expressed in the brain, but its function has not been fully elucidated yet. Most information about the role of SV2A in neuronal excitability comes from studies performed with knock-out mice. It appears that SV2A is not crucial for vesicle biogenesis or synaptic function, but modulates exocytosis of transmitter-containing vesicles. Mice lacking SV2A are characterized by a decrease in the calcium-dependent exocytotic burst, which is a measure of the availability of neurotransmitter vesicles ready to release their content. Furthermore, the absence of SV2A results in decreased action potential-dependent neurotransmission, while action potential-independent neurotransmission remains normal [Crowder et al., Proc Natl Acad Sci USA, 1999; Janz et al., Neuron, 1999, Xu and Bajjalieh, Nat Cell Biol, 2001].

It has been well established that the anticonvulsant potency of SV2A ligands in the audiogenic seizure-prone mice correlates with their binding affinity [Lynch et al., Proc Natl Acad Sci USA, 2004]. More recently, we have also confirmed the existence of strong correlation between SV2A binding affinity and anticonvulsant potency in three distinct preclinical models of both partial and generalized epilepsy, which reinforced the significance of this molecular target in the mechanism of action of the tested ligands [Kaminski et al., Neuropharmacology, 2008]. These data also imply that SV2A-mediated mechanisms are equally important in protection against seizures irrespective of the preclinical model, which consequently may extend to a broad spectrum clinical efficacy, as demonstrated by levetiracetam.

SV2A (−/−) mice develop strong seizure phenotype starting very early in their development and do not survive beyond 2–3 weeks after birth [Crowder et al., Proc Natl Acad Sci USA, 1999; Janz et al., Neuron, 1999], while SV2A (+/−) mice, which are deficient in the SV2A protein, develop normally after birth. Since SV2A (−/−) mice cannot be used in pharmacological in vivo experiments due to their premature lethality, we have performed a thorough characterization of SV2A (+/−) mice in binding experiments and several models of epilepsy [Kaminski et al., Epilepsia, 2009].

Binding experiments in SV2A (+/+) wild type and SV2A (+/−) heterozygous mice indicated that the affinity of SV2A ligands remained unaltered and the relative ex vivo SV2A occupancy curves for levetiracetam were completely overlapping. However, SV2A (+/−) mice displayed 50% less sites available for binding of levetiracetam consistently with its proposed mechanism of action [Lynch et al., Proc Natl Acad Sci USA, 2004]. The binding data was corroborated by immunolabeling studies indicating homoge-
nous reduction in SV2A protein expression throughout the brain of SV2A (+/−) mice [Kaminski et al., Epilepsia, 2009].

SV2A (+/−) mice displayed no spontaneous epileptiform activity during long-term video-EEG recordings, but accelerated amygdala kindling development and reduced afterdischarge threshold was observed. Similarly, their seizure thresholds for pilocarpine- and kainate-induced convulsions were lower as compared to wild-type littermates. The threshold dose of intravenous (iv) PTZ required to induce clonic convulsions was also significantly reduced in SV2A (+/−) mice. Thus, the pro-epileptic phenotype of SV2A (+/−) mice observed in kindling, pilocarpine, kainate, PTZ (iv) and 6 Hz models, but not in the MES model, matched very well with the protective activity of levetiracetam in those models [Kaminski et al., Epilepsia, 2009].

The key observation supporting SV2A-related mechanism of action of levetiracetam is the fact that it displayed reduced anticonvulsant efficacy in SV2A deficient mice [Kaminski et al., Epilepsia, 2009]. Levetiracetam failed to produce dose-dependent increases in the threshold for 6 Hz seizures in SV2A (+/−) mice, which contrasted with its effects observed in SV2A wild-type animals. Furthermore, valproate, which has SV2A-unrelated mechanism of action [Lošcher, Prog Neurobiol, 1999; Noyer et al., Eur J Pharmacol, 1995], produced the same dose-dependent 6 Hz threshold increase in both genotypes [Kaminski et al., Epilepsia, 2009].

Several lines of evidence indicate that SV2A protein is the main target for the anticonvulsant action of levetiracetam: 1) SV2A is the unique binding site for levetiracetam and plays an important role in synaptic vesicle function; 2) affinity-potency correlations in several models of partial and generalized epilepsy indicate that SV2A is a broad spectrum anticonvulsant target; 3) SV2A deficiency leads to increased seizure vulnerability together with accelerated epileptogenesis; 4) SV2A plays a key role in mediation of the anticonvulsant action of levetiracetam in vivo.

Seizures and substance abuse. Ethanol withdrawal seizures

Jolanta Kotlińska

Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Stażnica 4, Pl. 200581 Lublin, Poland

Drug abuse may induce many medical complications. One of them is an increased risk for seizures. They can be induced by a variety of mechanisms. Drugs may evoke seizures by indirect mechanisms, such as infection (cerebral complication of endocarditis or AIDS in parenteral drug users), trauma (as a consequence of intoxication or a violence associated with drug use), stroke (hemorrhagic or ischemic), or metabolic derangements (including hyponatremia, hypocalcemia, renal failure and, particularly in alcoholics, hypoglycemia). Depending on the drug type, direct mechanisms can involve either intoxication or, in a subject physically dependent on a drug – withdrawal [Brust, Neurology, 2006]. Overdose of opioids (heroin), psychostimulants (amphetamine-like agents and cocaine), marijuana, hallucinogens (mescaline, LSD), inhalants, phencyclidine or anticholinergics (plants containing scopolamine and atropine) can induce myoclonus or seizures [Brust, Neurology, 2006]. Seizures can also be a feature of withdrawal from sedative and hypnotic agents, such as barbiturates and benzodiazepines [Brust, Neurology, 2006] or ethanol [Victor and Brausch, Epilepsia, 1967].

