relate to the phenomenon of hypoglycemia-induced seizures? Perhaps the severity of hypoglycemia, with diminished output from glycogenolysis, might lead to severely reduced intracellular ATP levels, such that even activation of K_{ATP} channels under these conditions could not overcome the destabilization of the cellular membrane potential that results from the impairment of critical pumps, such as the Na^+, K^+ -ATPase.

Notwithstanding the usual concerns raised when detailed investigations of this type are conducted on normal nonepileptic neurons, this compelling study by Kawamura and colleagues provides yet another molecular target that may play a pivotal role in reducing seizures under conditions of reduced glycolytic flux and helps fill an important gap in understanding how metabolic changes or adaptations yield profound effects on neuronal excitability and integrity.

by Jong M. Rho, MD

References

- Freeman J, Veggiotti P, Lanzi G, Tagliabue A, Perucca E; Institute of Neurology IRCCS C. Mondino Foundation. The ketogenic diet: From molecular mechanisms to clinical effects. *Epilepsy Res* 2006;68:145–180.
- Muzykewicz DA, Lyczkowski DA, Memon N, Conant KD, Pfeifer HH, Thiele EA. Efficacy, safety, and tolerability of the low glycemic index treatment in pediatric epilepsy. *Epilepsia* 2009;50:1118–1126.
- Fontana L, Partridge L, Longo VD. Extending healthy life span—from yeast to humans. *Science* 2010;328:321–326.
- Maalouf M, Rho JM, Mattson MP. The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies. *Brain Res Rev* 2009;59:293–315.
- Garriga-Canut M, Schoenike B, Qazi R, Bergendahl K, Daley TJ, Pfender RM, Morrison JF, Ockuly J, Stafstrom C, Sutula T, Roopra A. 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. *Nat Neurosci* 2006;9:1382–1387.
- Lian XY, Khan FA, Stringer JL. Fructose-1,6-bisphosphate has anticonvulsant activity in models of acute seizures in adult rats. *J Neurosci* 2007;27:12007–12011.
- Gasior M, Yankura J, Hartman AL, French A, Rogawski MA. Anticonvulsant and proconvulsant actions of 2-deoxy-d-glucose. *Epilepsia*. 2010 May 17. [Epub ahead of print].
- Theodore WH, Gaillard WD. Neuroimaging and the progression of epilepsy. *Prog Brain Res* 2002;135:305–313.
- Pan JW, Williamson A, Cavus I, Hetherington HP, Zaveri H, Petroff OAC, Spencer DD. Neurometabolism in human epilepsy. *Epilepsia* 2008;49(suppl 3):31–41.
- Cunha RA. Different cellular sources and different roles of adenosine: A1 receptor-mediated inhibition through astrocytic-driven volume transmission and synapse-restricted A2A receptor-mediated facilitation of plasticity. *Neurochem Int* 2008;2:65–72.
- Dunwiddie TV, Diao L, Proctor WR. Adenine nucleotides undergo rapid, quantitative conversion to adenosine in the extracellular space in rat hippocampus. *J Neurosci* 1997;17:7673–7682.
- Boison D. The adenosine kinase hypothesis of epileptogenesis. *Prog Neurobiol* 2008;84:249–262.
- Bough KN, Wetherington J, Bjornar H, Pare JF, Garyluk JW, Greene JG, Shaw R, Smith Y, Geiger JD, Dingledine RJ. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Ann Neurol* 2006;60:223–235.
- MacVicar BA, Thompson RJ. Non-junction functions of pannexin-1 channels. *Trends Neurosci* 2010;33:93–102.
- Ma W, Berg J, Yellen G. Ketogenic diet metabolites reduce firing in central neurons by opening $K(ATP)$ channels. *J Neurosci* 2007;27:3618–3625.

NEUROSTEROIDS ON THE EPILEPSY CHESSBOARD—KEEPING SEIZURES IN CHECK

Endogenous Neurosteroid Synthesis Modulates Seizure Frequency. Lawrence C, Martin BS, Sun C, Williamson J, Kapur J. *Ann Neurol* 2010;67(5):689–693. Inhibitory neurosteroids, molecules generated in glia from circulating steroid hormones and de novo from cholesterol, keep seizures in check in epileptic animals. They can enhance inhibitory transmission mediated by γ -aminobutyric acid receptors and have anticonvulsant action.

