Neonatal seizures

Maria Roberta Cilio

Abstract

Epileptic seizures are the most frequent clinical manifestation of central nervous system dysfunction in the newborn. Current data from animal and human studies suggest that neonatal seizures themselves may lead to worsening brain injury, decrease the threshold for late seizures and result in poor long-term neurological outcome. Studies on the long-term consequences of status epilepticus during development have demonstrated status epilepticus-induced cell loss and mossy fibre sprouting within the hippocampus, and cognitive impairment and decreased seizure threshold, when the animals were tested as adults. In some cases, neonatal seizures are associated with an excellent neurodevelopmental outcome, as in benign familial neonatal seizure syndrome (BFNS), an autosomal dominantly inherited idiopathic seizure syndrome of the newborn characterised by partial or generalised seizures. The 2001 report of the International League against Epilepsy on classification and terminology classifies the BFNS syndrome among the familial autosomal dominant focal epilepsies. Mutations in either of two homologous potassium genes, KCNQ2 and KCNQ3, have been found in patients with BFNS. Because BFNS is associated with the loss of function of a potassium channel, the pathological neuronal hyperexcitability in this epilepsy syndrome is likely to be caused by impaired repolarisation. Gating changes may interfere with state-dependent drug binding. In particular, retigabine is a novel anticonvulsant that binds to a hydrophilic pocket of the activation gate. The elucidation of the molecular defect of monogenic epilepsies, such as BFNS, shows how genetic research leads to better understanding of the pathophysiology of more common disorders and opens the possibility of new therapeutic approaches.

Keywords animal studies; newborn; potassium channels; seizures

Epileptic seizures are the most frequent clinical manifestation of central nervous system dysfunction in the newborn, with an incidence as high as 57.5 per 1000 in infants with birth weights lower than 1500 g, but only 2.8 per 1000 for infants with birth weights of 2500–3999 g.1 The majority of neonatal seizures are occasional seizures, occurring as reactive events to acute insults, systemic diseases or disturbances, and subsiding soon after removal of the causative event. A significant percentage of neonatal seizures, however, are symptoms of severe or progressive brain disease and prelude the development of neurological deterioration and epilepsy.

A particular vulnerability of the developing brain of the newborn may relate to the rich expression in the developing brain of glutamate receptors, which appear to play an important role in neuronal differentiation and plasticity.2 This rich expression of glutamate receptors, important for normal development, may become a source of overexcitation or neuronal death with repeated or prolonged seizures. In addition, early in development, the principal inhibitory neurotransmitter gamma-aminobutyric acid (GABA) acts at the major post-synaptic GABA_A receptor to produce excitation rather than inhibition, as occurs later in development.3 Moreover, only the proconvulsant projection network of the substantia nigra (and not the later developing anticonvulsant network) functions early in brain development.

Current data from animal and human studies suggest that neonatal seizures themselves may lead to worsening brain injury, decrease the threshold for late seizures and result in poor long-term neurological outcome. Animal studies have demonstrated that the pathophysiological consequences of status epilepticus or recurrent seizures in the developing brain differ from those in the mature brain. In the adult animal, status epilepticus causes neuronal loss in hippocampal fields CA1 and CA3, and the dentate hilus.3,4 In addition to cell death, prolonged seizures in the adult brain lead to synaptic reorganisation with aberrant growth (sprouting) of granule cell axons (so-called mossy fibres) in the supragranular zone of the fascia and infrapyramidal region of CA3.5

Although the threshold for seizure generation is lower in immature brains than in adult brains, developing neurones are less vulnerable, in terms of neuronal damage and cell loss, than adult neurones to a wide variety of pathological insults. For example, immature hippocampal neurones will continue responding to synaptic stimuli in a fully anoxic environment for a longer duration than adult ones; likewise, longer anoxic episodes are required to irreversibly destroy the circuit in young animals. Young animals are less vulnerable than mature animals to cell loss in the hippocampus after a prolonged seizure.

