INOSITOL

Synthesis, Functions and Clinical Implications

BIOCHEMISTRY RESEARCH TRENDS

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Chapter 3

Inositols and Epilepsy

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Abstract

Epilepsy is a heterogeneous group of disorders. It is the most common neurological disorder after stroke. Despite achieved progress in the treatment of epilepsy, about one third of the patients with epilepsy are resistant to existing pharmacotherapies. The current treatment of epilepsy focuses exclusively on preventing or suppressing seizures, which are the symptoms of the underlying disease.

The most important challenge is to prevent epileptogenesis, the process by which brain becomes epileptic. Drugs that prevent the development of epilepsy are not yet available.

The central nervous system is characterized with relatively high concentrations of myo-inositol (MI) as well as the means to synthesize it. MI serves not only as a precursor molecule for inositol lipid synthesis, but also as a physiologically important osmolyte. Alterations in MI deposition may playrole in a number ofneuropathological conditions, either as a physiologically important osmolyte or as a precursor molecule for phosphoinositide synthesis.

Several lines of evidence indicate the involvement of changes in inositols and other osmolytes in epilepsy and the effects of inositols in the regulation of induced seizures. We have revealed that the water extract of the medicinal plant *Aquilegia vulgaris* (a plant widely used in Oriental folk medicine as antiepileptic and soporific treatments) contains compounds altering binding of ligands to the benzodiazepine andgamma-aminobuturic acid (GABA) binding sites of the GABA-A receptors. We have identified two such compounds of this extract: (1) (MI) and (2) sleep-inducing lipid oleamide. Further we have shown that MI and scyllo-inositol (SCI) pretreatment significantly decreases the severity of seizures induced either by pentylentetrazolium or kainic acid (KA). As these effects were achieved by physiological concentrations of the administered inositols, we have hypothesized that MI and SCI could represent endogenous anti-convulsants.

Our original data showed that MI also could interfere with the process of epileptogenesis. We have first induced status epilepticus by KA and then applied MI daily treatment. We have shown that a 28-day MI treatmentsignificantly attenuates biochemical changes associated with the process of epileptogenesis in the hippocampus, and restores the amount of some drastically reduced proteins to the normal level. To these proteins belongs the GLUR1 subunit of glutamate receptors, calcium/calmodulin dependent protein kinase II (CaMKII), a γ2 subunit of GABA-A receptors. The obtained results indicate that MI treatment could at least modify the epileptogenesis process induced bybrain insult. Further studies of MI and SCI action could lead to more successful translational research and development of inositols as future anti-epileptic compounds.

1. Introduction

Epilepsy is a common and diverse set of chronicneurological disorders characterized by seizures and the cognitive, psychosocial, and social consequences of this condition (for a recent review see Loescher and Brandt, 2010).It is the most common neurological disorder after stroke, and there is a 2–3% lifetime risk of being given a diagnosis of epilepsy (Browne and Holmes, 2001). Some definitions of epilepsy require that seizures be *recurrent* and *unprovoked* (Chang and Lowenstein, 2003), but others require only a single seizure combined with brain alterations which increase the chance of future seizures (Fisher et al., 2005).Seizures can be divided into two main groups 1. Partial (Focal or local) seizures and 2.Generalized (Convulsive or

non convulsive) seizures. In each of these groupsseveral subtypes of seizures are observed (Chang and Lowenstein, 2003)

The process by which a normal brain becomes epileptic is called epileptogenesis. It is a seizure-free interval lasting months to years between brain insults and the onset of symptomatic epilepsy (McNamaraet al., 2006;Jacobs et al., 2009).Historically, it was generally believed that epilepsy came about through a supernatural process. Even within the medical profession, it was not until the 18thcentury that ideas of epileptogenesis as a supernatural phenomenon were abandoned. However, biological explanations have also long existed, and sometimes explanations contained both biological and supernatural elements (Eadie and Bladin, 2001).Nowadays the widely accepted hypothesis holds that there is a cascade of poorly understood changes that transform the nonepileptic brain into one that generates spontaneous recurrent seizures (Jacobs et al., 2009; Pitkänen, 2010; Walker et al., 2002). Inepileptogenesis, an array of events occurs on molecular and cellular levels and causes neurons to fire in a disordered manner. Numerous possible mechanisms underlying this process have been suggested, but no consensus has emerged about which of the observed changes is causal or consequential and how they interact (Loescher and Brandt, 2010)

It is important to note that the brain tries to repair itself after damage, which may contribute to the fact that only a fraction of patients develop epilepsy after brain insults. It is thus vital to understand which of the molecular and cellular alterations induced by brain insults contribute directly to the development of epilepsy and which are involved in the attempt of the brain to repair the damage and recover lost function (Dichter, 2009; Jacobs et al., 2009). The latent period after brain insults may offer a window of opportunity in which an appropriate treatment may stop or modify the epileptogenic process induced by a brain insult (Jensen, 2009; Pitkanen, 2004).