As mentioned above, seizures are a prominent feature of ethanol withdrawal, most often within the first 36 hours of abstinence, and are frequently preceded by tremor [Victor and Brausch, Epilepsia, 1967]. They usually occur singly (generalized tonic-clonic seizures) or as a brief cluster although partial seizures also occur [Freedland et al., J Emerg Med, 1993; Victor and Brausch, Epilepsia, 1967].
Brust, Neurology, 2006]. Untreated patients sometimes progress to delirium tremens. Moreover, seizures related to alcohol can be caused by a neurotoxic effect of ethanol [Bartolomei et al., Epileptic Disord, 2004] and, very seldom, are induced by a direct effect of alcohol (convulsive inebriation) [Devetag et al., Ital J Neurol Sci, 1983; Hattemer et al., Epileptic Disord, 2008]. Rodent models that mimic human ethanol withdrawal-related tonic-clonic seizures have been useful in defining pathophysiological mechanisms underlying ethanol withdrawal. In these models, animals are exposed to alcohol by intragastric intubation [Majchrowicz, Psychopharmacology, 1975; Kotlinska and Langwinski, Drug Alcohol Depend, 1986; Adams et al., Alcohol Clin Exp Res, 1995], inhalation [Goldstein and Pal, Science, 1971] or feeding in a nutritionally complete liquid diet for periods of 2 to 21 days. The animals exhibit sound-evoked audiogenic seizures or handling-induced convulsions during the 1- to 3-day period after cessation of ethanol intake and may also experience spontaneous generalized seizures.

Audiogenic seizures are the best-studied type of ethanol withdrawal seizures. These symptoms are mediated largely in the brainstem, although the hippocampus may be involved after seizure initiation [Hunter et al., Pharmacol Biochem Behav, 1973]. In rodents [Hunter et al., Pharmacol Biochem Behav, 1973] and in humans [Sand et al., Acta Neurol Scand, 2002] the cortical EEG shows no sign of paroxysmal activity between episodes of alcohol withdrawal-related tonic-clonic seizures. In rodents, electrophysiological studies have demonstrated a critical role for the inferior colliculus (IC) in the initiation of audiogenic seizures. Neurons within the deep layers of the superior colliculus [Yang et al., Brain Res, 2001] and the periaqueductal gray [Yang et al., Neuropharmacology, 2003] also may play a role in the initiation of audiogenic seizures. The deep layers of the superior colliculus send projections directly to the spinal cord via the pontine reticular formation and the periaqueductal gray. The periaqueductal gray is thought to trigger clonic seizures, whereas the pontine reticular formation is implicated in the generation of the tonic phase of audiogenic seizures [Faingold, Prog Neurobiol, 2004]. Some evidence suggests that the IC plays a role in alcohol withdrawal seizures in humans [Hughes and Fino, J Clin Neurophysiol, 1985]. Although the action of ethanol on biological systems largely results from alterations in the fluidity of cell membrane (as a member of a group of anesthetic substances), it has been more recently postulated that some actions of ethanol are stereospecific and linked to direct protein interactions. Indeed, ethanol modifies the functional activity of many receptors and ion channels, including N-methyl-D-aspartate (NMDA) [Lovinger et al., Science, 1989; Lovinger et al., J Neurosci, 1990], kainate [Carta et al., Proc Natl Acad Sci, 2003], serotonin 5-HT3 [Lovinger and White, Mol Pharmacol, 1991], γ-aminobutyric acid (GABA)A [Davis, J Psychiatry Neurosci, 2003], and glycine [Mihic et al., Nature, 1997] receptors as well as G protein-coupled inwardly rectifying potassium channels [Kobayashi et al., Nat Neurosci, 1999] and calcium channels [Walter and Messing, Neurochem Int, 1999]. In most cases, alcohol affects these targets only at high, suprapharmacological concentrations. The brain maintains neurochemical balance through inhibitory (GABA) and excitatory (glutamate – mainly NMDA) neurotransmission. However, certain GABA receptor isoforms are exquisitely sensitive to alcohol at concentrations within the intoxicating range [Wei et al., J Neurosci, 2004; Hanchar et al., Life Sci, 2004].

Ethanol, at low concentrations can positively modulate the activity of some GABA receptors containing the δ subunit, such as α4β2 δ [Sundstrom-Poromaa et al., Nat Neurosci, 2002] and α6β2 δ [Wallner et al., Proc Natl Acad Sci USA, 2003]. Such δ subunit – containing GABA receptors are located largely perisynaptically or extrasynaptically, where they mediate tonic inhibition of neurons by ambient GABA. It has been speculated that extrasynaptic GABA receptors may be activated by a spillover of GABA when GABAergic interneurons are intensively activated, for instance during seizures discharge, thus producing negative feedback. Potentiation of extrasynaptic GABA receptors likely contributes to the anticonvulsant activity of ethanol, including its protective activity against alcohol withdrawal seizures [Rogawski, Epilepsy Curr, 2005].

In alcohol dependence a compensatory adaptation of GABA receptors (down-regulation of GABA receptors) to prolonged ethanol exposure plays a critical role [Morrow et al., Alcohol, 1990; Mhatre et al., J Neurochem, 1993]. Ablution of alcohol, in addition to a decrease in α1 [Charlton et al., J Neurochem, 1997] or γ2 [Follesa et al., Mol Pharmacol, 2003] subunits expression that occur with prolonged ethanol exposure, also leads to a rapid increase in the abundance of α4 subunits [Mahmoudi et al., J Neurochem,}
Generally, the changes in density of synaptic GABA<sub>A</sub> receptors and alterations in GABA<sub>A</sub> receptor subunit composition lead to the loss of GABA-mediated inhibition and predispose to alcohol withdrawal seizures. Because of such changes in subunit composition of GABA<sub>A</sub> receptor during ethanol withdrawal, benzodiazepines (drugs for treatment or prevention of alcohol withdrawal seizures) are relatively modestly active [Rogawski, Epilepsy Curr, 2005]. More effective is chlormethiazole, which has high efficacy in enhancing GABA<sub>A</sub> receptors containing α4 subunits [Usala et al., Br J Pharmacol, 2003]. Antiepileptic drugs, such as carbamazepine and phenytoine, are also weak or ineffective in protecting against the occurrence of seizures in alcoholics on withdrawal [Bayard et al., Am Fam Physician, 2004; Chance, Ann Emerg Med, 1991], although carbamazepine may be useful to treat alcohol craving [Bayard et al., Am Fam Physician, 2004].