In order to clarify the relation between age and seizure-related damage, we studied the long-term consequences of status epilepticus during development on hippocampal plasticity and cognitive functions. A variety of animal models developed in the past 20–25 years share important characteristics with human status epilepticus. Among many techniques using chemo-convulsants, the lithium–pilocarpine model has been especially popular because it replicates the pathology of temporal lobe epilepsy.6–8 The cholinergic agent pilocarpine produces limbic and generalised status epilepticus in rodents. Pretreatment with lithium has been shown to potentiate the epileptogenic action of pilocarpine, reduce mortality, and avoid many of the peripheral cholinomimetic side-effects of pilocarpine. This is a good experimental model for testing the long-term sequelae on hippocampal circuitry, particularly visuospatial learning and memory.

In our study,9 we used lithium–pilocarpine to induce status epilepticus at different age points during development (P12, P16 and P20), and evaluated the effects of this abnormal neural activity on spatial memory performance and seizure susceptibility in the animal beginning on P55, testing the animals for water maze performance10,11 and seizure susceptibility to flurothyl and kindling. It has been proposed that the 7–10-day-old rat may be equivalent to a human newborn.12 Although the exact correspondence between developmental stages across species is not readily describable, P12 in rats can be reasonably considered as

Maria Roberta Cilio  MD PhD  is faculty in the Division of Neurology, Bambino Gesù Children’s Hospital, Rome, Italy.
corresponding to the newborn period, and P16 and P20 to infancy and early childhood in humans respectively.

We demonstrated that status epilepticus at P12 did not result in any detectable structural or functional changes in adulthood, whereas status epilepticus at both P16 and P20 induced cell loss and mossy fibre sprouting within the hippocampus and cognitive impairment when the animals were tested as adults (Figure 1). Moreover, in those rats with status epilepticus at P20, we found a negative correlation between performance in the water maze and the degree of supragranular sprouting, suggesting a role of synaptic reorganisation in determining the cognitive impairment.

We chose to use two different models to assess seizure susceptibility — flurothyl inhalation, a model of generalised seizure, and kindling, a model of partial seizures with secondary generalisation — because we wished to determine whether seizures in the immature brain could ‘prime’ the brain for later seizure susceptibility. Although the seizure threshold was not altered when it was evaluated using flurothyl inhalation, a model of primarily generalised seizures, when seizure susceptibility was assessed with amygdala kindling, a model for focal and secondarily generalised seizures, the P20 status epilepticus animals demonstrated a lower seizure threshold as they needed fewer stimulations to reach each kindling stage and had a significantly lower afterdischarge threshold compared with controls.

Our study demonstrated that partial seizures at early age, primarily involving the limbic system, make the brain subsequently prone to seizures arising from the same modified circuitry. The specificity for a model of limbic seizure, such as kindling, suggests that the altered susceptibility to seizure as an adult is not the result of a general increase in neuronal excitability throughout the brain but rather reflects the seizure-promoting changes that we observed within the limbic system, on circuitry previously activated by pilocarpine.

These findings correlate well with data on outcome after neonatal seizures, indicating a quite unfavourable outcome in newborns with seizures, in terms of both psychomotor development and subsequent risk of epilepsy. However, in some cases, neonatal seizures are associated with an excellent neurodevelopmental outcome, for instance in benign familial neonatal seizure syndrome (BFNS), although newborns with BFNS may present later in life with an increased incidence of epilepsy (11%) compared with the general population (approximately 3%).

BFNS is a rare autosomal dominantly inherited idiopathic epilepsy of the newborn characterised by partial or generalised seizures, which occur during wakefulness and sleep. Clinically, it is characterised by clusters of generalised and partial seizures that typically start around day 3 of life and most often remit after several weeks or months and usually by approximately 4 months of age. However, about 10–15% of patients have febrile or afebrile seizures later in childhood. The diagnosis of BNFS rests entirely on the occurrence of similar events in relatives, consistent with an autosomal dominant transmission.

