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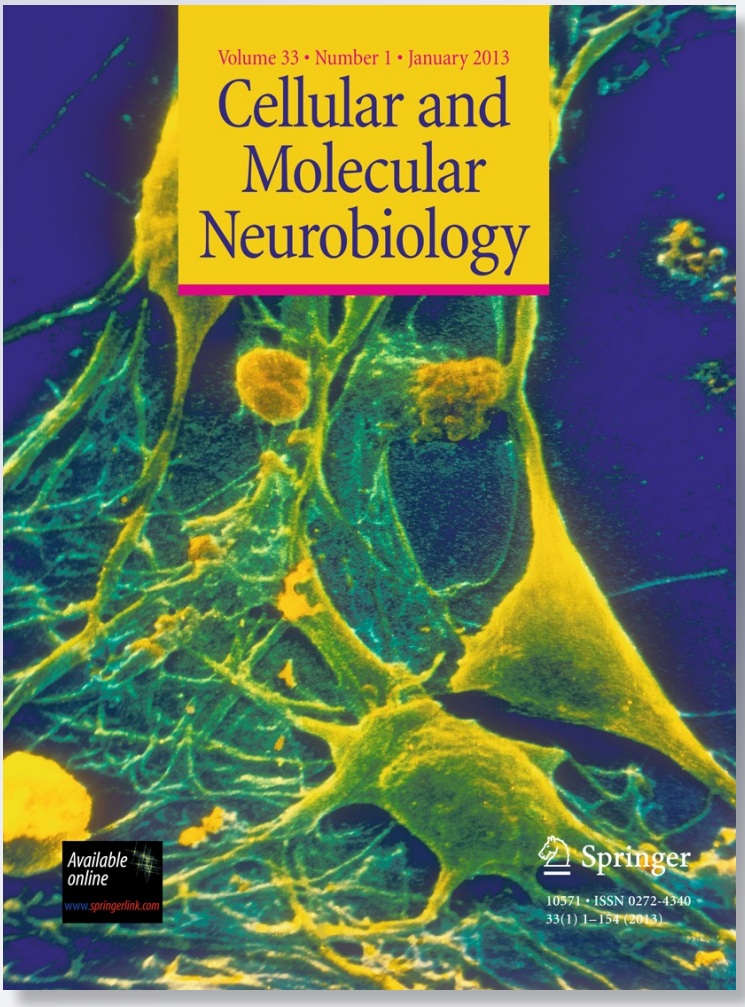


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ORIGINAL RESEARCH

# Myo-Inositol Treatment and GABA-A Receptor Subunit Changes After Kainate-Induced Status Epilepticus

**Revaz Solomonia · Nana Gogichaishvili · Maia Nozadze · Eka Lepsveridze · David Dzneladze · Tamar Kiguradze**

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**Abstract** Identification of compounds preventing the biochemical changes that underlie the epileptogenesis process is of great importance. We have previously shown that myo-Inositol (MI) daily treatment prevents certain biochemical changes that are triggered by kainic acid (KA)-induced status epilepticus (SE). The aim of the current work was to study the further influence of MI treatment on the biochemical changes of epileptogenesis and focus on changes in the hippocampus and neocortex of rats for the following GABA-A receptor subunits: $\alpha 1$, $\alpha 4$, $\gamma 2$, and $\delta$. After SE, one group of rats was treated with saline, while the second group was treated with MI. Control groups that were not treated by the convulsant received either saline or MI administration. 28–30 h after the experiment, a decrease in the amount of the $\alpha 1$ subunit was revealed in the hippocampus and MI had no significant influence on it. On the 28th day of the experiment, the amount of $\alpha 1$ was increased in both the KA− and KA + MI-treated groups. The $\alpha 4$ and $\gamma 2$ subunits were strongly reduced in the hippocampus of KA-treated animals, but MI significantly halted this reduction. The effects of MI on $\alpha 4$ and $\gamma 2$ subunit changes were significantly different between hippocampus and neocortex. On the twenty-eighth day after SE, a decrease in the amount of $\alpha 1$ was found in the neocortex, but MI treatment had no effect on it. The obtained results indicate that MI treatment interferes with some of the biochemical processes of epileptogenesis.

**Keywords** Epilepsy · Kainic acid · Epileptogenesis · Myo-Inositol · $\gamma$-Aminobutyric acid receptor subunits

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## Introduction

Epilepsy is a heterogeneous group of disorders. It is the most common neurologic disorder after stroke, with a 2–3 % life time risk that a patient may receive a diagnosis of epilepsy (Browne and Holmes 2001). The available therapy against epilepsy is only symptomatic and often ineffective (Loescher et al. 2008). The most important challenge is to prevent the process of epileptogenesis (Loescher et al. 2008), not merely to treat its symptoms. At present, there is no anti-epileptic drug (AED) that performs this function (Loescher et al. 2008). Thus, the search for truly anti-epileptogenic drugs is of outstanding importance for modern biomedical sciences.

A series of our previous experiments have revealed that a water extract of the medicinal plant *Aquilegia vulgaris* contains compounds that alter the binding of ligands to the benzodiazepine and $\gamma$-aminobutyric acid (GABA) binding sites of the GABA-A receptors (GABA-AR) (Solomonia et al. 2004). We have identified two main active compounds from this extract: (1) myo-Inositol (MI) and (2) Oleamide a—sleep-inducing lipid (Solomonia et al. 2004). MI was not previously known to be a compound acting on GABA-AR, and hence was not expected to influence the 3H-muscimol binding. Nevertheless, we confirmed that MI was indeed the compound inhibiting 3H-muscimol binding. We also showed that MI increases 3H-MK-801 (a NMDA receptor antagonist) binding to rat brain membranes (Solomonia et al. 2004).