COMMENTARY

The steroid hormone progesterone has long been known to have anticonvulsant properties. Seizure protection is

not conferred by progesterone itself, but rather by the progesterone metabolite allopregnanolone, a member of a class of endogenous steroids commonly referred to as neurosteroids (1). Neurosteroids do not have traditional hormonal activity. Rather, allopregnanolone and other similar neurosteroids act as positive allosteric modulators and direct activators of $GABA_A$ receptors. Like other such $GABA_A$ -receptor modulators,

allopregnanolone is a powerful anticonvulsant. Although neurosteroids act on all GABA_A-receptor isoforms, including those localized at synapses, they produce especially large effects on nonsynaptic δ -subunit containing GABA_A-receptor isoforms. Recent evidence indicates that neurosteroids are present predominantly, if not exclusively, in principal (excitatory) neurons in many brain regions that are relevant to focal epilepsies, including the hippocampus and neocortex (2). All of the enzymes required for neurosteroid synthesis are expressed in the brain. Although it had previously been speculated that brain neurosteroidogenesis occurs in astrocytes, new immunohistochemical evidence indicates that GABA_A-receptor modulatory neurosteroids may not be present in glia or in nonpyramidal neurons (i.e., interneurons). The highly restricted distribution of neurosteroids to excitatory neurons suggests that they are mainly derived from local synthesis and not from the circulation, although it is clear that peripheral neurosteroids do easily cross the blood-brain barrier.

The factors that regulate local neurosteroid synthesis are obscure. However, since GABA_A receptors are also localized to principal neurons, neurosteroids may serve an autocrine role to influence the functional activity of the very neurons in which they are synthesized. Recently, evidence has accumulated that neurosteroids influence GABA_A receptors by binding to discrete sites on the receptor complex located within the transmembrane domains of α and β GABA_A-receptor subunits (3) and that they access these sites by lateral membrane diffusion (4). Neurosteroids are viewed as high-potency modulators of GABA_A receptors, since they are effective at concentrations in the range of 1 μ M and below in aqueous solution. However, neurosteroid binding to the GABA_A receptor is of low affinity (K_d , $\sim$ 1 mM), and the high effective potency of neurosteroids results from partitioning of the lipophilic steroids within the plasma membrane, such that the concentrations presented to the receptor are orders of magnitude greater.

Given the emerging understanding of the role of neurosteroids as autocrine modulators of neuronal excitability, the question arises as to whether endogenous neurosteroids regulate seizure susceptibility. Lawrence et al. provide important new evidence that the availability of neurosteroids does indeed critically influence the propensity for seizures. These investigators used epileptic female rats that had experienced a prolonged bout of status epilepticus induced by lithium-pilocarpine treatment. Spontaneous seizure activity was monitored by continuous video-EEG recording. The epileptic animals exhibited about six seizures per day, each lasting approximately a minute. However, when neurosteroids were withdrawn, using the neurosteroid synthesis inhibitor finasteride, an enormous (more than ten-fold) increase in seizure frequency was observed. In contrast, finasteride—a selective inhibitor of

5 α -reductase, the first and rate-limiting enzymatic step in the synthesis of neurosteroids from their steroid hormone precursors (e.g., progesterone)—did not induce seizures in normal animals.

Similarly, there is no evidence that finasteride causes seizures in humans who do not have epilepsy. Finasteride is used clinically for the treatment of benign prostatic hypertrophy and male pattern hair loss. Seizures have not been reported as an adverse event of finasteride treatment, and given the long experience with the drug, it is safe to say that it does not cause epilepsy. It is also noteworthy that individuals with congenital 5 α -reductase deficiency, caused by a mutation in the 5 α -reductase type 2 gene (a condition with ambiguous genitalia), do not exhibit epilepsy. Although neurosteroid deficiency does not induce epilepsy, there is evidence that neurosteroids can modulate epileptogenesis (5).

While finasteride does not provoke seizures in the general population, there are no prospective studies to determine whether inhibition of 5 α -reductase by finasteride influences seizure susceptibility in individuals with epilepsy. There is a single anecdotal report of a woman with epilepsy taking finasteride for male pattern baldness who experienced an increase in seizure frequency and severity in association with finasteride use (6). The doses of finasteride used clinically are in the range of 1 to 5 mg per day, which is far less than the doses of 30 to 100 mg/kg used in rats in the study by Lawrence et al. Furthermore, in humans, finasteride is selective for the type 2 5 α -reductase isoform and less active on the type 1 enzyme that is the isoform predominantly present in the brain. This selectivity is not observed with the rat enzymes. In sum, finasteride, as administered clinically in humans, probably does not block neurosteroidogenesis sufficiently to influence seizure susceptibility under most circumstances.