On the other hand, it has been reported that a non-competitive NMDA-receptor antagonist, MK-801, is highly effective anticonvulsant in animal models of ethanol withdrawal seizures [Grant et al., Eur J Pharmacol, 1990; Morrisett et al., Eur J Pharmacol, 1990]. This results from the fact that ethanol, at concentrations associated with behavioral effects in humans, inhibits the NMDA receptor via a non-competitive mechanism [Lovingier et al., Science, 1989; Lovinger et al., Ann Med, 1990; Wirker et al., Naunyn Schmiedebergs Arch Pharmacol, 2000], which mediates the post-synaptic excitatory effects of glutamate. Tolerance to ethanol results in the up-regulation of the NMDA receptor so that abrupt withdrawal produces a hyperexcitable state that leads to seizures, delirium tremens, and excitotoxic neuronal death [Whittington et al., Alcohol Alcohol, 1995; Tsai and Coyle, Annu Rev Med, 1998]. Our experiments indicated that, besides non-competitive antagonists, also antagonists of other binding sites in the NMDA receptor are able to attenuate the ethanol withdrawal seizures. For example, glycine B site antagonist – L-701,324 and polyamine site antagonist – eliprodil attenuated ethanol withdrawal seizures [Kotlinska and Liljequist, Psychopharmacology, 1996]. Furthermore, non-competitive NMDA receptor antagonist – memantine and L-701,324 given before every ethanol administration during ethanol dependence prevented the development of ethanol withdrawal seizures in rats [Kotlinska, Pol J Pharmacol, 2001]. However, most of the NMDA antagonists display side effects, such as psychosis, nausea, vomiting, memory impairment, and neuronal cell death [Gardoni and Di Luca, Eur J Pharmacol, 2006] that preclude their clinical usefulness. Antagonists of group I metabotropic glutamate receptor (mGluR) show less side effects than NMDA receptor antagonists [Carroll, Ann NY Acad Sci, 2008] and group I mGluR antagonists are often found to produce behavioral effects that are essentially similar to those induced by NMDA antagonists [Homayoun et al., Neuropsychopharmacology, 2004]. Our unpublished study also pointed out that mGluR antagonists of group I (mGlu1 and mGlu5) such as EMQMCM and MTEP are also capable of attenuating the ethanol withdrawal seizures in rats. Until now, there is a lack of glutamatergic antagonists that fulfill requirements for being therapeutic agents in alcohol withdrawal seizures. Nevertheless, anticonvulsants with partial anti-glutamatergic mechanism, such as gabapentine [Rustembegovic et al., Med Arh, 2004] or topiramate [Rustembegovic et al., Med Arh, 2002] were effective in preventing seizures in human subjects undergoing withdrawal in clinical trials.
Nanotechnology perspectives on epilepsy treatment

Władysław Łasoñ1,2, Iga Bechyne3,4

1 Institute of Pharmacology, Polish Academy of Science, Szymańska 12, PL 31-043 Kraków, Poland; 2 Institute of Public Health, Collegium Medicum, Jagiellonian University, Kraków, Poland; 3 Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland; 4 UCB Pharma, Medical Affairs Department, Warszawa, Poland

The aim of nanotechnology as a science is to create structures and devices on an atomic scale, where the size limit is 100 nanometers (1 nm = 10^{-9} m). Such an approach yields new physical features which are a consequence of the high surface-to-volume ratio of these nanoparticles (e.g., gold becomes a liquid on a nano scale). There exist different carbon nanostructures, such as fullerences or nanotubes, which have enormous strength, as well as unique optical, electrical and thermal properties. Nanotechnology or molecular engineering was once initiated by H. Rohrer and I. Binnig, who constructed the first scanning tunneling microscope which enables the imaging and manipulating of individual atoms. Nanotechnology provides a basis for the so-called systemic medicine which views human organism as a system of cooperating molecular networks. Individual elements of such a system can be successfully manipulated by nanotechnology instruments which, in turn, may change the way of studying, diagnosing and treating diseases.

