

VALPROATE ENHANCES NEUROPEPTIDE Y EXPRESSION: MODULATING THE MODULATORS

Chronic Valproic Acid Treatment Triggers Increased Neuropeptide Y Expression and Signaling in Rat Nucleus Reticularis Thalami. Brill J, Lee M, Zhao S, Fernald RD, Huguenard JR. *J Neurosci* 2006;26:6813–6822. Valproate (VPA) can suppress absence and other seizures, but its precise mechanisms of action are not completely understood. We investigated whether VPA influences the expression of neuropeptide Y (NPY), an endogenous anticonvulsant. Chronic VPA administration to young rats (300–600 mg · kg⁻¹ · d⁻¹ in divided doses over 4 d) resulted in a 30–50% increase in NPY mRNA and protein expression in the nucleus reticularis thalami (nRt) and hippocampus, but not in the neocortex, as shown by real-time PCR, radioimmunoassay, and immunohistochemistry. No increased expression was observed after a single acute dose of VPA. Chronic treatment with the pharmacologically inactive VPA analog octanoic acid did not elicit changes in NPY expression. No significant expression changes could be shown for the mRNAs of the Y₁ receptor or of the neuropeptides somatostatin, vasoactive intestinal polypeptide, and cholecystokinin. Fewer synchronous spontaneous epileptiform oscillations were recorded in thalamic slices from VPA-treated animals, and oscillation duration as well as the period of spontaneous and evoked oscillations were decreased. Application of the Y₁ receptor inhibitor N²-(diphenylacetyl)-N-[(4-hydroxyphenyl)methyl]-D-arginine-amide (BIBP3226) enhanced thalamic oscillations, indicating that NPY is released during those oscillations and acts to downregulate oscillatory strength. Chronic VPA treatment significantly potentiated the effect of BIBP3226 on oscillation duration but not on oscillation period. These results demonstrate a novel mechanism for the antiepileptic actions of chronic VPA therapy.

COMMENTARY

The cellular basis of epileptic seizures often is referred to as an imbalance involving excess excitation and/or insufficient inhibition. While disturbed connectivity within circuits also is important, the concept of altered excitability is useful to understanding many features of epileptic seizures. Disruption of normal ion channel function and glutamate/GABA neurotransmission have well-documented roles in epilepsy, but other modulatory systems may help regulate the balance of excitability in neuronal circuits. Neuropeptide Y (NPY) is one particularly

promising endogenous antiepileptic peptide. The recent paper by Brill et al. builds on previous work to show that the ability of valproate to alter thalamocortical excitability involves regulation of NPY expression within the thalamus.

NPY is a 36-amino-acid peptide that is widely distributed throughout the CNS. In normal brain, this protein is expressed exclusively in inhibitory neurons. Investigations using a combination of intracerebroventricular administration of NPY, NPY overexpression, and knockout animals have shown that this neuromodulator helps control a variety of functions, including feeding, stress response, and reproduction. Six types of NPY receptors (referred to as Y₁–Y₆) have been proposed on the basis of pharmacological experiments, but only Y₁, 2, 4, and 5 actually have been cloned and shown to form functional receptors in native rat and primate tissue. The majority of NPY receptors

in the brain are of the Y1 or Y2 subtype, with lower levels of Y5 being expressed in some brain regions. As with other G-protein-coupled receptors, NPY receptors activate a variety of secondary messenger systems. However, as a rule, Y1 receptors in the thalamus and hippocampus act postsynaptically to activate G-protein coupled inwardly rectifying potassium channels, while Y2 receptors inhibit neurotransmitter release through suppression of presynaptic calcium channels.

In addition to its other functions, NPY helps regulate neuronal excitability and may be an important component in controlling the seizure threshold. As reviewed in a previous *Epilepsy Currents* commentary (1), work on multiple models of epilepsy has described the interrelationship between NPY and epileptic seizures. Intracranial administration of exogenous NPY suppresses seizures; similar results are obtained by using transgenic animals or recombinant viral vectors to overexpress NPY in the brain. Furthermore, inactivation of the *NPY* gene produces transgenic animals that, while largely normal, have lowered thresholds to both electrical and chemoconvulsant-induced seizures (2,3). Conversely, increased NPY expression is seen after acute seizures and chronic kindling in animal models of epilepsy (4) as well in tissue taken from epilepsy surgery patients with hippocampal sclerosis (5). Chronic epilepsy also is associated with more complex alterations of the NPY system, including upregulation of Y2 but decreased expression of Y1 receptors within the hippocampi (6). Finally, even the pattern of NPY expression is disturbed in the hippocampi of epileptic patients or animals. In normal subjects, NPY expression in the dentate gyrus is restricted primarily to hilar interneurons, with projections that include CA3 and the dentate molecular layer. Following status epilepticus, while inhibitory neurons of the dentate hilus are lost, there actually is increased NPY expression in the dentate molecular layer. As part of the pathological remodeling that occurs during temporal lobe epileptogenesis, dentate granule cells develop recurrent mossy fiber projections that express NPY *de novo*. This unique expression of NPY by a glutamatergic neuron may help restrain the hyperexcitable dentate granule cells through presynaptic inhibition of glutamate release (7). Indeed, consistent with the efficacy of NPY to suppress seizures, the recurrent excitation of dentate granule cells in slices from epileptic animals is reduced by application of Y2 agonists and enhanced by Y2 antagonists. While Y5 analogs also may have anticonvulsant activity, it is unclear how much of this effect actually is due to nonspecific activation of Y2 receptors (3). In contrast, similar studies have suggested that activation of Y1 receptors may lower the seizure threshold. Thus, NPY may either increase or decrease excitability, depending on the specific cell type and the NPY receptors involved.

