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# Distinct regulation of metabotropic glutamate receptor (mGluR1 alpha) in the developing limbic system following multiple early-life seizures

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**Abstract**

The effects of repeated neonatal seizures on metabotropic glutamate receptors (mGluRs) during critical periods of brain development are unknown. Therefore, we characterized the expression of Group I (mGluR1 and mGluR5) and Group II (mGluR2/3) metabotropic glutamate receptor proteins in the developing limbic system in response to a varied neonatal seizure history. Status epilepticus was induced with kainic acid (KA) either once (1× KA) on postnatal (P) day (P13), twice (2× KA) on P6 and P9 or P13, or three times (3× KA) on P6, P9, and P13. In control hippocampus, mGluR1α protein expression differed at all stages of development examined, whereas mGluR2/3 and mGluR5 protein expression patterns were mature by P15. After KA-induced status epilepticus, there was a significant elevation in mGluR1α protein expression within a select group of inhibitory interneurons of the CA1 stratum oriens–alveus that was enhanced with increasing number of neonatal seizures. mGluR2/3 and mGluR5 subtypes were unchanged. Increases were also observed within neurons of the amygdala and piriform cortex. Selective increases of mGluR1α subtypes within limbic structures may contribute to the resistance and tolerance of the immature hippocampus from damage. This may occur by excessive stimulation of excitatory synapses to collectively enhance the inhibitory drive of the immature brain by increasing GABA release. Data suggest that the mGluR1α subtype plays an important role in regulating hippocampal network activity after early-life seizures.


*Keywords:* Metabotropic; Glutamate; Seizures; Epilepsy; Hippocampus; Amygdala; Piriform cortex; Neonatal; Development

## Introduction

Experimental epilepsy models have shown that the immature brain is relatively resistant to seizure-induced brain damage until a critical stage in development (Albala et al., 1984; Nitecka et al., 1984; Holmes and Thompson, 1988; Friedman et al., 1997). However, mechanisms underlying the reduced vulnerability of developing neurons to epileptic seizures remain unknown. Previous research by us and others has elucidated the effects of a single episode of KA-induced status epilepticus on ionotropic glutamate receptors (iGluRs) in the mature (Pollard et al., 1993; Friedman, 1998; Friedman et al., 1994; Grooms et al., 2000) and immature brain (Friedman et al., 1997). However, studies on metabotropic glutamate receptor (mGluR) expression after single and multiple seizures are lacking in the developing brain.

Unlike iGluRs, mGluRs mediate slow synaptic responses due to their coupling to intracellular G proteins and second messengers were divided into three groups (Nakanishi, 1994). Group I includes mGluR1 and mGluR5 subtypes, which exist in a number of alternatively spliced forms (mGluR1α, β, c, d and mGluR5 a, b) and are coupled to $IP_3/Ca^{2+}$ signal transduction. Group II includes mGluR2 and mGluR3 subtypes. Group III includes mGluR4 and mGluR6–mGluR8 subtypes. Both Group II and III subtypes are negatively coupled to adenylate cyclase (Nakanishi, 1994; Pin and Duvoisin, 1995; Conn and Pin, 1997). These receptors also play a role in synaptic plasticity, modulation of neural excitability, and neurotransmitter release (Pin and Duvoisin, 1995; Wong et al., 2002). Moreover, mGluRs play a role in learning, specifically with the induction of long-term potentiation (LTP) and long-term depression

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(LTD) in hippocampus and limbic cortex (Harberly and Price, 1978; Bashir et al., 1993; Anwyl, 1999; Stripling and Patneau, 1999; Sugitani et al., 2004).

It is well known that mGluR mRNA and protein expression is developmentally regulated in the hippocampus (Catania et al., 1994; Defagot et al., 2002; Lopez-Bendito et al., 2002). Differential spatiotemporal patterns are particularly notable for the mGluR1α subtype isoform (Lopez-Bendito et al., 2002). Furthermore, mGluR1α subtypes are co-localized within specific subclasses of GABAergic interneurons of the hippocampus with somatostatin (Baude et al., 1993) or vasointestinal neuropeptide (Ferraguti et al., 2001). In the mature brain, it is known that hippocampal CA1 inhibitory interneurons control excitation and synchronization of pyramidal cells. These interneurons also participate in hippocampal synaptic plasticity (Freund and Buzsaki, 1996; Buzsaki and Chrobak, 1995). However, the functional role of these interneurons in the neonatal brain is less established.