Epilepsy is one of the most common neurological disorders and is believed to affect around 1% of the total population. It is caused by abnormal electrical discharges originating from a well-defined focus (partial or focal epilepsy), or affecting both hemispheres of the brain at the same time (generalized seizures). One of the major limitations to current epilepsy treatment is an inefficient delivery of antiepileptic drugs to the affected part of the brain. The blood-brain barrier is permeable by small (< 1000 Da), lipophilic molecules only. When an antiepileptic drug enters the brain, it may be partially brought back to the capillary lumen by multidrug resistance proteins. Moreover, most of the orally delivered antiepileptic drugs influence the whole organism, thus causing peripheral toxicity and numerous adverse reactions. Some medicinal products may also undergo phagocytosis by macrophages in the reticulo-endothelial system. Overall, new strategies of drug delivery should enhance the efficacy and safety of treatment of different diseases, including epilepsy. Such innovative approaches as, e.g., drug transport nanosystems, pro-drugs or inhibition of MRP proteins, would enable antiepileptic drugs to reach and maintain their therapeutic concentration in the brain. Liposomes, polymeric nanoparticles or stealth polymers can act as carrier vehicles. Spherical liposomes contain a phospholipid and cholesterol bilayer. Hydrophilic agents can be encapsulated within their aqueous core, whereas hydrophobic and amphiphilic molecules can be bound directly to the lipid bilayer [Bannout et al., Drugs, 2009]. More advanced polymeric nanoparticles ensure better stability and security against biodegradation, as well as a controlled release of their content in time. One of the most widely used nanoparticles is PLGA (poly(lactic-co-glycolic acid)), which appears to be safe, biocompatible and has currently been recommended by FDA [Bennewitz and Saltzmann, Neurotherapeutics, 2009]. The pro-drug concept is based on the creation of a substance which circulates in blood in an inactive form and undergoes a metabolic process in a target tissue; therefore locally releasing the active molecule. Currently, phosphatidylycholine-valproic acid (DP-VPA) is under pre-clinical development. Theoretically, this pro-drug is cleaved by phospholipase at a seizure focus, thus releasing active VPA [Bialer et al., Epilepsy Res, 2001]. It is not unlikely that in future such pro-drugs will be administered in nanoparticle encapsulated forms. Other nanotechnological approaches have also been tested in a pre-clinical setting. For example, when carbamazepine in the form of nanoemulsion was injected intravenously to mice, it exhibited beneficial pharmacokinetic parameters [Madhusudhan et al., J Drug Target, 2007]. Similarly, the oxidative metabolism of primidone was reduced when that antiepileptic drug was entrapped in nanocapsules [Ferranti et al., Drug Metabol Drug Interact, 2001]. Some other studies investigated the functional effect of new delivery nanosystems on epileptic seizures in rodents. It was shown that phenytoin-loaded liposomes locally suppressed the epileptic focus affected by cAMP/EDTA stimulation in rats [Mori et al., Brain Res, 1995]. When the non-competitive antagonist of NMDA receptor, MRZ 2/576, was administered intravenously, being incor-
porated into nanoparticles composed of poly(butylcyanoacrylate) with polysorbate 80, its anticonvulsant activity was prolonged more than 10 times compared to free MRZ 2/576 [Friese et al., Eur J Pharm Biopharm, 2000]. An intranasal delivery of nanoparticles is another innovative approach. To this end, Kubek et al. [Neurotherapeutics, 2009] investigated the effect of TRH-PLA (thyrotropin-releasing hormone-poly(lactide) nanoparticles, delivered intranasally, on kindling development in mice. The latter study showed that those nanomolecules significantly attenuated epileptogenesis and reduced seizure severity when administered 7 days prior to or during kindling. Nanotechnology may also have an impact on the diagnosis of epilepsy. Using a rodent model of temporal lobe epilepsy, Akhtari et al. [Epilepsia, 2008] demonstrated that nanodevices can be developed to indicate localization of the epileptic focus. Furthermore, non-radioactive magnetonanoparticles, bound covalently to non-radioactive alpha methyl tryptophan (AMT), were proven to be capable of crossing the blood-brain barrier, of cumulating in the epileptogenic tissue and of being detected by MRI. Although this new nanoapproach appears to be very promising, safety concern is likely to grow. Some nanoparticles may be highly reactive, or may generate potentially cell-destructive free radicals. In conclusion, nanotechnology approaches seem to be very promising in the treatment of epilepsy, as they may turn out to be more efficacious than the already known antiepileptic drugs. At the same time, they are likely to have a restrained negative impact on non-target tissues. Nevertheless, since the results of pre-clinical research cannot be related directly to humans, further studies need to be undertaken.

Role of calcium in neurodegeneration

Jerzy W. Łazarewicz, Elżbieta Ziemińska, Elżbieta Salińska

Laboratory of Pharmaconeurochemistry, Department of Neurochemistry, Mostowski Medical Research Centre, Polish Academy of Sciences, Pawirskiego 5, PL 02-106 Warszawa, Poland

Calcium is an extracellular cation and its excessive influx to the cells is highly toxic. This view is supported by a large ratio of calcium concentrations in the extracellular and intracellular compartments of mammals, reaching value of $10^4$. In the tissue fluids $\text{Ca}^{2+}$ concentration remains relatively stable at about $10^{-3}$ M, while its steady-state level in the cytosol is kept below $10^{-6}$ M. Similar very low intracellular calcium concentration was found in prokaryota, which indicates that the mechanisms releasing calcium from cells are old and evolutionary conserved. Explanation of this phenomenon is based on the notion, that evolution of life on Earth utilizes phosphates as intracellular anions highly important in energy and lipid metabolism regulating protein functioning and serving as the linkers in replicating molecules RNA and DNA. Magnesium serves as a counter-ion in these processes. A prolonged increase in the intracellular $\text{Ca}^{2+}$ content is lethal because these ions compete with magnesium, and calcium phosphates are practically insoluble in water. To avoid the lethal precipitations inside the cells intracellular $\text{Ca}^{2+}$ concentration cannot exceed the level of $10^{-5}$ M. This is the fundamental reason, why calcium is dangerous to the cells [Kretsinger, in Intracellular Calcium Regulation, 1990].

There are interesting evolutionary aspects of $\text{Ca}^{2+}$ homeostasis. Concentration of $\text{Ca}^{2+}$ ions in the contemporary oceans varies in the range of $10^{-2} – 10^{-3}$ M. However, most probably the evolution of the earliest forms of life and even of eukaryota took place in waters of much lower, $10^{-4}$M $\text{Ca}^{2+}$ level. About 1 billion years ago $\text{Ca}^{2+}$ concentration in oceans increased to the present values and the cells had to adapt to this strong $\text{Ca}^{2+}$ pressure [Kempe and Degens, Chem Geol, 1985]. As a result of evolution, the contemporary prokaryota use two basic mechanisms of releasing $\text{Ca}^{2+}$ from the cells: the plasma membrane $\text{Ca}^{2+}$ ATPase, which directly depends on availability of ATP, and $\text{Na}^+/\text{Ca}^{2+}$ exchanger, which depends on the normal sodium gradient generated by $\text{Na}^+/K^+$ ATPase. Eukaryota utilize the same mechanisms, which are also supported by the sophisticated systems of in-
tracellular Ca\(^{2+}\) buffering. They include accumulation of Ca\(^{2+}\) in the intracellular organelle: endoplasmic reticulum (ER) and mitochondria. The former transport uses a specific Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA) that accumulates Ca\(^{2+}\) with high affinity and supports maintaining its very low level in the cytosol. However, capacity of Ca\(^{2+}\) stores in ER is rather limited. Energized mitochondria accumulate Ca\(^{2+}\) with lower affinity but high capacity. Still, the process of extensive Ca\(^{2+}\) uptake by mitochondria may trigger dangerous secondary mechanisms (see below). One should also keep in mind the high buffering potential of the numerous cellular Ca\(^{2+}\) binding proteins and still unclear but probably very important active role of nucleus in Ca\(^{2+}\) homeostasis.

