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Zonisamide regulates basal ganglia transmission via astroglial kynurenine pathway

Kouji Fukuyama [a,1], Shunske Tanahashi [a], Masamitsu Hoshikawa [b], Rika Shinagawa [b], Motohiro Okada [a,*,1]

[a] Department of Neuropsychiatry, Division of Neuroscience, Graduate School of Medicine, Mie University, 2-174 Edobashi, Tsu, Mie 514-8507, Japan
[b] Discovery Research Laboratories I, Ono Pharmaceuticals Co., Ltd., Japan

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ABSTRACT

To clarify the anti-parkinsonian mechanisms of action of zonisamide (ZNS), we determined the effects of ZNS on tripartite synaptic transmission associated with kynurenine (KYN) pathway (KP) in cultured astrocytes, and transmission in both direct and indirect pathways of basal ganglia using microdialysis. Interactions between cytokines [interferon-γ (IFNγ) and tumor-necrosis factor-α (TNFα)] and ZNS on astroglial releases of KP metabolites, KYN, kynurenic-acid (KYNA), xanthurenic-acid (XTRA), cinnabarinic-acid (CNBA) and quinolinic-acid (QUNA), were determined by extreme liquid-chromatography with mass-spectrometry. Interaction among metabotropic glutamate-receptor (mGluR), KP metabolites and ZNS on striato-nigral, striato-pallidal GABAergic and subthalamo-nigral glutamatergic transmission was examined by microdialysis with extreme liquid-chromatography fluorescence resonance-energy transfer detection. Acute and chronic ZNS administration increased astroglial release of KYN, KYNA, XTRA and CNBA, but not QUNA. Chronic IFNγ administration increased the release of KYN, KYNA, CNBA and QUNA, but had minimal inhibitory effect on XTRA release. Chronic TNFα administration increased CNBA and QUNA, but not KYN, KYNA or XTRA. ZNS inhibited IFNγ-induced elevation of KYN, KYNA and QUNA, but enhanced IFNγ-induced that of CNBA. TNFα-induced rises in CNBA and QUNA were inhibited by ZNS. ZNS inhibited striato-nigral GABAergic, striato-pallidal GABAergic and subthalamo-nigral glutamatergic transmission via activation of groups II and III mGluRs. ZNS enhanced astroglial release of endogenous agonists of group II mGluR, XTRA and group III mGluR, CNBA. Activated endogenous mGluR agonists inhibited transmission in direct and indirect pathways of basal ganglia. These mechanisms contribute to effectiveness and well tolerability of ZNS as an adjunct treatment for Parkinson's disease during L-DOPA monotherapy.

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1. Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder after Alzheimer's disease with an estimated incidence of 0.5–1% among people older than 65 years (Toulouse and Sullivan, 2008). PD is a complex multifactorial disease marked by extensive neuropathology in brain with prominent and progressive loss of dopaminergic neurons within substantia nigra pars compacta. Both clinical and pre-clinical studies have demonstrated that activation of pro-inflammatory cytokines, e.g., interferon-γ (IFNγ) and tumor-necrosis factor-α (TNFα), and dysfunction of synaptic transmission are important factors in the pathomechanism of PD (Boka et al., 1994; Hunot et al., 1996; Dobbs et al., 1999; Mogi et al., 2007; Chakrabarty et al., 2011; Zadori et al., 2012). For example, activation of IFNγ and TNFα enhances glial kynurenine (KYN) pathway (KP) (Guillemin et al., 2001; Asp et al., 2011). KP synthesizes various endogenous neuroactive metabolites. Kynurenic acid (KYNA) is broad-spectrum endogenous AMPA- and NMDA-receptors antagonists (Stone, 2001), whereas quinolinic-acid (QUNA) is endogenous NMDA-agonist (Stone, 2001). Electrophysiological studies of other KP metabolites have so far failed to determine the direct effects of these metabolites on neuronal activity (Stone, 2001); however, recently xanthurenic acid (XTRA) and cinnabarinic acid (CNBA) have been identified as endogenous agonists of group II (II-mGluR) and group III (III-mGluR) metabotropic glutamate receptors (mGluRs), respectively (Fazio et al., 2012; Copeland et al., 2013). Activation of pre-synaptic

* Corresponding author. Tel.: +81 59 2315018; fax: +81 59 2315208.
E-mail address: okadamot@clin.medic.mie-u.ac.jp (M. Okada).
[1] Kouji Fukuyama and Motohiro Okada contributed equally to this work.

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II-mGluR and III-mGluR reduce neurotransmitter release via inhibition of adenylyl cyclase and voltage-sensitive Ca^{2+} channels, respectively (Cenci, 2007; Duty, 2010). Several pre-clinical studies have suggested that activation of both II-mGluR and III-mGluR is potentially important drug targets to provide both symptom relief and neuroprotection in PD (Duty, 2010; Nicoletti et al., 2011).

