

THE UPS AND DOWNS OF HIPPOCAMPAL METABOTROPIC GLUTAMATE RECEPTORS: RAMIFICATIONS FOR EPILEPTOGENESIS AND COGNITIVE IMPAIRMENT FOLLOWING STATUS EPILEPTICUS

Loss of Metabotropic Glutamate Receptor-Dependent Long-Term Depression via Downregulation of mGluR5 after Status Epilepticus. Kirschstein T, Bauer M, Müller L, Rüschemschmidt C, Reitze M, Becker AJ, Schoch S, Beck H. *J Neurosci* 2007;27(29):7696–7704. Synaptic plasticity is thought to be a key mechanism of information storage in the CNS. Different forms of synaptic long-term potentiation have been shown to be impaired in neurological disorders. Here, we show that metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD), but not NMDA receptor-dependent LTD at Schaffer collateral–CA1 synapses, is profoundly impaired after status epilepticus. Brief application of the group I mGluR agonist (*R,S*)-3,5-dihydroxyphenylglycine (100 μ M; 5 min) induced mGluR LTD in control, but not in pilocarpine-treated rats. Experiments in the presence of selective inhibitors of either mGluR5 [2-methyl-6-(phenylethynyl)-pyridine] or mGluR1 [7-(hydroxyimino)cyclopropachromen-carboxylate ethyl ester and (*S*)-(+)-amino-4-carboxy-2-methylbenzeneacetic acid] demonstrate that loss of mGluR LTD is most likely attributable to a loss of mGluR5 function. Quantitative real-time reverse transcription PCR revealed a specific downregulation of mGluR5 mRNA, but not of mGluR1 mRNA in the CA1 region. Furthermore, we detected a strong reduction in mGluR5 protein expression by immunofluorescence and quantitative immunoblotting. Additionally, the scaffolding protein Homer that mediates coupling of mGluR5 to downstream signaling cascades was downregulated. Thus, we conclude that the reduction of mGluR LTD after pilocarpine-induced status epilepticus is the result of the subtype-specific downregulation of mGluR5 and associated downstream signaling components.

Functional Role of mGluR1 and mGluR4 in Pilocarpine-Induced Temporal Lobe Epilepsy. Pitsch J, Schoch S, Gueler N, Flor PJ, van der Putten H, Becker AJ. *Neurobiol Dis* 2007;26(3):623–633. Altered expression and distribution of neurotransmitter receptors, including metabotropic glutamate receptors (mGluRs), constitute key aspects in epileptogenesis, impaired hippocampal excitability and neuronal degeneration. mGluR1 mediates predominantly excitatory effects, whereas mGluR4 acts as inhibitory presynaptic receptor. Increased hippocampal expression of mGluR1 and mGluR4 has been observed in human temporal lobe epilepsy (TLE). In this study, we address whether genetic mGluR1 upregulation and mGluR4 knock-down influence seizure susceptibility and/or vulnerability of hippocampal neurons by analyzing transgenic animals in the pilocarpine TLE model. Therefore, we generated transgenic mice expressing mGluR1-enhanced green fluorescent protein (EGFP) fusion protein under control of the human cytomegalovirus (CMV) immediate early promoter. Status epilepticus (SE) was induced in 1) mice overexpressing mGluR1-EGFP and 2) mice deficient for mGluR4 (mGluR4 KO) as well as littermate controls. In the acute epileptic stage after pilocarpine application, mGluR4 KO mice showed a significant increase of severe seizure activity, in contrast to mGluR1 transgenics. Analysis of both transgenic mouse lines in the chronic epileptic phase, using a telemetric EEG-/video-monitoring system, revealed a significant increase in seizure frequency only in mGluR1-EGFP mice. In contrast, enhanced neuronal cell loss was only present in the hippocampus of epileptic mGluR4 KO mice. Our results suggest a role for mGluR1 in promoting seizure susceptibility as well as for mGluR4 to counteract excitatory activity and seizure-associated vulnerability of hippocampal neurons. Therefore, our data strongly recommend both mGluRs as potential drug targets to interfere with the development of hippocampal damage and seizure activity in TLE.

