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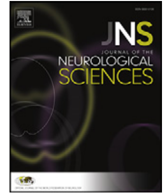

Aldehyde dehydrogenase 2 deficiency increases resting-state glutamate and expression of the GluN1 subunit of N-methyl-D-aspartate receptor in the frontal cortex of mice

Mostofa Jamal [a,*], Junichiro Ono [b], Kiyoshi Ameno [a], Gotaro Shirakami [b], Naoko Tanaka [a], Ayaka Takakura [a], Hiroshi Kinoshita [a]

[a] Department of Forensic Medicine, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki, Kita, Kagawa 761-0793, Japan
[b] Department of Anesthesiology and Emergency Medicine, Faculty of Medicine, Kagawa University, Japan

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ABSTRACT

Our previous study showed that Aldh2-knockout (Aldh2-KO) mice, an animal model of inactive aldehyde dehydrogenase 2 (ALDH2), have better spatial memory when compared with wild-type (WT) mice. Given that the neurotransmitter glutamate has been associated with learning and memory, the goal of the present study was to investigate whether the strain-dependent difference in spatial memory was associated with changes in glutamate transmitter levels or receptor function in the frontal cortex of Aldh2-KO and WT mice. Thus, we first measured extracellular glutamate levels in free-moving mice using microdialysis. Second, we studied protein expression of the N-methyl-D-aspartate (NMDA) receptor (GluN1) subunit and the α-amino-3-hydroxy-5 methylisoxazole-4-propionic acid (AMPA) receptor (GluA1) subunit in lipid raft fractions using Western blot (WB). The samples were collected for WB, and lipid rafts were prepared from the insoluble fraction of homogenate tissue. Protein concentration was measured in the whole cell lysate (WCL) and in five separate lipid raft fractions. Cholesterol was also measured in all fractions 1–5. The microdialysis study revealed that basal glutamate concentration in the dialysates was approximately three-fold (0.27 ± 0.12 μM) higher in Aldh2-KO mice than in WT (0.10 ± 0.03 μM) mice. We also found an increase in the expression of GluN1 in Aldh2-KO mice compared with WT mice, both in the WCL and fraction 5, but GluA1 levels were unchanged as measured by WB. Our novel findings provide the first evidence for the role of ALDH2 in glutamate release and GluN1 protein expression in the frontal cortex. The observed strain differences in glutamate levels and GluN1 expression may suggest that enhanced glutamatergic function facilitates improved spatial memory in Aldh2-KO mice and such observation deserves further investigation.

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1. Introduction

Glutamate plays an important role in cognitive function, particularly in memory and attention [19,26]. Generally, neuronal glutamate receptors activate to take glutamate into the cells, but too much accumulation of glutamate is toxic to neurons [20]. The number of glutamate receptor sites on the neuronal surface is an important determinant of the level of glutamate [28]. Alterations in glutamate transmission are implicated in pathologies ranging from neurotoxicity to neuropsychiatric disorders [9,17,31]. Glutamate exerts its effects by binding to specific receptors on nerve cells and once it is released into the synapse, it is rapidly cleared by glutamate transporters, limiting glutamate excitotoxicity [21].

Glutamate receptors have been pharmacologically classified as ionotropic and metabotropic. The former includes the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5 methylisoxazole-4-propionic acid (AMPA) and kainate families of receptors. Most AMPA receptors are impermeable to Ca^{2+} and contribute to fast synaptic transmission. In contrast, NMDA receptors are highly permeable to Ca^{2+}, but their voltage-dependent block by Mg^{2+} tends to result in slow gating kinetics. These features make NMDA receptors more suitable for mediating plastic changes in the brain, such as those involved in learning [10]. Indeed, NMDA receptor activation is required for some forms of long-term potentiation (LTP) [15] and learning and memory [2,11]. High protein expression levels of the NMDA receptor subunits GluN1 and GluN2B correspond to improve spatial learning in four- to five-month-old mice [25,34].

