

DOES THE DEVELOPMENT OF A GABAERGIC PHENOTYPE BY HIPPOCAMPAL DENTATE GYRUS GRANULE CELLS CONTRIBUTE TO EPILEPTOGENESIS?

Monosynaptic GABAergic Signaling from Dentate to CA3 with a Pharmacological and Physiological Profile Typical of Mossy Fiber Synapses

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Mossy fibers are the sole excitatory projection from dentate gyrus granule cells to the hippocampus, where they release glutamate, dynorphin, and zinc. In addition, mossy fiber terminals show intense immunoreactivity for the inhibitory neurotransmitter GABA. Fast inhibitory transmission at mossy fiber synapses, however, has not previously been reported. Here, we show that electrical or chemical stimuli that recruit dentate granule cells elicit monosynaptic GABA(A) receptor-mediated synaptic signals in CA3 pyramidal neurons. These inhibitory signals satisfy the criteria that distinguish mossy fiber-CA3 synapses: high sensitivity to metabotropic glutamate receptor agonists, facilitation during repetitive stimulation, and NMDA receptor-independent long-term potentiation. GABAergic transmission from the dentate gyrus to CA3 has major implications not only for information flow into the hippocampus but also for developmental and pathological processes involving the hippocampus.

Seizures Induce Simultaneous GABAergic and Glutamatergic Transmission in the Dentate Gyrus-CA3 System

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Monosynaptic and polysynaptic responses of CA3 pyramidal cells (PC) to stimulation of the dentate gyrus (DG) are normally blocked by glutamate receptor antagonists (GluRAs). However, after kindled seizures, GluRAs block the monosynaptic excitatory postsynaptic potential (EPSP) and isolate a monosynaptic inhibitory postsynaptic po-

tential (IPSP), suggesting that mossy fibers release GABA. However, kindling epilepsy induces neuronal sprouting, which can underlie this fast inhibitory response. To explore this possibility, the synaptic responses of PC to DG stimulation were analyzed in kindled epileptic rats, with and without seizures, and in nonepileptic rats, immediately after a single pentylenetetrazol (PTZ)-induced seizure, in which sprouting is unlikely to have occurred. Excitatory and inhibitory synaptic responses of PC to DG stimulation were blocked by GluRAs in control cells and in cells from kindled nonseizing rats, confirming that inhibitory potentials are disynaptically mediated. However, a fast IPSP could be evoked in kindled epileptic rats and in nonepileptic rats after a single PTZ-induced seizure. The same response was induced after rekindling the epileptic nonseizing rats. This IPSP has an onset latency that parallels that of the control EPSP and is not altered under low Ca^{2+} medium or halothane perfusion. In addition, it was reversibly depressed by L(+)-2-amino-4-phosphonobutyric acid (L-AP4), which is known to inhibit transmitter release from mossy fibers. These results demonstrate that seizures, and not the synaptic rearrangement due to an underlying epileptic state, induce the emergence of fast inhibition in the DG-CA3 system, and suggest that the mossy fibers underlie this plastic change.

Kindling Induces Transient Fast Inhibition in the Dentate Gyrus-CA3 Projection

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The granule cells of the dentate gyrus (DG) send a strong glutamatergic projection, the mossy fibre tract, toward the hippocampal CA3 field, where it excites pyramidal cells and neighbouring inhibitory interneurons. Despite their excitatory nature, granule cells contain small amounts of GAD (glutamate decarboxylase), the

main synthetic enzyme for the inhibitory transmitter GABA. Chronic temporal lobe epilepsy results in transient upregulation of GAD and GABA in granule cells, giving rise to the speculation that following overexcitation, mossy fibres exert an inhibitory effect by release of GABA. We therefore stimulated the DG and recorded synaptic potentials from CA3 pyramidal cells in brain slices from kindled and control rats. In both preparations, DG stimulation caused excitatory postsynaptic potential (EPSP)/inhibitory postsynaptic potential (IPSP) sequences. These potentials could be completely blocked by glutamate receptor antagonists in control rats, while in the kindled rats, a bicuculline-sensitive fast IPSP remained, with an onset latency similar to that of the control EPSP. Interestingly, this IPSP disappeared 1 month after the last seizure. When synaptic responses were evoked by high-frequency stimulation, EPSPs in normal rats readily summate to evoke action potentials. In slices from kindled rats, a summation of IPSPs overrides that of the EPSPs and reduces the probability of evoking action potentials. Our data show for the first time that kindling induces functionally relevant activity-dependent expression of fast inhibition onto pyramidal cells, coming from the DG, that can limit CA3 excitation in a frequency-dependent manner.