COMMENTARY

The cholinergic agent pilocarpine has long been used in animal studies to elicit limbic status epilepticus (1). As the hippocampus receives strong cholinergic inputs, it perhaps is not surprising that hyperactivation of these excitatory

pathways can elicit acute seizures. However, the usefulness of this model is that, following a latent period of days to weeks, the vast majority of animals develop chronic recurrent seizures of temporal lobe origin usually associated with hippocampal sclerosis. This model, therefore, is commonly employed to study both temporal lobe epilepsy and hippocampal epileptogenesis (2).

Epileptogenesis, a gradual permanent transformation, results in a persistently lowered seizure threshold and predisposes

the individual to recurrent seizures. At present, it is not possible to accurately predict which patients who endure status epilepticus will go on to develop epilepsy. However, it is noteworthy that differences in seizure threshold and susceptibility of different strains of mice to developing recurrent seizures and hippocampal sclerosis following status epilepticus have been correlated with baseline differences in metabotropic glutamate receptor (mGluR) expression: those strains with reduced expression of group I mGluRs seem to have a greater resistance to the development of epilepsy (3).

The discovery of the mGluRs over the past few decades has led to a better understanding of the potential long-term consequences of excessive glutamatergic transmission. These G-protein coupled receptors activate intracellular cascades of events, resulting in long-lasting modifications of cellular and network excitability. The group I mGluRs (mGluR1 and mGluR5), in particular, are localized primarily on the postsynaptic membrane and are predominantly excitatory. Therefore, the mGluRs have become an area of intense interest in studies of synaptic plasticity (e.g., long-term potentiation [LTP] and long-term depression [LTD]) as well as network plasticity (e.g., epileptogenesis). Two recent papers attempt to correlate the presence of various mGluR subtypes with the long-term sequelae of pilocarpine-induced status epilepticus on hippocampal functioning in rodents.

The set of detailed experiments by Kirschstein et al. reviewed here examined changes in group I mGluRs in rats that became epileptic following pilocarpine-induced status epilepticus. In these studies, rats that developed at least three spontaneous stage 5 seizures, beginning 2 or more weeks after pilocarpine-induced status epilepticus, were recorded from weeks 4 to 10 and compared with sham control rats that received saline instead of pilocarpine and, thus, never experienced status epilepticus. The authors found that the epileptic rats remained capable of producing LTD via NMDA activation, but they could not produce NMDA-independent LTD that is normally mediated by group I mGluRs. Further studies utilizing mGluR1 and mGluR5 antagonists suggested that the impairment was specifically due to deficient mGluR5 activation, with mGluR1 remaining intact. Quantitative measurements of group I mGluR proteins revealed selective downregulation of mGluR5 and its membrane anchoring protein, Homer, which was out of proportion to the hippocampal neuronal loss. These findings are consistent with previously reported kindling studies in which transient mGluR5 downregulation was observed as well (4).

Acknowledging the role of mGluR-dependent LTD in learning (5), Kirschstein et al. suggested that loss of mGluR5-dependent LTD might explain some of the cognitive deficits seen during induction of epilepsy. They also proposed that loss of this form of LTD might promote axonal sprouting during

epileptogenesis, as it has been previously reported that LTD is necessary for the pruning of glutamatergic synapses (6). An additional implication of the data, not mentioned by the authors, is the possibility that some of the cognitive side effects of currently used antiepileptic drugs might result from an interference with NMDA-dependent LTD. If this is the only form of LTD available in some patients with epilepsy, any agent compromising the NMDA-dependent pathway ought to have greater negative impact on cognition than it would in normal controls.

