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Valproic Acid Leads New Neurons Down the Wrong Path

Ectopic Neurogenesis Induced by Prenatal Antiepileptic Drug Exposure Augments Seizure Susceptibility in Adult Mice

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Epilepsy is a neurological disorder often associated with seizure that affects ~0.7% of pregnant women. During pregnancy, most epileptic patients are prescribed antiepileptic drugs (AEDs) such as valproic acid (VPA) to control seizure activity. Here, we show that prenatal exposure to VPA in mice increases seizure susceptibility in adult offspring through mislocalization of newborn neurons in the hippocampus. We confirmed that neurons newly generated from neural stem/progenitor cells (NS/PCs) are integrated into the granular cell layer in the adult hippocampus; however, prenatal VPA treatment altered the expression in NS/PCs of genes associated with cell migration, including CXC motif chemokine receptor 4 (Cxcr4), consequently increasing the ectopic localization of newborn neurons in the hilus. We also found that voluntary exercise in a running wheel suppressed this ectopic neurogenesis and countered the enhanced seizure susceptibility caused by prenatal VPA exposure, probably by normalizing the VPA-disrupted expression of multiple genes including Cxcr4 in adult NS/PCs. Replenishing Cxcr4 expression alone in NS/PCs was sufficient to overcome the aberrant migration of newborn neurons and increased seizure susceptibility in VPA-exposed mice. Thus, prenatal exposure to an AED, VPA, has a long-term effect on the behavior of NS/PCs in offspring, but this effect can be counteracted by a simple physical activity. Our findings offer a step to developing strategies for managing detrimental effects in offspring exposed to VPA in utero.

Commentary

Exposure of the developing fetus to anti-seizure drugs (ASDs) in pregnant mothers with epilepsy has been an area of significant concern for decades. Clinical studies have established that a number of ASDs can have negative effects on the fetus. Among these drugs, valproic acid (VPA) stands out as being particularly harmful.[1] Valproic acid is known to increase levels of the inhibitory neurotransmitter GABA in the brain, targeting sodium channels, and acting as a histone deacetylase inhibitor. Use during pregnancy, however, is associated with increased risk of congenital malformations, including neural tube defects, cognitive delay, language delay, and autism. Based on these risks, the US Food and Drug Administration now states that VPA is contraindicated for pregnant women with migraine (pregnancy category X) and should only be used if other medications are not effective for pregnant women with epilepsy (category D). Despite the concerns, the Australian Register of Antiepileptic Drugs in Pregnancy reported that 20% of pregnant women in their study were treated with VPA in 2016.[1]

The mechanisms by which VPA disrupts development have yet to be fully elucidated. Recent work by Sakai and colleagues, however, provides new insights: They found that prenatal exposure to VPA in mice increased the susceptibility of the exposed animals to chemoconvulsant-induced seizures in adulthood. Increased seizure susceptibility was paired with a reduction in the rate of adult neurogenesis, and an increase in the density of hippocampal dentate granule cells that were malpositioned in the dentate hilus. This increase in ectopic granule cells has potential mechanistic relevance because these abnormal neurons are implicated in disrupted cognition[2] and temporal lobe epileptogenesis.[3] To identify the mechanism by which VPA exposure promotes the accumulation of ectopic granule cells, Sakai and colleagues examined gene expression in the animals and found that Cxcr4 was downregulated. Chemokine receptor 4 is expressed by granule cell progenitors and regulates neuronal migration. Chemokine receptor 4 deletion in mice reduces adult neurogenesis and leads to the appearance of ectopic granule cells.[4,5] To confirm the role of Cxcr4, the investigators overexpressed the gene in granule cell progenitors using a retroviral strategy, which reduced the density of ectopic cells and prevented the increase in seizure susceptibility.