Despite the progress in the pharmacotherapy of epilepsy, about one-third of epileptic patients are resistant to current treatments (Löscher et al.,2008; Sasa, 2006). Available therapy is only symptomatic and often ineffective (Löscher et al., 2008; Pitkänen, 2002). At present there is no anti-epileptic drug (AED) which could effectively prevent or modify epileptogenesis. Thus the search for a truly anti-epileptogenic drug is a problem of outstanding importance for modern biomedical sciences.

Several medicinal plant extracts have been tried and some of them are used as epilepsy treatments (e.g. for review seeKumar et al., 2012).

More than 15 years ago our attention became focused on a medicinal plant *Aqulegia vulgaris,* which belongs to genus *Aquilegia* and was widely used in

Chinese and Tibetan folk medicine as antiepileptic and soporific treatments (Shreter, 1975). We have hypothesized that the water extract of this plant contains a compound acting on gamma-aminobutiric acid type A (GABA-A) receptors and we have tested it experimentally *in vitro*. Indeed we have shown that low-molecular weight (<1Kda) aqueous fraction of this plant increases nearly two-fold the binding of 3H-flunitrezepam(agonist for benzodiapezine site of GABA-A receptors) and completely abolishes the binding of 3H-muscimol (GABA-A receptor agonist) to rat brain membranes *in vitro* (Solomonia et al., 2004).

Two rounds of High performance liquid chromatography (HPLC) and Gas chromatography/mass spectrometry detection revealed two compounds from the extract of*Aquilegia vulgaris* that influenced the binding of specific ligands with GABA-A receptors. These compounds were *myo*-inositol (MI)and oleamide-sleep inducing lipid (Solomonia et al., 2004).

Oleamide was originally isolated from the cerebrospinal fluid of sleep-deprived cats and was named as a sleep-inducing lipid (Cravatt et al., 1995). Sleep induction time is reduced by oleamide without altering the duration of rapid eye movement sleep (Basile et al., 1999; Mendelson and Basile, 1999). Oleamide is environmentally ubiquitous, being found in a variety of vegetable oils, and is used as an industrial lubricant in polyolefin manufacturing (Molnarand Erucamide1974). We have shown that oleamide exhibits antiepileptic activity and significantly decreases the degree of pentylenetetrazole-induced seizures (Solomonia et al., 2008).

The inositols are the nine isomeric forms of cyclohexanehexol, a group of small and chemically stable polar molecules that have versatile properties (Fisher et al., 2002; Michell et al., 2008).MI is the most abundant isomer and is a ubiquitous component of all eukaryotic cells. The central nervous system (CNS) is characterized with relatively high concentrations of MI as well as the means to synthesize it. MI serves not only as a precursor molecule for inositol lipid synthesis, but also as a physiologically important osmolyte (Fisher et al., 2002).MI could be directly up-taken from extracellular spaces by the specific sodium/myo-inositol cotransporter (SMIT) and H+/myo-inositol cotransporter (HMIT). In addition to this mechanism mammalian cells have two pathways by which they synthesize MI. The first is by recycling MI in a multistep dephosphorylation of inositol polyphosphate species, such as inositol trisphosphate, liberated from membrane-associated phosphatidylinositol 4,5-bisphosphate. The second is through de novo synthesis from glucose phosphate, producing MI monophosphate.

The final step in both biochemical pathways is the dephosphorylation of MI monophosphate, which is mediated by myo-inositol monophosphatase (IMPase: EC 3.1.3.25) and free MI is produced (reviewed in Fisher et al., 2002)

Alteration in MI deposition may play a role in the number of neuropathological conditions, where MI acts either as a physiologically important osmolyte or as a precursor molecule for phosphoinositide synthesis (Fisher et al., 2002).