Should the discrepancy between mGluR1 and mGluR5 identified in these studies be surprising? Although mGluR1 and mGluR5 are members of the same family, their respective roles in seizure production and epileptogenesis differ. In vitro studies have shown that transient activation of group I mGluRs will result in long-lasting, spontaneous ictal discharges (7) and that, while mGluR1 and mGluR5 work together in both the induction and maintenance phases, it is primarily mGluR5 that drives the epileptogenesis in the induction phase and mGluR1 that maintains the ongoing expression of the seizure discharges (8). The selective down-regulation of mGluR5 observed by Kirschstein et al. in the chronic phase in part may explain the increasingly important role recognized for mGluR1 in the ongoing expression of seizure discharges following epileptogenesis.

The recent studies by Pitsch et al. further implicate mGluR1 in exacerbating epileptic seizures. In one set of experiments, transgenic mice overexpressing mGluR1 were subjected to pilocarpine-induced status epilepticus then recorded for the next 4 weeks. While the severity and latency of the acute convulsive responses in these mice were no different from those of control mice, the chronic recurrent seizures that developed were progressively worse than those of control animals in both frequency and severity. As the acute seizures in this model are initiated by direct cholinergic activation, it seems sensible that mGluR1 activation has no significant contribution to their expression. However, the contribution of mGluR1 to the expression of chronic recurrent seizures is revealing, implicating mGluR1 as a major player not only in the seizures that follow Group I mGluR-induced epileptogenesis (8) but likely bearing broader relevance to other forms of epileptogenesis as well. Some pharmacoresistant patients may have hyperexcitable mGlu1 receptors; such patients could find relief from new therapies that target this receptor subtype.

Unlike the group I mGluRs, group II (mGluR2 and mGluR3) and group III (mGluR4 and mGluRs 6–8) are primarily presynaptic in localization and tend to suppress neurotransmission. A separate set of experiments by Pitsch et al. used mGluR4 knockout mice. These studies showed that mice lacking mGluR4 experienced no change in seizure frequency following pilocarpine; however, they did have increased seizure severity, both during the acute stage (in immediate response to pilocarpine injection) and the subsequent chronic stage, as

well as increased hippocampal neuronal loss. This finding too would not be unexpected: mGluR4 reduces transmitter release and thereby should help reduce all seizure activity, acute or chronic. Absence of mGluR4s, therefore, should and did exacerbate seizures. As for the exacerbation of hippocampal cell loss, the authors propose that it may be due to NMDA-mediated neurotoxicity, which has previously been shown to be reduced in the presence of group III mGluR agonists, indicating that mGluR4 can serve a neuroprotective role (9). However, one flaw in their explanation remains: the worsened neuronal loss may simply be a consequence of more severe seizure activity and not related to mGluR4 function per se or lack thereof. It is likely that anything minimizing the severity of the acute seizures also would protect the hippocampus from increased damage—it need not be via mGluR4 activation. So while mGluR4 may be a useful target for the suppression of seizures and excitotoxic damage (10), the resultant reduced neurotransmission may come with excessive toxicities in human studies. The important implication of the data, however, is that patients who have deficient baseline mGluR4 function may be more vulnerable to the development of some forms of epilepsy.

One key issue not addressed in these studies is whether the mGluR changes observed underlie the epileptogenesis or result from it. Changes observed in epileptic animals may be compensatory as opposed to contributory. What conferred protection to those few animals that received pilocarpine but did not develop epilepsy? Furthermore, none of these studies examined the progressive changes during the latent period—such studies might be better at determining causal versus compensatory changes and might be of greater use in the development of agents with antiepileptogenic potential.

A better understanding of the contribution of mGluRs to seizure susceptibility, cognitive deficits in epilepsy, and the expression of epileptic seizures, as provided by these studies and others, will certainly assist development of a broader range of anticonvulsants, allowing for better seizure control in refractory patients and possibly minimizing the cognitive side effects endured by many who take currently available agents. However, more importantly, results obtained from studies examining the

early role of group I mGluRs in the epileptogenic process may provide hope that these receptors will serve as useful targets in the development of antiepileptogenic agents.

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