Rational Polypharmacy: When Two Old Drugs Are Better Than One

Bumetanide Enhances Phenobarbital Efficacy in a Neonatal Seizure Model. Dzhala VI, Brumback AC, Staley KJ. *Ann Neurol* 2008;63(2):222–235. OBJECTIVES: High levels of expression of the Na^+-K^+-$2Cl^-$ (NKCC1) cotransporter in immature neurons cause the accumulation of intracellular chloride and, therefore, a depolarized Cl^- equilibrium potential (E_{Cl}). This results in the outward flux of Cl^- through $GABA_A$ channels, the opposite direction compared with mature neurons, in which $GABA_A$ receptor activation is inhibitory because Cl^- flows into the cell. This outward flow of Cl^- in neonatal neurons is excitatory and contributes to a greater seizure propensity and poor electroencephalographic response to GABAergic anticonvulsants such as phenobarbital and benzodiazepines. Blocking the NKCC1 transporter with bumetanide prevents outward Cl^- flux and causes a more negative GABA equilibrium potential (E_{GABA}) in immature neurons. We therefore tested whether bumetanide enhances the anticonvulsant action of phenobarbital in the neonatal brain. METHODS: Recurrent seizures were induced in the intact hippocampal preparation in vitro by continuous 5-hour exposure to low-Mg^{2+} solution. The anticonvulsant efficacy of phenobarbital, bumetanide, and the combination of these drugs was studied. RESULTS: Phenobarbital failed to abolish or depress recurrent seizures in 70% of hippocampi. In contrast, phenobarbital in combination with bumetanide abolished seizures in 70% of hippocampi and significantly reduced the frequency, duration, and power of seizures in the remaining 30%. INTERPRETATION: Thus, alteration of Cl^- transport by bumetanide enables the anticonvulsant action of phenobarbital in immature brain. This is a mechanistic demonstration of rational anticonvulsant polypharmacy. The combination of these agents may comprise an effective therapy for early-life seizures.

COMMENTARY

Although traditionally assigned the role as the main inhibitory influence that prevents excessive excitation and seizures in the brain, the neurotransmitter GABA plays a much more complex role than initially imagined. As reviewed in recent *Epilepsy Currents* commentaries (1,2), depending on the intracellular chloride content of postsynaptic neurons, GABA may also be excitatory, even promoting epileptiform activity. The postsynaptic effect of GABA stems from the opening of chloride channels within the $GABA_A$-receptor complex: when intracellular chloride is kept low because of its extrusion by the K^+/Cl^--cotransporter isoform 2, KCC2, $GABA_A$-receptor activation causes the flow of negatively charged chloride ions into the cell. The resulting hyperpolarization of the neuronal membrane is the basis for the inhibitory effect on postsynaptic neurons and for the antiepileptic effects of drugs (e.g., benzodiazepines and barbiturates) that enhance GABAergic neurotransmission. However, when intracellular chloride is high, which occurs when KCC2 expression is reduced or when the $Na^+/K^+/Cl^-$-cotransporter isoform 1 (NKCC1) is expressed, $GABA_A$-activated chloride flow is reversed, and GABA is depolarizing, even surpassing the threshold for action

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potential generation. Because the reversal potential for $GABA_A$-activated chloride currents is never as depolarized as that for the glutamate-receptor activated currents, GABA can still have a "shunting" effect on excitatory potentials and remain a relatively stabilizing influence on excitability.

During the early postnatal period in rodents and likely during the late prenatal and early postnatal period in humans, NKCC1 is expressed highly in the brain, while KCC2 expression is low. Under these conditions, GABA release contributes to the initiation of giant depolarizing potentials (GDPs) in populations of neurons. These GDPs not only serve as a marker of GABAergic excitatory influence but also play a critical role in the development of mature synapses. As the brain matures, NKCC1 expression is downregulated; however, recent evidence points to a recapitulation of this early developmental pattern of chloride transporter expression in neurons exposed to injury and in hippocampal neurons from patients with mesial temporal lobe epilepsy.