Further, we have demonstrated that intraperitoneal pretreatment of animals with MI significantly decreases the severity of SE seizures that are induced either by kainic acid (KA) or by pentylentetrazol (PTZ) (Solomonia et al. 2007). Moreover, our recent experiments have shown that another representative of inositols, namely scyllo-inositol (SCI), also significantly reduces the severity score and duration of PTZ-induced convulsions (Nozadze et al. 2011). Our electron-microscopic studies indicate that MI pretreatment before KA-induced SE exerts a strong neuroprotective action on the neuronal degeneration in hippocampus that was induced by KA (Zhvaniia et al. 2007). As the doses of administered inositols in all our experiments (Nozadze et al. 2011; Solomonia et al. 2007, 2010; Zhvaniia et al. 2007) were within the range of physiologic concentrations, we have proposed that, in addition to other known functions in the brain (Fisher et al. 2002), MI and SCI may act as endogenous regulators of seizure activity (Nozadze et al. 2011).

To explore whether MI exerts its action not only on acute SE seizures but also on SE-induced epileptogenesis, we first induced SE by KA, and then initiated daily MI treatment 6 h later. The daily MI treatment (30 mg/kg) over the course of 28 days attenuated certain biochemical changes which were associated with the process of epileptogenesis. Namely, we have demonstrated that KA treatment decreases the levels of the GLUR1 subunit of AMPA-glutamate receptors, as well as the levels of calcium-calmodulin-dependent protein kinase II (CaMKII) in the hippocampus. These alterations were almost completely reversed by daily MI treatment (Solomonia et al. 2010).

A number of molecular changes take place during the early and latent phases after KA-induced SE, which may contribute to the process of epileptogenesis (Brooks-Kayal et al. 2009; Majores et al. 2007; McNamara et al. 2006). Therefore, the candidate compounds for epileptogenesis prevention should be studied thoroughly for their effects. In this paper, we examine the influence of MI daily treatment on changes in GABA-AR subunit amounts after KA-induced SE.

GABA-ARs are heteropentamers formed by the assembly of multiple subunits that generate a wide array of receptors (for a recent review see Olsen and Sieghart 2009). The process of epileptogenesis is associated with changes in the expression and function of GABA-ARs (Friedman et al. 1994; Kharlamov et al. 2011; Sperk et al. 1998; for a review see Gonzalez and Brooks-Kayal 2011). Various drugs that enhance GABAergic inhibition are commonly used as AEDs. Identification of the mechanisms involved in GABA-AR malfunction during epileptogenesis and the ability to reverse this malfunction are crucial steps toward developing specific and effective therapies.

Among the numerous subunits of GABA-ARs, we decided to focus on the following ones: $\alpha 1$, $\alpha 4$, $\gamma 2$, and $\delta$. The reasons for this selection are (i) the expression of these subunits undergoes time-dependent changes during epileptogenesis and following epilepsy (Friedman et al. 1994; Gonzalez and Brooks-Kayal 2011; Sperk et al. 1998) and (ii) $\alpha 1$ and $\gamma 2$ are found in synapses and participate in phasic inhibition (Mangan et al. 2005; Nusser and Mody 2002), whereas $\alpha 4$ and $\delta$ subunits are crucial for tonic inhibition and are predominantly expressed in extrasynaptic location (Jia et al. 2005; Wei et al. 2003). Both types of inhibitions are modified in epilepsy (Pavlov et al. 2011; Zhang et al. 2007).

## Materials and Methods

### Animal Treatment

#### *KA Treatment*

Male Wistar rats of 2.5–3 months in age received an intraperitoneal injection of KA (10 mg/kg, Sigma) dissolved in saline. Each animal was placed into an individual plastic cage for observation lasting 4 h. Seizures were scored according to a modified Racine scoring system from 0 to 6 (Clement et al. 2003; Racine 1972; Solomonia et al. 2010). Twenty-eight animals with seizures of grades 4–6 were selected. Four KA-treated animals died within the first few days and experiments were done on 24 animals. The same amount of control rats received saline injections.

#### *MI Treatment and Time Schedule*

Experiments were carried out on four groups of Wistar rats. Each group was divided in two subgroups according to the time of decapitation (the first or the twenty-eighth day after the start of the experiment). The first and second groups each consisted of 24 animals. Six hours following KA treatment, the first group received a saline injection (KA + SAL group), while the second group received an MI (30 mg/kg) injection (KA + MI group). Each of these two groups was divided further into two subgroups according to the time of decapitation. The next morning, half of the KA + SAL group received a saline injection, while half of the KA + MI group received MI injections. Animals from both subgroups were killed 10 h afterward [time corresponding to the 28–30 h following KA treatment (first day)]. Remaining rats were treated twice daily either by saline (KA + SAL group) or MI (KA + MI group) during the following 27 days and then decapitated.

Spontaneous seizures in KA-treated animals during the first month are rare, but could take place prior to the

twenty-eighth day (Williams et al. 2009). If such seizures take place a few days before the second time-point decapitation, it is possible that the detected changes are the consequence of acute seizure, but not of the long-term process of epileptogenesis. Therefore, starting on the twenty-fourth day of the experiment, the rats were monitored by infrared video system for 24 h a day. In the experimental animals, no seizures were observed.

The third and fourth groups of animals were not treated by KA. Rats in one group received saline (CON + SAL), while another group received MI injections (30 mg/kg, CON + MI) twice a day. They were killed according to the same time schedule as groups treated with KA. The diagram of experimental design is provided in Fig. 1. During the whole experiment, the rats were housed in cages with free access to water and food. All experiments were carried out at the I.Beritashvili Center of Experimental Biomedicine and were performed in compliance with approved institutional animal care guidelines.

