

PILOCARPINE-INDUCED SEIZURES REVISITED: WHAT DOES THE MODEL MIMIC?

Antagonism of Peripheral Inflammation Reduces the Severity of Status Epilepticus. Marchi N, Fan Q, Ghosh C, Fazio V, Bertolini F, Betto G, Batra A, Carlton E, Najm I, Granata T, Janigro D. *Neurobiol Dis* 2009;33(2):171–181. Status epilepticus (SE) is one of the most serious manifestations of epilepsy. Systemic inflammation and damage of blood-brain barrier (BBB) are etiologic cofactors in the pathogenesis of pilocarpine SE while acute osmotic disruption of the BBB is sufficient to elicit seizures. Whether an inflammatory-vascular-BBB mechanism could apply to the lithium-pilocarpine model is unknown. LiCl facilitated seizures induced by low-dose pilocarpine by activation of circulating T-lymphocytes and mononuclear cells. Serum IL-1 β levels increased and BBB damage occurred concurrently to increased theta EEG activity. These events occurred prior to SE induced by cholinergic exposure. SE was elicited by lithium and pilocarpine irrespective of their sequence of administration supporting a common pathogenetic mechanism. Since IL-1 β is an etiologic trigger for BBB breakdown and its serum elevation occurs before onset of SE early after LiCl and pilocarpine injections, we tested the hypothesis that intravenous administration of IL-1 receptor antagonists (IL-1ra) may prevent pilocarpine-induced seizures. Animals pre-treated with IL-1ra exhibited significant reduction of SE onset and of BBB damage. Our data support the concept of targeting systemic inflammation and BBB for the prevention of status epilepticus.

COMMENTARY

Experimental models of seizures and epilepsy have been invaluable for understanding of the basic mechanisms underlying ictogenesis and epileptogenesis as well as for developing new therapeutic options. It is, therefore, of utmost importance to know the pathophysiological basis of the experimental model and which human pathological situations it mimics.

One of the earliest and most frequently used models for evoking experimental status epilepticus and subsequent epilepsy is the systemic injection of pilocarpine in rodents (1). Treatment of rats and mice with low doses of lithium chloride (LiCl, 3 mEq/kg), followed by pilocarpine (30 mg/kg) or with a high dose of pilocarpine alone (300–350 mg/kg), results in a severe seizure-brain damage syndrome. The use of LiCl was devised to improve animal survival, because with this combination, the dose of pilocarpine can be decreased by about 10-fold. Since pilocarpine acts on muscarinic receptors (2) and its convulsant actions are evoked in the presence of the peripherally acting muscarinic antagonist, methyl-scopolamine (1), the activation of the cholinergic system by pilocarpine in the brain is believed to be the initiating factor for triggering seizures. Various studies have characterized pilocarpine-induced seizures using both EEG analysis and behavioral observations. Although there is still debate on the initiation site(s) of seizures (i.e., the hippocampus and related limbic regions vs ventral forebrain) and the involvement of brain regions in neuropathology (i.e., the hippocampus as compared to predominant neocortical damage), these initial studies nevertheless garnered a consensus among basic scien-

tists that this model is representative of temporal lobe epilepsy precipitated by convulsive status epilepticus.

Recently, novel findings raise the possibility that pilocarpine-induced seizures are not only the result of a direct cholinergic system activation in the brain, but also are derived from primary proinflammatory actions of pilocarpine involving the periphery. In 2007, Marchi and colleagues showed that pilocarpine causes acute peripheral proinflammatory changes leading to blood-brain barrier (BBB) leakage, prior to the onset of status epilepticus (3). Additionally, they showed that pilocarpine is poorly permeable to the BBB and fails to produce seizure activity *in vitro* when applied at concentrations identical to, or higher than, drug levels measured in the brain, *in vivo*. In brain slices or isolated guinea pig brain, seizure-like events could be evoked by pilocarpine only in the co-presence of elevated K⁺ concentrations, mimicking actions that occur when BBB permeability function is compromised (3,4). Based on the premises established from previous work and showing that pilocarpine can directly activate muscarinic receptors on brain microvasculature and white blood cells (5,6), Marchi and colleagues reported the following three interesting findings:

- The proinflammatory mediator interleukin (IL)-1 β is increased four-fold on average in serum shortly after pilocarpine injection and before the onset of status epilepticus; this effect was a consequence of white blood cell activation by pilocarpine.
- At the same time, there was a significant reduction in the number of CD4-expressing cells. These peripheral effects were not antagonized by methyl-scopolamine at the dose used to prevent the systemic actions of pilocarpine.

- Before the onset of status epilepticus, BBB damage was observed as assessed by leakage of serum albumin into the brain parenchyma (3).