When neurosteroid levels fluctuate, loss of seizure control can occur. A key clinical situation in which neurosteroids are a factor in seizure control is perimenstrual catamenial epilepsy. Rodents have a 4 to 5 day estrous cycle and studies of fluctuations in seizure susceptibility in cycling female rodents have not led to results that are relevant to the human menstrual cycle. In order to study the neurobiological basis of catamenial epilepsy and develop treatment approaches, a rat model was developed both to simulate the prolonged exposure to high levels of estrogen and progesterone that occur in the luteal phase of the 28-day human menstrual cycle as well as to replicate the withdrawal of allopregnanolone that occurs in conjunction with the fall in progesterone levels at the time of menstruation (7). Prolonged elevated sex hormone levels are induced by gonadotrophin treatment, and 11 days later allopregnanolone is withdrawn by treatment with finasteride. Lawrence et al. utilized the catamenial epilepsy model in epileptic rats. They found an even greater exacerbation of seizures than ordinarily occurs

in epileptic animals. This finding suggests that prolonged exposure to neurosteroids (and perhaps other hormonal changes in the catamenial epilepsy model) leads to heightened excitability in the epileptic brain that is revealed when the neurosteroids are withdrawn. A key alteration that has been associated with chronic neurosteroid exposure and could be a contributing factor is down-regulation of δ -subunit containing nonsynaptic GABA_A receptors (8).

Lawrence et al. conducted a final experiment in ovariectomized epileptic animals that had low serum progesterone levels maintained by subcutaneous implantation of a progesterone pellet. Unexpectedly, these animals also exhibited a huge (25-fold) increase in seizure frequency following finasteride treatment. Unfortunately, the authors did not study animals in which peripheral progesterone was completely eliminated. Nevertheless, the results of this last experiment suggest that the exacerbation of seizures that occurs following finasteride treatment is due to the inhibition of brain neurosteroid synthesis and is not caused by suppression of the conversion of peripheral (ovarian) progesterone to allopregnanolone. This important conclusion focuses attention squarely on brain neurosteroids as critical regulators of seizures in epilepsy. Clearly, more needs to be learned about the role of locally synthesized neurosteroids in epileptic brain circuits.

The paper by Lawrence et al. also has important implications for therapy. The authors demonstrate that allopregnanolone treatment rapidly terminates the finasteride-induced exacerbation of seizures, providing additional evidence that the increase in seizure frequency is because of a finasteride-induced reduction in neurosteroids and not some other action of the drug. More importantly, it supports the concept that neurosteroid replacement may be useful in the treatment of seizures

associated with neurosteroid fluctuations, such as catamenial epilepsy (7). In catamenial epilepsy, breakthrough seizures occur despite treatment with antiepileptic drugs. Previous studies (reviewed in 7) and the new results from Lawrence et al. support the potential of neurosteroids as a novel treatment approach for these pharmacoresistant seizures.

by Michael A. Rogawski, MD, PhD

References

1. Kokate TG, Banks MK, Magee T, Yamaguchi S, Rogawski MA. Finasteride, a 5α -reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. *J Pharmacol Exp Ther* 1999;288:679–684.
2. Saalmann YB, Kirkcaldie MT, Waldron S, Calford MB. Cellular distribution of the GABA_A receptor-modulating 3α -hydroxy, 5α -reduced pregnane steroids in the adult rat brain. *J Neuroendocrinol* 2007;19:272–284.
3. Hosie AM, Clarke L, da Silva H, Smart TG. Conserved site for neurosteroid modulation of GABA_A receptors. *Neuropharmacology* 2009;56:149–154.
4. Chisari M, Eisenman LN, Covey DF, Mennerick S, Zorumski CF. The sticky issue of neurosteroids and GABA_A receptors. *Trends Neurosci* 2010;33:299–306.
5. Biagini G, Baldelli E, Longo D, Pradelli L, Zini I, Rogawski MA, Avoli M. Endogenous neurosteroids modulate epileptogenesis in a model of temporal lobe epilepsy. *Exp Neurol* 2006;201:519–524.
6. Herzog AG, Frye CA. Seizure exacerbation associated with inhibition of progesterone metabolism. *Ann Neurol* 2003;53:390–391.
7. Reddy DS, Rogawski MA. Neurosteroid replacement therapy for catamenial epilepsy. *Neurotherapeutics* 2009;6:392–401.
8. Maguire J, Mody I. Steroid hormone fluctuations and GABA_AR plasticity. *Psychoneuroendocrinology* 2009;34(suppl 1):S84–S90.