For instance, at least part of the pathogenesis of Down's syndrome was supposed to be related to the function of inositol as an osmolyte in the nervous system. Band q22 of the human chromosome 21, which contains the gene encoding the sodium *myo*-inositol transporter (SMIT), is triplicated (Berry et al., 1995) in Down's syndrome patients and the inositol concentrations in the brain and cerebrospinal fluid (CSF) are 30–50% higher than in healthy controls (Berry, et al., 1999; Shetty, et al., 1995).

Contrary to Down's syndrome, the CSF of both unipolar and bipolar depressed patients are significantly lower in concentrations of inositol as compared to healthy control subjects (Barkai, et al., 1978). Inositol is widely used for the treatment of human neuropsychiatric disorders such as depression (Levine et al., 1995), panic disorder (Benjamin et al., 1995), and obsessive-compulsive disorder (Fux et al., 1996).

The presence ofMI in the plant extracts that possessed anti-epileptic properties prompted us to study the role of inositol in epilepsy. Our data provide ample evidence indicating that inositol could be involved in the treatment of epilepsy. Below we summarize and discuss the data about the involvement of inositol in epilepsy.

2. Changes in Metabolism of Inositols and Epilepsy

Changes in MI metabolism could be involved in different types of epilepsy, and are observed in various animal epilepsy models as well as in humans.

Temporal lobe epilepsy (TLE) patients are characterized with MI abnormalities; MI changes distinguish between the seizure focus, where MI is increased, and areas of seizure spread where MI is decreased.

The temporal lobe,ipsilateral to seizure origin in hippocampal sclerosis TLE (HS-TLE), but not late onset TLE (LO-TLE),had reduced N-acetylaspartate (NA) and elevated MI levels. Frontal lobe MI level was low in both patient groups. Ipsilateral frontal lobes had lower MI than contralateral frontal lobes (Wellard et al., 2003).

Research done on identification of novel loci and genes associated with susceptibility to febrile seizure (FS) revealed that the gene coding for MI monophosphatase (IMPase) 2 is likely to be the FS susceptibility gene. This study included participants that were the FS probands and family members of 59 Japanese nuclear families (223 members including 112 affected children). Forty-eight of these families had at least two affected children for which genome-wide linkage screening was carried out. Obtained data suggested linkage of FSs to chromosome 18p11.2. This region includes the IMPA2 gene, which encodes MI monophosphatase 2 (Nakayama et al., 2004). In the phosphatidylinositol-signaling pathway, IMPase is inhibited by lithium, which has a proconvulsant effect, and is stimulated by carbamazepine, an anticonvulsant.

The role of the IMPA2 gene was also studied in a cohort of 96 unrelated Caucasian subjects with a history of FS (Blair et al., 2007). No significant differences in genotypes of cases and matched controls were identified; no mutations or non-synonymous polymorphisms were detected in these individuals. These data suggest that the genetic variants in the IMPA2 gene are not associated with a risk of FS in Caucasian patients and patients from various genetic groups are likely to have different genetic causes of FS (Blair et al., 2007).

Kainic acid (KA) induced status epilepticus (SE)is followed by time-dependent changes in SMIT gene expression (Nonaka et al., 1999). SMIT mRNA began to increase in the brain 2 h after onset of seizure, and peaked at 12 h. In situ hybridization revealed a rapid increase of SMIT mRNA (2 h of seizure) in the CA3 hippocampal pyramidal cells and in the dentate granular cells. Then, at 4-6 h SMIT mRNA expression was observed in the other limbic structures such as the amygdala and piriform cortex. Finally, in the neocortex and in CA1 pyramidal cells, SMIT mRNA was slowly increased and peaked at 12 h. Microautoradiogram demonstrated that cells expressing SMIT mRNA were mainly neurons. These results suggest that SMIT mRNA is upregulated by KA-induced seizure primarily in structures involved in seizure activity (Nonaka et al., 1999).

MI is not the onlymolecule with osmolyte features that is involved in epilepsy.