Seizures observed in these newborns are brief and of a mixed type, starting with tonic posture, symptoms, apnoea and other autonomic features. The seizures often progress to clonic movements and motor automatisms. The postictal state is brief, and interictically the neonates look well. Seizures are frequently recurrent with up to 20 seizures per day. The ictal EEG pattern with generalized suppression of amplitude on onset may be unique. Interictal EEG is either normal or shows transient minimal focal or multifocal abnormalities. The neonates are neurologically normal. Neurocognitive development is usually normal. According to the 2001 report of the International League against Epilepsy (ILAE) on classification and terminology, the BFNS syndrome is classified among the familial autosomal dominant focal epilepsies.

BNFS is the first seizure syndrome for which a gene could be identified. BFNS is a monogenic epilepsy inherited via an autosomal dominant trait with high penetrance. Among monogenic epilepsies, BFNS represents so far one of the best recognised disease models of idiopathic epilepsies; therefore, investigation of the molecular mechanism by which the genetic alterations found in affected patients cause BFNS is of fundamental relevance also for the treatment of idiopathic epilepsy in the adult population. Genetic linkage studies have mapped two disease genes for BFNS: KCNQ2 on chromosome 20q13.3 and KCNQ3 on chromosome 8q24, indicating a genetically heterogeneous disorder. Genetic studies on most families in which the disorder occurs show a link to chromosome 20. Seizures are characterised by paroxysmal neuronal excitability.

Timm staining with thionin counterstaining reveals more dark labelling in the stratum oriens (arrow) of the CA3 region in a rat subjected to lithium–pilocarpine status epilepticus at P20 a than in a control rat b. Calibration 50 μm.

Figure 1
Potassium channels are important for repolarising action potentials. KCNQ2 and KCNQ3 are expressed in the brain and belong to a subfamily of potassium channel genes that have been implicated in other diseases. Mutation in the KCNA1 potassium channel causes episodic ataxia, a non-epileptic disorder with paroxysmal cerebellar symptoms. Because BFNS is associated with the loss of function of a potassium channel, the pathological neuronal hyperexcitability in this epilepsy syndrome is likely to be caused by impaired repolarisation.

The identification of mutation in the homologous KCNQ2 and KCNQ3 potassium channel genes in a single disorder support the hypothesis that these two potassium channel genes may make up a single functional entity. Importantly, it has been recently demonstrated that the M-current, a tonic inhibitory potassium current, is made up of the KCNQ2 and KCNQ3 proteins. The M-current has a major role in controlling excitability because it regulates the ability of a neurone to fire an action potential. Pharmacological reduction of the M-current results in the excessive firing of action potentials typical of an epileptic seizure.

A recent study has expanded the naturally occurring mutation spectrum of KCNQ2 and supported the previous findings that the C-terminus is the region that contains the most mutations. Importantly, at least one individual in every family with a KCNQ2 or KCNQ3 mutation has an onset of seizures during the first week of life—a hallmark of the BFNS disorder.

Despite a wealth of studies addressing the molecular mechanisms responsible for BFNS pathogenesis, little is known about the cellular and developmental basis of the convulsive manifestation in the affected individuals. We recently found a novel missense mutation in the KCNQ2 gene in a family affected by BFNS. This mutation involved an uncharged residue, conserved among all KCNQ members except for KCNQ3, located in the N-terminal of the S4 voltage sensor protein. Electrophysiological analysis of the functional changes prompted by this genetic variant revealed that KCNQ2 A/V subunits introduced dramatic gating changes reflecting true mutation-induced modifications of channel behavior. Gating changes may interfere with state-dependent drug binding. In particular, retigabine is a novel anticonvulsant that binds to a hydrophobic pocket of the activation gate formed by the S5 and S6 segments of neuronal KCNQ channels, thus stabilising the open channel conformation.

Recognising the BFNS is a challenge, first of all because the syndrome has a benign outcome, suggesting that not all neonatal seizures have a poor prognosis, and second for genetic studies. Channel function in complex genetic and acquired epilepsies is much more difficult to study and much remains to be elucidated. The future of anticonvulsant therapy includes the identification of molecular targets that will interfere with epileptogenesis. The elucidation of the molecular defect of monogenic epilepsies, such as BFNS, shows how genetic research leads to a better understanding of the pathophysiology of more common disorders (acquired epilepsies) and opens the possibility of new therapeutic approaches.

**Conflict of interest**

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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