

SYNCHRONIZED GABAERGIC INHIBITION DRIVES EPILEPTIFORM ACTIVITY

Synchronization of Kainate-induced Epileptic Activity via GABAergic Inhibition in the Superfused Rat Hippocampus In Vivo

Khazipov R, Holmes GL

J Neurosci 2003;23:5337–5331

We studied cellular mechanisms of synchronization of epileptiform activity induced by kainic acid in a novel preparation of superfused rat hippocampus in vivo. Under urethane anesthesia, kainate induced epileptic population spikes occurring at 30 to 40 Hz. Pyramidal cells fired exclusively during population spikes, with an average probability of 0.34 on rebound of rhythmic γ -aminobutyric acid (GABA)_A-mediated inhibitory postsynaptic events. Excitatory synaptic events contributed little to seizure activity. Rhythmic epileptiform activity was suppressed by blocking GABA_A receptors and was slowed by barbiturates. Thus GABAergic inhibition is instrumental in synchronizing kainate-induced epileptiform rhythmic activity in the γ frequency band in the intact hippocampus in vivo.

Excitatory GABA Input Directly Drives Seizure-like Rhythmic Synchronization in Mature Hippocampal CA1 Pyramidal Cells

Fujiwara-Tsukamoto Y, Isomura Y, Nambu A, Takada M

Neuroscience 2003;119:265–725

γ -Aminobutyric acid (GABA), which generally mediates inhibitory synaptic transmissions, occasionally acts as an excitatory transmitter through intense GABA_A-receptor activation, even in adult animals. The excitatory effect results from alterations in the gradients of chloride, bicarbonate, and potassium ions, but its functional role still remains a mystery. Here we show that such GABAergic excitation participates in the expression of seizure-like rhythmic synchronization (afterdischarge) in the mature hippocampal CA1 region. Seizure-like afterdischarge was induced by high-frequency synaptic stimu-

lation in the rat hippocampal CA1-isolated slice preparations. The hippocampal afterdischarge was completely blocked by selective antagonists of ionotropic glutamate receptors or of GABA_A receptor and also by gap-junction inhibitors. In the CA1 pyramidal cells, oscillatory depolarizing responses during the afterdischarge were largely dependent on chloride conductance, and their reversal potentials (average, -38 mV) were very close to those of exogenously applied GABAergic responses. Moreover, intracellular loading of the GABA_A-receptor blocker fluride abolished the oscillatory responses in the pyramidal cells. Finally, the GABAergic excitation-driven afterdischarge has not been inducible until the second postnatal week. Thus excitatory GABAergic transmission seems to play an active functional role in the generation of adult hippocampal afterdischarge, in cooperation with glutamatergic transmissions and possible gap-junctional communications. Our findings may elucidate the cellular mechanism of neuronal synchronization during seizure activity in temporal lobe epilepsy.

COMMENTARY

The highlighted articles are the latest in a series of investigations that question the function of γ -aminobutyric acid (GABA) as an “inhibitory” transmitter. Sufficient data suggest that GABAergic systems play an important role in mediating rhythmic activity in hippocampus and neocortex under normal conditions. Thus it is not so surprising that hyperexcitability within inhibitory networks would be capable of sustaining hypersynchronous discharges, recognizable as aberrant rhythmic activity that is characteristic of ictal-like events. Clearly, GABA can act as an excitatory transmitter, particularly in immature animals but also in mature ones, especially with high-frequency activation. In immature animals, this finding mainly is due to changes in the chloride gradient. In mature animals, the excitation that occurs is likely the result of alterations in the chloride gradient, secondary to chloride accumulation and redistribution of bicarbonate ions. Further, it is known that inhibitory systems can generate rhythmic, “epileptiform” activity, which is characterized by an initial burst followed by afterdischarge, even in the absence of ionotropic excitatory drive (shown by

many investigators, by using the 4-aminopyridine model) (1). Increasingly and not so surprisingly, investigators also are finding that GABA antagonists can block such rhythmic activity and the afterdischarge. Gap-junction blockers (presumably via their actions on electrotonic transmission, especially among synchronized inhibitory interneurons but also principal cells) are likewise capable of antagonizing this activity. Dependence on such an “interneuron-based” mechanism has been implicated in several models of rhythmic and epileptiform activity, including 4-aminopyridine, low magnesium, carbachol, metabotropic glutamate, tetanic stimulation, and kainate.