This complex mechanism of the cellular Ca\(^{2+}\) homeostasis has been developed in various ways in different cells to meet their specific functional demands. In the excitable cells, particularly in neurons, diverse mechanisms of the controlled influx of Ca\(^{2+}\) to the cytosol have been developed. They comprise voltage-sensitive and receptor-operated Ca\(^{2+}\) channels as well as store-operated channels present in the plasma membrane. In addition there are mechanisms of Ca\(^{2+}\) release from the intracellular stores in ER using IP3 and ryanodine receptors/channels. These mechanisms are instrumental in the evolutionary achievement of eukaryota, which is the use of Ca\(^{2+}\) in the intracellular signaling [Berridge, Neuron, 1998]. Why the evolution selected Ca\(^{2+}\) as an intracellular messenger? Probably because of particular physicochemical properties of this cation, the presence of already developed efficient mechanisms of a rapid Ca\(^{2+}\) extrusion from the cytosol, and due to abundant and easy available Ca\(^{2+}\) stores in the extracellular and intracellular compartments.

The sequential mechanisms of generating Ca\(^{2+}\) signals in neurons are initiated by the extracellular messages, including depolarization and chemical neurotransmission. They induce activation of voltage-operated (e.g. mainly post-synaptic L-channels) or ligand-gated Ca\(^{2+}\) channels, like NMDA receptors (NMDARs), resulting in the influx of Ca\(^{2+}\) to the cytosol. An initial temporary increase in the Ca\(^{2+}\) concentration in cytosol may be prolonged and potentiated by the secondary release of Ca\(^{2+}\) from ER stores via ryanodine receptors in the mechanism of Calcium Induced Calcium Release (CICR). The alternative mechanism of generating Ca\(^{2+}\) transients comprises stimulation of the G-protein coupled receptors, including group I metabotropic glutamate receptors (mGluRs GI), resulting in the activation of phospholipase C, the release of IP3, activation of IP3 channels and mobilization of Ca\(^{2+}\) from the intracellular stores in ER. Excitatory amino acid receptors, particularly NMDARs and mGluRs GI, are normally activated by their universal endogenous ligand glutamate, but are also sensitive to other potentially pathogenic endogenous amino acids like homocysteine. As has been demonstrated in the numerous in vitro experimental systems, these receptors utilize the above mentioned mechanisms inducing Ca\(^{2+}\) transients and Ca\(^{2+}\) signaling [review: Salinska et al., Folia Neuropathol, 2005]. Also our previous studies using brain microdialysis technique coupled with measurement of radioactive Ca\(^{2+}\) efflux from the previously pre-labeled pool, demonstrated these phenomena in vivo. We observed the NMDA receptor dependent decrease of the extracellular Ca\(^{2+}\) concentration in the rabbit hippocampus, reflecting influx of Ca\(^{2+}\) to neurons or homocysteine-induced mGluRs GI-mediated mobilization of Ca\(^{2+}\) from the IP3 sensitive stores [Lazurewicz et al., Neurochem Res, 2003], and the NMDA receptor dependent release of Ca\(^{2+}\) from the intracellular stores in the rat hippocampus using the CICR mechanism [Lazurewicz et al., J Neurosci Res, 1998]. For encoding the message, the intracellular Ca\(^{2+}\) signaling uses specific characteristics of Ca\(^{2+}\) transients including their amplitude, frequency, duration and intracellular localization.

The next step in Ca\(^{2+}\)-mediated signal transduction employs different classes of sensors which respond to increase of Ca\(^{2+}\) in the cytosol. Calcium ions directly or indirectly interfere with activity of several enzymes, including proteases, lipases, protein kinases and nucleotidases. Moreover they bind to regulatory proteins like calmodulin, that subsequently modulate the next signaling proteins, particularly protein kinases and phosphatases, as well as nitric oxide synthase. In turn, a number of Ca\(^{2+}\) binding proteins play a role of intracellular buffers damping Ca\(^{2+}\) signaling. These signaling events are extremely complex and far from complete understanding. They play a crucial role not only in several processes, which are basic for general cell physiology, but also in the specific functions of neuronal cells including synaptic transmission (both pre- and postsynaptic events) and plastic changes.

Maintaining the intracellular level of Ca\(^{2+}\) within the regulatory limits is a prerequisite for the physio-
logical Ca$^{2+}$ signaling. In turn, imbalance in Ca$^{2+}$ homeostasis may lead to pathological events. They include dysfunction resulting from disturbed Ca$^{2+}$ signaling or even necrotic/apoptotic neuronal death. Thus, on top of previously mentioned danger of precipitation of calcium phosphates and antagonism of Ca$^{2+}$ with magnesium, appears the second and more important level of Ca$^{2+}$ neurotoxicity, based on disturbances in cellular signaling mechanisms. This Ca$^{2+}$ imbalance may be caused by excessive stimulation of neurons (e.g. in the excitotoxic insult) leading to Ca$^{2+}$ influx to neurons via receptor-operated and voltage-sensitive channels, and by the reversal of the activity of Na$^+$/Ca$^{2+}$ exchanger. Under some experimental conditions the primary event is the disturbance in energy metabolism. It may switch of the mechanisms of Ca$^{2+}$ removal from the cytosol and secondarily activates its uncontrolled influx to neurons [Berridge et al., Nature, 1998].