Functional studies carried out in adult rats show that Group I mGluR agonists have proconvulsant effects (Rutecki and Yang, 1997) whereas Group II and III agonists have anticonvulsant effects (Abdul-Ghani et al., 1997). In contrast, slice recordings from immature rats (P9–P16) are developmentally regulated such that the Group I mGluR agonist 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) and the broad-spectrum antagonist alpha-methyl-4-carboxyphenylglycine (MCPG) have both biphasic and time-dependent effects on bicuculline-evoked spontaneous epileptiform events (Burke and Hablitz, 1995). For example, ACPD facilitated evoked spontaneous epileptiform discharges at low concentrations but reduced them at high doses. Group II mGluR antagonists did not reduce spontaneous epileptiform discharges as observed in adults (Burke and Hablitz, 1995). Another example of age-dependent regulation is dihydroxyphenyl glycol (DHPG) enhanced post-synaptic potentials in CA1 neurons prepared from adults slices; but reduced them in neonatal preparations (Ross et al., 2000). In adults, mGluR II subtype-induced hyperpolarization responses in amygdala neurons were decreased after kindling (Holmes et al., 1996a). No such study has been carried out in neonates. Thus, it is postulated that such alterations may further perpetuate seizures in adulthood but not in infancy due to age-dependent effects.

Since the expression of certain mGluR subtypes changes within the developing hippocampus, it is first necessary to elucidate the spatiotemporal pattern of expression to determine the role of these receptors in developmental epilepsy. We questioned whether changes in expression of glutamate receptor classes may depend upon the frequency of early-life seizures. Therefore, the present study used immunohistochemical techniques to examine developmental localization of Group I and Group II mGluRs within the hippocampus and then examine changes in mGluR expression following repetitive episodes of status epilepticus induced in the early postnatal period. Marked increases of mGluR1α expression were observed in CA1 interneurons and other limbic structures whereas other subtypes were unaffected. This suggests that activation of presynaptic GABAergic terminals as well as dendrites of interneurons via the mGluR1α subtype may play a unique role in the treatment of pediatric epilepsy.

## Materials and methods

### *Developmental expression of mGluRs in absence of seizures*

Brains from Sprague–Dawley rats ranging in age from postnatal (P) day P6 to adulthood were used. Animals were grouped as follows: P6 (8–10 g, $n=3$), P9 (12–15 g, $n=3$), P15 (25–32 g, $n=3$), P20 (40–50 g, $n=3$), P30 (55–65 g, $n=3$), and adult (275–325 g, $n=3$). Animals were given food and water ad libitum and kept on a 12-h light/dark cycle at room temperature (55% humidity) in our own accredited animal facility in accordance with NIH guidelines.

### *Kainic acid administration*

To examine the expression of mGluRs after single or multiple neonatal seizures, kainic acid (KA) was administered to rat pups at P6, P9, and P13. Appropriate doses of KA were used to induce status epilepticus for each age as previously described (Friedman et al., 1997; Liu et al., 2003; Liu et al., 2006). Animals treated with only one injection of KA (1× KA) ($n=5$) were administered a dose of 3.5 mg/kg, i.p. on P13. Rat pups that received two injections of KA (2× KA) ($n=5$) were administered a dose of 1.8 mg/kg on P6 and a second dose of 2 mg/kg subcutaneously on P9 or 3.5 mg/kg, i.p. on P13. Three injections of KA (3× KA) ($n=8$) were given on P6, P9, and on P13 as previously described (Liu et al., 2003). Age-matched control littermates ($n=8$) were administered equivalent volumes of phosphate-buffered saline (0.1 M, pH 7.4). After KA injection, rats were placed into a clean and comfortable cage and their epileptic behavior was monitored every 5 min for 2 h. Only rats that exhibited status epilepticus (approximately 90%) were used in this study as described (Liu et al., 2006). Following the observation period, pup rats were returned to their lactating mother. All animals were sacrificed on P15.

### *Immunohistochemistry*

To examine the expression of mGluR1α, mGluR2/3, and mGluR5 proteins during development and following a single or multiple seizures, immunohistochemistry was performed as described (Vissavajjhala et al., 1996; Friedman, 1998; Friedman and Veliskova, 1998). Pups were killed by transcardial perfusion on P15. Vibratome (40 μm) sections were prepared from 1× KA, 2× KA, and 3× KA treated rat pups at the level of the hippocampus. Free-floating sections were washed in PBS (2×), then incubated with 0.5% $H_2O_2$ for 15 min to remove endogenous peroxides. Sections were washed (4× for 10 min each) and blocked in 5% goat serum/0.5% BSA–PBS. The anti-mGluR1α, anti-mGluR2/3, and anti-mGluR5 (1:200) primary antibodies were incubated for 48 h at 4°C. Tissue sections were washed (3× for 10 min each) in PBS to remove primary antibodies. Secondary biotinylated anti-rabbit IgG (diluted