Betaine is an organic osmolyte whichhas been suggested to be a protective osmolyte in the brain (see e.g. Bitoun and Tappaz 2000). Betaine is transported together with GABA by betaine/GABA transporter (BGT1) (Yamauchi et al., 1991; Matskevitchet al., 1999). KA induced SE is followed by strong upregulation of BGT1in glial cells at 1 week after kainate injection and with decline 3 weeks post KA injection (Zhu and Ong, 2004). Opposite to the increase in BGT-1 1 week after KA treatment, a decrease in the amounts of GABA transportersGAT-1 and GAT-3 were detected, whereas no changes were observed for GAT-2. Specific changes in expression of BGT-1 might result in alterations in the levels of GABA/betaine in the extracellular space, with consequent effects on neuronal excitability or osmolarity (Zhu andOng, 2004).

Changes in another small organic osmolyte – taurine is also associated with epilepsy. Taurine (2-aminoethanesulfonic acid) is a β-amino acid, which is not incorporated into proteins and exists in a free condition in the body. Taurine is involved in osmoregulation (Nagelhus et al., 1993, Law, 1998), membrane stabilization (Wright et al., 1986) and neuroprotection from excitotoxic cell death (Huxtable 1992).Taurine deficiency causes the prolongation of seizure activities and the persistence of a state of hyperexcitability in the brain (Mutani et al., 1977). In the human brain, the taurine level is lower than normal in the most hyperexcitable areas (Van Gelder et al., 1972). Six months after KA induced SE in the group of rats with strong behavioral response, increased taurine levels were found in the hippocampus and caudate nucleus. It is suggested that increased taurine levels in the hippocampus may involve processes for membrane stabilization, thus favoring recovery after neuronal hyperactivity (Baran 2006).

Pilocarpine induced SE is also followed by the time-dependent changes in osmoprotective genes expression (Rowley et al., 2011). Expression of BGT1, SMIT and taurine transporter werealready increased 24h after SE. From these osmoprotective genes, SMIT upregulated expression is the strongest and is considered long-term (Rowley et al., 2011).

3. Influence of Inositolson Acute Seizures

Lithium pretreatment in rats potentiates the epileptogenic effects of pilocarpine and other cholinergic agonists (Kofman et al., 1993).Lithium

inhibits the enzyme inositol monophospatase and could act through a decreasedMI level (inositol depletion hypothesis Berridge et al., 1989).

This assumption was the main reason for studying MI influence on a lithium-pilocarpine model of seizures. The anti-convulsant potential of MI has been shown in experimentson lithium-pilocarpine induced seizures. In order to determine if this effect of lithium could be reversed by MI, rats were pretreated with intracerebroventricular (ICV) injections of MI, artificial CSF or L-chiro-inositol. Lithium chloride, 3 mg/kg was administered intraperitoneally 20-24 h prior to the subcutaneous injection of pilocarpine, (20 or 30 mg/kg). At both doses of pilocarpine, MI significantly prolonged the latency to the appearance of clonic seizures and lowered the pilocarpine induced seizure score (Kofman et al., 1993). MI prevented the development of clonic seizures in 50% of the rats receiving pilocarpine, 20 mg/kg. The levels of cortical MI in rats injected with MI were approximately double those of the CSF and L-chiro-inositol groups (Kofman et al., 1993). In another series of experiments besides pilocarpine, the agonist for 5HT2/5HT1c serotonergic receptor (DOI) was included and the hippocampal and cortical EEG was recorded. Administration of DOI (8 mg/kg) or pilocarpine (30 mg/kg) to rats pretreated with lithium acutely (3 mmol/kg) or chronically (dietary, 4 weeks) resulted in seizures, whereas these doses did not cause seizures without lithium pretreatment. This indicated that lithium most likely affects a signal transduction process common to both systems, which is the phosphoinositide second messenger system. To examine the potential influence of altered inositol levels on these responses, the effects of infusions (10 mg, i.c.v.) of MI, and of epi-inositol 30 min before pilocarpine or DOI were tested. Administration of MI slightly reduced the incidence of seizures induced by acute lithium plus DOI but almost completely blocked seizures induced by acute lithium plus pilocarpine. Epi-inositol also blocked seizures under both conditions but it was less effective than MI after treatment with acute lithium plus pilocarpine. Epi-inositol is an inositol isomer which is not used for phosphoinositide synthesis. Thus these data indicated that a mechanism other than phosphoinositide repletion contributed to the response of MI and epi-inositol (Williams and Jope 1995).In the same series of experiments peripheral administration of a high dose of MI blocked seizures induced by acute lithium plus pilocarpine, but the inositol treatment itself was toxic and caused seizures prior to pilocarpine administration, so the mechanism of action cannot simply be attributed to increased brain inositol levels (Williams and Jope 1995).Existence of MI in the water extract of *Aquilegia vulgaris,* its effects on 3H-muscimol binding in vitro (Solomonia et al., 2004) and the above

discussed data prompted us to carry out a special series of experiments of MI effects on acute seizures.