The results of our early studies concerning brain ischemic pathology suggested that Ca$^{2+}$ imbalance may participate in the mechanisms of neuronal damage, involving also mitochondrial injury and oxidative stress [Lazarewicz, Nauropat Pol, 1978; Majewska et al., Brain Res, 1978]. The general “calcium hypothesis” presented in 1981 argued, that disturbances in Ca$^{2+}$ homeostasis not only accompany brain ischemia and injury, hypoglycemia and epilepsy but also play a key role in pathogenesis of brain neuronal damage under these conditions [Siesjö, J Cereb Blood Flow Metab, 1981]. For the last 30 years this hypothesis was generally accepted and developed. It is clear, that disturbances in Ca$^{2+}$ homeostasis resulting in the increase in Ca$^{2+}$ concentration in the cytosol, activate Ca$^{2+}$-sensitive catabolic enzymes proteases and lipases, leading to membrane pathology. Activation of endonucleases leads to DNA damage. However, these processes, as well as uncontrolled activation of protein kinases and phosphatases, reflect a lethal disturbance in the intracellular signaling. The processes normally utilized by neurons for the intracellular signal transduction turn into deadly destructive mechanisms. Not surprisingly, depending on other conditions accompanying given pathology, particularly on availability of energy, Ca$^{2+}$ imbalance may lead to necrotic or apoptotic neuronal death.

There are several important components of the Ca$^{2+}$-induced neurodegeneration. Its pathogenesis comprises the most obvious pro-necrotic mechanisms like destruction of the membranes and the cytoskele-ton, caused by lipo- and proteolysis and oxidative stress. The role of the latter component is hard to overestimate, since Ca$^{2+}$ imbalance triggers several processes resulting in production of free radicals, which in turn potentiate disturbances in Ca$^{2+}$ metabolism. One of the most important elements of Ca$^{2+}$-induced cell pathology is mitochondrial injury caused by Ca$^{2+}$ overload of these organelle. Resulting activation of megachannels and deenergization of mitochondria may lead either to necrosis or to apoptosis, the latter due to release of the pro-apoptotic proteins. In fact, a complex pathological mechanism connected with excitotoxicity and Ca$^{2+}$ imbalance, which is active in brain ischemia or epilepsy represents the necrotic/apoptotic continuum.

Although the scenario of the involvement of Ca$^{2+}$ in neurodegeneration seems to be clear, nevertheless there are several examples of experimental data indicating that such a model is oversimplified. Our data from the in vitro experiments using a model of primary culture of rat cerebellar granule cells, demonstrated that homocysteine induces damage to these neurons, which is mediated simultaneously by NMDARs and mGluRs GI. This effect is accompanied by mitochondrial swelling and release of cytochrome c but without visible symptoms of Ca$^{2+}$ influx to neurons or of its increased level in the cytosol [Ziemińska et al., Neurochem Int, 2003; ibid 2006]. We tend to ascribe the mechanism of this phenomenon to effects of interactions between NMDARs, mGluRs GI and IP3 receptors mediated by postsynaptic density proteins, and to a massive Ca$^{2+}$ recompartmentation from ER to mitochondria without detectable intracellular Ca$^{2+}$ transients.

The conventional model of the role of Ca$^{2+}$ in excitotoxicity postulates, that the entrance of Ca$^{2+}$ to neurons via NMDARs plays a key role in its mechanism. Calcium neurotoxicity does not correlate with its maximal intracellular concentration, but rather with the total Ca$^{2+}$ load [Salińska et al., Folia Neuropathol, 2005]. However, there are opinions revising this dogma. Among them there are data pointing to the role of zinc as a cation, which may be also responsible for neurotoxicity. Toxic effects of zinc were described in several in vitro studies [review: Capasso et al., J Alzheimers Dis, 2005]. Moreover, the observations of intracellular Ca$^{2+}$ transients with Ca$^{2+}$-sensitive fluorescent probes, detected also changes in the intracellular zinc, suggesting that the role of Ca$^{2+}$ may be overestimated [Stork and Li, J Neurosci, 2006].
Although the exact mechanism of zinc neurotoxicity remains unclear, these data indicate that toxic effects ascribed exclusively to Ca$^{2+}$ may result from more complex pathogenic factors. Other studies demonstrated, that the uncontrolled release of Ca$^{2+}$ from ER stores, evoked e.g. by thapsigargin, may be highly injurious to neurons, although NMDARs are not involved [Silverstein and Nelson, Neurosci Lett, 1992]. Our recent results indicate, that in cultured neurons the environmental toxin tetrabromobisphenol-A (TBBPA), the representative of a large group of brominated flame retardants, also releases Ca$^{2+}$ from the ryanodine-sensitive intracellular stores with only minor component of the extracellular Ca$^{2+}$ influx. This results in excessive increase in the intracellular Ca$^{2+}$ concentration and neuronal death [Ziemińska et al., in abstracts of internationals conference: Disturbances of Cerebral Function Induced by Food and Water Contaminants, Valencia, 2010]. Moreover, new data suggest that physical interactions between these receptors and NOS using PSD-95 proteins, may play a key role in the execution of Ca$^{2+}$ neurotoxicity induced by the excitotoxic challenge, plays the secondary increase of the intracellular Ca$^{2+}$ concentration evoked by activation of the unselective cation channels [Manev et al., Mol Pharmacol, 1989]. There are indications, that the Transient Receptor Potential Channels Melastatin (TRPM) type 2 and 7 may be responsible for this secondary Ca$^{2+}$ influx, and that they represent promising targets for new neuroprotective efforts [Aarts et al., Cell, 2003; Nicotera nd Bano, Cell, 2003; Aarts and Tymianski, Pflugers Arch – Eur J Physiol, 2005]. To sum up, although from the beginning of evolution of life the cells had to extrude Ca$^{2+}$ to avoid deadly Ca$^{2+}$ homeostasis started to be particularly dangerous in eukaryota, which use Ca$^{2+}$ for intracellular signal transduction. These Ca$^{2+}$-dependent signaling mechanisms play a key role in nerve cell physiology, however their disturbance may result in the necrotic and apoptotic cell death. Because of physiological importance of Ca$^{2+}$ signaling, potential drugs interfering with Ca$^{2+}$ homeostasis or signaling either directly or indirectly, like glutamate receptor antagonists, have serious site effects and their utility as neuroprotecting agents has been disputed. Thus new therapeutic strategies averting neurodegeneration are urgently needed, which would prevent or reverse neurotoxic effects of Ca$^{2+}$, while avoiding interference with intracellular Ca$^{2+}$ signaling.

