Nevertheless, the role of snapin in regulating late endosomal trafficking and lysosomal fusion could be related to the function of BLOC-1 and novel forms of HPS. The perinatal lethality of *snapin*-null mice, which may be related to such a multivalent role of snapin in coordinating several membrane transport/fusion systems, has hampered elucidation of the specific roles of snapin in vivo. Therefore, it would be important to clarify the physiological and pathological role of snapin in regulating the late endosome-lysosome pathway in vivo by using snapin-L99K knockin mice.

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VGLUTs—Potential Targets for the Treatment of Seizures?

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Vesicular glutamate transporters (VGLUTs) load glutamate into synaptic vesicles. In this issue of *Neuron*, Juge et al. report that ketone bodies compete with chloride-dependent activation of VGLUTs, leading to suppression of glutamate release and seizures. These findings provide a surprising explanation for the efficacy of the ketogenic diet in controlling epilepsy.

The functioning of the mammalian CNS depends on a delicate balance between excitatory and inhibitory synaptic activities. Glutamate is the major excitatory neurotransmitter in the brain, and its release is tightly regulated. At a given synapse, the amount of glutamate released per action potential depends on factors such as past firing patterns, activity of neighboring synapses, retrograde signaling, and second-messenger cascades. Most commonly, the number of synaptic vesicles undergoing exocytosis is regulated, and a vast amount of studies have addressed this mechanism of synaptic plasticity. However, it is becoming apparent that transmitter output may also be controlled by changing the transmitter content of synaptic vesicles (for review see Ahnert-Hilger et al., 2003; Edwards, 2007).

Loading of synaptic vesicles with glutamate is mediated by a small family of vesicular glutamate transporters termed VGLUTs1–3 (Edwards, 2007). Transport is driven by a proton electrochemical gradient across the vesicle membrane, which is generated by a vacuolar ATPase (Edwards, 2007). In contrast to the well-characterized Na^+-dependent glutamate transporters in the plasma membrane, our knowledge about VGLUTs is lagging behind because VGLUTs can only be studied in vesicles that contain an active proton pump, greatly limiting experimental flexibility. To deter researchers even further, VGLUTs expressed in nonneuronal cells display low activities, and once incorporated in the plasma membrane they "moonlight" as Na^+-dependent transporters for inorganic phosphate (Aihara et al., 2000; Ni et al., 1994).

Since the early days of measuring vesicular glutamate uptake (Disbrow et al., 1982; Naito and Ueda, 1985), it is known that uptake depends on chloride ions at millimolar concentrations. However, it has not been easy to distinguish

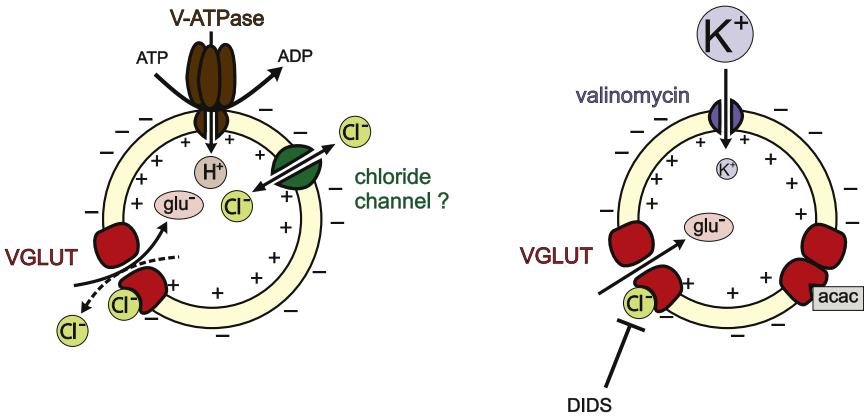

Figure 1. Multiple Roles for Chloride Ions in VGLUT-Mediated Filling of Synaptic Vesicles
Left: synaptic vesicles possess an electrogenic proton pump (V-ATPase). The resulting inside-positive membrane potential fuels glutamate transport. At high Cl^- concentrations, Cl^- ions enter the vesicle and balance the charge of protons, thus generating a pH gradient and dissipating the membrane potential. Under physiological conditions, the initial intravesicular Cl^- concentration is expected to be high, raising the possibility that Cl^- leaves the vesicle in exchange for glutamate, either via a vesicular chloride channel or directly via VGLUT.
Right: reconstitution of VGLUT into proteoliposomes as described by Juge et al. (2010). A transient inside positive membrane potential is generated by creating an inwardly directed K^+ diffusion potential, which drives glutamate uptake. No Cl^- transport is observed. However, glutamate uptake depends on allosteric activation by Cl^- of VGLUT at a DIDS-sensitive binding site. Acetoacetate (acac) competes with Cl^- binding and prevents activation of VGLUT.