The rational basis of our design was to use intraperitoneal (i.p.)injections of MI in the range of physiological concentrations and test the effects of the inositols on chemically induced seizures according to the classically approved schemes, e.g. 30 minute before convulsant application (see Vogel 2002).In the first series of experiments we used two types of chemical convulsants: pentilentetrazolium (PTZ) and KA. MI in the dose of 20 mg/kg was injected i.p. 30 minutes before chemical convulsant administration. MI pretreatment induced a significant decrease in the seizure score for both types of chemical convulsant induced seizures (Solomonia et al., 2007).

In the next series of experiments we focused only on PTZ induced convulsions, but more parameters were scored and more compounds were tested (Nozadze et al., 2011).In the control group design we added a group treated with mannitol – polyol of the same molecular weight as inositols. Besides MI, the effect of another inositol –scyllo-inositol (SCI) was investigated. SCI is present in the human brain in concentrations estimated to be between 5 to 12% of MI[MI concentration in the rat brain is in the range of 7-11.5 mmol/kg wet wt, see review Fisher et al., 2002].In rat and rabbit brains, MI is converted into SCI by specific epimerase (Sherman et al., 1968, Fisher et al., 2002).The existence of such specific enzyme for SCI synthesis suggests SCI's important physiological role in the brain.

Half an hour after treatment with MI (30 mg/kg) or SCI (5 mg/kg) seizures were induced in Wistar rats with PTZ (60 mg/kg). Control animals were treated either by normal saline or mannitol (30 mg/kg), given at the same time interval before PTZ injection, as MI/SCI groups. The anticonvulsant effects of MI/SCI treatment were assessed by the latent period (the time from PTZ-injection to the onset of first seizures), and the duration and severity (score) of seizures. Both MI and SCI treatment significantly reduced the seizure score and seizure duration as compared to control groups treated either by saline or mannitol (Nozadze et al., 2011).Both inositiols effectively increased the latency of seizure onset. Inositol pretreatment did not change the mortality rate (Nozadze et al., 2011).

Despite a six times higher concentration of MI compared to SCI in these experiments, no significant differences were observed between MI+PTZ and SCI+PTZ groups. Inositol stereoisomers have a different distribution of hydroxyl groups across the surfaces of the sugar ring and these differences could account for different effective concentrations of MI and SCI on PTZ induced seizures.

The effects of MI and SCI in these experiments revealed that they are specific to MI and SCI as inositols.

No statistically significant differences were found between two sham groups: saline and mannitol treated, and both groups were significantly different from the MI and SCI groups with respect to nearlyall of the studied parameters (Nozadze et al., 2011). These results have convincingly demonstrated that MI and SCI, in the range of physiological concentrations, possess anticonvulsant properties on PTZ induced convulsions. Based on these and some of our other results we have proposed that, in addition to other functions, MI and SCI are endogenous anticonvulsants (Nozadze et al., 2011).

Other osmolytes of the organism also revealed anti-seizure activities. Betaine was found to block the induction of convulsions by electroconvulsive shock and by PTZ (Freed et al., 1979). Taurine displays anti-convulsive activitiesin the model of ouabain-induced seizures in rats (Barbeau and Donaldson 1974). Taurine pretreatment (150 mg/kg) before KA application significantly reduces convulsions for the following 2 hours and has neuroprotective effects. KA induced molecular effects, measured on the third day after SE, also disappear with taurine pretreatment (Junyent et al., 2009).

4. Inositols and Epileptogenesis

Ourexperiments and those by other authors'(above) have described experiments where inositol or other osmolyte pretreatments were applied before chemical convulsant induced brain insult (e.g. Solomonia et al., 2007, Nozadze et al., 2011, Junyent et al., 2009, Freed et al., 1979, Williams and Jope 1995). Such design is not applicable for testing inositols as an antiepileptogenic compound (see Loesher and Brandt 2010). As it was demonstrated inositol pretreatment attenuates the severity and shortens the duration of SE (Solomonia et al., 2007, Nozadze et al., 2011)and thereby could reduce the long-term consequences of the brain insult.