Previous studies have shown that the excitatory effect of GABA is at least partially responsible for the increased susceptibility to seizures in neonates (3). Bumetanide, a loop diuretic that potently inhibits NKCC1 transporter, impairs the intracellular accumulation of chloride and thereby, may convert GABA from excitatory to inhibitory. Accordingly, bumetanide abolishes GDPs in neonatal brain and prevents experimental seizures both in vitro and in vivo (3). Not surprisingly, because phenobarbital potentiates the effects of GABA, it is ineffective in reducing seizure activity in the same seizure model.

In clinical practice as well, phenobarbital fails to control acute seizures in more than half of neonates (4). Nevertheless, many physicians continue to choose barbiturates or benzodiazepines as first line agents, perhaps because of their familiarity and lack of compelling data for alternate therapies. In the present study by Dzhala et al., a possible new treatment strategy was tested in the established in vitro low-magnesium seizure model, using intact hippocampi from neonatal rats. This study design may provide a more robust model of hippocampal seizures because the hippocampal circuitry remains intact and the induction of seizures did not involve alteration of ion gradients that may influence chloride homeostasis. The authors hypothesized that if inhibition of NKCC1 with bumetanide alters the chloride gradient of neonatal hippocampal neurons such that GABA becomes more hyperpolarizing, then phenobarbital will gain the seizure suppressive effect that it has in mature brains.

To test their hypothesis, the authors first showed the effects of these drugs on chloride homeostasis and neuronal activity. The reversal potential for $GABA_A$-activated currents in immature pyramidal neurons was –63 mV, compared with a resting potential of –70 mV, indicating that GABA was in fact depolarizing. Consistent with this result, a $GABA_A$-receptor agonist (i.e., isoguvacine) exerted a net excitatory effect, increasing neuronal firing. As expected, although phenobarbital augmented $GABA_A$-activated currents, it did not reduce its excitatory effect. When bumetanide was added, the reversal potential for GABA shifted to –73 mV, suggesting that GABA had become inhibitory. Importantly, the administration of phenobarbital in combination with bumetanide now suppressed neuronal firing.

The authors next asked whether bumetanide could alter the effect of phenobarbital on seizure activity. Bathing intact hippocampi from neonatal rats in a low-magnesium solution induced recurrent seizure-like discharges, which was probably related to hyperexcitability induced when the magnesium block of the NMDA receptor was relieved. In this model, phenobarbital alone stopped seizure-like events in 30% of hippocampi and decreased frequency in others; however, it also increased duration and amplitude of the power spectra of the population activity in those hippocampi, indicating that by some measures the remaining seizures were worsened. Bumetanide alone had some efficacy against seizures: although it abolished seizures in only 20% of hippocampi, it decreased frequency, duration, and amplitude of power spectra in the others. The combination of bumetanide with phenobarbital produced a much stronger effect: seizures were abolished in 70% of hippocampi, and their frequency, duration, and power spectra were reduced in the remaining hippocampi more than with bumetanide alone. These findings support the clinical observation that phenobarbital alone is unlikely to stop seizures in neonates, and bumetanide, although itself weakly anticonvulsant, is also unlikely to produce a clinically satisfactory effect. However, the combination of increasing the inhibitory efficacy of GABA with bumetanide and potentiating its effect with phenobarbital, even when administered simultaneously, has the potential to be a powerful anticonvulsant therapy for neonatal seizures.