Androsterone, a metabolite of testosterone, has antiepileptic properties and enhances the protective activity of some antiepileptic drugs in mice

Katarzyna Mróz, Tomasz Mróz, Marian Wielosz, Piotr Tutka

Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Jacezkowskiego 8, PL 20-090 Lublin, Poland

There are still too frequent failures in the pharmacological treatment of epilepsy. It makes an impulse to look for more efficacious and safe methods of therapy. Nowadays much attention is paid for the role of steroid sex hormones as substances modifying the epileptic activity in CNS [Reddy, Neuroscience, 2004; Veliškova, Neuroscience, 2006]. The role of male sex hormones (androgens) in seizure excitability, both in animals and humans, still remains elusive. It is known that in epileptic men free testosterone and its major metabolites, etiocholanolone and androsterone (5α-androstan-3α-ol-17-one, AND), plasma lev-
els are reduced [Herzog et al., Arch Neurol, 1986; Bauer et al., Neurology, 2004]. AND was found in adult brain. It was protective in some animal seizure models [Kaminski et al., Epilepsia, 2005].

The aim of the study was: 1) to determine the effects of AND in two commonly used seizure models (pentylenetetrazole-(PTZ) and maximal electroshock-induced seizures (MES)), 2) to determine the influence of AND on the protective activity of two conventional (carbamazepine, phenobarbital) and one newer (gabapentin) antiepileptic drugs against convulsions caused by MES in mice. The effects of AND on motor coordination impairment induced by the antiepileptic drugs were also examined. In order to determine a nature of interactions between AND and the antiepileptic drugs, the measurement of plasma and total brain concentrations of the antiepileptic drugs given alone or in combination with AND was undertaken.

AND administered intraperitoneally (i.p.) dose-dependently inhibited the convulsive action of PTZ, increasing its CD_{50} (convulsive dose_{50}) for clonic convulsions caused by PTZ + saline to 93.9 mg/kg (PTZ + AND 40 mg/kg)(p < 0.05) and 113.9 mg/kg (PTZ + AND 60 mg/kg)(p < 0.001). In mice pretreated with AND in a dose of 60 mg/kg, the CD_{50} for tonic convulsions induced by PTZ increased from 102 to 127.6 mg/kg (p < 0.01). AND also exhibited dose-dependent protection against tonic-clonic convulsions caused by MES with ED_{50} (effective dose_{50}) of 272 mg/kg.

In the next part of experiments using MES, AND was given i.p. in a single subthreshold dose of 40 mg/kg. The compound enhanced the protective activity of carbamazepine, phenobarbital, and gabapentin lowering their ED_{50} (effective dose_{50}) from 8.59, 20.86, and 419.91 mg/kg to 6.05 (p < 0.05), 10.0 (p < 0.001), and 111.51 (p < 0.05) mg/kg, respectively. AND in a dose of 40 mg/kg did not affect the total brain levels of three antiepileptics. Thus, the effects of AND on the anticonvulsive activity of phenobarbital, gabapentin, and carbamazepine may depend on the direct action of the drug, and do not appear to have a pharmacokinetic nature. Only in case of carbamazepine, the pharmacokinetic interaction was found in plasma, but not in the brain. In the chimney test, AND given in a dose enhancing the anticonvulsive activity of the antiepileptics (which alone was without effect on motor performance of mice) did not affect impairment of motor coordination produced by the antiepileptics. The TD_{50} (toxic dose_{50}) for i.p. carbamazepine (56.03 mg/kg), phenobarbital (89.39 mg/kg), and gabapentin (978.15 mg/kg) were not significantly changed with concurrent administration of AND (47.15, 85.06, and 871.19 mg/kg for respective antiepileptic drugs). Protective indices, calculated by dividing the respective TD_{50} value by the correlating ED_{50} value, for carbamazepine, phenobarbital, and gabapentin given alone were 6.5, 4.3, and 2.3, and for these drugs given with androsterone were 7.8, 8.5, and 7.8, respectively (approximately 1.2-, 2-, and 3.4-fold increase).

Our findings indicate that AND: 1) protects against convulsions in PTZ and MES models of seizures, 2) increases the anticonvulsive activity of carbamazepine, phenobarbital, and gabapentin in mice. An increase of the anticonvulsive activity of the antiepileptics caused by AND together with a lack of pharmacokinetic interaction between AND and the antiepileptics in brain tissue recommend further preclinical and clinical research on the drug in question. If AND increases the antiepileptic activity of carbamazepine, phenobarbital, and gabapentin in humans, this may constitute a rationale to consider modification of dosage of these drugs in epileptic patients with a deficiency of androgens. Moreover, it appears reasonable to conduct further studies on AND in respect of its use as adjuvant therapy in the management of epilepsy.
The pathophysiology of status epilepticus

Konrad Rejdak, Ewa Papuć, Zbigniew Stelmasiak

Department of Neurology, Medical University of Lublin, Jacekowskiego 8, PL-20-090 Lublin, Poland

Epilepsy is the most common serious neurological disorder, affecting approximately 1 in 150 people, and status epilepticus (SE) is sometimes described as the maximal expression of epilepsy, being associated with both short- and long-term significant mortality and morbidity [Sander, Neurology, 2003]. There are almost as many types of status as there are of seizures. SE describes a unique pathological state, during which seizures tend to become self-perpetuating. The definition of SE was evolving and still there is no general consensus how to describe this pathological condition. Different time of duration was used: 30 min in the guidelines of the Epilepsy Foundation of America’s Working Group on Status Epilepticus [Treatment of convulsive status epilepticus, JAMA, 1993], 20 min [Bleck T, Clin Neuropharmacol, 1991] and 10 min in the VA Cooperative Trial [Treiman D et al., N Engl J Med, 1998]. Additionally, the operational definition of SE was proposed with 5 min of seizures in order to emphasize the need of early treatment [Lowenstein DH et al., Epilepsia 1999]. Early therapeutic intervention diminishes the risk of SE-induced neuronal injury [Wasterlain CG, Epilepsia, 1974; Fujikawa DG, Brain Res, 1996] and of the time-dependent development of pharmacoresistance [Mazarati AM et al., Brain Res, 1998; Kapur J et al., J Neurosci, 1997]. Our current understanding of the basic mechanisms of SE in animal models and in clinical situations is in accordance with the Clark’s description of three phases of SE (Clark LP et al., Am J Insanity, 1903), which we call impending SE, established SE, and subtle SE [Chen JW et al., Lancet Neurol, 2006].