Therefore in the next series of experiments we have first induced the SE by KA, and then tried MI daily treatment. With such design of experiment antiepileptogenic properties of the compound could be explored. It is clear that treatment strategies that could interfere with the process of epileptogenesis would have significant benefits over the current approach of AEDs (see e.g, Loscher and Brandt, 2010) and will be of great importance for epilepsy treatment.

The diagram of our behavioral and biochemical experimental design is provided on Figure1. Briefly, experiments were carried out on four groups of Wistar rats.

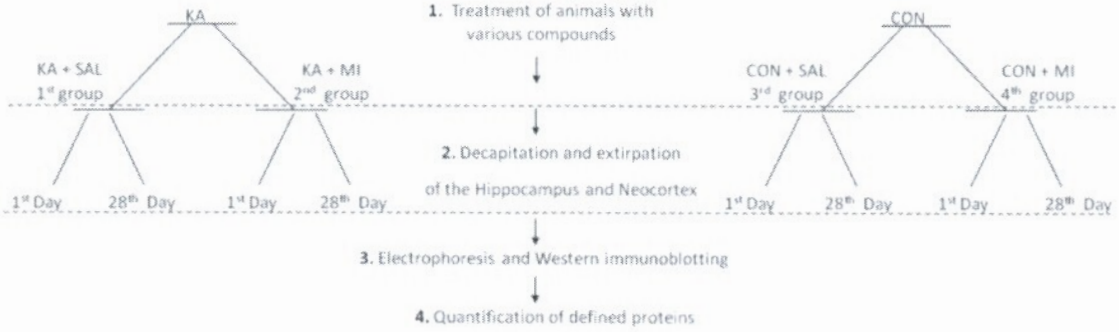

Figure 1.General diagram of experimental design.

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, see Figure1).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). 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 these subgroups were killed 10 h afterwards [time corresponding to the 28–30 h following KA treatment (first day – Figure1). 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 (28^{th} day of experiment).

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 from the twenty-fourth day of the experiment, the rats were monitored by an infrared video system for 24 h a day. The third and fourth groups of animals were control groups and were not treated by KA. Rats in the third group received saline (CON+SAL), while the fourth 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.

After decapitation, two regions - hippocampus and neocortex- were removed from each brain. The electrophoresis and quantitative Western Immunoblotting of nuclear free homogenate fractions from obtained tissue samples were carried as described in our work (Solomonia et al., 2010, 2013).

In the first series of experiments we focused on the changes of the following proteins: α-subunit of calcium-calmodulin-dependent protein kinase II (αCaMKII); (ii) GLUR1 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) type glutamate receptors (GLUR1) and (iii) heat shock protein 90 (HSP90). The reasons for choosing these proteins were the following: (i) there is a strong evidence that decreased expression of αCaMKII is sufficient to induce limbic epilepsy(Butler et al., 1995; Churn et al., 2000; reviewed in McNamara et al., 2006); (ii) the amount of GLUR1 immunopositive neurons is known to be drastically reduced after SE (Tang et al., 2005); (iii) whereas the amount of HSP90 is not changed (Gass et al., 1995).

The amount of neither protein was changed significantly by experimental conditions in the hippocampus or neocortex1 day after beginning theexperiment or at the 28th day in the neocortex.

The mean amount of the GLUR1 subunit was drastically reduced (more than 95%) in the hippocampus of the KA+SALgroup of rats and was significantly lower as compared to two groups of controlrats. The mean amount ofGLUR1 AMPA receptors in the KA+MI group was in the middle between the KA and control groups: significantly lower as compared to two control groups but significantly higher as compared to the KA group (Figure2). Thus MI treatment significantly halts the drastic reduction of GLUR1 AMPA receptors induced by KA evoked SE (Solomonia et al., 2010).

The changes for CaMKII in general were similar to those observed for the GLUR1 AMPA receptor. The mean amount of CaMKII in the KA+Sal group was significantly decreased (by approximately 60%) as compared to two control groups.

The mean amount of CaMKII in the KA+MI group was significantly more as compared to the KA+SAl group,which was closer and not different from two control groups. The mean amounts in the two control groups were not different from each other (Figure2). The mean amounts of HSP90 protein did not differ significantly between the experimental groups (Figure2).Thus the results of these series of experiments revealed that long-term MI treatment can halt the biochemical processes induced by KA induced SE (Solomonia et al., 2010).

