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The Effects of Kainic Acid-Induced Seizure on Gene Expression of Brain Neurotransmitter Receptors in Mice Using RT² PCR Array

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ABSTRACT

Introduction: Kainic acid (KA) induces neuropathological changes in specific regions of the mouse hippocampus comparable to changes seen in patients with chronic temporal lobe epilepsy (TLE). According to different studies, the expression of a number of genes are altered in the adult rat hippocampus after status epilepticus (SE) induced by KA. This study aimed to quantitatively evaluate changes in the gene expression of brain neurotransmitter receptors one week after administration of kainic acid in the mouse hippocampus.

Methods: We used 12 BALB/c mice in this study and randomly divided them into 2 groups. To both groups, saline (IP) was administered for 7 days, and on the last day, KA (10 mg/kg, IP) was injected 30 minutes after administration of saline. Subsequently, behavioural changes were observed in mice. Then, in one group (1 day group), 2 hours and in another group (7 days group), 7 days after KA administration, the hippocampus tissue of mice was removed and used for gene expression analyses. Total brain RNA was isolated and reversely transcribed. We performed qPCR using RT² Profiler ™PCR Array Mouse Neurotransmitter Receptors and Regulators (QIAGEN) containing primers for 84 genes. In this regard, we selected 50 related genes for KA model.

Results: Our results showed significant changes in the gene expression of $GABA_A$ subunits receptors, including α1-α3, α5, α6, β2, β3, γ1, ρ, and rho1-2 on day 7 compared with the day 1.

Conclusion: Expression of both inhibitory and excitatory receptors changed after one week. Further studies are needed to find more molecular changes in the gene expression of brain neurotransmitter receptors and regulators over longer periods of time in KA models using RT² PCR array.

1. Introduction

Kainic acid (KA) is an excitatory amino acid and its systemic or intracerebral injections cause epileptiform seizures in the CA3 region of the hippocampus (Ben-Ari & Cossart, 2000). These seizures propagate to other limbic structures and its region-specific neuropathological changes in hippocampus are comparable to those of patients with chronic temporal lobe epilepsy (TLE) (Ben-Ari & Cossart, 2000; Obeid et al., 2010;

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Laurén Lopez-Picon, Brandt, Rios-Rojas, & Holopainen, 2010; Sloviter, Zappone, Harvey, & Frotscher, 2006).

γ-Amino butyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain. GABAergic neurons are distributed in CNS and have important role in processing and integration of all brain functions. Several different and differentially distributed GABA receptors have been spotted throughout the brain. $GABA_A$ receptors are composed of 5 subunits that form a chloride channel. Thus far, a total of six α, four β, three γ, one δ, one ε, one π, one θ, and three ρ subunits of $GABA_A$ receptors have been identified in the mammalian nervous system (Sperk, Wieselthaler-Hoelzl, & Drexel, 2009).

Dysfunction of the GABAergic system may have a crucial role in the propagation of acute seizures and manifestation of epilepsy syndromes (Sperk et al., 2009). Also, several studies have shown that expression of a number of genes are altered in the adult rat hippocampus after status epilepticus (SE) induced by KA (Laurén Lopez-Picon, et al., 2010; Sperk, Schwarzer, Tsunashima, & Kandlhofer, 1998).

Anticonvulsant and neuroprotective effects have been reported for some neuropeptides, such as galanin, neuropeptide Y, and somatostatin (Wilson et al., 2005). On the other hand, excitatory and proconvulsive effects have been reported for neuropeptide tachykinin 1 (Wilson et al., 2005). Thus, we aimed to quantitatively evaluate the possible effects of KA-induced seizure on gene expression of brain neurotransmitter receptors in mice by RT^2 PCR array method.

2. Methods

2.1. Animals

Twelve male BALB/c mice (weight 20–25 g) were provided from Razi Institute (Karaj, Iran). All animals were housed under standard laboratory conditions. They were kept at room temperature (21±2°C) with a 12:12 h light-dark cycle. They had free access to food and water.

