



# Myeloid EP2 Receptors in Status Epilepticus—Peripheral But Not Negligible

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## Peripheral Myeloid Cell EP2 Activation Contributes to the Deleterious Consequences of Status Epilepticus

Varvel NH, Espinosa-Garcia C, Hunter-Chang S, Chen Di, Biegel A, Hsieh A, Blackmer-Raynolds L, Ganesh T, Dingledine R. *J Neurosci*. 2021;41(5):1105-1117. doi: 10.1523/JNEUROSCI.2040-20.2020

A multidimensional inflammatory response ensues after status epilepticus (SE), driven partly by cyclooxygenase-2-mediated activation of prostaglandin EP2 receptors. The inflammatory response is typified by astrocytosis, microgliosis, erosion of the blood–brain barrier (BBB), formation of inflammatory cytokines, and brain infiltration of blood-borne monocytes. Our previous studies have shown that inhibition of monocyte brain invasion or systemic administration of an EP2 receptor antagonist relieves multiple deleterious consequences of SE. Here, we identify those effects of EP2 antagonism that are reproduced by conditional ablation of EP2 receptors in immune myeloid cells and show that systemic EP2 antagonism blocks monocyte brain entry in male mice. The induction of hippocampal interleukin-6 after pilocarpine SE was nearly abolished in EP2 conditional knockout (KO) mice. Serum albumin levels in the cortex, a measure of BBB breakdown, were significantly higher after SE in EP2-sufficient mice but not in EP2 conditional KOs. The EP2 deficiency in innate immune cells accelerated the recovery from sickness behaviors following SE. Surprisingly, neurodegeneration was not alleviated in myeloid conditional KOs. Systemic EP2 antagonism prevented monocyte brain infiltration and provided broader rescue of SE-induced effects than myeloid EP2 ablation, including neuroprotection and broader suppression of inflammatory mediators. Reporter expression indicated that the cellular target of CD11b-driven Cre was circulating myeloid cells but, unexpectedly, not microglia. These findings indicate that activation of EP2 receptors on immune myeloid cells drives substantial deficits in behavior and disrupts the BBB after SE. The benefits of systemic EP2 antagonism can be attributed, in part, to blocking brain recruitment of blood-borne monocytes. **SIGNIFICANCE STATEMENT** Unabated seizures reduce quality of life, promote the development of epilepsy, and can be fatal. We previously identified activation of prostaglandin EP2 receptors as a driver of undesirable consequences of seizures. However, the relevant EP2-expressing cell types remain unclear. Here we identify peripheral innate immune cells as a driver of the EP2-related negative consequences of seizures. Removal of EP2 from peripheral immune cells was beneficial, abolishing production of a key inflammatory cytokine, accelerating weight regain, and limiting behavioral deficits. These findings provide evidence that EP2 engagement on peripheral immune and brain endothelia contributes to the deleterious effects of SE, and will assist in the development of beneficial therapies to enhance quality of life in individuals who suffer prolonged seizures.

## Commentary

Status epilepticus is a life-threatening condition characterized by continuous seizure activity. The immediate goal of acute treatment is to stop the seizures as soon as possible to prevent further brain injury caused by prolonged seizures; however, current medications frequently fail to terminate status epilepticus for hours or even days.<sup>1</sup> Status epilepticus is associated with high short-term and long-term mortality, as well as cognitive deficits and epilepsy. Novel therapeutic strategies are thus urgently needed but critically depend on a better understanding of the underlying mechanisms.

One of the more recently discovered pathological mechanisms potentially contributing to status epilepticus and epilepsy

is neuroinflammation. It is well described that status epilepticus is associated with systemic and brain inflammation.<sup>2</sup> This association is bidirectional, that is, an acute infection can lead to status epilepticus, as seen in febrile seizures in children, and vice versa, status epilepticus, even without underlying infection, causes neuroinflammation. Studies in mouse models and in resected tissue from humans have shown that pro-inflammatory factors such as interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$ , IL-6, and cyclooxygenase-2 (Cox-2) are upregulated after status epilepticus or in epilepsy in the brain, and genetic or pharmacological inhibition of these signaling pathways can shorten status epilepticus and reduce the subsequent occurrence of epilepsy in mice.<sup>2</sup> Interestingly, the



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induction of pro-inflammatory factors is not only detectable in the brain but also in the blood suggesting systemic inflammation.<sup>3</sup> The relative contributions of brain versus peripheral inflammation to disease sequelae after status epilepticus, such as overall sickness, weight loss, neurodegeneration, and cognitive deficits are unknown. In particular, studies that assess the contribution of peripheral inflammation are scarce. Recent pre-clinical work by Varvel, Dingledine, and colleagues starts filling this gap by providing interesting and much-needed insight into the role of peripheral immune activation in status epilepticus.<sup>4</sup>