Lipid rafts are specific microdomains within the cell membrane that are particularly rich in sphingolipids and cholesterol [29]. The rafts are associated with several post-synaptic proteins, including NMDA, AMPA and acetylcholine receptors [4,13,14]. Lipid rafts have recently received considerable attention because they are thought to be involved in many cellular functions, for example, signal transduction for

* Corresponding author. Tel.: +81 87 891 2140; fax: +81 87 891 2141.
E-mail address: jamal@kms.ac.jp (M. Jamal).

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extracellular stimuli. There is increasing evidence that the membrane localization of distinct neurotransmitter receptors within lipid rafts can influence their function by affecting neurotransmitter binding, receptor trafficking and clustering [1,32].

ALDH2 (aldehyde dehydrogenase 2) is known to decrease oxidative stress in mitochondria, a major source of aldehyde [8,12,22]. ALDH2 is also required for the detoxification of acetaldehyde produced by alcohol metabolism. Approximately half of the Asian population has an inactive ALDH2, and this deficiency may lead to several diseases such as cancer [5,33] and coronary heart disease [30]. A single nucleotide polymorphism of ALDH2*2, acts as a dominant-negative gene and is found in Asian population [12]. This ALDH2*2 allele exhibits the alcohol-flushing syndrome attributable to an elevated blood acetaldehyde level. ALDH2 deficiency is proposed to contribute to age-dependent neurodegeneration accompanying memory loss in mice after 1 year of age [23]. Furthermore, ALDH2-deficient 10–12 week-old mice have better spatial memory than do ALDH2-normal mice [16].

Glutamate plays an important role in cognitive function, however, the role of ALDH2 in the glutamatergic system remains elusive. To explore the mechanism of this relationship, we investigated whether a genetic deficiency in ALDH2 affects extracellular glutamate and protein expression of GluN1 and GluA1 subunits in the frontal cortex of Aldh2-knockout (Aldh2-KO) and wild-type (WT) mice. Accordingly, first, we measured extracellular glutamate release in vivo using microdialysis. Second, we examined GluN1 and GluA1 protein expression in the lipid raft fractions using western blot (WB).

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Kagawa University Animal Investigation Committee. Aldh2-KO mice were generated as previously reported [18]. These mice were maintained and backcrossed with the C57BL/6 J strain for more than 10 generations. They have the same genetic background, except for Aldh2. Two eight-week-old male/female pairs were obtained from the Department of Environmental Health at the University of Occupational and Environmental Health in Japan and were bred at the Kagawa University animal facility. Breeding pairs from this strain were used to generate the experimental groups. WT mice have the same genetic background as C57BL/6 J mice. Control mice were purchased from CLEA Japan (Tokyo, Japan). All experiments were conducted with male mice that were 10–12 weeks of age and that weighed 24–28 g. All animals were housed in controlled temperature (21 ± 3 °C), humidity (50–70%) and light (12-h light–dark cycle) conditions.

2.2. In vivo microdialysis with HPLC-ECD

Aldh2-KO and WT were (n = 6 from each) anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus. A guide cannula was stereotaxically implanted unilaterally in the frontal cortex (coordinates relative from bregma: anterior, 2.0 mm; lateral, 1.4 mm; height, 1.4 mm from the dural surface). After a 24-h recovery, the mice underwent a microdialysis study in the morning. A microdialysis probe (A-I-4-2, Eicom) was introduced through the guide cannula, and the probe was perfused with a Ringer's solution (147 mM NaCl, 4 mM KCl, 2.25 mM CaCl2, 1 mM MgCl2) at 1.0 μl/min using a 1.0-ml gas-tight syringe (Hamilton, USA). A glutamate internal standard (IS) solution (1.0 μM) was perfused at 1.0 μl/min using a 1-ml gas-tight syringe (Hamilton).