Zonisamide (ZNS), 3-sulfamoylmethyl-1,2-benzisoxazole, which was developed by Dainippon Pharma (Osaka, Japan: currently Dainippon-Sumitomo Pharma), is used as an antiepileptic drug in Japan, South Korea, USA and Europe (Seino and Leppik, 2007). Several clinical studies have reported the wide clinical spectrum of ZNS against psychiatric and neurological disorders, including epilepsy (Seino and Leppik, 2007), mood-disorders (McElroy et al., 2005), schizophrenia (Nakagawa et al., 2012), essential-tremor (Bermejo, 2007), and its protective effects against ischemic cerebral damage (Willmore, 2004). Especially, ZNS is effective in PD at doses lower than the therapeutic doses for epilepsy (Epi-dose: 200–600 mg/day) (Murata, 2004; Murata et al., 2007; Seino and Leppik, 2007). Japanese clinical study has demonstrated that ZNS (25 and 50 mg/day) improved motor symptoms of PD patients treated with L-DOPA, and 50 mg/day ZNS improved L-DOPA-induced dyskinesia (Murata et al., 2007). However, the exact mechanism of action of ZNS when used at the therapeutic dose in PD (PD-dose: 25–50 mg/day) remains to be clarified (Yamamura et al., 2009). The major mechanisms of antiepileptic actions of ZNS (Rogawski and Porter, 1990) are considered to be inhibition of voltage-gated Na^+ channel (Rogawski and Porter, 1990), T-type voltage-sensitive Ca^{2+} channel (Suzuki et al., 1992) and Ca^{2+}-induced Ca^{2+}-releasing system (Yoshida et al., 2005, 2007). Furthermore, candidate mechanisms of anti-parkinsonian effects of ZNS are inhibition of monoamine-oxidase (Okada et al., 1992, Okada et al., 1995), enhancement of dopamine turnover (Okada et al., 1992, 1995), neuroprotection via activation of glutathione (Asanuma et al., 2010) and inhibition of oxidative stress-induced expression of caspase-3 (Yurekli et al., 2013). However, the PD-dose of ZNS does not affect these targets (Okada et al., 1992, 1995; Yamamura et al., 2009). We have already demonstrated that at the PD-dose, ZNS inhibits striato-pallidal GABAergic-transmission via activation of striatal δ1-receptor (Yamamura et al., 2009). However, the exact mechanisms of the inhibitory effects of ZNS on subthalamo-nigral glutamatergic-transmission remain obscure.

To determine the anti-parkinsonian effects of ZNS at the PD-dose, we examined the effects of ZNS on astroglial KP transmission associated with cytokine activation, employing primary cultured astrocytes, and measuring transmission in both direct and indirect pathways of basal ganglia using microdialysis.

2. Materials and methods

Animal care and the experimental procedures described in this report complied with the Ethical Guidelines established by the Institutional Animal Care and Use Committee at Mie University. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (McGrath et al., 2010).

2.1. Chemical agents

Zonisamide was provided by Dainippon-Sumitomo Pharma (Osaka, Japan). Synaptobrevin inhibitor, tetanus-toxin (TeNT) (Tanahashi et al., 2012; Yamamura et al., 2013), and glial-toxin, fluorocitrate (FLC) (Alexander et al., 2011), 3-hydroxykynurenine (3H-KYN), XTRA and CNBA were obtained from Sigma (St. Louis, MO). Voltage-sensitive Na^+ channel inhibitor, tetrodotoxin (TTX) (Alexander et al., 2011) was obtained from Wako Chemicals (Osaka, Japan). Rat recombinant IFNγ and TNFα were purchased from Biolegend (San Diego, CA) and R&D system (Minneapolis, MN), respectively. II-mGluR agonist, LY354740 (Alexander et al., 2011), II-mGluR antagonist, LY341495 (Alexander et al., 2011), III-mGluR agonist, L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) (Alexander et al., 2011), and III-mGluR antagonist, (RS)-α-cyclopropyl-4-phosphonophenyl glycine (CPPG) (Alexander et al., 2011) were obtained from Tocris Bioscience (Bristol, UK).