After decapitation, two regions were removed from each brain—hippocampus and neocortex—and frozen immediately on dry ice. The number of animals used was estimated to be the minimum required for adequate statistical analysis (Solomonia et al. 2010).

## Electrophoresis and Immunoblotting

The nuclear-free homogenate fractions from brain tissue samples were obtained as described earlier (Solomonia et al. 2010). In all fractions, the protein concentration was determined in quadruplicate using a micro bicinchoninic acid protein assay kit (Pierce). Aliquots containing 30 μg of protein and of equal volume were applied to the gels. SDS gel electrophoresis and Western blotting were carried out as described previously (Solomonia et al. 2010). After the protein had been transferred onto nitrocellulose membranes, the membranes were stained with Ponceau S solution to confirm the transfer and the uniform loading of the gels.

For the detection and quantification of GABA-ARs subunits, the following antibodies were used: (i) for α1-rabbit polyclonal to GABA-AR α1 (ABCAM-ab 33299); (ii) for α4-rabbit polyclonal to GABA-A receptor α4 (ABCAM-ab 73874); (iii) for γ2-rabbit polyclonal to GABA-AR γ2 (ABCAM-ab 4073); and (iv) for δ-rabbit polyclonal to GABA-AR δ (ABCAM-ab 37363). The molecular weights of the GABA-AR subunits studied are in close proximity to each other (see "Results"). Therefore, separate electrophoresis and blotting were carried out for the δ + γ2 subunits and the α1 + α4 subunits. The nitrocellulose membranes were cut into two parts. In all cases, to detect the expression of total actin—an internal loading control—the lower parts of the membranes were stripped, reblocked, and stained with a primary monoclonal anti-actin antibody (Santa Cruz Biotechnology Inc, sc-81760).

Standard immunochemical procedures were carried out using peroxidase-labeled secondary antibodies and Super-Signal West Pico Chemiluminescent substrate (Pierce) (Solomonia et al. 2010). The optical densities of bands corresponding to the α1, α4, δ, and γ2 subunits and actin were measured using LabWorks 4.0 (UVP). The autoradiographs were calibrated by including in each gel four standards comprising the homogenate fraction from the brain of untreated rats. Each standard contained 15–60 μg total protein. Optical density was proportional to the amount of α1, α4, δ, and γ2 (see Fig. 2). To obtain the data given in Fig. 3 the optical density of each band from an experimental sample was divided by the optical density which, from the calibration of the same autoradiograph, corresponded to 30 μg of total protein in the standard (Solomonia et al. 2010). Data expressed this way will be referred to as "relative amount" of subunits. Data from the experimental stained bands were not normalized with respect to actin staining because such normalization has been found to be an unreliable loading control (Dittmer and Dittmer 2006).

## Statistical Analysis

Overall statistical analysis was done by factorial analysis of variances with the following factors: 1. experimental