These initial investigations highlighting the peripheral proinflammatory effects of pilocarpine anticipated the present study by Marchi and collaborators, which demonstrates that peripheral inflammation is indeed the initial and required mechanism by which pilocarpine, or LiCl plus pilocarpine, triggers status epilepticus. Marchi and colleagues describe changes in peripheral markers of inflammation after the injection of 3 mEq/kg of LiCl alone, which are very similar to those previously described using full convulsant doses (350 mg/kg) of pilocarpine (3). In particular, Marchi and colleagues report a decrease in CD4:CD8 positive cells ratio and an increase in monocytes/macrophages, at 3 hours and 20 hours after LiCl injection, thus reflecting profound changes in peripheral immune cells. Moreover, a concomitant increase in serum IL-1 β level was found to be similar to that induced by a full convulsant dose of pilocarpine; this phenomenon was associated with the evidence of albumin brain extravasation as an index of BBB leakage. These changes were accompanied by alteration in the EEG pattern consisting of a pronounced increase in 4–5 Hz activity and spike amplitude, as assessed 3 hours after LiCl administration. While the EEG synchronized to a burst firing pattern over time, no clinical seizures were observed.

Since IL-1 β is elevated in serum early after pilocarpine or LiCl administration, it is an etiological factor in BBB breakdown (7), and possesses proconvulsant activity when applied to the brain (8), the authors set out to antagonize the effects of increased serum levels of IL-1 β and to investigate the impact of this cytokine on pilocarpine's effects. They used the IL-1 receptor antagonist (IL-1Ra), which is an endogenous competitive antagonist of IL-1 type 1 receptors that inhibit the biological action of IL-1 β . It has been previously shown that IL-1Ra mediates powerful anticonvulsant actions in different models of seizures (8) and that transgenic mice overexpressing IL-1Ra in astrocytes are less susceptible to seizures (9,10), thus establishing the crucial role of elevated brain levels of IL-1 β in the mechanisms of ictogenesis (8,9). Marchi and colleagues used a systemic single bolus injection of IL-1Ra 2 hours before pilocarpine administration to antagonize the peripheral actions of IL-1 β ; they found a drastic reduction in the number of rats showing status epilepticus as well as in the severity of status epilepticus in those rats that still developed seizures in spite of the IL-1Ra treatment. Moreover, IL-1Ra prevented BBB breakdown following pilocarpine.

In summary, this set of data demonstrates that pilocarpine induces status epilepticus, via a primary peripheral effect on white blood cells, leading to elevated serum levels of IL-1 β , which in turn alters BBB permeability. The ionic imbalance due to K⁺ accumulation in the extracellular space, induced by BBB

leakage, is required for pilocarpine to produce its convulsant activity by stimulation of muscarinic receptors in the brain. The possibility also exists that elevated serum levels of IL-1 β contribute to lower seizure threshold to pilocarpine by a direct action of IL-1 β on neurons (8). Thus, serum IL-1 β may enter the brain because of the BBB leakage or may prime its own synthesis and release by activating IL-1R type 1 on endothelial cells and adjacent perivascular astrocytes (10).

These findings prompt reconsideration of the use of pilocarpine injections for evoking prolonged seizures as a model of peripheral inflammation prodromic to the onset of status epilepticus. The concept that peripheral inflammatory reactions contribute to lower seizure threshold was established a long time ago in studies demonstrating that systemic lipopolysaccharide (a component of the bacterial wall of Gram-negative bacteria mimicking infection) reduces the threshold to seizures in adult rodents (11). Recently, lipopolysaccharide or intracolonic administration of 2,4,6-trinitrobenzene sulfonic acid (in a model of bowel disease), delivered to immature rats (postnatal day 7 or 14), was reported to induce long-lasting increases in seizure susceptibility and seizure-associated brain damage (12,13). Thus, systemic inflammation is associated in experimental models with modifications in seizure susceptibility that, when evoked in early infancy, persists well into adulthood.

Systemic inflammation may occur in a clinical setting and is often associated with fever, which subsequently may provoke seizures in children. Activation of the IL-1 β system has been implicated in the mechanisms underlying experimental febrile seizures (14). Pathological events, such as systemic or CNS infection, and febrile seizures appear to be associated with an increased risk for late unprovoked seizures, therefore warranting further investigations of the underlying mechanisms of increased brain excitability. In light of this novel information, the pilocarpine model of status epilepticus should be revisited for the study of status epilepticus associated with systemic inflammatory processes. In this framework, it may be a useful tool to investigate pharmacological interventions to stop seizures precipitated by systemic inflammatory causes (8,10,15).

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