whether chloride acts directly on the transporter or whether it acts indirectly by affecting the proton electrochemical gradient. The V-ATPase transports H^+ ions in an electrogenic manner, resulting in a high, inside positive, membrane potential (see Figure 1). Chloride serves as counter-ion entering the vesicle via a chloride channel, which allows for the accumulation of protons and the generation of a pH gradient at the expense of the membrane potential. Since glutamate transport is primarily driven by the membrane potential, uptake is reduced at increasing chloride concentrations (Maycox et al., 1988). Furthermore, chloride is sequestered during vesicle endocytosis under physiological conditions and may serve as counter-ion in exchange for glutamate, thus maintaining osmotic balance and charge neutrality during vesicle refilling (see Figure 1). However, none of these mechanisms explains the strong activation of glutamate transport by a few millimolar Cl^-, suggesting the presence of an allosteric regulatory binding site.

Why should anyone except for a few aficionados of vesicular transporters care about the intricacies of VGLUT regulation by chloride ions? After all, the intracellular chloride concentration is comfortably above the needs for VGLUT activation. In this issue of *Neuron*, Juge et al. (2010) give a surprising answer by showing that the allosteric activation of VGLUTs by anions may be modulated by energy metabolites, which has major relevance for the treatment of common neurological diseases.

Juge and colleagues (2010) used a classical approach for their investigation of VGLUTs: they purified the transporter and then incorporated it into artificial membrane vesicles. To circumvent the need for an electrogenic proton pump as energy source, the authors generated transient, inwardly directed K^+ diffusion potentials (Figure 1, right panel). In this simplified system, VGLUT-mediated transport can be studied without interference by other proteins such as fickle, multisubunit proton ATPases (see Figure 1). The data are extraordinarily clean, with a signal-to-noise that was beyond our dreams two decades ago when we reconstituted glutamate and GABA uptake from crude vesicle extracts using the same technique (Hell et al., 1991). The authors show that VGLUTs are allosterically activated by chloride ions in a highly cooperative manner: no transport without chloride, initial activation at 2 mM and saturation at 5 mM chloride, respectively. In search for inhibitors the authors noted that the ketone bodies acetoacetate and β-hydroxybutyrate, at concentrations reached during physiological ketogenesis (see below), directly compete with chloride-dependent activation of VGLUTs. These findings parallel an earlier report in which branched-chain α-keto acids where shown to compete with chloride activation of glutamate transport (Reis et al., 2000). Furthermore, incubation of cultured neurons with acetoacetate reversibly decreased depolarization-induced glutamate release and reduced the amplitude of miniature excitatory postsynaptic potentials whereas miniature inhibitory potentials were not affected. These findings are exciting since acetoacetate is well known for its anticonvulsant activity (Hartman et al., 2007). To explore this connection further, the authors induced seizures in rats by local injection of a K^+-channel blocker and then measured transmitter release. Indeed, coinjection of acetoacetate suppressed convulsions, and glutamate but not dopamine release was significantly reduced.

Together, these findings provide an unexpected molecular explanation for the hitherto enigmatic efficacy of ketone bodies in controlling epileptic seizures. Ketone bodies are energy metabolites derived from acetyl CoA that can substitute for glucose in aerobic oxidation. Under physiological conditions, ketone body production is increased when glucose and insulin levels are low, i.e., during starvation or when the body derives its energy primarily from breakdown of fat. Unlike tissues such as liver and muscle, the brain cannot oxidize fatty acids and is thus dependent on ketone bodies produced by the liver. When the brain shifts to burning ketone bodies at low glucose conditions, the cytoplasmic concentration of ketone bodies increases, resulting in an inhibition of VGLUTs by blocking chloride binding and thus in a global dampening of glutamatergic transmission. The model also explains why the neuroprotective effects of a ketogenic diet (rich in fat, low in proteins and carbohydrates) are thwarted by the ingestion of sweets (Hartman et al., 2007): carbohydrates immediately decrease circulating ketone body levels, thus lifting the inhibition of VGLUTs and increasing synaptic glutamate output. Certainly, VGLUTs are now on the map as potential drug targets.

The study shows in an exemplary fashion that even moderate changes in vesicular neurotransmitter levels may have major consequences for brain function. There is still much to learn about how transmitter content depends on transporter activity and energy gradients, how transport activity is controlled by metabolic and signaling pathways, and how ion and osmotic balance is maintained during vesicle filling. Despite the exciting progress provided by this study, the roles of chloride ions in glutamate uptake are far from clear. For instance, the authors show convincingly that chloride is not transported by VGLUT, which contrasts with a previous study providing similarly compelling evidence for chloride transport and chloride:glutamate exchange by VGLUT (Schenck et al., 2009). Furthermore, chloride dependence of VGLUT appears to be regulated by the trimeric GTPase $G\alpha_{o2}$ (Winter et al., 2005), but the underlying molecular mechanism is not known. I am sure that clean biochemical approaches as in the study of Juge et al. (2010) will be instrumental in providing answers to these important questions.

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