This retroviral rescue experiment is intriguing for several reasons. Firstly, retrovirus selectively infects granule cell progenitors, telling us that this specific cell population is key to the rescue. Secondly, VPA was given between embryonic days 12

to 14, while retroviral transfection was conducted when the pups were 4 weeks old. This demonstrates that delayed treatment can be effective. Finally, only a single treatment was needed. This is somewhat surprising, as retrovirus only infects a subset of progenitor cell stages—so a single treatment would presumably leave many untransfected progenitors.

Exactly how and why Cxcr4 expression produces its effects is still not entirely clear. The most parsimonious explanation is that Cxcr4 overexpression restores the migratory cues needed for immature granule cells to correctly integrate into the cell body layer, reducing the density of hyperexcitable ectopic cells. This fits with the established role of Cxcr4 in regulating granule cell migration[5] and the growing body of literature indicating that ectopic granule cells are hyperexcitable and contribute to epileptogenesis.[3] So how might VPA disrupt Cxcr4 signaling? Valproic acid is known to increase GABA levels in the brain, and Guyon and colleagues[6] demonstrated that GABA, along with other GABA agonists, can bind to and activate Cxcr4. Indeed, work by Bhattacharyya and colleagues[7] demonstrated that the Cxcr4 ligand Cxcl12 is likely coreleased with GABA onto granule cell progenitors by hippocampal interneurons. By disrupting GABA signaling in the neurogenic niche, therefore, VPA may interfere with Cxcr4-regulated migration of immature granule cells. Negative feedback pathways leading to Cxcr4 downregulation could produce long-lasting changes.[5] Consistent with this interpretation, Koyama and colleagues[8] found that treatment with the GABA agonist phenobarbital increased the density of ectopic granule cells in normal rat pups; conversely, treatment with the GABA antagonist picrotoxin decreased ectopic cell density in a rat febrile seizure model.

An alternate possibility is that Cxcr4 overexpression acts to increase the rate of neurogenesis in the animals, and this increase in neurogenesis mediates the beneficial effects of treatment. Cxcr4 regulates dentate neurogenesis rates in rodents,[4,5] and in prior work with the VPA model, the investigators found that embryonic VPA treatment led to a reduction in adult neurogenesis.[9] Work by Iyengar and colleagues[10] found that reducing granule cell neurogenesis increased susceptibility to the convulsant kainic acid; the same drug used to assess seizure susceptibility in the Sakai study. Reduced neurogenesis, therefore, could be a key part of the mechanism by which VPA exposure induces its effects on the developing brain. Consistent with this interpretation, wheel running—which increases granule cell neurogenesis—prevented the neurocognitive and pro-convulsant effects of embryonic VPA exposure.[9,11] Complicating the story, however, wheel running also increased Cxcr4 levels. Whether Cxcr4 overexpression acts by preventing ectopic cell migration, promoting neurogenesis, or some other process remains to be fully resolved.

The study raises the question of whether children exposed to VPA during development have an increased risk of seizures. Despite extensive evidence linking valproate exposure to other abnormalities, there is little evidence in the clinical literature to suggest an increased seizure risk. That said, VPA-exposed mice only exhibited increased susceptibility to evoked seizures and not epilepsy per se; a similar change in humans would be difficult to detect. An effect might manifest as an increased incidence and/or severity of febrile seizures, which could be included as an outcome measure in future pregnancy registries. More likely, however, ectopic granule cells and impaired neurogenesis might contribute to the cognitive impairments, including memory dysfunction, associated with VPA exposure. Either or both could contribute to the intellectual deficits observed in exposed children. Notably, because of the protracted period of granule cell neurogenesis (third trimester through at least childhood in humans), both gestational exposure and postnatal exposure in breast milk could be detrimental. Finally, it is promising that exercise (wheel running) mitigated the negative effects of VPA exposure in rodents,[9,11] but whether behavioral modification and exercise therapies could be developed to produce similar effects in children is not certain. Nonetheless, further development of therapeutic strategies to offset the negative effects of VPA exposure in mothers without other good options for seizure control is warranted.

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