2.2. Drugs

KA was purchased from Sigma (Sigma, St. Louis, MO, USA) and was dissolved in saline. Other drugs included xylazine 2% (Loughrea, Co. Galway, Ireland) and ketamine 10% (Rotexmedica, GmbH, Germany).

2.3. Kainic acid administration and experimental design

Mice were divided into two groups of 6 animals each. Both groups received an intraperitoneal injection (IP) of saline (10 mL/kg) daily for 7 days, and on the last day, KA (10 mg/kg, IP) was injected 30 minutes after administration of saline. After KA injection, behavior of animals were observed for 2 hours and rated based on these scores: 0) no change, 1) no movement, 2) increase in muscle tone at rest, 3) head bobbing/scratching or and circling, 4) clonus/rearing/falling of forelimb, 5) repetitive behavior of 4, 6) severe tonic-clonic seizures (Morrison et al., 1996).

Only mice exposing the entire behavioral changes (score 3-5) were included in the study. Then, in one group (1 day group), 2 hours and in another group (7 days group), 7 days after KA administration, mice were anaesthetized with IP injection of ketamine/xylazine (60 mg/kg and 6 mg/kg, respectively) and then were killed. Next, their hippocampal tissues were dissected, quickly removed, and cleaned with chilled saline (0.9%). One hippocampus from each brain was snap frozen and kept at -80°C to be later used for gene expression analysis (Hemmati et al., 2013).

2.4. Real-time PCR and comparative threshold cycle method

All hippocampus tissues were homogenized and the total RNA extraction kit was used to extract total RNA (Jena Bioscience, GmbH, Jena, Germany). Next, these RNAs were reverse transcribed.

The cDNA was mixed with RT^2 SYBR Green/ROX qPCR Master Mix (Qiagen, Maryland, USA) and the mixture was added into a 96-well RT^2 mRNA PCR Array (SABiosciences) that contained primers for 84 tests and 5 housekeeping genes according to manufacturer's instruction. Thermal cycling was performed using ABI-7500 (Applied Biosystems, Foster, CA, USA) with an initial denaturation at 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 60°C for 1 minute. A signal was acquired at 60°C during each cycle. Values of cycle threshold (Ct) obtained in quantification were used for calculations of fold changes in mRNA abundance using $2^{-\Delta\Delta Ct}$ method.

2.5. Data analyses

Data were expressed as fold change. Fold change ($2^{-\Delta\Delta Ct}$ method) is the normalized gene expression ($2^{-\Delta Ct}$) in the test sample divided by the normalized gene expression ($2^{-\Delta Ct}$) in the control sample. Differences among experimental groups were analysed by Student's t-test and used for comparisons with RT^2 Profiler PCR Array data analysis software version 3.5 (SABiosciences). $P<0.05$ was considered to be statistically significant.

3. Results

Five housekeeping genes were used in this study as follows: β-glucuronidase, hypoxanthine-guanine phosphoribosyltransferase 1, heat shock protein 90 α class B member 1, glyceraldehydes 3-phosphate dehydrogenase, and β-actin.

3.1. Effects of KA on $GABA_A$-subunits gene expression

Gene expression of subunits α1, α2, α3, α5, and α6 on the 7 days group was significantly decreased compared to 1 day group (P<0.05, P<0.001, P<0.05, P<0.01, P<0.05, respectively) (Table 1). Gene expression of subunits β2, β3, and γ1 were significantly decreased in 7 days group compared to 1 day group (P<0.05, P<0.001, respectively) (Table 1). Also, gene expression of subunits of rho1 and rho2 decreased on day 7 group compared to day 1 group. There was no difference between the groups (1 day and 7 days) with regard to gene expression of subunits α4, δ, γ2, and θ (P>0.05) (Table 1). The expression of glutamic acid decarboxylase 1 (GAD 1) gene did not alter in 7 days group compared to 1 day group (P>0.05).