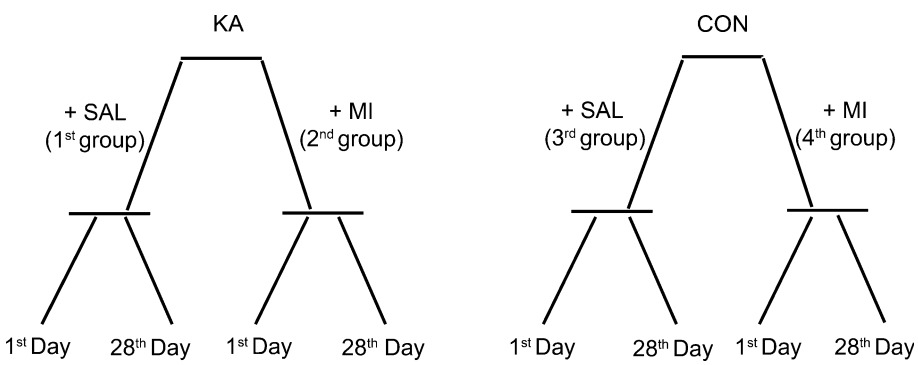


**Fig. 1** The diagram of experimental design

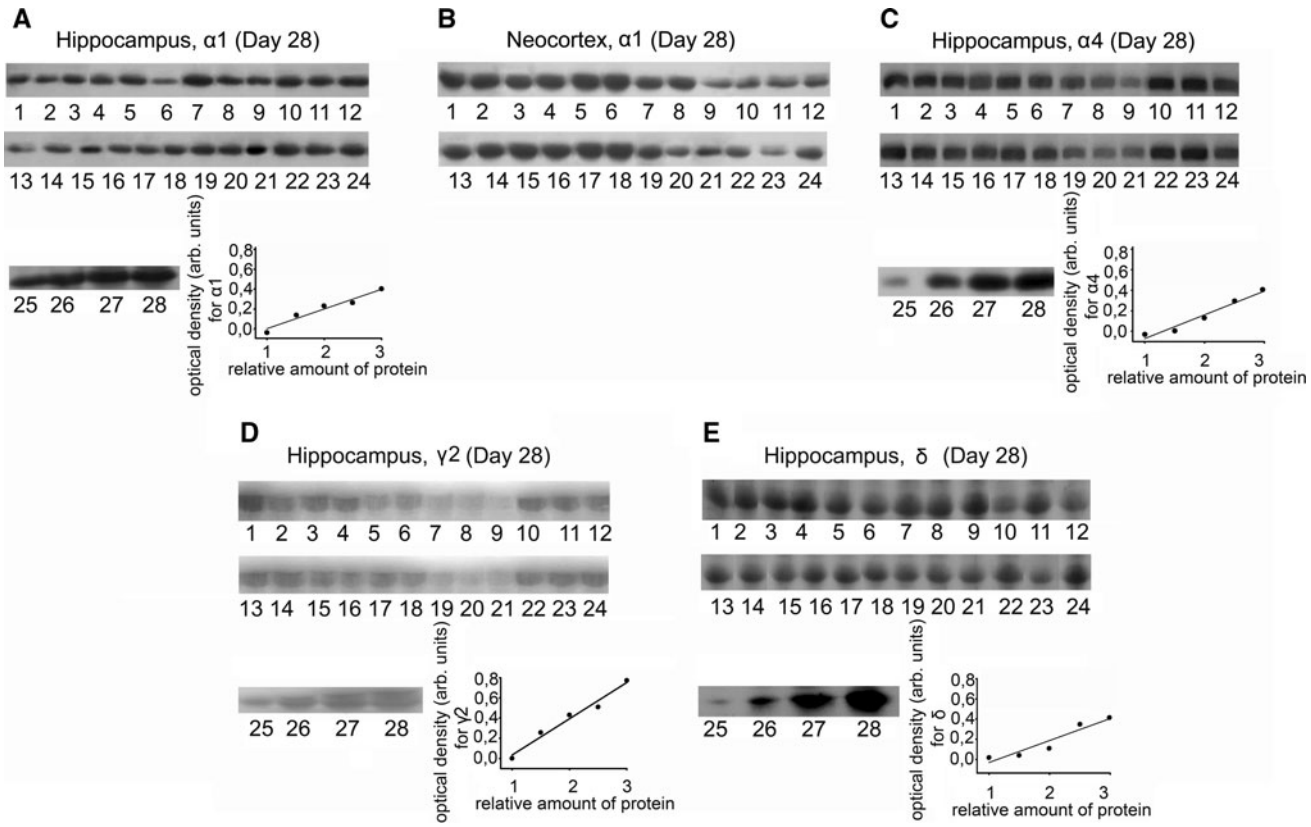


**Fig. 2** Representative Western blot autoradiographs of homogenate fractions of rat brain hippocampus and neocortex from four groups of rats on 28th day of the experiment (see Animal Treatment). Blots were stained with antibodies against α1 (**a**, **b**), α4 (**c**), γ2 (**d**), and δ (**e**). The optical densities of the bands were linearly related to the amount of proteins in the bands (see calibration plots for α1, α4, γ2, and δ). Each *lane* was derived from a single sample. *Lanes 1–3* and *13–15* are samples from the CON + SAL group (**a–e**); *lanes 4–6*and *16–18* are from the CON + MI group (**a–e**); *lanes 7–9* and *19–21* are from the KA + SAL group (**a–e**); *lanes 10–12* and *22–24* from the KA + MI group (**a–e**)

condition (CON + SAL; CON + MI; KA + SAL; and KA + MI); 2. subunit (α1, α4, γ2, and δ); 3. brain region (Hippocampus and Neocortex); and 4. time (1st day and 28th day).

Further statistical analysis was done by the one-way analysis of variances (one-way ANOVA) for each subunit, region, and time point separately. Planned comparisons were made between all groups (CON + SAL, CON + MI, KA + SAL, and KA + MI) at a defined time point, region, and subunit (e.g., hippocampus, 28th day , α1 subunit) by *t* tests.

To compare whether MI treatment effects are significantly different between the hippocampus and neocortex following type of analysis was carried out, data from KA + MI groups were expressed as a percentage of the mean of the corresponding KA + SAL group and obtained data compared between the regions by two-sample *t* test. All statistical tests were two tailed and all significant differences are reported.

## Results

### Immunostaining Patterns

The polyclonal anti-GABA-AR α1-specific antibody detected immunoreactive bands at 51 kDa, which corresponded to the size of the α1 subunit protein (De Blas 1996, Fig. 2a, b). The polyclonal anti-GABA-AR α4 antibody bound to specific immunoreactive band at 67 kDa, which corresponded to the size of the α4 subunit protein (Bencsits et al. 1999, Fig. 2c). The polyclonal anti-GABAAR γ2 antibodies detected immunoreactive bands at 46 kDa, which corresponded to the size of the γ2 subunit (Tretter et al. 1997. Fig. 2d). Finally, antibodies against the GABA-AR δ subunit reacted with the specific immunoreactive band at 55 kDa, corresponding to the size of the δ subunit protein (Joshi and Kapur 2009, Fig. 2e). The optical densities of the bands were linearly related to the amounts of proteins in the bands (see calibration plots for α1, α4 γ2, and δ, Fig. 2a–e).

