Commentary
A truism of pharmacology is that no drug is specific. Scientists and clinicians ignore this fact at their peril. Lack of specificity does not prevent drugs from being powerful tools in experiments and in medicine, but it does necessitate cautious interpretations and prescriptions. A case in point is carbenoxolone and its role in studies of the cellular mechanisms of seizures.

Carbenoxolone (3β-hydroxy-11-oxoolean-12-en-30-oic acid 3-hemisuccinate) is a glycyrrhetinic acid derivative with a steroid-like structure, similar to substances found in the flavorful root of the licorice plant. The drug has a motley pharmacologic history (1). It influences endogenous glucocorticoids by potently inhibiting 11β-hydroxysteroid dehydrogenase (11β-HSD), and it has been used clinically to treat gastric ulcers and other types of inflammation. Electrolyte imbalance is a serious side effect of carbenoxolone when used systemically.

Carbenoxolone is best known in cellular physiology as a modestly potent, reasonably effective, water-soluble blocker of gap junctions (2). Gap junctions are fast-track intercellular communication routes. They comprise clusters of transcellular channels, each a multimeric complex of connexin proteins, that allow inorganic ions and small organic signaling molecules to diffuse rapidly and directly from the cytoplasm of one cell to that of another. Gap junctions that interconnect two neurons are called electrical synapses because ionic currents passing through the junctions allow each cell to influence the membrane potential of the other. Carbenoxolone at concentrations of 50 to 100 µM nearly eliminates the electrical coupling that occurs among a variety of central neurons.

Electrical synapses are widely expressed in the mammalian brain. Within the neocortex and hippocampus, many types of inhibitory interneurons are selectively interconnected by gap junctions that require connexin36 (Cx36), a neuronal connexin (3). There is more limited evidence for electrical synapses between excitatory neurons of the cerebral cortex (4). Gap junctions also ubiquitously interconnect glial cells. Glial gap junctions may be important in seizures, but that is a story for another essay.

The normal functions of electrical synapses in the forebrain are uncertain. One thing they do indisputably well, however, is to synchronize the spikes and subthreshold electrical activity of coupled neurons. Voltage differences between adjacent cells lead to a rapid flow of current through gap junction channels, thus an action potential in one cell may quickly increase the probability of spiking in its neighbors. This is where seizures...
come in. A long-standing hypothesis is that electrical synapses play some sort of role in the basic mechanisms of epileptic seizures (5, 6). It is a compelling idea with a clear rationale: seizures are generally defined by an over-abundance of neural synchrony; electrical synapses enhance synchrony; neuronal gap junctions are common in the forebrain where seizures often originate; perhaps some seizures are enhanced or caused by gap junctions that are overexpressed or poorly regulated. Alternatively, more electrical synapses among inhibitory neurons could have an anticonvulsant effect under some scenarios.

Testing this hypothesis has not been easy. One common strategy has been to measure the probability and characteristics of experimental seizures before and after reducing gap junction function. The easiest way to block gap junctions is to use a drug, and the most commonly used blocker has been carbenoxolone. Carbenoxolone reduces seizure-related synchronization in a variety of model systems, both in vivo and in vitro (5–7). The anticonvulsant effects of carbenoxolone and other gap junction–blocking drugs are frequently interpreted as evidence that electrical synapses play a critical role in seizure generation. Is this a reasonable conclusion?

The value of carbenoxolone as a diagnostic probe for gap junctions rests entirely on the specificity of its actions, but several studies imply that it is exceedingly nonspecific. Some reports found that carbenoxolone does not influence neuronal excitability and GABA responses (6, 8). Others, however, suggest that in addition to blocking gap junctions and 11β-HSD, carbenoxolone also reduces excitatory synaptic currents through a presynaptic effect, blocks postsynaptic NMDA receptors, reduces inhibitory synaptic currents through a direct effect on GABAA receptors, suppresses action potentials, decreases input resistance, and blocks calcium channels (9–12). On balance, it appears that an effect of carbenoxolone on a physiological process is not, by itself, reliable evidence that gap junctions are involved.