3.2. Effect of KA on cholinergic receptor gene expression

Gene expressions of M1, M3, M4, and M5 receptors were significantly decreased in 7 days group compared to 1 day group (P<0.05, P<0.001, P<0.05, P<0.001, respectively) (Table 2). There was no difference between two groups regarding gene expression of M2 receptor (P>0.05) (Table 2).

Gene expressions of Nα1 (P<0.05), Nα2 (P<0.01), Nα3 (P<0.05), Nα5 (P<0.05), Nα7 (P<0.05), Nβ1 (P<0.001), Nβ2 (P<0.05), Nβ3 (P<0.01), Nβ4 (P<0.05), Nδ (P<0.001), Nβε (P<0.001), and Nγ (P<0.001) receptors were significantly decreased in 7 days group compared to day 1 group (Table 2).

3.3. Effect of KA on neuropeptides gene expression

Gene expressions of NPY1, NPY2, and NPY5 receptors were significantly decreased in 7 days group compared to 1

Table 1. The effects of KA on the gene expression of $GABA_A$ and $GABA_c$ subunits receptors and GAD1 in the hippocampus of mice.

Gene symbol	Neurotransmitter receptors	Fold change (7)
GABA receptors		
Gabra1	$GABA_A$ receptor, α1	0.0528*
Gabra2	$GABA_A$ receptor, α2	0.0326***
Gabra3	$GABA_A$ receptor, α3	0.0016*
Gabrb4	$GABA_A$ receptor, α4	2.3241
Gabra5	$GABA_A$ receptor, α5	0.1564**
Gabra6	$GABA_A$ receptor, α6	0.2097*
Gabrb2	$GABA_A$ receptor, β2	0.1267*
Gabrb3	$GABA_A$ receptor, β3	0.1449***
Gabrd	$GABA_A$ receptor, δ	2.9147
Gabrg1	$GABA_A$ receptor, γ1	0.1493***
Gabrg2	$GABA_A$ receptor, γ2	0.1597
Gabrp	$GABA_A$ receptor, ρ	0.3601**
Gabrq	$GABA_A$ receptor, θ	0.0227
Gabrr1	$GABA_C$ receptor, rho1	0.22316**
Gabrr2	$GABA_C$ receptor, rho2	0.0941***
Neurotransmitter biosynthesis		
Gad1	Glutamic acid decarboxylase 1	0.6659

Data are displayed as fold change (n=3, with 3 technical replicate per n) by student's t-test. NEUROSCIENCE

*P<0.05, **P<0.01, ***P<0.001.

Table 2. The effects of KA on the gene expression of muscarinic and nicotinic acetylcholinereceptors in the hippocampus of mice.

Gene symbol	Neurotransmitter receptors	Fold change (7)
Muscarinic Acetylcholine Receptors		
Chrm1	Cholinergic receptor, M1	0.2333*
Chrm2	Cholinergic receptor, M2	1.2599
Chrm3	Cholinergic receptor, M3	0.0859***
Chrm4	Cholinergic receptor, M4	0.1166*
Chrm5	Cholinergic receptor, M5	0.1239***
Nicotinic acetylcholine receptors		
Chrna1	Cholinergic receptor, Nα1	0.2449*
Chrna2	Cholinergic receptor, Nα2	0.0488**
Chrna3	Cholinergic receptor, Nα3	0.1436*
Chrna4	Cholinergic receptor, Nα4	1.5333
Chrna5	Cholinergic receptor, Nα5	0.1847*
Chrna6	Cholinergic receptor, Nα6	0.0547
Chrna7	Cholinergic receptor, Nα7	0.2176*
Chrnb1	Cholinergic receptor, Nβ1	0.1227***
Chrnb2	Cholinergic receptor, Nβ2	0.1318*
Chrnb3	Cholinergic receptor, Nβ3	0.0294**
Chrnb4	Cholinergic receptor, Nβ4	0.2553*
Chrnd	Cholinergic receptor, Nδ	0.315***
Chrne	Cholinergic receptor, Nβε	0.2007***
Chrng	Cholinergic receptor, Nγ	0.1285***

Data are displayed as fold change (n=3, with three technical replicate per n) by Student's t-test. NEUROSCIENCE

*P<0.05, **P<0.01, ***P<0.001.

day group (P<0.001). There was no difference between two groups regarding gene expression of NPY6 (P>0.05).