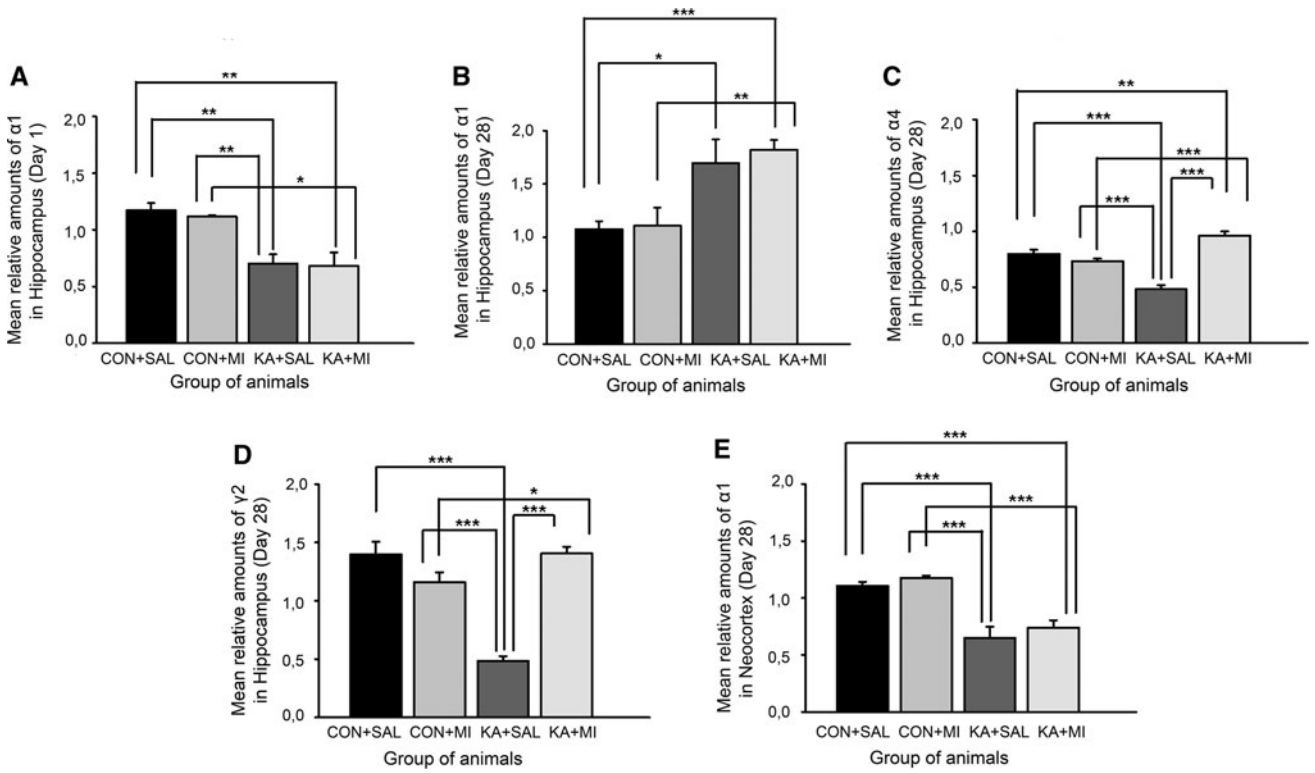


**Fig. 3** Mean relative amounts of α1 (**a, b**), α4 (**c**), and γ2 (**d**), in the rat brain hippocampus and α1 of neocortex (**e**) from four group of rats on the first (**a**) and twenty-eighth (**b**–**e**) day of the experiment. The *error bars* represent standard errors of the means. **a** Hippocampus α1 subunit, first day of experiment; **b** hippocampus α1 subunit, twenty-eighth day of experiment; **c** hippocampus α4 subunit, twenty-eighth day of experiment; **d** hippocampus γ2 subunit, twenty-eighth day of experiment; **e** neocortex α1 subunit, twenty-eighth day of experiment; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. For more detailed information about the significance of these differences see the "Results" section

## Overall Statistical Analysis

Factorial analysis of variances with the following factors: 1. experimental condition, 2. Subunit, 3. brain region, and 4. time, revealed that the effects of experimental condition and subunit were significant (correspondingly $F_{3,320} = 3.40$, $p = 0.018$ and $F_{3,320} = 7.00$, $p = 0.0001$). Interaction between all 4 factors (experimental condition × subunit × region × time) was also significant $F_{9,320} = 2.30$, $p = 0.0163$. This was the reason for applying separate analysis for each subunit within the defined brain region at a given time point.

## Hippocampus: Day One

Among all the proteins studied only the amount of the α1 subunit was significantly influenced by experimental conditions (Table 1). The mean amount of the α1 subunit was significantly reduced in the hippocampus of the KA + SAL and KA + MI groups as compared to the two control groups (KA + SAL vs. CON + SAL, $t = 4.51$, $p = 0.001$; KA + SAL vs. CON + MI, $t = 3.01$, $p = 0.013$; KA + MI vs. CON + SAL, $t = 3.65$, $p = 0.004$; KA + MI vs. CON + MI, $t = 2.69$, $p = 0.023$; for all comparisons df = 10), (Fig. 3a).

## Neocortex: Day One

No significant changes were detected for any of the proteins studied.

## Hippocampus: Day Twenty-eight

### *α1 Subunit*

The amount of α1 subunit in the hippocampus was significantly influenced by the experimental condition (Table 1). The mean amount of the subunit was significantly higher in the KA + SAL and KA + MI groups than in the control groups (KA + SAL vs. CON + SAL, $t = 2.69$, $p = 0.023$; KA + MI vs. CON + SAL, $t = 6.18$, $p = 0.0001$; KA + MI vs. CON + MI, $t = 3.72$, $p = 0.004$; for all comparisons df = 10). The difference between the KA + SAL and CON + MI groups was significant only on the one-tailed test ($t = 2.14$, $p = 0.029$).

**Table 1** Summary of one-way ANOVA results

| | Hippocampus | | Neocortex | |
|---|---|---|---|---|
| | Day 1 | Day 28 | Day 1 | Day 28 |
| $\alpha 1$ $F_{3,20}$ | 7.43 | 6.70 | 1.39 | 17.41 |
| $p$ | 0.002 | 0.003 | 0.274 | <0.0001 |
| $\alpha 4$ $F_{3,20}$ | 1.98 | 32.74 | 2.01 | 0.26 |
| $p$ | 0.15 | <0.0001 | 0.147 | 0.854 |
| $\gamma 2$ $F_{3,20}$ | 0.1 | 30.20 | 1.17 | 1.80 |
| $p$ | 0.960 | <0.0001 | 0.346 | 0.180 |
| $\delta$ $F_{3,20}$ | 1.71 | 0.75 | 0.73 | 0.78 |
| $p$ | 0.197 | 0.536 | 0.546 | 0.517 |

$F$ variance ratio from one-way ANOVA, $p$ probability

There were no differences between KA-treated groups or the two control groups (Fig. 3b)

