

CLOSING THE GAP: ELECTROTONIC JUNCTIONS IN SEIZURE CONTROL

Involvement of Gap Junctions in the Manifestation and Control of the Duration of Seizures in Rats In Vivo

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Epilepsia 2003;44:1596–1600

PURPOSE: The possible role of gap junctions in the manifestation and control of the duration of seizures was tested on the 4-aminopyridine-induced epilepsy model in rats in vivo, by using electrophysiologic, pharmacologic, and molecular biologic techniques.

METHODS: In electrophysiologic experiments, the functional states of the gap junctions were manipulated with a specific blocker (carbenoxolone) or opener (trimethylamine) at the already active focus of adult, anesthetized rats, 60 min after the induction of the first seizure, which was repeated spontaneously thereafter. Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) amplification was used to measure the levels of connexin (Cx) 32, 43, and 36 messenger RNAs (mRNAs) prepared from the areas of the already active primary and mirror foci.

RESULTS: After repeated seizures, the expression levels of Cx32, Cx43, and Cx36 mRNAs at the epileptic foci were increased significantly. Blockade of the gap junctions with carbenoxolone shortened the duration of seizures and decreased the amplitude of the seizure discharges, whereas their opening with trimethylamine lengthened the duration and increased the amplitude. Secondary epileptogenesis was facilitated when the gap junctions were opened.

CONCLUSIONS: Our findings support the idea that, in epileptic foci, the gap junctions are involved in the expression of rhythmic ictal discharges and in the control of the duration and propagation of the individual seizures in vivo.

Through gap junctions, electrical activity can spread rapidly, synchronizing a local neuronal network. Such rapid coupling has obvious implications for the synchronization and spread of seizure activity (1,2). The involvement of gap junctions in epilepsy is appealing but controversial. Although fast, nonsynaptic electrical transmission can potentially facilitate the generation and spread of seizure activity in the brain, it has long been debated whether gap junctions are sufficiently plentiful and strategically placed to enhance neuronal synchrony to the extent that epileptic firing is augmented (3).

Two lines of investigation may shed light on this controversy. First, rapidly increasing knowledge exists about the molecular biology of gap junctions. Gap junctions are composed of connexins—intramembranous protein complexes from adjoining cell membranes (neurons or glia), the pore of which allows intercellular passage of current (usually as K⁺ ions) and other small molecules. At least 10 different connexins are expressed in the mammalian central nervous system, and this diversity endows gap junctions with a panoply of physiological properties, depending on the type and stoichiometry of the particular connexins (4,5). Other new techniques, such as cloning of connexin genes, in situ hybridization, and production of transgenic mice have allowed investigators to begin unraveling the complexities and functions of gap junctions in the brain. Gap junctions can now be visualized in vivo and in vitro, complementing the electron microscopy studies that initially verified their existence.

The second research trend is the discovery that gap junctional transmission is rapidly modifiable. Electrical synapses do not have fixed properties; rather, rapid changes in coupling strength have now been shown in response to a variety of neurotransmitters, phosphorylation, second messengers, and other modulators, such as nitric oxide. In particular, the availability of agents that rapidly open or close gap junctions has permitted investigators to probe the functional role of gap junctions in a variety of physiological conditions, including epilepsy (6).

In this article and in previous work (7), Gajda et al. exploited these recent experimental advances to study the role of gap junctions during a seizure in intact animals. They induced repetitive seizures in anesthetized adult rats, by using local application of 4-aminopyridine (4-AP) on somatosensory neocortex. Electrographic seizure activity was recorded from the injection site (primary focus) and from homotopic cortex in the opposite hemisphere (mirror focus). Electrographic seizure frequency, duration, and amplitude were documented

COMMENTARY

Gap junctions in the mammalian brain allow rapid intercellular communication via a nonsynaptic mechanism.

and compared after application of carbenoxolone (a gap junction closer), trimethylamine (a gap-junction opener), or both drugs sequentially. Seizure duration was significantly increased by the gap-junction opener, trimethylamine, and decreased by the gap-junction closer, carbenoxolone. The probability of seizure spread to the contralateral cortex (mirror focus) was increased severalfold by trimethylamine.

To evaluate whether seizure-induced hyperexcitability alters connexin gene expression, the investigators used reverse transcriptase–polymerase chain reaction (RT–PCR) to examine mRNAs for connexins (Cx) Cx32, Cx43, and Cx36 at the primary and mirror foci. Cx32 is primarily localized on oligodendrocytes and neurons, Cx43 is found on neurons and astrocytes, and Cx36 is believed to be primarily neuronal in location (4,5). The mRNAs for each of these connexins increased significantly after a seizure, at both the primary focus and at the mirror focus. These results imply that gap junctions are increased in response to a seizure, even in distant cortex (mirror focus). Increased connexin gene expression is prevented by treatment with the gap junction closer, carbenoxolone.

The mechanisms for these findings are unclear, but the ability of a gap-junction blocker to alter both connexin gene expression and ictal manifestations over a rapid time course (less than 1 hour) attests to the involvement of gap junctions in the modulation of ongoing seizures. Some caveats are warranted. Although the gap-junction opening and closing agents might act primarily at the level of electrical synapses, it is difficult to exclude more widespread effects at the membrane and intracellular levels (8). It is uncertain why homotopic cortex is preferentially activated and whether other cortical areas also respond to seizures by upregulation of connexins. Therefore these results are intriguing but must be interpreted cautiously.

The most exciting aspect of these experiments is the possibility of an entirely new therapeutic strategy, involving direct

manipulation of gap junctions in the pre- or periictal periods. By decreasing synchrony, gap-junction closers could prevent the generation or spread of hypersynchronous electrical activity that may underlie a seizure. Further investigation of systemic and nonsystemic side effects of carbenoxolone and other gap-junction closers must be undertaken before they can be considered for clinical use—which is one gap that certainly needs closing.

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