The process of epileptogenesis is associated with changes in the expression and function of GABA-A receptors (GABA-AR)(Friedman et al. 1994; Kharlamov et al. 2011; Sperk et al. 1998; for a review see GonzalezandBrooks-Kayal 2011).

Various drugs that enhance GABAergic inhibition are commonly used as AEDs. In the next series of experiments we have examined the influence ofdaily MI treatment by the same design (see Figure1) on changes in GABA-AR subunit amounts.

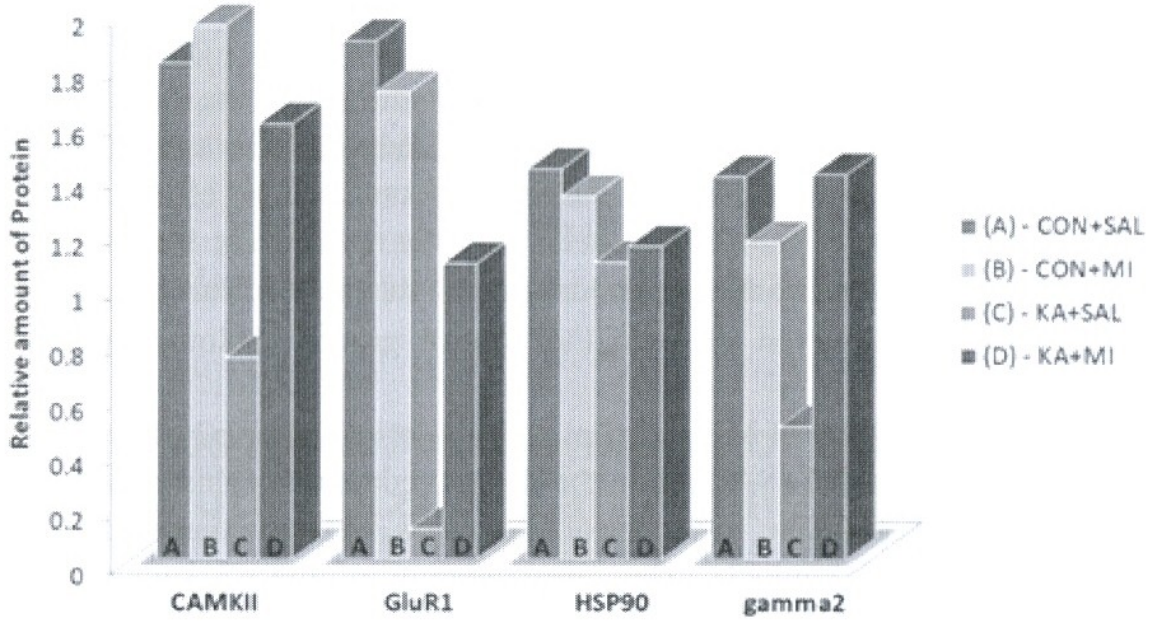

Figure 2.Mean relative amounts of CamKII, GluR1, HSP90 and gamma2 proteins in of rat brain hippocampus from four groups of rats on 28th day of experiment. Columns "A" correspond to CON+SAL group, columns "B" – to CON+MI, columns "C" to KA+SAL and columns "D" to KA+MI groups. For the details of calculations of relative amounts of proteins see Solomonia et al., 2011; 2013.

Among the numerous subunits of GABA-ARs we decided to focus on the following ones: α1, α4, γ2 and δ. The reasons for this selection were: (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) α1 and γ2 are found in synapses and participate in phasic inhibition (Mangan et al. 2005; Nusser and Mody 2002) whereas α4 and δ 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).

One day after the beginning of the experiment the significant changes were observed only for the α1 subunit – the protein amount was reduced in the hippocampus of the KA+SAL and KA+MI groups as compared to the two control groups and MI did not exert any influence on KA treated animals. 28 days later the mean amount of the α1 subunit in the hippocampus was not significantly higher in the KA+SAL and KA+MI groups than in the control groups. The late increase of the α1 subunit could account for a compensatory increase of the α1 subunit containing GABA-ARs. There were no differences between KA treated groups or the two control groups.

MI treatment does not have any additional influence on this late increase of α1 subunits (Solomonia et al., 2013).

