Altered Neuronal Chloride Homeostasis and Excitatory GABAergic Signaling in Human Temporal Lobe Epilepsy

Cation-Chloride Cotransporters and GABAergic Innervation in the Human Epileptic Hippocampus. Munoz A, Mendez P, DeFelipe J, Alvarez-Leefmans FJ. *Epilepsia* 2007;48(4):663–673. Intracellular chloride concentration, $[Cl]_i$, determines the polarity of $GABA_A$-induced neuronal Cl currents. In neurons, $[Cl]_i$ is set by the activity of Na^+, K^+, 2Cl cotransporters (NKCC) such as NKCC1, which physiologically accumulate Cl in the cell, and Cl extruding K^+, Cl cotransporters like KCC2. Alterations in the balance of NKCC1 and KCC2 activity may determine the switch from hyperpolarizing to depolarizing effects of GABA, reported in the subiculum of epileptic patients with hippocampal sclerosis. We studied the expression of NKCC (putative NKCC1) and KCC2 in human normal temporal neocortex by Western blot analysis and in normal and epileptic regions of the subiculum and the hippocampus proper using immunocytochemistry. Western blot analysis revealed NKCC and KCC2 proteins in adult human neocortical membranes similar to those in rat neocortex. NKCC and KCC2 immunolabeling of pyramidal and nonpyramidal cells was found in normal and epileptic hippocampal formation. In the transition between the subiculum with sclerotic regions of CA1, known to exhibit epileptogenic activity, double immunolabeling of NKCC and KCC2 revealed that approximately 20% of the NKCC-immunoreactive neurons do not express KCC2. In these same areas, some neurons were distinctly hyperinnervated by parvalbumin (PV) positive hypertrophic basket formations that innervated mostly neurons expressing NKCC (74%) and to a lesser extent NKCC-immunonegative neurons (26%). Hypertrophic basket formations also innervated KCC2-positive (76%) and -negative (24%) neurons. The data suggest that changes in the relative expression of NKCC1 and KCC2 in neurons having aberrant GABAergic hyperinnervation may contribute to epileptiform activity in the subicular regions adjacent to sclerotic areas of the hippocampus.

Perturbed Chloride Homeostasis and GABAergic Signaling in Human Temporal Lobe Epilepsy. Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C. *J Neurosci* 2007;27(37):9866–9873. Changes in chloride (Cl^-) homeostasis may be involved in the generation of some epileptic activities. In this study, we asked whether Cl^- homeostasis, and thus GABAergic signaling, is altered in tissue from patients with mesial temporal lobe epilepsy associated with hippocampal sclerosis. Slices prepared from this human tissue generated a spontaneous interictal-like activity that was initiated in the subiculum. Records from a minority of subicular pyramidal cells revealed depolarizing $GABA_A$ receptor-mediated postsynaptic events, indicating a perturbed Cl^- homeostasis. We assessed possible contributions of changes in expression of the potassium–chloride cotransporter KCC2. Double in situ hybridization showed that mRNA for KCC2 was absent from 30% of CaMKII (calcium/calmodulin-dependent protein kinase II)-positive subicular pyramidal cells. Combining intracellular recordings with biocytin-filled electrodes and KCC2 immunochemistry, we observed that all cells that were hyperpolarized during interictal events were immunopositive for KCC2, whereas the majority of depolarized cells were immunonegative. Bumetanide, at doses that selectively block the chloride-importing potassium–sodium–chloride cotransporter NKCC1, produced a hyperpolarizing shift in $GABA_A$ reversal potentials and suppressed interictal activity. Changes in Cl^- transporter expression thus contribute to human epileptiform activity, and molecules acting on these transporters may be useful antiepileptic drugs.

COMMENTARY

Novel remedies are needed for temporal lobe epilepsy (TLE) because current pharmacotherapeutics frequently are ineffective or wrought with side effects. Medically intractable TLE, a common clinical entity, often necessitates the removal of epileptic foci, with the risks of morbidity accompanying a major neurosurgical procedure. Mesial temporal sclerosis (MTS) is a common histological and radiographic finding in the brains of patients with TLE and is characterized by cell loss and gliosis in the CA1 area (i.e., Sommer's sector) of the hippocampal formation with an apparently intact subiculum. Despite years of research, the mechanism underlying epileptogenesis in TLE associated with medial sclerosis is still unknown.

