CURRENT LITERATURE

GAP JUNCTIONS AND FAST OSCILLATIONS: A ROLE IN SEIZURES AND EPILEPTOGENESIS?

A Possible Role for Gap Junctions in Generation of Very Fast EEG Oscillations Preceding the Onset of, and Perhaps Initiating, Seizures

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We propose an experimentally and clinically testable hypothesis, concerning the origin of very fast (> approximately 70 Hz) EEG oscillations that sometimes precede the onset of focal seizures. These oscillations are important, as they may play a causal role in the initiation of seizures. Subdural EEG recordings were obtained from children with focal cortical dysplasias and intractable seizures. Intra- and extracellular recordings were performed in rat hippocampal slices, with induction of population activity, as follows: (a) bath-applied tetramethylamine (an intracellular alkalinizing agent, that opens gap junctions); (b) bath-applied carbachol, a cholinergic agonist; and (c) focal pressure ejection of hypertonic K+ solution. Detailed network simulations were performed, the better to understand the cellular mechanisms underlying oscillations. A major feature of the simulations was inclusion of axon-axon gap junctions between principal neurons, as supported by recent experimental data. Very fast oscillations were found in children before seizure onset, but also superimposed on bursts during the seizure, and on interictal bursts. In slice experiments, very fast oscillations had previously been seen on interictal-like bursts; we now show such oscillations before, between, and after epileptiform bursts. Very fast oscillations were also seen superimposed on gamma (30-70 Hz) oscillations induced by carbachol or hypertonic K+, and in the latter case, very fast oscillations became continuous when chemical synapses were blocked. Simulations replicate these data, when axonal gap junctions are included. Electrical coupling between principal neurons, perhaps via axonal gap junctions, could underlie very fast population oscillations, in seizure-prone brain, but possibly also in normal brain. The anticonvulsant potential of gap-junction blockers such as carbenoxolone, now in clinical use for treatment of ulcer disease, should be considered.

Local Generation of Fast Ripples in Epileptic Brain

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Aperiodic high-frequency oscillations (>100 Hz) reflect a short-term synchronization of neuronal electrical activity. It has been shown in the epileptic brain that spontaneous oscillations in the frequency range of 250-600 Hz reflect action potential population bursts of synchronously discharging neuronal clusters. These oscillations occur in the early stages of epileptogenesis in areas adjacent to the brain lesion and may trigger the formation of seizure-generating neuronal networks. We studied the extent of the area generating oscillations in the frequency range of 250-600 Hz [fast ripples (FRs)] in intrahippocampal kainic acid-treated rats with spontaneous seizures, by analyzing voltage versus depth profiles of FRs in hippocampal and parahippocampal areas in freely moving animals and by spatial mapping in hippocampal slice preparations in vitro. The strength of inhibition was compared in areas with and without FRs using a paired-pulse paradigm. The extent of the areas generating FRs did not exceed 1 mm(3). The areas generating FRs became broader after the application of the GABA(A) receptor antagonist bicuculline. Paired-pulse fast inhibition at 15-30 msec intervals was similar in areas generating FRs and areas not generating FRs. Our data illustrate that hypothesized clusters of highly interconnected neurons are capable of overcoming interneuron feedback inhibition, resulting in generation of epileptiform bursts, eventually leading to seizure activity.

COMMENTARY

The role of gap junctions, electrotonic coupling, and fast electrical oscillations in seizure onset and epileptogenesis has a long and contentious history, and these two articles highlight two current avenues of investigation along this theme. Electrotonic coupling through gap junctions, electrical field effects via the extrmacellular space, and activity-dependent alterations in the concentration of extracellular and intracellular ions have long been considered to play hypothetical roles in the synchronization of epileptiform activity (i.e., prolonged and repetitive hypersynchronous burst discharges). These hypothetical mechanisms have often been considered "non-synaptic" because they are independent of active chemical synaptic transmission, but many workers in the field would consider electrotonic junctions to represent electrical synapses. Electrotonic junctions alone, without a mechanism for excitation (e.g., action potentials and/or excitatory postsynaptic potentials), would not be expected to produce seizure activity; that is, electrotonic coupling per se would be expected to underlie the hypersynchrony rather than the hyperactivity of a seizure. Because of their higher speed of transmission compared to chemical transmission, their unique ability to transmit subthreshold signals in a reciprocal manner, and their tendency to shunt synaptic inputs as part of their synchronizing mechanism (1), a neuronal network with electrotonic coupling through gap junctions tends to behave in an all-or-none manner with a high threshold, which is a property often considered to be characteristic of epileptic seizures.

