Diuretics as Antiepileptic Drugs: Should We Go with the Flow?

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Recent epidemiological and experimental studies have suggested that certain diuretics may have significant anticonvulsant actions. Potential anticonvulsant mechanisms are discussed in light of the effects of these diuretics on electrolyte balance and synaptic signaling.

Although acetazolamide has been used as a fourth-line anticonvulsant since the days when there were only three other anticonvulsants from which to choose (1), diuretics are not the first class of drugs that come to mind as alternative anticonvulsants. After all, clinicians know that thiazide use can be complicated by hyponatremic seizures (2), and electrophysiologists know that furosemide is an antagonist of the inhibitory $GABA_A$ receptor (3–5). However, two intriguing findings compel us to take another look at the anticonvulsant effects of diuretics. In 1995, the Schwartzkroin laboratory discovered that very high concentrations of the loop diuretic furosemide had pronounced anticonvulsant effects *in vitro* (6). This finding was confirmed *in vivo* using the kainate and the posthypoxic audiogenic seizure models (7,8). Now epidemiological studies have demonstrated that diuretics exert a measurable anticonvulsant effect in human patients (9,10). These anticonvulsant effects appear robust and raise this question: Are these phenomena understandable in terms of what we know about the actions of diuretics?

Diuretic Actions on Electrolyte Balance

Three diuretics have been shown to have anticonvulsant effects: acetazolamide, furosemide, and the thiazides. Acetazolamide is a carbonic anhydrase inhibitor. Carbonic anhydrase catalyzes the dehydration and rehydration of HCO_3^- (11). Dehydrated HCO_3^- plus a proton is simply CO_2, which is membrane permeable. Thus, carbonic anhydrase, when present on both sides of a cell's membrane, enables rapid diffusion of HCO_3^- (and a proton) across the cell's membrane. In the kidney, the proton that is transported across the membrane in this process is exchanged in the proximal tubule for a sodium ion. Carbonic anhydrase inhibition by acetazolamide reduces the availability of this proton and thereby limits sodium reabsorption from urine in the proximal tubule (12), as well as sodium extrusion into the cerebrospinal fluid in the choroid plexus (13).

Furosemide also inhibits carbonic anhydrase at micromolar concentrations (14). However, its principal effect is an inhibition of chloride cotransport. The electroneutral K^+-Cl^- cotransporter exports Cl from cells using the "downhill" electrochemical K^+ transmembrane gradient to supply the energy for the "uphill" export of Cl. The protein that accomplishes this is termed KCC2, which is an acronym for K^+-Cl^- cotransporter type 2 (15,16). Furosemide inhibits KCl exchange at high micromolar concentrations. At low micromolar concentrations, furosemide inhibits NKCC1, the electroneutral Na^+-K^+-Cl^- cotransporter type 1, which is known in nonhuman species as BSC2 (17). This protein is widely expressed in tissues, including the brain, and functions to import Cl^- into cells by using energy from the Na^+ transmembrane gradient. The diuretic effect of furosemide derives from its inhibition of the renal isoform of this protein, which is known as NKCC2 (or BSC1). The net effect of KCC2 is the removal of salt (KCl) and thus water and volume from the cell, whereas the net effect of NKCC1 is inhibition of salt and water influx and consequent increase in cellular volume. Thus, furosemide limits cellular volume regulation as well as both Cl import and export. Furosemide also inhibits Cl^-/HCO_3^- exchange at the same concentrations that inhibit K^+Cl^- cotransport (18).

Thiazides inhibit NaCl cotransporter protein, NCC. NCC is only expressed in the kidney. Thiazides inhibit neither K^+Cl^- cotransport nor $Na^+K^+2Cl^-$ cotransport, but they do inhibit carbonic anhydrase (19).

Diuretic Actions on Neurons

Furosemide has been widely used as an inhibitor of neuronal Cl^- transport (4,20) and as an antagonist of neuronal $GABA_A$ receptors (3–5). At the typically used concentration of 500 μmol/L furosemide inhibits K^+-Cl^- exchange, Na^+-K^+-Cl^-

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cotransport, carbonic anhydrase, Cl^-/HCO_3^- exchange, and at least some $GABA_A$ receptors. Because it inhibits so many enzyme systems, it is not always easy to identify the locus of action of furosemide. For example, if cells are loaded with chloride, the principal effect of furosemide is inhibition of Cl^- export, but if neurons are Cl^- depleted, the principal effect is inhibition of Cl^- uptake (21). The neurotransmitter GABA inhibits adult neurons by opening a membrane channel that is permeable to HCO_3^- and Cl^- (22). Although HCO_3^- leaves the neuron through the $GABA_A$ channel, quite a bit more Cl^- enters, and the resultant net influx of negative charge hyperpolarizes the neuron. Thus, in chloride-loaded cells, furosemide may make the $GABA_A$ reversal potential more negative, whereas in chloride-depleted cells, the reversal potential may become more positive. In both cases, the $GABA_A$ conductance is somewhat decreased (3,4,23), and thus, current-voltage analyses are necessary to interpret the effect of furosemide on the GABA system.

Thiazides have not been widely investigated in the nervous system, principally because the thiazide-sensitive transporter is only expressed in the kidney.

Acetazolamide limits the rate at which HCO_3^- can diffuse back into the neuron as CO_2 after it has left the neuron through the $GABA_A$ channel. When the GABA receptor is persistently active, this limits the total amount HCO_3^- that leaves the neuron. Because egress of negatively charged HCO_3^- depolarizes the neuron, the net effect of acetazolamide is to make large and long-lasting $GABA_A$ currents more hyperpolarizing (24).