One interesting hypothesis, based on the findings by Cohen et al., maintains that seizures in TLE result from excitatory GABAergic signaling that is due to perturbed neuronal chloride transport (1). In temporal lobe slices resected from medically intractable TLE patients with MTS, Cohen and colleagues demonstrated the presence of spontaneous interictal depolarizations originating in GABAergic pyramidal neurons in the subiculum and its transitional area with CA1. GABA, normally an inhibitory transmitter in most regions of the adult brain (including the subiculum), paradoxically triggered excitatory impulses in subicular pyramidal neurons in TLE patients. The authors suggested these depolarizing impulses serve as the nidus of spontaneous interictal discharges that spread

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to adjacent regions of the temporal lobe during seizures. The mechanism behind the "switch" that makes GABA excitatory rather than inhibitory in these neurons, until recently, had been a matter of conjecture. However, it had been known that an excitatory-to-inhibitory switch in GABA signaling, as occurs shortly after birth in the rodent and human cortex, is achieved in immature neurons via upregulation of the K^+-Cl^- cotransporter, KCC2 (2).

The direction and magnitude of GABA-induced chloride flux through $GABA_A$ receptor-associated chloride channels is determined by the intracellular Cl^- concentration ($[Cl^-]_i$), which, in turn, is established via the coordinated activities of the Na^+-K^+-$2Cl^-$ cotransporter NKCC1 and of KCC2 (3). NKCC1 and KCC2 are related *SLC12A* family cation chloride cotransporters (CCCs) that are the primary mediators of neuronal chloride influx and efflux, respectively (3). These electroneutral cotransporters utilize the electrochemically favorable cellular sodium or potassium gradients established via the Na^+-K^+ ATPase to drive the influx or efflux of chloride via secondary active transport. In principal, the GABA hyperpolarizing-to-depolarizing shift occurring with TLE could result from any process that increases neuronal $[Cl^-]_i$ by augmenting the relative activity of NKCC1 to KCC2 such that the opening of chloride-permeable $GABA_A$ channels produces passive outflow of negatively charged chloride ions, thereby depolarizing the neuron's membrane and upon reaching threshold, triggering action potentials. The contribution of altered NKCC1 and KCC2 activity to epileptogenesis has been documented in several model systems: 1) high expression of NKCC1 in the developing brain contributes to the pathogenesis of neonatal seizures (4,5); 2) *KCC2* knockout mice exhibit hippocampal hyperexcitability and generalized seizures, dying shortly after birth (6,7); and 3) pharmacological inhibition of CCCs block epileptiform activity in vitro and in vivo (4,5,8).

Two recent reports provide insight into the mechanism underlying the paradoxical excitatory action of GABA in TLE. Munoz et al., using double-labeling immunofluorescence with antibodies specific for NKCC1 and KCC2, demonstrated a greater than 95% colocalization of NKCC1 and KCC2 in neurons in the subiculum (i.e., subiculum CA1, CA1, and CA4 areas in hippocampal tissue) of healthy individuals. In contrast, while a similar high percentage of neurons coexpressed NKCC1 and KCC2 in the nonsclerotic CA1 and CA4 areas of tissue from patients with TLE associated with hippocampal MTS, greater than 20% of NKCC1-expressing pyramidal neurons in the sclerotic subicular/CA1 transitional zone not only lacked KCC2 but exhibited increased NKCC1 expression as well. These data corroborated earlier experimental findings by Palma et al. that demonstrated a 20% downregulation of *KCC2* and a twofold upregulation of *NKCC1* mRNA in the subiculum, compared with the hippocampus proper or the temporal lobe neocortex, in patients with TLE associated with hippocampal MTS (9).

Huberfeld et al. examined the functional significance of altered CCC expression in TLE associated with hippocampal sclerosis. Similar to previous reports (9), in situ hybridization and immunohistochemistry revealed down-regulation of KCC2 in subicular pyramidal neurons relative to adjacent hippocampal areas in diseased patients. Confirming the seminal findings by Cohen et al. (1), Huberfeld and colleagues found that $GABA_A$-mediated IPSPs reversed at depolarizing values in greater than 20% of pyramidal neurons from patients with TLE; these neurons were anatomically and electrically similar to neighboring subicular neurons that displayed hyperpolarizing (inhibitory) GABAergic potentials. The authors combined electrophysiological recordings with biocytin-filled electrodes and KCC2 immunochemistry to demonstrate that all neurons that were hyperpolarized during interictal events stained positively for KCC2, whereas many (but not all) neurons that exhibited interictal depolarizations did not express KCC2. Most importantly, bumetanide, a loop diuretic related to furosemide that selectively blocks NKCC1 in the 5–10 μM range, produced a hyperpolarizing shift in reversal potentials of $GABA_A$ receptor currents and suppressed epileptic activity in hippocampal slices from TLE patients over a period of about 30 minutes, a time consistent with that which is needed to re-establish a new intracellular steady state of chloride. These findings were reminiscent of an earlier study demonstrating that 1) $GABA_A$ reversal potentials—elicited in *Xenopus* oocytes by injecting subicular neuron cell membranes from TLE patients that harbored $GABA_A$ receptors and CCCs—were more depolarized compared with the reversal potentials of $GABA_A$ receptor currents elicited in membranes derived from the temporal neocortex or hippocampus proper of these same patients and 2) bumetanide was able to shift these depolarizing $GABA_A$ receptor current reversal potentials to more negative values (9).