In the early 1980s, several articles from at least three different laboratories nearly simultaneously showed that robust seizure-like activity could be initiated in hippocampal slices when active chemical synaptic transmission was blocked by any of several ionic and pharmacological treatments (2-4; reviewed in references 5-8). The role of electrotonic coupling between principal neurons in cortical structures (e.g., hippocampal pyramidal cells), and more recently interneurons, continues to be analyzed. Considerable progress has been made in the molecular characterization of the connexins in gap junctions of the nervous system, and the evidence for and potential relevance of gap junctions between hippocampal pyramidal cells and among interneurons remains a controversial subject. The two articles that form the basis of this commentary have provided independent lines of evidence that gap junctions between principal cells (i.e., hippocampal and cortical pyramidal cells) synchronize action potentials and support high-frequency oscillations (>100 Hz), and that these events arise from discrete regions of epileptogenic cortex. The hypothetical mechanism attributes high-frequency oscillations in EEG recordings to the field potentials from synchronous action potentials superimposed on synaptic potentials, which have a lower frequency of oscillation.

The article by Traub et al. (9) concerning the possible role of gap junctions in fast EEG oscillations and seizure onset extends a long-standing line of computer modeling research on cell and network activity in the hippocampus. The previous research by Traub and his colleagues has focused on the contribution of different ionic conductances and neuronal connections in the production of oscillatory patterns in normal brain tissue, and on how these mechanisms may be involved in the generation of epileptiform events. The authors first note that fast oscillations in the field potentials are present at seizure onset and during the interictal period between the seizures in the subdural EEG recordings from children with focal cortical dysplasia and intractable epilepsy. Incidentally, the presence of these electrographic events may not have been realized previously because classical EEG techniques would have filtered out these high-frequency signals. Other experimental data in this paper are based on electrophysiological experiments in hippocampal slices that have found fast oscillations appearing as small "population spikes" (i.e., synchronized action potentials from hippocampal pyramidal cells). Several treatments that promote seizure-like activity are shown to be associated with the fast oscillations, including ones that increase the conductance of gap junction channels. The critical conceptual result, built on a solid foundation of similar computer modeling studies, is that the optimal model for simulating these fast oscillations involves the presence of gap junctions between principal cells, and specifically between the axons of pyramidal cells. Recent electrophysiological and tracer coupling experiments further support this idea (10). Gap junctions have not yet been seen on the axons of pyramidal cells, and most previous evidence and speculation had proposed them to be on dendrites and somata.

Most of the early electrophysiological evidence suggested that only a few percent of the pairs of cells show evidence for coupling, but how would such a diffuse system of interconnections contribute to synchronization? The proposed answer, which needs additional supporting data, is similar to that found in networks interconnected with chemical synapses (e.g., the CA3 area): if each neuron is only connected to a few other neighboring neurons, but most or all neurons participate in the connections, then the neurons can become synchronized when they become active and have membrane potentials near threshold (e.g., see reference 11). When electrotonic junctions are combined with recurrent excitatory synapses, even in a diffuse pattern of connections, the two local circuits appear to act synergistically to create the potential for a hypersynchronous condition. Experimental data to test this hypothesis will continue to be difficult to obtain because the percentage of electrotonically coupled pairs is expected to be quite small, even if all neurons participate in the hypothetically coupled network. Furthermore, recent studies from dual whole-cell recordings between coupled pairs of corti-

cal interneurons suggest that the coupling coefficient can be extremely small (below 0.1), and yet still play a role in the synchronization of neuronal activity. Probably more difficult is the problem of ensuring that artifactual coupling through potential damage from recording electrodes does not occur during these experiments.