*α4 Subunit*

The effect of the experimental condition on the amount of α4 subunit was highly significant (Table 1). KA-induced SE was followed by a decrease of the mean amount of protein as compared to all three other groups (KA + SAL vs. CON + SAL, $t = 5.99$, $p = 0.0001$; KA + SAL vs. CON + MI, $t = 5.46$, $p = 0.0001$ and KA + SAL vs. KA + MI, $t = 8.90$, $p < 0.0001$). MI treatment after KA-induced SE not only restored the amount of α4 but also increased it significantly compared to the control groups (KA + MI vs. CON + SAL, $t = 3.09$, $p = 0.011$ and KA + MI vs. CON + MI, $t = 5.46$, $p = 0.0001$) (Fig. 3c). No differences were observed between the control groups. MI effect in hippocampus for α4 subunit at 28th day was significantly different as compared to the neocortex ($t = 6.38$, $p < 0.0001$, df = 10, see "Statistical Analysis").

*γ2 Subunit*

The experimental condition significantly influenced the amount of γ2 subunits in the group of rats (Table 1). In the KA + SAL group, the drastic reduction of this subunit was observed compared to the two control groups (KA + SAL vs. CON + SAL $t = 7.71$, $p = 0.0001$; KA + SAL vs. CON + MI, $t = 7.10$, $p = 0.0001$) as well as compared to the KA + MI group ($t = 12.94$, $p = 0.0001$). The mean amount was higher in the KA + MI group compared to the CON + MI group ($t = 2.37$, $p = 0.039$, Fig. 3d). No significant differences were observed between the control groups (Fig. 3d). Thus, MI treatment restored the amount of this subunit to the normal level. MI effect in hippocampus for γ2 subunit at 28th day was significantly different as compared to the neocortex ($t = 9.37$, $p < 0.0001$, df = 10, see "Statistical Analysis").

*δ Subunit*

No significant changes were found by ANOVA for this subunit.

Neocortex: Day Twenty-eight

*α1 Subunit*

The amount of the α1 subunit in the neocortex was significantly influenced by the experimental condition (Table 1). KA treatment decreased the amount of protein in both the KA + SAL and KA + MI groups as compared to the control groups (CON + SAL vs. KA + SAL, $t = 4.38$, $p = 0.001$; CON + SAL vs. KA + MI, $t = 4.91$, $p = 0.001$; CON + MI vs. KA + SAL, $t = 5.22$, $p = 0.0001$; CON + MI vs. KA + MI, $t = 6.25$, $p = 0.0001$). There was no difference between KA-treated groups or between the control groups. Thus, in the neocortex, MI has no effect on KA-induced changes in the amount of the α1 subunit (Fig 3e).

*α4, Gamma-2 and Delta Subunits*

No significant changes were found for any of these proteins.

## Discussion

In our previous work, with the same type of experimental design, we have shown that MI treatment significantly halts the drastic reduction of CaMKII and GLUR1 in the hippocampus of KA-treated rats (Solomonia et al. 2010). In the current work, we have further investigated MI effects on the molecular changes of KA-induced epileptogenesis. Now, we have targeted the subunits of GABA-ARs. Numerous changes in GABA-AR subunits occur in animal models and humans with temporal lobe epilepsy (Gonzalez and Brooks-Kayal 2011). These changes are time-dependent, complex, and include both increased and decreased expression of several GABA-AR subunits. If in our previous studies the MI effect was simply opposite to the changes taking place during epileptogenesis, in the current case the MI effects were selective and more complex.

α1 Subunit

In the hippocampus, the α1 subunit was decreased 1 day after KA-induced SE and increased 28 days later. The MI was without any evident changes on this process. According to our experimental design, MI treatment is started 6 h after KA injection. Before decapitation, the

animals received MI injections only twice, which probably cannot compensate for this decrease. The late increase of the α1 subunit could account for a compensatory increase of the α1 subunit containing GABA-ARs. MI treatment does not have any additional influence on it.

In the neocortex, decrease of the α1 subunit is observed on the 28th day after SE. MI has no influence on it. These data indicate that either MI effects are region specific or MI treatment does not interfere with α1 subunit expression. For both brain regions, no differences were observed between the CON + SAL and CON + MI groups.

### α4 Subunit

In the hippocampus, on the 28th day after KA treatment, a decrease in the amount of α4 subunit is observed in the KA + SAL group. MI treatment not only restores this subunit level; however, in the KA + MI group, it is significantly higher compared to the control groups. No differences were observed between CON + SAL and CON + MI groups. The α4 subunit is linked to benzodiazepine resistance (Olsen and Sieghart 2009). At present, it is not clear if enhanced expression of the α4 subunit after MI treatment contributes to the prevention of epileptogenesis or not.

### γ2 Subunit

In the hippocampus of the KA + SAL-treated group, the amount of γ2 subunit is drastically reduced on the 28th day after SE. It has been shown that the γ2 subunit is mutated and reduced in some forms of epilepsy (reviewed in Macdonald et al. 2010). MI treatment restored the amount of this subunit to normal level. γ2 subunits are important for the assembly of GABA-ARs, their accumulation at synapses, surface stability, and synaptic clustering (Alldred et al. 2005; Essrich et al. 1998). Hence, restoration of its level should normalize the function of the GABA-AR system. As in the case of the other subunits, no differences were observed between the control groups.

The GABA-AR subunit distribution and changes in the hippocampus after KA-induced SE have been studied by various groups (e.g. Sperk et al. 1998; Friedman et al. 1994). The observed alterations in various subunit expression were complex, time-related, and depended on subregion of the hippocampus. Hence, direct comparison with our results cannot be made since we have explored the whole hippocampus and not the separate subregions.