Vesicular GABA Transporter mRNA Expression in the Dentate Gyrus and in Mossy Fiber Synaptosomes

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In the normal granule cells of the dentate gyrus, glutamate and both gamma-aminobutyric acid (GABA) and glutamic acid decarboxylase (GAD) coexist. GAD expression is increased after seizures, and simultaneous glutamatergic and GABAergic neurotransmission from the mossy fibers to CA3 appears, supporting the hypothesis that GABA can be released from the mossy fibers. To sustain GABAergic neurotransmission, the amino acids for the presence and regulation of expression of the vesicular GABA transporter (VGAT) mRNA in the dentate gyrus and in mossy fiber synaptosomes of control and kindled rats. We found trace amounts of VGAT mRNA in the dentate gyrus and mossy fiber synaptosomes of control rats. In the dentate gyrus of kindled rats with several seizures and of control rats subject to one acute seizure, no changes were apparent either 1

or 24 h after the seizures. However, repetitive synaptic or antidromic activation of the granule cells in slices of control rats *in vitro* induces an activity-dependent enhancement of VGAT mRNA expression in the dentate. Surprisingly, in the mossy fiber synaptosomes of seizing rats, the levels of VGAT mRNA were significantly higher than in controls. These data show that the granule cells and their mossy fibers, besides containing machinery for the synthesis of GABA, also contain the elements that support its vesiculation. This further supports the notion that local synaptic molecular changes enable mossy fibers to release GABA in response to enhanced excitability.

COMMENTARY

The possibility that excitatory glutamatergic neurons can switch their phenotype and become inhibitory GABAergic neurons flies in the face of conventional wisdom. However, this is exactly what has been proposed for dentate gyrus granule cells of the hippocampus, which are neurons that are generally considered to be glutamatergic but also synthesize low levels of GABA. The expression of GABA and its synthetic enzyme glutamic acid decarboxylase (GAD) greatly increases after seizures, suggesting that these neurons could become functionally inhibitory, particularly in the context of epilepsy. Although hard to predict how this would impact hippocampal function, and even more difficult to understand how “inhibitory” granule cells would affect hippocampal excitability in temporal lobe epilepsy, it is first important to clarify what data support this claim.

Originally, the neurotransmitter used by granule cells was thought to be an excitatory transmitter, most likely glutamate (1,2). In 1991, Sandler and Smith provided evidence using electron microscopy that granule cells synthesized GABA, but the finding did not garner much attention (3). After it was shown that GABA and GAD increased in granule cells after seizures (4–9), the onus fell on physiologists to prove that the considerable pool of granule cell GABA had functional implications. However, all previous studies of granule cell function were consistent with an excitatory action. Indeed, there is a long history of hippocampal recordings *in vivo* and *in vitro*, which demonstrates that granule cells are the first part of the excitatory hippocampal trisynaptic circuit. Nevertheless, recent studies suggest that GABA synthesized in granule cells could have a role as a neurotransmitter.

A series of articles have demonstrated in hippocampal slices that a short latency (presumably monosynaptic) inhibitory synaptic potential/current (IPSP or IPSC) could be recorded in granule cell targets (CA3 pyramidal cells) upon stimulation of granule cells. Data from Gutierrez (10) and Gutierrez and

Heinemann (11) mainly used kindled rats or animals that had a single pentylenetetrazol-induced seizure. A synaptic potential that appeared similar in reversal potential and pharmacology to a conventional GABA_A receptor-mediated IPSP was recorded upon electrical stimulation of the granule cell layer in the presence of ionotropic glutamate receptor antagonists (10,11). The IPSP had the pharmacological profile of mossy fiber transmission, that is, sensitivity to the metabotropic receptor antagonist L-AP4 (10). Naysayers may still require evidence, beyond the short latency, of monosynaptic transmission and may be cautious in considering whether L-AP4 defines mossy fiber transmission unequivocally, particularly after seizures when receptors can change radically. However, additional evidence was recently provided in normal tissue, in which an apparently monosynaptic IPSC could be recorded by using either electrical stimulation or glutamate application to granule cells (12). The IPSC had typical characteristics of a GABA_A receptor-mediated IPSC. It showed metabotropic receptor sensitivity and additional characteristics of mossy fiber synaptic transmission, such as robust facilitation and NMDA receptor-independent long-term potentiation.

Subsequent studies have provided evidence from reverse transcription-polymerase chain reaction of synaptosomes that granule cell terminals express the vesicular GABA transporter (VGAT) (13), although the true levels of terminal expression are hard to predict given the amplification associated with this method and the impurity of the synaptosome preparation. After seizures, expression of VGAT appears to increase (13). Other studies suggest that GABA may instead be released by reverse action of a GABA transporter, such as GAT-3, which increases after kainic acid-induced seizures (14).

So can glutamatergic neurons switch their phenotype? This probably is not a general rule in the central nervous system, as granule cells appear to be somewhat unique in their ability to synthesize both GABA and glutamate. Even in the case of granule cells, it seems that the GABAergic signal is normally masked by glutamate excitation. It remains to be determined whether physiologically relevant inhibition can be generated by granule cells, but the possibility that this occurs, particularly in epilepsy, must be seriously considered. If granule cell GABA does not serve in a neurotransmitter role, could it have other functions? One possibility is that granule cell GABA could buffer cytosolic pH and thus contribute to the widely observed resistance of granule cells to insults. Indeed, GABA may have such a role in plants (15). It is also possible that the enhanced GABA synthesis in granule cells produced by seizures could recapitulate a developmental program (8,15). In any case, plasticity of neurotransmitter phenotype could have important implications for understanding epileptogenesis in the hippocampus.

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