Two different models are used to activate epileptiform discharges in the articles considered presently—kainate superfusion of rat hippocampus *in vivo* and repetitive electrical stimulation. Khazipov and Holmes used a novel preparation of superfused hippocampus *in vivo* that permits stable extracellular and patch-clamp recordings and pharmacologic manipulations. The technique, which limits pulsation artifacts and instability, uses a chamber-like device that is mounted onto dorsal hippocampus, into which electrodes and various solutions can be introduced. Kainate application induced the expected epileptic population spikes in CA3, blocked by the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate glutamate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Individual pyramidal cells were studied by using cell-attached and loose cell-attached recordings. Firing of putative CA3 pyramidal cells was tightly locked to the population spikes. Typically, pyramidal cells fired no more than one action potential during the population spikes, which were phase-locked to rhythmic GABA_A fast inhibitory events. Gamma frequency range activity was suppressed by the GABA antagonist bicuculline and reduced by barbiturates. The authors propose an interesting model to explain their results. Accordingly, kainate produces tonic depolarization of the hippocampal neurons and increases their firing rate. With GABAergic inhibition intact, neuronal activity is locked by synchronous inhibition provided by a collective discharge of interneurons. At the end of the collective GABA_A-mediated inhibitory events, the probability of pyramidal cell firing increases, and approximately one third of the cells fire rebound action potentials (as was seen experimentally), giving rise to the next population spike. Synchronization of interneuronal discharge would rely on a similar mechanism, but direct recordings from interneurons must confirm this theory.

Fujiwara et al. used repetitive extracellular stimulation at 100 Hz for 0.5 seconds, applied to isolated hippocampal CA1

slices, to induce repetitive spikes on an initial wave of depolarization followed by afterdischarge, lasting many seconds. Such activities have been produced in hippocampus under a variety of conditions, even when all synaptic activity has been blocked (presumably owing to potassium accumulation, ephaptic interactions, gap-junction communication, and so on). In this study, physiologic medium was used, and the source of the abnormal discharges induced by tetanic stimulation was exhaustively investigated by the authors. Under this condition, it was found that *N*-methyl-D-aspartate (NMDA) blockers could shorten the afterdischarge, but AMPA/kainate antagonists completely suppressed the activity. Interestingly, GABA_A antagonists and the carbonic anhydrase inhibitor, acetazolamide, abolished the abnormal activity, and GABA_B blockers prolonged the afterdischarge. Gap-junction blockers also suppressed the afterdischarge. The authors found that the reversal potential of the afterdischarge was between -25 mV and -50 mV, close to that of exogenously applied GABA. They propose that intense GABA_A-receptor activation causes a transient chloride accumulation inside the postsynaptic pyramidal cells, which, given a shift in reversal potential, changes the GABAergic effect from inhibition to excitation. The extent of excitation would be determined primarily by the balance between intracellular chloride accumulation via GABA_A receptors and subsequent chloride extrusion by cation-dependent chloride transporters, as well as by inwardly rectifying chloride channels.

The clinical relevance of GABAergic interneurons in the synchronization of rhythmic epileptiform activity in hippocampus is unclear, although it seems that the mechanisms examined must have some role in human epilepsy and other states that involve hyperexcitability. Even in the absence of excitatory synaptic transmission, the GABAergic network can be recruited into firing synchronous, rhythmic activity that can be recorded in extracellular fields. Thus the suggestion by Khazipov and Holmes that synchronization via inhibition may operate during the preictal phase in patients with epilepsy, manifested as the increase in the power in the γ frequency band, seems quite reasonable.

by Larry S. Benardo, M.D., Ph.D.

Reference

1. Yang L, Benardo LS. Laminar properties of 4-aminopyridine-induced synchronous network activities in rat neocortex. *Neuroscience* 2002;111:303–313.