As emphasized above, no differences were observed for either of the studied subunits between the CON + SAL and CON + MI groups, whereas MI treatment had strong and significant effects on α4 and γ2 subunit changes in KA-treated animals. Thus, MI treatment at physiologic concentrations targets the biochemical processes initiated by KA-induced SE and not those of a normal brain. Our current experiments were focused on biochemical changes after SE and MI treatment and we have monitored all experimental rats for the last 5 days. We cannot exclude the possibility that before this there were some seizures in each group or there were some seizures detectable only by electroencephalographic analysis. However, by analyzing animals which were without behavioral seizures for last 5 days, we have excluded the chance that the observed differences are caused by acute changes of generalized seizures. Indeed, changes in GABAAR on first day differ from changes at 28th day of experiment (e.g., decrease in α1 on 1st day versus increase on 28th day).

Our previous work has shown the anti-convulsant effects of MI against KA-induced seizures (Solomonia et al. 2007). Thus, the possibility exists that the differences seen in receptor expression on the 28th day time- point could be due to an anti-seizure effect of MI versus an anti-epileptogenic effect (i.e., non-MI-treated animals had more seizures than MI treated during the first 24 days). We do not favor this possibility since even a single pretreatment of rats with MI before KA-induced SE exerts a strong neuroprotective effects on cell damage in the hippocampus on the fourteenth day of the experiment (Zhvaniia et al. 2007).

What could be the possible mechanism of MI action on the molecular processes of epileptogenesis after SE? During intense neuronal excitation, a massive intracellular influx of $Na^+$, $Ca^{2+}$, and $Cl^-$ takes place, which leads to water inward flow, cellular swelling, and extracellular space reduction (Choi 1987; Dietzel et al. 1982; Rothman 1985). Under such conditions, various enzymes cannot function normally. In addition, reduced extracellular space enhances neuronal excitability (Dudek et al. 1990; Schwartzkoin et al. 1998). To compensate for this condition, neuronal and other cells increase the intracellular concentration of small organic osmolytes by upregulation of osmoprotective gene expression (Rowley et al. 2011), which does not perturb the function of the intracellular enzymes. One such important osmolyte appears to be MI (Fisher et al. 2002) and one possible mechanism of MI action could be osmoprotection, which prevents the process of epileptogenesis. However, based on our previous work demonstrating influence of MI on 3H-muscimol and 3H-MK801 binding in vitro (Solomonia et al. 2004), we propose that inositols could also exert their anti-epileptic action by the modulation of neuronal activity through direct influence on GABA-ergic or/and glutamatergic synaptic transmission, in particular by modulatory action on the respective postsynaptic receptors.

The process of epileptogenesis is associated with changes in the expression of myriad genes (Brooks-Kayal et al. 2009; Majores et al. 2007; McNamara et al. 2006). One of

the major challenges to the epilepsy research community has been to determine which of these changes contribute to epileptogenesis, which are compensatory, and which are non-contributory (Brooks-kayal et al. 2009). Taking into account the various actions of MI, such as the neuroprotective influence on KA-induced SE and following cell degeneration (Zhvaniia et al. 2007) the anti-convulsive effects on PTZ and KA-induced seizures (Solomonia et al. 2007; Nozadze et al. 2011), rescuing ability from the drastic decrease of neuronally important proteins after KA-induced SE (Solomonia et al. 2010 and current data) and general osmoprotective properties (see Fisher et al. 2002), we propose that MI treatment could have an inhibitory effect on the processes of epileptogenesis.

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**Conflict of interest** The authors declare that they have no conflict of interest.

## References


Alldred MJ, Mulder-Rosi J, Lingenfelter SE, Chen G, Luscher B (2005) Distinct gamma2 subunit domains mediateclustering and synaptic function of postsynaptic GABA-A receptors and gephyrin. J Neurosci 25:594–603

Bencsits E, Ebert V, Tretter V, Sieghart W (1999) A significant part of native gamma-aminobutyric acid A receptors containing alpha4 subunits do not contain gamma or delta subunits. J Biol Chem 274:19613–19616

Brooks-Kayal AR, Raol YH, Russek SL (2009) Alteration of epileptogenesis genes. Neurotherapeutics 6:312–318

Browne TR, Holmes GL (2001) Epilepsy. N Engl J Med 344:1145–1151

Choi DW (1987) Ionic dependence of glutamate neurotoxicity. J Neurosci 7:369–379

Clement AB, Hawkins A, Lichtman AH, Cravatt BF (2003) Increased seizure susceptibility and proconvulsant activity of anandamide in mice lacking fatty acid amide hydrolase. J Neurosci 23:3916–3923

De Blas AL (1996) Brain GABAA receptors studied with subunit specific antibodies. Mol Neurobiol 12:55–71

Dietzel I, Heinenmann U, Hofinier G, Lux HD (1982) Stimulus-induced changes in extracellular $Na^+$ and $Cl^-$ concentration in relation to changes in the size of the extracellular space. Exp Br Res 46:73–84

Dittmer A, Dittmer J (2006) Beta-actin is not a reliable loading control in western blot analysis. Electrophoresis 27:2844–2845

Dudek FE, Obenaus A, Tasker JG (1990) Osmolality induced changes in extracellular volume after epileptiform burst independent of chemical synapses in the rat: importance of non-synaptic mechanisms in hippocampal epileptogenesis. Neurosci Lett 120:267–270

Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B (1998) Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. Nat Neurosci 1:563–571

Fisher KS, Novak JE, Agranoff BW (2002) Inositol and higher inositol phosphates in neuronal tissues: homeostasis, metabolism and functional significance. J Neurochem 82:736–754