Expressions of somatostatin genes, including receptors 1(P<0.01), 2(P<0.001), 3(P<0.001), 4(P<0.01), and 5 (P<0.01) were significantly reduced in 7 days group compared to 1 day group (Table 3). Gene expressions of tachykinin receptors, including 2 and 3 were significantly reduced in 7 days group compared to 1 day group (P<0.001) (Table 3). Furthermore, gene expressions of galanin receptors, including 1, 2, and 3 were significantly reduced on 7 days group compared to 1 day group (P<0.01, P<0.001, respectively) (Table 3).

4. Discussion

In the present study, we investigated the effects of KA-induced seizure on gene expression of brain neurotransmitterreceptors by using RT^2 PCR array in mice. Our results regarding GABAergic system showed that gene expressions of $GABA_A$ subunits, including α1, α3, α5, α6, β2, β3, γ1, ρ, and rho1–2 decreased 7 days after KA administration compared to 1 day time in the hippocampus of studied mice. Decreased mRNA levels may be due to significant neurodegeneration after KA administration (Drexel, Kirchmair, & Sperk, 2013). It seems that neuronal loss was most severe 24 hours after KA injection than other times (Drexel, Preidt, & Sperk, 2012).

An initial decrease in mRNA expressions of α1 and γ2 subunits in the several hippocampal areas have been reported after KA-induced seizure. Moreover, it was shown that mRNA levels of almost all other subunits (α2, α3, α4, β2, and β3) transiently decreased after KA-induced seizure (Drexel, et al., 2013). Interestingly, rapid overexpression of subunit γ2 mRNA and protein in all subfields of the hippocampus (up to

Table 3. The effects of KA on the gene expression of neuropeptide Y, somatostatin, tachykinin and galanin receptors in the hippocampus of mice.

Gene symbol	Description	Fold change (7)
Neurotransmitter receptors		
Neuropeptide Y receptors		
Npy1r	Neuropeptide Y, receptor Y1	0.0829***
Npy2r	Neuropeptide Y, receptor Y2	0.1615***
Npy5r	Neuropeptide Y, receptor Y5	0.1153***
Npy6r	Neuropeptide Y, receptor Y6	0.8586
Somatostatin receptors		
Sstr1	Somatostatin receptor 1	0.1413**
Sstr2	Somatostatin receptor 2	0.1429***
Sstr3	Somatostatin receptor 3	0.0292***
Sstr4	Somatostatin receptor 4	0.0183**
Sstr5	Somatostatin receptor 5	0.1801**
Tachykinin receptors		
Tacr1	Tachykinin receptor 1	1.8575
Tacr2	Tachykinin receptor 2	0.3085***
Tacr3	Tachykinin receptor 3	0.207***
Galanin receptors		
Galr1	Galanin receptor 1	0.1601**
Galr2	Galanin receptor 2	0.1303**
Galr3	Galanin receptor 3	0.009***

Data are displayed as fold change (n=3, with three technical replicate per n) by student's t-test. NEUROSCIENCE

*P<0.05, **P<0.01, ***P<0.001.

24 hours after KA injection) has been reported as a potential compensation for the initial losses (Schwarzer et al., 1997).

However, overexpression of subunit γ2 mRNA was reported in the dentate gyrus (DG), sector CA1, layer II of the entorhinal cortex (EC), and perirhinal cortex (PRC) at late intervals, i.e. 30-90 days after KA-induced seizure (Drexel, et al., 2013). Also, upregulation of subunits α1 and α3 mRNAs were observed in the hippocampus 30-90 days after KA- induced seizure (Drexel, et al., 2013). This condition has been reported for subunit α4 mRNA in the DG area 30-90 days after KA-induced seizure. Moreover, subunits α5 and δ mRNAs were downregulated in most areas of brain (Drexel, et al., 2013).