While Dzhala et al. used an animal model to demonstrate the efficacy of bumetanide and phenobarbital to decrease artificially induced seizures, the application of these two drugs to the treatment of human epilepsy is logical. Presumably, the strategy will work with benzodiazepines as well. The authors argue that bumetanide has demonstrated safety in neonates, and in vivo studies suggest effective penetration into brain (3); however, Dzhala and colleagues are careful to note the uncertain consequences of altering the delicate balance of inhibitory and excitatory influences in the developing brain using bumetanide. As previously mentioned, depolarizing GABA responses such as GDPs play an important role in the development of mature synapses, therefore long-term administration of bumetanide could have unintended and potentially untoward effects that may manifest later in life. Phenobarbital itself has been implicated in deleterious effects on neuron survival (5) and in persistent cognitive deficits after use during critical developmental stages (6). Therefore, greater

efficacy of a drug combination against typically treatment-resistant neonatal seizures may allow for use of lower doses or shorter duration of phenobarbital therapy. In addition, the benefit of providing earlier control of seizure activity may itself reduce potential long-term consequences of neonatal seizures. Finally, because of mounting evidence for altered chloride homeostasis in adult epileptic brain, the prospective use of this combination therapy may extend beyond neonates to rational combinations of bumetanide and GABA-enhancing medications in treatment-resistant adult epilepsy.

by Gregory C. Mathews, MD, PhD

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VEGF As a Target for Neuroprotection

Vascular Endothelial Growth Factor Is Up-Regulated after Status Epilepticus and Protects against Seizure-Induced Neuronal Loss in Hippocampus. Nicoletti JN, Shah SK, McCloskey DP, Goodman JH, Elkady A, Atassi H, Hylton D, Rudge JS, Scharfman HE, Croll SD. *Neuroscienc*e 2008;151(1):232–241. Vascular endothelial growth factor (VEGF) is a protein factor which has been found to play a significant role in both normal and pathological states. Its role as an angiogenic factor is well-established. More recently, VEGF has been shown to protect neurons from cell death both in vivo and in vitro. While VEGF's potential as a protective factor has been demonstrated in hypoxia–ischemia, in vitro excitotoxicity, and motor neuron degeneration, its role in seizure-induced cell loss has received little attention. A potential role in seizures is suggested by Newton et al.'s [Newton SS, Collier EF, Hunsberger J, Adams D, Terwilliger R, Selvanayagam E, Duman RS (2003) Gene profile of electroconvulsive seizures: Induction of neurotrophic and angiogenic factors. *J Neurosci* 23:10841–10851] finding that VEGF mRNA increases in areas of the brain that are susceptible to cell loss after electroconvulsive-shock induced seizures. Because a linear relationship does not always exist between expression of mRNA and protein, we investigated whether VEGF protein expression increased after pilocarpine-induced status epilepticus. In addition, we administered exogenous VEGF in one experiment and blocked endogenous VEGF in another to determine whether VEGF exerts a neuroprotective effect against status epilepticus-induced cell loss in one vulnerable brain region, the rat hippocampus. Our data revealed that VEGF is dramatically up-regulated in neurons and glia in hippocampus, thalamus, amygdala, and neocortex 24 h after status epilepticus. VEGF induced significant preservation of hippocampal neurons, suggesting that VEGF may play a neuroprotective role following status epilepticus.

COMMENTARY

Vascular endothelial growth factor (VEGF) is a vascular growth factor that induces angiogenesis, increases vascular permeability, and promotes inflammation in the CNS (1,2). VEGF, originally considered as an endothelial-specific growth factor and a potent mitogen for endothelial cells derived from arteries, veins, and lymphatics, has recently been shown to have direct effects on different cell types, including neurons, Schwann cells, astrocytes, neural stem cells, and microglia. Increased levels of VEGF in the brain have been measured after a variety of insults, including hypoxia/ischaemia and seizures. In particular, following seizure induction in experimental models, VEGF was expressed mainly by neurons and astrocytes (3; Nicoletti et al.), while in human temporal lobe epilepsy specimens, prominent VEGF immunostaining was found also in the microvasculature. The identification of VEGF receptors in epileptogenic tissue—not only on endothelial cells of blood vessels (3) but also on astrocytes and neurons (1,2,4)—raised

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