Varvel et al studied the influence of the peripheral immune system on status epilepticus-mediated sickness by using a novel conditional knockout mouse that blocked the prostaglandin E2 (PGE2)-dependent immune response specifically in blood myeloid cells while leaving brain cells largely intact. Prostaglandin E2 functions through the EP2 receptor and is the main product of Cox-2, which is increased after status epilepticus. Previous studies from this group have confirmed a major role of this pathway in status epilepticus-induced brain injury by showing that systemic inhibition of EP2 signaling using brain-permeable antagonists is neuroprotective and reduces mortality.<sup>5</sup> A similar effect was seen with forebrain neuron-specific depletion of Cox-2,<sup>6</sup> initially suggesting that the effects of EP2 signaling are solely mediated through the brain. The current study sought to test this hypothesis using myeloid cell-specific, and thus mainly peripheral, EP2 receptor deletion in mice. Pharmacologically induced status epilepticus in these mice was behaviorally indistinguishable from their wild-type littermates but elicited reduced behavioral deterioration and improved recovery of body weight. This supports the interesting hypothesis that peripheral neuroinflammation mediated through the Cox-2/PGE2 pathway after status epilepticus contributes to its detrimental effects on health.

Careful evaluation of the conditional knockout mouse model revealed that EP2 receptors were effectively deleted from blood monocytes and only a small subset of brain microglia and brain endothelial cells. A hallmark of status epilepticus is a compromised blood–brain barrier, which can lead to infiltration of the brain with cells and proteins from the periphery and is believed to contribute to its damaging effects. Activation of inflammatory signaling in the brain can thus occur via factors either produced in the brain or via peripheral factors and cells invading the brain through a compromised blood–brain barrier. The largely peripheral deletion of EP2 receptors in this study slightly improved blood–brain barrier integrity, as measured by brain infiltration with serum albumin. It is conceivable that these rather subtle protective effects on the blood–brain barrier were mediated through EP2 receptor loss on brain endothelial cells as opposed to peripheral myeloid cells, which could have contributed to the beneficial effects on overall health. Treatment with the blood–brain barrier-permeable EP2 receptor antagonist TG6-10-1 led to a more complete restoration of blood–brain barrier integrity and faster weight gain after status epilepticus than peripheral EP2 receptor deletion, further supporting this hypothesis. Other important factors

affecting the blood–brain barrier could be minor differences in status epilepticus in the conditional knockout mice. Varvel et al did not detect differences in behavioral assessments of pharmacologically induced status epilepticus but more sensitive techniques, such as EEG recording are required to entirely rule out altered status epilepticus as a confounding factor in the effects of peripheral EP2 receptor knockout.


It is important to note that peripheral EP2 receptor deletion did not rescue status epilepticus-induced neurodegeneration. The long-term effects on the development of spontaneous seizures and cognition were not assessed but the lack of amelioration of status epilepticus-induced neurodegeneration suggests that cognition and behavior will not be improved with peripheral EP2 receptor deletion.

Another interesting aspect is the selective loss of pathological IL-6 induction in the hippocampus with myeloid cell-specific EP2 knockout, while other inflammation markers, for example, IL-1 $\beta$  and transforming growth factor  $\beta$ , were still induced. By contrast, the EP2 receptor antagonist TG6-10-2 equally reduced induction of all of these factors in the hippocampus and also prevented the infiltration of monocytes into the brain after status epilepticus. Brain monocyte infiltration in the conditional EP2 receptor knockout mice was not tested but given the subtle effects on blood brain barrier integrity—most likely still occurred. Inhibition of the IL-6 receptor with a specific antibody (Tocilizumab) showed some initial success in a small trial in individuals with new-onset refractory status epilepticus,<sup>7</sup> and IL-6 deficiency in either brain-resident cells or in infiltrating monocytes in mice with Theiler murine encephalomyelitis-induced epilepsy reduces seizure frequency.<sup>8</sup> This suggests that IL-6 and/or monocyte infiltration of the brain may contribute to epileptogenesis, which could be tested using the conditional EP2 knockout mice.

The time-dependent role of peripheral EP2 receptor signaling after status epilepticus is another important factor in understanding the contributions of peripheral inflammation to its detrimental effect on overall health. In fact, early neuroinflammation after status epilepticus may be neuroprotective,<sup>9</sup> and Dingledine and colleagues have previously shown that there are distinct therapeutic windows for Cox-2 inhibition following status epilepticus.<sup>10</sup> As a next step, inducible myeloid cell-specific EP2 receptor knockout mice could be used to identify the most beneficial time window to block peripheral EP2 signaling for improvement of recovery after status epilepticus.

The study by Varvel and colleagues is an important step toward elucidating the role of the peripheral immune response after status epilepticus. However, the role of the peripheral immune response in contributing to clinically significant functions in the brain, including brain injury and epileptogenesis remains to be seen. Follow-up studies, including those outlined above, are needed to further understand the exciting prospects of the peripheral immune system as a pathological mechanism of and treatment target for status epilepticus and epilepsy.

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