GABA is synthesized in brain by GAD. GAD has two isoforms of GAD1 and GAD2 encoded by two different genes (Trifonov, Yamashita, Kase, Maruyama, & Sugimoto, 2014). The expression of Gad1 gene has been observed in the hippocampus and frontal of cortex in mice; however, the level of expression in these regions seems to be lower than other examined regions (Trifonov et al., 2014). GAD is the rate-limiting enzyme for synthesizing GABA and its expression is increased in patients with TLE and in rats after KA-induced limbic seizures. GAD increase in these conditions lead to enhanced transmission of GABA. Apparently, this increment may have anticonvulsive effect (Schwarzer & Sperk, 1995).

In our study, the level of expression of Gad1 did not decrease 7 days after administration of KA. This issue may indicate the lasting anticonvulsive effects of GAD1 even one week after seizure in the hippocampus. However, this elevation in animal study was contrary to our study, which was transient.

In our study on cholinergic system, gene expression of M1, M3, M4, and M5 decreased in 7 days group compared

to 1 day group. Furthermore, gene expression of Nα1, Nα2, Nα3, Nα5, Nα7, Nβ1, Nβ2, Nβ3, Nβ4, Nδ, Nβε, and Nγ significantly decreased 7 days after the administration of KA compared to the day 1. To our knowledge no other study has examined the levels of subunits of muscarinic and nicotinic receptors at different time points after injection of KA. However, the change in expression of muscarinic receptors mRNA in different kinds of seizures such as pilocarpine and electroconvulsive has been reported (Hamilton et al., 1997; Mingo et al., 1998).

Overexpression of receptors of NPY5 with NPY in the hippocampus could suppress seizure. However, upregulation of NPY2 receptor alone could suppress epileptic activity in both electrical kindling and KA models of seizures (Woldbye et al., 2010).

A transient elevation in gene expression of galanin and somatostatin in KA samples of hippocampus, 24 hours post-seizure has been reported in P30 rats (Wilson et al., 2005). Similarly, this gene expression occurred for NPY in KA-induced seizure after 24 and 72 hours in both P15 and P30 rats (Wilson et al., 2005). It seems that galanin has a neuroprotective role in the CNS, and could modulate neural excitability in the hippocampus. Also, it was reported that both GalR1 and GALR2 could mediate anticonvulsant effects of galanin (Mazarati & Lu, 2005). Similar to this study, GALR1 deficiency could influence susceptibility to KA-induced injury in C57BL/6J mice (Schauwecker, 2010).

Recently, the role of neuropeptides such as galanin neuropeptide Y, and somatostatin were discussed as targets for the development of anticonvulsant drugs in the brain (Clynen, Swijsen, Raijmakers, Hoogland, & Rigo, 2014). Substance P as a member of tachykinin family has an important role in the maintenance of SE. This substance is expressed in the hippocampus and could increase the glutamate release (Liu, Mazarati, Katsumori, Sankar & Wasterlain, 1999).

In this study, we observed that the expression of most neuropeptides, including NPY, somatostatin, tachykinin, and galanin receptors reduced one week after systemic injection of KA (except NPY6). Thus, it seems that the amount of expression both anticonvulsive and proconvulsive peptide genes decreased. Gene express reduction of above receptors had not been reported before using RT^2 PCR array technique.

In summary, we reported for the first time the alterations in the gene expression of 50 brain neurotransmitter receptors and regulators one week after KA systemic injection in hippocampus of studied mice by using RT^2 PCR array. Further studies are needed to find other changes in gene expression of brain neurotransmitter receptors and regulators in other time periods after KA administration using RT^2 PCR array.

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Conflict of Interests

The authors declared no conflict of interest.

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