Friedman LK, Pellegrini-Giampietro DE, Sperber EF, Bennett MVL, Moshe SL, Zukin RS (1994) Kainate-induced status epilepticus alters glutamate and GABA-A receptor gene expression in adult rat hippocampus:an in situ hybridization study. J Neurosci 14:2697–2707

Gonzalez MI, Brooks-Kayal A (2011) Altered GABA-A receptor expression during epileptogenesis. Neurosci Lett 497:218–222

Jia F, Pignataro S, Schofield CM, Yue M, Harrison NL, Goldstein PA (2005) An extrasynaptic GABAA receptor mediates tonic inhibition in thalamic VB neurons. J Neurophysiol 94:4491–4501

Joshi S, Kapur J (2009) Slow intracellular accumulation of GABA(A) receptor delta subunit is modulated by brain-derived neurotrophic factor. Neuroscience 164:507–519

Kharlamov EA, Lepsveridze E, Meparishvili M, Solomonia RO, Lu B, Miller ER, Kelly KM, Mtchedlishvili Z (2011) Alterations of GABA(A) and glutamate receptor subunits and heat shock protein in rat hippocampus following traumatic brain injury and in posttraumatic epilepsy. Epilepsy Res 95:20–34

Loescher W, Gernert M, Heinemann U (2008) Cell and gene therapies in epilepsy–promising avenues or blind alleys? Trends Neurosci 31:62–73

Macdonald RL, Kang JQ, Gallagher MJ (2010) Mutations in GABAA receptor subunits associated with genetic epilepsies. J Physiol 588:1861–1869

Majores M, Schoch S, Lie A, Becker AJ (2007) Molecular neuropathology of temporal lobe epilepsy: complementary approaches in animal models and human disease tissue. Epilepsia 48:4–12

Mangan PS, Sun C, Carpenter M, Goodkin HP, Sieghart HP, Kapur J (2005) Cultured hippocampal pyramidal neurons express two kinds of GABAA receptors. Mol Pharmacol 67:775–788

McNamara JO, Huang YZ, Leonard AS (2006) Molecular signaling mechanisms underlying epileptogenesis. Sci STKE. doi: 10.1126/stke.3562006re12

Nozadze M, Mikautadze M, Lepsveridze E, Mikeladze E, Kuchiashvili N, Kiguradze T, Kikvidze M, Solomonia R (2011) Anticonvulsant activities of myo-Inositol and scyllo-Inositol on pentylenetetrazol induced seizures. Seizure: European. J Epilepsy 20:173–176

Nusser Z, Mody I (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J Neurophysiol 87:2624–2628

Olsen RW, Sieghart W (2009) GABAA receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56:141–148

Pavlov I, Huusko N, Drexel M, Kirchmair E, Sperk G, Pitkanen A, Walker MC (2011) Progressive loss of phasic, but not tonic GABA-A receptor-mediated inhibition in dentate granule cells in a model of post-traumatic epilepsy in rats. Neuroscience 194:208–219

Racine RJ (1972) Modification of seizure activity by electrical stimulation II. Motor seizure: Electroencephalogram. Clin Neurophysiol 32:281–294

Rothman SM (1985) The neurotoxicity of excitatory amino acid is produced by passive chloride influx. J Neurosci 5:1483–1489

Rowley NM, Smith MD, Lamb JG, Schousboe A, White S (2011) Hippocampal betaine/GABA transporter mRNA is not regulated by inflammation or dehydrations post-statues epilepticus. J Neurochem 117:82–90

Schwartzkoin PA, Baraban SC, Hochmann DW (1998) Osmolarity, ionic flux and changes in brain excitability. Epilepsy Res 32:275–285

Solomonia R, Kuchiashvili N, Berulava A, Pkhakadze V, Trapaidze N, Zhvania M, Abesadze I, Kojima H, Dalakishvili N (2004) Purification and identification of components of the *Aquilegia*

*vulgaris* extract fraction exhibiting anti-epileptic activity. J Biol Phys Chem 4:187–192

Solomonia R, Nozadze M, Kuchiashvili N, Bolkvadze T, Kiladze M, Zhvania M, Kiguradze T, Pkhakadze V (2007) Study of effects of myo-Inositol on pentylentetrazol and kainic acid induced convulsions in rats. Bull Exp Biol Med 143:58–60

Solomonia R, Mikautadze E, Nozadze M, Kuchiashvili N, Lepsveridze E, Kiguradze T (2010) Myo-Inositol treatment prevents biochemical changes triggered by kainate-induced status epilepticus. Neurosci Lett 468:277–281

Sperk G, Schwarzer K, Tsunashima K, Kandlhofer S (1998) Expression of GABA-A receptor subunits in the hippocampus of the rat after kainic acid-induced seizures. Epilepsy Res 32:129–139

Tretter V, Ehya N, Fuch K, Sieghart W (1997) Stoichiometry and assembly of a recombinant GABAA receptor subtype. J Neurosci 17:2728–2737

Wei W, Zhang N, Peng Z, Houser CR, Mody I (2003) Perisynaptic localization of delta subunit-containing GABA-A receptors and their activation by GABA spillover in the mouse dentate gyrus. J Neurosci 23:10650–10661

Williams PA, White AM, Clark S, Ferraro SJ, Swiercz W, Staley KJ, Dudek FE (2009) Development of spontaneous recurrent seizures after kainate-induced status epilepticus. J Neurosci 29:2103–2112

Zhang N, Wei W, Mody I, Houser CR (2007) Altered localization of GABA-A receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. J Neurosci 27:7520–7531

Zhvaniia MG, Solomonia RO, Bikashvili TZ, Kotaria NT, TsG Chkhikvishvili, Kiladze MT, Nozadze MB, Dzhaparidze ND (2007) The influence of myo-inositol on the ultrastructure of hippocampal CA1 area in kainate treated rats. Tsitologia 49:939–943