Together, these data suggest that a decrease in the functional activity of the chloride extruder KCC2, along with the concurrent presence of a robustly active chloride entry pathway via NKCC1, creates a chloride equilibrium potential within subicular neurons and the subicular/CA1 transitional area that is much more positive than the resting membrane potential. This renders $GABA_A$ receptor-mediated neurotransmission *depolarizing* (and thus potentially excitatory) as a result of the passive efflux of chloride through the $GABA_A$ receptor chloride ion channel. These depolarizing discharges could serve as pacemakers that generate seizures after currents spread to adjacent hippocampal tissue. Excitatory GABAergic signaling also would help explain why anticonvulsants that promote GABA release or enhance the $GABA_A$ receptor chloride current (e.g., benzodiazpines and barbiturates) are often ineffective or even detrimental to patients with TLE. Not surprisingly, the futility of

GABA agonists is seen as well with neonatal seizures, in which a high expression of NKCC1 relative to KCC2—a phenomenon typical of early cortical development—also exists. Consistent with the underlying pathophysiology of NKCC1 overactivity in the disorder, bumetanide quells epileptic activity in mouse models of neonatal seizures (4,5).

A question left open is the nature of the interaction between NKCC1 and KCC2. While both studies clearly indicate that most neurons express both proteins, these are high capacity transporters that, if oppositely directed, have the potential to burn through millimolar concentrations of chloride, sodium, and potassium (and therefore ATP!) every second. To maintain cellular energetics, a more sophisticated mechanism than a chloride tug of war must be operative. Understanding how the activities of these transporters are regulated may illuminate a related question: is altered CCC expression/activity a cause of or a secondary consequence of TLE that is associated with hippocampal sclerosis? Investigation into the complex mechanisms that regulate the activity of the CCCs, such as phosphorylation by the chloride-sensitive WNK/SPAK kinase complex, is needed to answer this question (10). Perhaps seizures themselves produce long-term depolarizing shifts in $GABA_A$ receptor current reversal potentials by triggering activity-dependent downregulation of KCC2. Sustained epileptic activity in hippocampal slices downregulates KCC2 in CA1 pyramidal neurons, an effect mediated by brain-derived neurotrophic factor acting on the tyrosine receptor kinase B (11). Downregulation of the tyrosine phosphorylation and activity of KCC2 also is seen after neuronal trauma (12). In both TLE and neurotrauma, injured neurons appear to revert to an immature neuronal pattern of CCC expression and associated depolarizing GABAergic currents (13). While excitatory GABAergic neurotransmission during development and after trauma leads to the activation of voltage-gated calcium channels and subsequent increases in intracellular calcium that play a role in neuronal growth, maturation, and repair (13), such excitatory GABA neurotransmission likely propagates seizure activity in TLE.

It seems that the time is ripe to translate these basic scientific findings into clinical practice. Bumetanide is a drug that has been used for over 30 years with minimal side effects, has well-known pharmacokinetics and pharmacodynamics, and exhibits a lipid:water partition coefficient of approximately 4:1 at physiological pH, supporting the idea that this drug is capable of crossing the blood–brain barrier (4,5). The specific and robust effects of bumetanide on seizure activity (4,5) indicates that this drug could be explored in clinical trials as a potential treatment not only for neonatal seizures but also for TLE associated with hippocampal sclerosis, particularly for patients whose clinical and molecular phenotype mimics that of seizures in neonates. Furthermore, if a human trial of bumetanide as an anticonvulsant is to be initiated, it seems that a prudent place to begin would be in adults with medically intractable and surgically nonresectable TLE that is associated with hippocampal sclerosis.

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