Where does this leave the idea that neuronal gap junctions have an important role in seizure mechanisms? Beaumont and Maccarelli performed a very thorough investigation of carbenoxolone's effects on a well-studied model of epileptiform activity—the acute actions of 4-aminopyridine (4-AP) on hippocampus in vitro. The potassium channel blocker 4-AP causes robust, synchronized, epileptiform discharge of inhibitory interneurons. Numerous studies had demonstrated that carbenoxolone and other gap junction blockers reduce 4-AP–mediated epileptiform events both in vivo and in vitro. Beaumont and Maccarelli reproduced this effect of carbenoxolone, and they also performed several critical control experiments. First, they showed that a major group of inhibitory interneurons in the hippocampus is electrically coupled in wild-type mice, and that these cells lack electrical synapses in Cx36 knock-out mice. Next, and most crucially, they demonstrated that spontaneous GABAergic epileptiform events were essentially identical in wild-type and Cx36 knock-out animals. The presence of electrical synapses between interneurons had no influence on the events. Consistent with this finding, carbenoxolone reduced epileptiform events similarly in neurons regardless of Cx36 genotype. Further experiments showed that carbenoxolone antagonized postsynaptic GABAA receptors, whether GABA was applied from pipettes or released from endogenous synapses. Finally, application of mefloquine, an effective, chemically distinct blocker of neuronal gap junctions (13), did not affect the size or frequency of 4-AP–induced epileptiform events.

The inescapable conclusion from the work of Beaumont and Maccarelli is that Cx36-dependent electrical synapses are irrelevant for 4-AP–induced seizures, and that carbenoxolone suppresses these seizures via an action that does not involve electrical synapses. Carbenoxolone's anticonvulsant effect, at least in this model, is most likely due to its antagonism of GABAA receptors, not gap junctions.

Does this mean that electrical synapses are not important for either suppressing or enhancing seizures? Hardly. The convulsant effect of 4-AP on the hippocampus is just one specialized model of seizures, and it may be a poor model of any human condition. There are other bits of evidence implicating neuronal gap junctions in seizures (5–7), but definitive tests of gap junction involvement have yet to be done. Selective genetic techniques, which can conditionally target particular connexin subtypes in specific tissues, offer the best hope.

The history of carbenoxolone in studies of electrical synapses is a cautionary tale with implications for all gap junction blockers. These drugs are a chemically diverse lot, notorious for their limited potency, efficacy, reversibility, and selectivity (1, 2). Their broad effects on the brain are poorly understood, and they have been full of surprises. Mefloquine is a quinine derivative used clinically as an antimalarial agent; it also happens to be a gap junction blocker with partial selectivity for Cx36 over other connexin subtypes (13). Mefloquine is well known for its tendency to induce exceptionally vivid dreams, and in rare cases it has been associated with neuropsychiatric symptoms. Carbenoxolone is said to have nootropic effects. In a study of healthy and diabetic humans, several weeks of carbenoxolone treatment improved verbal fluency and verbal memory (14). Because carbenoxolone is several orders of magnitude more potent as an inhibitor of 11β-HSD than as a blocker of gap junctions, the authors suggested that the verbal effects were caused by changes in glucocorticoids. In an intriguing recent study, microinjections of carbenoxolone or mefloquine into the dorsal hippocampus of rats impaired context-dependent fear learning, memory, and theta rhythms (15). The authors provocatively concluded, “gap junction–mediated neuronal transmission is a prominent feature underlying emotional memories.”

Do these interesting cognitive effects of mefloquine and carbenoxolone have anything at all to do with their ability to block gap junctions? Considering the drugs’ multiple sites of action, it is impossible to say.

by Barry W. Connors, PhD

References


**American Epilepsy Society**  
*Epilepsy Currents Journal*  
Disclosure of Potential Conflicts of Interest

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