Although the issue of electrotonic coupling through gap junctions between hippocampal pyramidal cells has been debated for many years, and these recent experimental results strengthen the support for this hypothesis, several issues still need to be addressed. Are ultrastructurally defined gap junctions actually on the axons of hippocampal pyramidal cells? Are they also present on pyramidal cells in other cortical structures that generate fast oscillations? The important contribution of this paper and its computer simulation approach is that the conceptual experiments can be performed as an important initial test of the hypothesis: can a limited number of gap junctions among axons actually synchronize action potentials? This paper and other related studies lead to the next question of how few and how small may these gap junctions be? Probably the most important question for the readers of *Epilepsy Currents* is: Does the process of epileptogenesis involve an increase in gap junctional communication between principal cells and/or interneurons in hippocampus and cortex?

A large body of electrophysiological data, derived primarily from intact animal preparations, has suggested that fast oscillations play an important role in several sensory and cognitive mechanisms of information processing (12–14). Bragin et al. (15) have analyzed the hypothetical role of fast oscillations (also referred to as "fast ripples") in seizure generation of epileptogenic cortex. Rats were given intrahippocampal injections of kainate to produce an animal model of temporal lobe epilepsy, which was used to analyze oscillations of 250–600 Hz in extracellularly recorded events from the hippocampus and parahippocampal regions. The hypothesis investigated was that a distributed network of "pathologically interconnected clusters of neurons" (PIN clusters) generates fast ripples as a fundamental component of epileptogenesis in highly localized regions. In a series of in vivo recordings from freely behaving animals, fast oscillations were localized to discrete areas of cortex. Comparable experiments were conducted in hippocampal slices from these animals, and fast oscillations were also localized in vitro to discrete areas. Furthermore, pharmacological depression of $GABA_A$-receptor mediated inhibition with bicuculline unmasked these fast oscillations, and they could be recorded over a larger area. The studies of Bragin et al. (15) suggest the importance of interconnectivity of hippocampal granule cells and pyramidal cells. Whether by new recurrent excitatory synapses or gap junctions, the essential difficulty in this line of investigation is to link together the electrographic observations in freely behaving animals with a quantitative assessment of cellular mechanisms in the particular structures thought to be important in temporal lobe epilepsy. Is there actually an increase in the number and/or strength of gap junctions between dentate granule cells and among hippocampal pyramidal cells? If so, does this occur immediately after an injury and contribute to the process of epileptogenesis, or is there a progressive increase in the number of gap junctions? On the other hand, how good is the evidence that gap junctions couple principal cells in mammalian cortex, and is there actually a change in the number of gap junctions? Other mechanisms may unmask the abnormalities that are the potential basis of increased interconnectivity. For example, does focal loss of interneurons or loss of input to interneurons create localized regions with less inhibition that then manifests an apparent increase in interconnectivity through glutamatergic synapses and/or gap junctions, which in turn contribute to fast oscillations? Do electrical field effects through extracellular space (i.e., ephaptic interactions) also contribute to the synchronization of action potentials?

The two articles, by Traub et al. (9) and by Bragin et al. (15), provide two independent lines of investigation suggesting that nontraditional forms of neuronal communication, such as gap junctions, appear to be the most likely way to generate fast oscillations in cortical structures (9; see also, ref. 16), and these fast oscillations have a conspicuous presence in localized regions of hippocampus and surrounding cortex (15). In spite of this and more than 20 years of research on this topic, new experimental approaches that are more demanding are needed to evaluate further the hypothesis that electronic coupling through gap junctions contributes to fast oscillations that are important for epileptic seizure activity. If this hypothesis is correct, however, pharmacological agents specific for neuronal gap junctions may represent a new way to treat seizures.

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