Dialysate samples were assayed with HPLC coupled to an electrochemical detector (ECD-300, Eicom). Two auto-injectors (EAS-20, EAS-10, Eicom) were used: one for the glutamate IS and the other for the dialysate sample. The glutamate IS and dialysate were automatically injected into the auto-injector for 20 min regarded as a 1-sample set and then sent to the separation column with a 1.0-min interval. The operative conditions of HPLC, in short, were as follows: precolumn (CH-GEL, 4.0 × 5.0), a separation column (GU-GEL; 4.6 mm × 150 mm; Eicom), enzyme column (E-ENZPACK; 3.0 × 4.0 mm, Eicom), a platinum working electrode, column temperature 33 °C, detector and electrode potential (+450 mV against Ag/AgCl reference electrode). The enzymatic post-column reactor containing glutamate oxidase oxidized glutamate to hydrogen peroxide, which was identified at the platinum electrode surface. The detection limit for this methodology is <3 nM for any sample medium. The mobile phase contained 3.2 g/l ammonium chloride and 250 mg/l hexadecyltrimethylammonium bromide, adjusted to pH 7.2 with an ammonium solution delivered at a rate of 0.370 ml/min. Injections were automatically performed on the analytical column during the experimental period. The perfused dialysates were collected by an autoinjector connected to an automated HPLC–ECD. The chromatograms were recorded with a PowerChrom (ADInstruments, Sydney, Australia). Dialysate samples and glutamate IS were collected every 20 min until a stable baseline was obtained. Glutamate levels were calculated by dividing peak areas from dialysate samples with glutamate IS.

2.3. Isolation of lipid rafts

Aldh2-KO and WT mice (n = 4 from each) were killed by cervical dislocation and then decapitated. Brains were quickly removed and were washed in cold saline twice. Frontal cortical tissue (approximately 120 mg) was homogenized in 500 μl of lysis buffer with detergent [1× PBS (pH 8.0), 1% Triton-X 100, 1% phosphatase inhibitor]. The homogenate was sheared through a 23-gauge needle with 12–15 complete passes, and an additional 0.5 ml of buffer was added for a total sample volume of 1.0 ml. A 200 μl aliquot was sonicated and used for total protein concentration and stored at −80 °C. This aliquot served as the whole cell lysate (WCL) fraction. A 500 μl (10 mg/ml) sample of homogenized tissue was subjected to ultracentrifugation for fractionation using 100% sucrose as the gradient. To this, 2.5 ml of 35% sucrose was overlaid, followed by 1.5 ml of 5% sucrose. The tubes were centrifuged at 52,000 rpm for 16 h at 4 °C. After centrifugation, the mixture was clear except for a distinct, cloudy band at the interface between the 5% and 35% sucrose layers. Five sequential fractions of 1 ml each were gently removed from the top of the tube and individually aliquoted. The fractions 1–5 were used for next steps or stored at −80 °C until use.

2.4. Protein assay

The total protein concentration was measured in the WCL at 570 nm by a bovine serum albumin protein assay (Bradford) using a microplate reader, and a protein concentration of 10 mg/ml was used for lipid raft preparation. A 5 μl aliquot of each fraction was used for the protein assay as measured by a microplate reader.

2.5. Cholesterol assay

The lipid raft fractions were thawed on ice, and 50 μl of each fraction was used to determine the amount of cholesterol according to manufacturer instructions (Wako Chemicals). Total cholesterol was measured at an emission wavelength of 570 nm using a microplate reader.

2.6. Western blot analyses

Western blot analyses were performed on the WCL and fraction 5 of raft fractions. Prior to gel loading, WCL and fraction 5 samples were heated to 95 °C for 5 min. Equal volumes of WCL and fraction 5 protein samples were electrophoretically separated on an SDS-10% polyacrylamide gel. Nitrocellulose membranes (Bio-Rad, Hercules, CA) were blocked with 5% non-fat dry milk in 0.05% Tween-20/Tris-buffered saline and then incubated with a primary antibody overnight at 4 °C.

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