2.2. Primary astrocyte culture

Astrocytes were prepared using a protocol described previously (Tanahashi et al., 2012; Yamamura et al., 2013) with some modification. Neonatal Sprague-Dawley rats (SLC, Shizuoka, Japan) ($n = 16$) were sacrificed by decapitation at 0–24 h of age and the cerebral hemispheres were removed under dissecting microscope, followed by preparation of cortical astrocyte cultures. Briefly, the cortical tissue was chopped into fine pieces using scissors and then triturated briefly with a micropipette. Suspension was filtered using 70 μm nylon mesh (BD, Franklin Lakes, NJ) and centrifuged. Pellets were re-suspended in 10 mL Dulbecco's modified Eagle's medium containing 10% fetal calf serum (fDMEM) (repeated three times). After 14 day culture (DIV14), contaminating cells were removed by shaking in standard incubator for 16 h at 200 rpm. On DIV21, astrocytes were removed from flasks by trypsinization and seeded onto translucent PET membrane (1.0 μm) with 12- or 24-well plates (BD) directly at a density of 10^5 cells/cm^2 for experiments (Tanahashi et al., 2012; Yamamura et al., 2013). During DIV21-DIV28, the culture medium was changed twice a week, and cultured cells were treated by the test agent (detailed methods are described under "Treatment of astrocytes and study design"). On DIV28, the cultured astrocytes were washed out using artificial cerebrospinal fluid (ACSF, in mM: NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, and glucose 5.5, and buffered with 20 mM HEPES buffer to pH 7.3) (repeated three times). After the wash-out, astrocytes were incubated in ACSF (100 μL/translucent PET membrane) at 35 °C for 60 min in a CO_2 incubator. Each collected 100 μL ACSF sample was treated with 100 L of ethyl-4-OH-2-quin (20 nM) for internal standards, and filtrated by Vivaspin 500-3K (Sartorius, Goettingen, Germany). Each experiment was performed in triplicate using cultures derived from brains of three different rats. The filtered samples were freeze-dried and stored at −80 °C until analysis. The freeze-dried sample was treated with 50 μL acetonitrile containing 5% ammonium (vol/vol) for extreme liquid chromatography (xLC) with mass-spectrum (xLCMS) analysis.

2.3. Treatment of astrocytes and study design

2.3.1. Treatment with IFNγ, TNFα and 3H-KYN

To study the effects of IFNγ, TNFα and 3H-KYN on KP metabolites, astrocytes collected at DIV21 were incubated on translucent PET membrane for 7 days (from DIV21 to DIV28) in fDMEM with or without IFNγ (100 U/mL), TNFα (100 U/mL) or 3H-KYN (1, 10 and 100 μM) (Tanahashi et al., 2012; Yamamura et al., 2013).

2.3.2. Treatment with TeNT, FLC and TTX

To study the effects of TeNT on KP metabolites, astrocytes collected at DIV27 were incubated on translucent PET membrane for 1 day (from DIV27 to DIV28) in fDMEM with or without TeNT (3 μg/mL) (Tanahashi et al., 2012; Yamamura et al., 2013). To study the effects of FLC on KP metabolites, astrocytes collected at DIV28 were incubated on translucent PET membrane for 8 h in fDMEM with or without FLC (1 mM) (Tanahashi et al., 2012; Yamamura et al., 2013). To study the effects of TTX on KP metabolites, astrocytes collected at DIV28 were washed out with ACSF, then incubated on translucent PET membrane for 1 h in ACSF with or without TTX (1 μM) (Tanahashi et al., 2012; Yamamura et al., 2013).

2.3.3. Treatment with ZNS

To study the effects of long-term treatment with ZNS on KP metabolites, astrocytes collected at DIV21 were incubated for 7 days (from DIV21 to DIV28) on translucent PET membrane in fDMEM with or without ZNS (10, 30, 100 μM) (Tanahashi et al., 2012; Yamamura et al., 2013). To study the acute effects of ZNS on KP metabolites, astrocytes were collected at DIV28, washed out using ACSF, then incubated on translucent PET membrane for 2 h in ACSF with or without ZNS (10, 30, 100 μM) (Tanahashi et al., 2012; Yamamura et al., 2013).

2.4. Preparation of microdialysis system

Male Sprague–Dawley rats, weighing 250–300 g ($n = 108$), were placed in a stereotaxic frame and kept under 1.8% isoflurane anesthesia. Two concentric I-shaped direct insertion type dialysis probes were implanted; D-I-7-02 (0.22 mm diameter, 2 mm exposed membrane: Eicom) in lateral globus pallidus (LGP: $A = -1.6$ mm, $L = -3.5$ mm, $V = -7.2$ mm, relative to bregma) and D-I-9-01 in substantia nigra pars reticulata (SNr: $A = -5.6$ mm, $L = -2.3$ mm, $V = -8.4$ mm, relative to bregma) (Paxinos and Watson, 1998). Perfusion experiments commenced 18 h after recovery from anesthesia (Tanahashi et al., 2012; Yamamura et al., 2013). The perfusion rate was set at 1 μL/min in all experiments, using modified Ringer's solution (MRS) composed of (in mM) 145 Na^+, 2.7 K^+, 1.2 Ca^{2+}, 1.0 Mg^{2+}, and 154.4 Cl^-, buffered to pH 7.4 with 2 mM phosphate buffer and 1.1 mM Tris buffer (Tanahashi et al., 2012; Yamamura et al., 2013). Perfusion commenced using MRS alone. Extracellular levels of L-glutamate and GABA were measured at 6 h after the start of perfusion. After collection, each dialysate sample was injected immediately into xLCMS. When the coefficient of variation of the level of each neurotransmitter was less than 5% over 60 min (stabilization period), control data were obtained over