In contrast to temporal lobe seizures, very little is known about the role on NPY in idiopathic generalized epilepsy. Spike-wave discharges, the electrical hallmark of absence seizures, are

generated in the thalamocortical circuit, which includes the thalamic relay nuclei, neocortex, and the nucleus reticularis of the thalamus (nRT). Thalamic relay neurons send ascending excitatory projections to the cortex as well as to the nRT. Cortical neurons then send descending excitatory inputs back to the nRT. The nRT form a shell of exclusively GABAergic neurons around the rest of the thalamus. Each nRT neuron forms inhibitory synapses upon many thalamic relay cells. This recurrent circuit allows the simultaneous inhibition of many thalamic relay neurons, followed by a brief volley of rebound action potentials, thereby producing the synchronous, slow thalamocortical rhythms of sleep. Within the nRT itself, there are inhibitory interconnections that, when disrupted, can produce the hypersynchronous thalamocortical discharges of absence seizures. In addition to GABA, nearly all nRT neurons express NPY, and the nRT is the primary source of NPY input to the rest of the thalamus. The physiological role of NPY in the thalamus currently is unknown; however, recent work has sought to clarify this system. Investigators used a combination of NPY knockout animals with NPY analogs to show that burst firing in nRT neurons releases NPY, which subsequently activates Y1 receptors, causing a slow hyperpolarization via activation of G-protein inwardly rectifying potassium channels within the nRT neurons. Furthermore, application of NPY or the Y1 preferring peptide, [Leu³¹Pro³⁴] NPY, partially suppressed the thalamic network oscillations induced by electrical stimulation in bicuculline-treated brain slices. The opposite effects were seen with application of the selective nonpeptide Y1 receptor antagonist, BIBP3226, suggesting that NPY is released endogenously during burst firing, thereby limiting the duration and/or synchrony of these bursts (8).

Idiopathic generalized epilepsies are unusual in that they are often insensitive to, or even exacerbated by, many of the more commonly used antiepileptic drugs. Valproate is one of the few medications that are efficacious for these patients. The mechanism of valproate action is still unclear, but it may involve changes in the activity of certain transcription factors that, thereby, regulate the expression of key neuronal proteins. Along these lines, preliminary work in cell culture had suggested that valproate might alter NPY expression. The paper by Brill et al. expands on earlier findings to explore the effect of subacute valproate treatment to alter NPY modulation of thalamocortical circuits. Following 4 days of valproate administration, there was an increase in NPY expression in the nRT and hippocampus but not in the neocortex. The physiological significance of these changes was explored in thalamocortical slice preparations taken from animals treated with valproate or the biologically inactive analog, sodium octanoate. While acute application of valproate to brain slices did not alter burst firing, slices from valproate-treated animals had reduced burst duration as well as reduced synchrony among cells during a burst.

Furthermore, BIBP3226 increased the duration of thalamic oscillations in control and valproate-treated animals, suggesting a tonic activation of Y1 receptors in burst firing nRT neurons. Moreover, the magnitude of this effect was significantly greater in slices from valproate-treated animals. Since there was no detectable change in Y1 receptor expression following valproate treatment, these effects likely are related to increased expression and/or release of NPY.

While intracerebroventricular injection of NPY suppresses spike-wave discharges in the genetic absence epilepsy rats from Strasbourg (GAERS) model of absence epilepsy (9), the role of NPY in idiopathic generalized epilepsy otherwise is almost completely unknown. It will be interesting to see whether genetic models of absence have disrupted NPY function, especially if they are responsive to clinically relevant antiepileptic medications. Conversely, while NPY knockout mice have seizure-like behavioral events (2), it is unclear whether disruption of NPY expression in specific brain regions can cause absence seizures. Furthermore, it is entirely possible that other, unknown components of the NPY system may help to regulate thalamocortical function. The work by Brill et al. focused on Y1 because it is the predominant NPY receptor type within the thalamus. However, Y2 and Y5 receptors also are present (10), and activation of thalamic Y2 receptors lowers the frequency of inhibitory postsynaptic currents through suppression of N/P-type calcium channels (11). Given that Y1-receptor activation may lower the threshold for some seizure types, it would be useful to know if Y2-receptor activation also limits thalamocortical excitability. Thus, while NPY may be important in normal thalamocortical functioning and epilepsy, much work remains to be done.

In addition to being a broad-spectrum antiepileptic medication, valproate causes a number of other therapeutic as well as adverse effects. In some patient populations, the weight gain associated with valproate treatment is particularly troublesome. Given the importance of NPY in the regulation of feeding, it is tempting to speculate that the orexic effects of valproate also involve enhanced NPY expression. Valproate treatment did not alter NPY expression in the hypothalamic paraventricular nucleus, a key player in the regulation of food intake. However, expression of NPY or its receptors were not determined in other feeding-related hypothalamic nuclei, and it remains unknown which, if any, of valproate's diverse effects actually are mediated by enhanced NPY expression. Furthermore, although NPY clearly has anticonvulsant effects on a variety of seizure

models, the clinical utility of those finding is far from obvious. The lack of available NPY-specific drugs and the diverse actions of NPY on a variety of physiological functions make it unlikely that direct manipulation of NPY receptors will be useful in treating epilepsy in the near future. The findings of Brill et al. help expand our understanding of the role of neuropeptides to determine neuronal excitability, especially as it relates to the treatment of epilepsy. Perhaps more important, the ability of valproate to induce upregulation of a specific neuromodulatory peptide in specific brain regions provides an exciting alternative approach to the study and treatment of epilepsy patients.

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