Impending status epilepticus is defined as continuous seizures or intermittent seizures without full recovery of consciousness between seizures lasting more than 5 minutes. Established status epilepticus is defined as clinical or electrographic seizures lasting more than 30 min without full recovery of consciousness between seizures. The transformation from impending SE to established SE is probably a continuum, but there is good support in the clinical and experimental literature for a cut-off at 30 min: this is the time constant of transformation from prolonged seizure to SE [Chen JW et al., Lancet Neurol, 2006; De Lorenzo R et al., Epilepsia, 1999]. This also corresponds with the results of experimental studies when SE become self-sustained in animals [Mazarati AM et al., Brain Res, 1998], when SE-induced damage becomes evident [Fujikawa DG, Brain Res, 1996], and when pharmacoresistance to anticonvulsants may develop [Treiman DM et al., Epilepsy Res, 1990; Treiman D et al., N Engl J Med, 1998]. The term subtle status epilepticus was coined by Treiman [Treiman D et al., N Engl J Med, 1998] to describe the late, burned-out stage of SE during which both the motor and electroencephalographic (EEG) expression of seizures becomes less evident. In most electrical and chemical models of SE initiated in conscious, unanesthetized animals, seizures rapidly become self-sustaining despite the withdrawal of the epileptogenic stimulus. Human data are far less clear, but show that seizures which last more than 30 min rarely stop spontaneously [De Lorenzo RJ et al., Epilepsia 1999]. Cerebral metabolic demand is generally sustained during phase 1 tonic-clonic status, but not during phase 2, which is accompanied by profound metabolic complications. The combination of metabolic decompensation and the direct neurotoxic effects of ongoing seizure activity contribute to an association between long duration of status and poor outcome.

Experimental data show that after 30 min of intermittent stimulation of an excitatory glutamatergic pathway in the rat, stopping the stimulation no longer stops electrographic or behavioral seizures, which self-perpetuate for many hours and eventually become subtle [Mazarati AM et al., Brain Res, 1998]. Vicedomini and Nadler showed that these properties are shared by many other excitation pathways [Vicedomini JP, Brain Res, 1990]. Another unique feature of self-sustaining SE (SSSE) is the progressive, time-dependent development of pharmacoresistance: the potency of benzodiazepines may decrease 20-fold in 30 min of SSSE; phenytoin also loses potency, but more slowly [Mazarati AM et al., Brain Res, 1998]. By contrast, even late in its course, NMDA blockers continue to be effective in stopping SSSE [Mazarati A et al., Neurosci Lett, 1999]. The same dose of benzodiazepine which easily
blocks SE when given early is far less effective when given late. However, ketamine easily terminates established SE.

**Pathophysiological and biochemical cascades of self-sustained SE**

Repeated seizures produce complex cascades of pathophysiological and biochemical changes in the brain. The first milliseconds to seconds are dominated by the release of neurotransmitters and modulators, the activation and inactivation of ion channels, and receptor phosphorylation and desensitization. Within seconds to minutes, receptor trafficking, mainly of the GABA and glutamate receptors, is responsible for the key adaptations. The existing receptors can move from the synaptic membrane into endosomes, or be mobilized from storage sites to the synaptic membrane. This process enhances excitability by decreasing the number of inhibitory receptors and increasing the number of excitatory receptors in the synaptic cleft [Naylor DE et al., J Neurosci, 2005; Liu H et al., Proc Natl USA, 1999]. In the minutes to hours, neuropeptide modulators often increase the expression of proconvulsive neuropeptides and decrease the availability of inhibitory neuropeptides [Liu H et al., Proc Natl USA, 1999; Mazarati AM et al., J Neurosci, 1998; Mazarati A et al., Neuroscience, 1999], and this maintains enhanced excitability. Finally, in the hours to perhaps days to weeks following seizures, long-term changes in gene expression occur. The changes in gene expression are the combined effects of repeated seizures, of seizure-induced neuronal death, and of the subsequent neuronal reorganization. Some of the gene expression represents plastic adaptation to seizure activity. In addition to significant case fatality, status (particularly tonic-clonic status) is associated with considerable morbidity, in terms of cognitive and neurological deficit.

**Management of tonic-clonic status epilepticus**

The inappropriate treatment of non-epileptic seizures carries a significant risk of iatrogenic complications, including respiratory failure or arrest and admission to ITU. However, delayed treatment of tonic-clonic status can be equally dangerous. Lorazepam is the drug of choice among the first line drugs, in all current guidelines [Royal College of Physicians Edinburgh 2003; Scottish Intercollegiate Guidelines Network 2003; NICE 2004]. Intravenous diazepam has been used traditionally, and although it has a rapid onset of action, it is quickly redistributed into fatty tissue often leading to rebound seizures. If benzodiazepines fail, currently approved treatment for established status include: phenytoin, phenobarbitone and fosphenytoin. Intravenous valproate is an important option among II line drugs, and has been shown to be safe and effective [Wheless et al., Neurology, 2004; Limdi et al., Neurology, 2005]. When it comes to refractory status, expert consensus and all current guidelines advise that if treatment is still not controlling seizures at this stage (between 30 and 60 min), the patient should be transferred to ITU for general anaesthesia, both to suppress seizures and for the management of the systemic adverse effects of the epilepsy and the drugs being used to suppress it. Three agents are in common use at present: thiopentone, propofol and midazolam. If seizures recur, the diagnosis should be revisited, both in terms of whether this is truly epilepsy (necessitating EEG in the unconscious patient), and with respect to aetiology. Even in patients with known epilepsy, magnetic resonance imaging, and cerebrospinal fluid (CSF) examination, should be undertaken or repeated to exclude new pathology.