Diuretic Actions on Seizures

The limitation of HCO_3^- efflux from the GABA channel by acetazolamide substantially increases the efficacy of GABA-mediated inhibition, especially when the GABA channel is open for prolonged periods, as may occur during seizures or during treatment with anticonvulsants such as the barbiturates and benzodiazepines that prolong the GABA channels open time (25,26). Thus, a potential anticonvulsant mechanism of acetazolamide is an increase in the efficacy of GABA-mediated inhibition. This mechanism suggests that it should be possible to increase the anticonvulsant efficacy of barbiturates and benzodiazepines by combining these agents with acetazolamide, and experimental data support the feasibility of this strategy (27,28).

An anticonvulsant effect of the thiazides is not likely to be effected through inhibition of NCC because this enzyme is present in only the kidney. However, thiazides also inhibit carbonic anhydrase, and thus, the epidemiological data that suggest an anticonvulsant effect of the thiazides may be due to an acetazolamide-like effect. Of course, it is also possible that the anticonvulsant effects of the thiazides are mediated through systemic alterations of electrolyte balance or by inhibition of neuronal or glial enzymes that have not yet been discovered.

Because furosemide inhibits so many enzymes, it is difficult to isolate the mechanism of the anticonvulsant effects observed both experimentally (6–8) and epidemiologically (9,10). One potential mechanism involves reversal of the direction of Cl^- transport during seizures as extracellular potassium concentrations increase (29). Under these conditions, the transmembrane potassium gradient may favor NKCC2 transport of Cl^- into, rather than out of, the neuron. A higher neuronal Cl^- concentration would result in a positive shift in the reversal potential for GABA, which would weaken the inhibitory effect of $GABA_A$ receptor activation (30). However, inhibition of K^+-Cl^- cotransport by furosemide would leave no direct way for Cl^- to be transported out of the neuron. Cl^- would accumulate in the cell as a consequence of $GABA_A$ receptor activity, and it is not clear that there would be a net increase in the efficacy of inhibition in the presence of furosemide.

Another potential anticonvulsant mechanism of furosemide is inhibition of NKCC2 activity, which would limit Cl^- transport into the neuron. This would decrease the neuronal Cl^- concentration and thus make the $GABA_A$ reversal potential more negative, which would increase the efficacy of GABA-mediated inhibition. NKCC2 inhibition would also decrease the cell's volume by diminishing net salt and water intake, resulting in a net increase in extracellular space. Increases in the volume of the extracellular space reduces seizures by decreasing capacitive coupling (ephaptic transmission) between neurons (31); this effect is thought to underlie the anticonvulsant effects of mannitol and other osmotic agents (32). NKCC2 inhibition might be effective in the immature nervous system, where Cl is actively transported into cells and the GABAA reversal potential is so positive that cells are excited rather than inhibited by GABA (33). However, NKCC2 expression falls to very low levels as synaptic activity increases in the developing nervous system (34), and thus, furosemide's inhibition of NKCC2 is likely to be of only minor functional significance in the mature brain.

Other Potential Diuretic Actions

Carbonic anhydrase inhibition is the only mechanism shared by the three diuretics that are known to reduce seizures in human patients, and this may underlie the epidemiological findings (9,10). However, the experimental anticonvulsant effects of furosemide are much stronger than what has been observed for acetazolamide and are thus unlikely to be due solely to carbonic anhydrase inhibition. Several clues to the nature of the additional anticonvulsant effects of furosemide are available. The first is the concentration at which the anticonvulsant effects are observed. Anticonvulsant effects are seen at concentrations of several millimolar, approximately 10 times the concentration required to block Cl^- transporters and the GABA

receptor. The widespread nature of furosemide's enzyme inhibition is thought to be due to its interaction at the Cl binding site that most of the furosemide-inhibited enzymes (and perhaps the GABAA receptor) have in common. Thus, at high concentrations, furosemide may be acting at a Cl^- binding site that is only distantly related to those on the Cl^- transport enzymes. A second clue is the time course of the effect, which is substantially slower than the GABA antagonist effect of furosemide (6). The Schwartzkroin laboratory recently provided a third clue, which is that very low-chloride solutions inhibit epileptiform activity with the same time course as high concentrations of furosemide (35). The final clue is that although spontaneous epileptic activity is blocked by furosemide, abbreviated epileptiform responses can still be evoked by low-frequency stimulation (every 20 seconds or so).

One mechanism suggested by these clues is interference with the packaging of the excitatory neurotransmitter glutamate into synaptic vesicles. Chloride flux is a consistent component of glutamate transport (36), and glutamate transport from the neuronal cytoplasm to the synaptic vesicle is facilitated by physiological concentrations of chloride (37–39). Thus, furosemide or prolonged incubation in low-Cl^- media may diminish the rate at which glutamate can be concentrated into synaptic vesicles. Furosemide at high concentrations also interferes with release of glutamate into the extracellular space from glia, by interaction with either the chloride binding site or even the glutamate binding site on the glial glutamate transporter (40). An inhibition of vesicular glutamate transport would reduce the ability of a terminal to release glutamate, which would lead to the observed abbreviation of evoked responses and inhibition of spontaneous epileptiform activity. Interference with glial glutamate transport would inhibit the glial-neuronal glutamate recycling that is necessary for sustained synaptic transmission (41), which would produce the observed effects on evoked and spontaneous epileptiform activity. Inhibition of the availability of releasable glutamate is a particularly attractive anticonvulsant mechanism, in that low-frequency synaptic transmission may be less affected than high-frequency epileptiform activity, providing the possibility of effective anticonvulsant action with minimal side effects.

The links between furosemide and glutamate release, as well as the potential synergy of acetazolamide and GABAergic anticonvulsants, deserve further scrutiny. Although it is premature to prescribe furosemide as an anticonvulsant, there is clearly a great deal still to be learned about the anticonvulsant effects of diuretics.

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