An interesting effect was observed for the α4 subunit on the 28th day after starting the experiment in the hippocampus. KA-induced SE was followed by a decrease of the mean amount of protein as compared to all three other groups. MI treatment after KA induced SE not only restored the amount of α4, but increased it significantly even compared to the control groups. No differences were observed between the control groups (Solomonia et al., 2013).

The strongest effect of MI treatment was observed for the γ2 subunitin the hippocampus. In the KA+SAL group the drastic reduction of this subunit was observed compared to the two control groups as well as compared to the KA+MI group (see Figure2). The mean amount of the γ*2* subunitin the KA+MI group is not different from the CON+SAL group and is even significantly higher as compared to the CON+MI group. Thus MI treatment as in case of CaMKII and GluR1 restored the amount of the γ*2*subunit to the normal level. The MI effect in the hippocampus for the γ2 subunit at the 28th day was significantly different as compared to the neocortex (see Solomonia et al., 2013).γ2 subunits are important for the assembly of GABA-A receptors, 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-A receptor system. No significant changes were found for the *δ* subunit.

For the α1 subunit, significant changes were also observed in the neocortex on the 28th day of experiment. KA treatment decreased the amount of protein in both the KA+SAL and KA+MI groups as compared to the control. 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 (Solomonia et al., 2013). The data obtained indicate that either MI effects are region-specific or MI treatment

does not interfere with α1 subunit expression, neither in the necortex nor in the hippocampus.

MI pretreatment before KA induced SE exerts a strong neuroprotective effect on hippocampal cell loss during the process of epileptogenesis(Kotaria et al., 2013). As discussed above MI pretreatment evokes the decrease of KA-induced seizure severity (Solomonia et al., 2007).Thus MI treated groups were expected to have lower seizure severity grades as compared to SAL+KA treated groups. Therefore it was possible that differences revealed between the groups by hippocampal cell loss analysis would partially reflect the condition for less severe seizures in these animals. To avoid this pitfall, the rats were selected so that SAL+KA and MI+KA groups had the same seizure score.

The strongest neuroprotective effect was observed in the pyramidal layer of CA1, radial and oriental layers of CA3 fields. Ultrastructural alterations were described in CA1, 14days after treatment. The structure of neurons, synapses, and porosomes are well preserved in the rats pretreated with MI compared to rats treated with saline and then by KA (Kotaria et al., 2013)

5. Possible Mechanisms of MI Action on Acute Seizures and Epileptogenesis

What could be the mechanisms of MI action on acute seizures and on epileptogenesisprocesses? MI is the precursor of phosphoinositode biosynthesis and an initial assumption for the study of inositols influence on a lithium-pilocarpine induced seizure was "inositol-depletion hypothesis".

However this mechanism of inositol action on acute seizures was declined, as epi-inositol, an isomer that is not used for phosphoinosotide biosynthesis, also possesses anti-convulsive features on lithium-pilocarpine induced seizures (Williams and Jope 1995).

Another mode of inositol action on seizures and epileptogenesis may be linked with its osmolytic properties.During intense neuronal excitation a massive influx of Na^+, Ca^{2+} and Cl^- takes place, which leads to inward water flow and cellular swelling (Choi 1987; Rothman 1985).

Under such conditions various enzymes cannot function in the normal way (Yanceyet al., 1993). 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 (e.g. Rowley et al. 2011), which restores the conditions for normal functioning of the intracellular enzymes. One such important osmolyte appears to be MI (Fisheret al. 2002). Upregulation of SMIT after KA induced seizures also indicates the beneficial effects of MI accumulation for the normalization of cellular functions (Nonaka et al., 1999). We suggest thatone of the possible mechanisms of MI action could be osmoprotection, which prevents/modifies the biochemical processes of epileptogenesis.

Based on our previous work demonstrating the influence of MI on 3H-muscimol and 3H-MK-801 binding in vitro (Solomonia et al. 2004), we additionally 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.

Conclusion

Current evidence convincingly demonstrates that alterations in the metabolism ofinositols and some other organic osmolytes are involved in epilepsy. These molecules are able to reduce acute seizures induced by various types of convulsants. Moreover, and most importantly, MI is able to prevent/modify the process of epileptogenesis on a molecular level.

Further investigations are needed to gather more evidence about mechanisms of inositol actions which could lead to more successful translational research and development of inositols as future anti-epileptogenic compounds.

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INOSITOL

Synthesis, Functions and Clinical Implications

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