Neuroscience Letters 609 (2015) 18–22

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Propofol effects on cerebellar long-term depression

Kwan Young Lee[a,b], Young Im Kim[a,c], Se Hoon Kim[a], Hyung Seo Park[a], Youn Joon Park[d], Myung Sook Ha[e,f], Yunju Jin[g], Dong Kwan Kim[a,f,*]

[a] Department of Physiology, Konyang University, College of Medicine, Daejeon, South Korea
[b] Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[c] Department of Anesthesiology, Yeosu Chonnam Hospital, Yeosu, South Korea
[d] Department of Pediatric Surgery, Dankook University, College of Medicine, Cheonan, South Korea
[e] Department of Ophthalmology, Konyang University Hospital, Daejeon, South Korea
[f] Myunggok Medical Research Institute, Konyang University College of Medicine, Daejeon, South Korea
[g] Department of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

HIGHLIGHTS

- The effect of propofol was studied in cerebellar parallel fiber-Purkinje cell synapses.
- Propofol impaired cerebellar long-term synaptic plastcitiy.
- Propofol suppressed mGluR1 activity in cerebellar Purkinje cells.

ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form
27 September 2015
Accepted 30 September 2015
Available online 9 October 2015

Keywords:
Cerebellum
Metabotropic glutamate receptor 1
Long-term depression
Propofol
Purkinje cell
Parallel fiber

ABSTRACT

Propofol is an intravenously administered anesthetic that induces γ-aminobutyric acid-mediated inhibition in the central nervous system. It has been implicated in prolonged movement disorders. Since the cerebellum is important for motor coordination and learning, we investigated the potential effects of propofol on cerebellar circuitry. Using the whole-cell patch-clamp technique in Wister rat cerebellar slices, we demonstrated that propofol administration impaired long-term depression from the parallel fiber (PF) to Purkinje cell (PC) synapses (PF–LTD). Also, propofol reduced metabotropic glutamate receptor 1 (mGluR1)-mediated and group I mGluR agonist-induced slow currents in PCs. These results suggest that the propofol-induced PF–LTD impairment may be related to an alteration in mGluR1 signaling, which is essential to motor learning.

1. Introduction

Propofol (2,6-diisopropylphenol) is a widely used short-acting intravenous anesthetic that enhances γ-aminobutyric acid type A (GABAA) receptors activity [23]. Previous studies by other researchers have demonstrated that propofol may also modulate glutamate release and receptors in hippocampal and cortical synaptic transmission [19,22]. In addition, propofol produces a hypnotic effect by suppressing neuronal activity, and an amnesic effect via impaired hippocampal long-term potentiation and facilitated long-term depression (LTD) [21]. Especially, propofol has other adverse effects that cause prolonged movement disorders including dystonia, myoclonus and ataxia [3,5,25,27]. However, the mechanisms of these movement disorders have not been identified.

Abbreviations: aCSF, artificial cerebrospinal fluid; DHPG, (RS)-3,5-dihydroxyphenylglycine; DMSO, dimethyl sulfoxide; GABAA, γ-aminobutyric acid type A; LTD, long-term depression; LTP, long-term potentiation; mGlyRl, metabotropic glutamate receptor 1; PC, Purkinje cell; PF, parallel fiber; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; PKC, Protein kinase C; D-Ser, D-serine; GluD2, δ2 glutamate receptor; TRPC, transient receptor potential cation channels.

* Corresponding author at: Department of Physiology, Konyang University, College of Medicine, 158 Gwanjeodong-ro, Seo-gu, Daejeon 302-718, South Korea. Fax: +82 42 600 6314.
E-mail addresses: lky@life.illinois.edu (K.Y. Lee), thinker96@hanmail.net (Y.I. Kim), sehkim@konyang.ac.kr (S.H. Kim), hspark@konyang.ac.kr (H.S. Park), psurgeon@naver.com (Y.J. Park), hmseye@hanmail.net (M.S. Ha), yjin4@jhmi.edu (Y. Jin), 92dodong@gmail.com (D.K. Kim).

http://dx.doi.org/10.1016/j.neulet.2015.09.037
0304-3940/

Since the cerebellum is important for coordinating motor activities and the acquisition of novel motor skills, propofol-induced movement disorders may be a result of cerebellar dysfunction [9,10]. Purkinje cells (PCs) are the sole output of the cerebellar cortex and provide signals required for motor planning, execution, and coordination in their neuronal activity [12,24]. Granule cell axons, known as parallel fibers (PFs), synapse onto PCs and changes in the strength of PF–PC synapses can influence appropriate motor outputs [12,20,24]. LTD at PF–PC synapse (PF–LTD) is one of the mechanisms underlying motor learning in the cerebellum [4,12,24]. Previous studies have identified PF–LTD impairments in animal models with motor dysfunction [1,11,17]. Therefore, we hypothesize that propofol may affect PF–LTD, which consequently could cause dysfunction of cerebellar circuits and motor tone disorders. In essence, this study aims to clarify the actions of propofol on PF–LTD in the cerebellar cortex using whole-cell patch-clamp recordings.

2. Materials and methods

2.1. Cerebellar slice preparation

Experiments were performed on postnatal Wister rats aged between 15 and 25 days. All animal procedures were carried out in accordance with the regulations of the Institutional Animal Care and Use Committee in Konyang University (Daejeon, Korea). The rats were decapitated after being anesthetized with ether, and the cerebella were rapidly removed and dissected in an ice-cold solution containing (in mM) 220 sucrose, 2.5 KCl, 1 Na_2HPO_4, 2.5 $MgCl_2$, 0.5 $CaCl_2$, 25 $NaHCO_3$, and 20 D-glucose, bubbled with 95% O_2 and 5% CO_2. Parasagittal slices (200-μm thick) from the cerebellar hemisphere were prepared using a VT-1000 M vibratome (Leica, Germany). All experiments were performed at 30–32 °C (TC-324B; Warner Instrument, USA) after initial 1-h incubation at 34 °C.

2.2. Whole cell patch-clamp recording

After incubation, the recording chamber was continuously perfused at a rate of 1.5 ml/min with oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM) 125 mM NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 25 D-glucose bubbled with 95% O_2 and 5% CO_2. Picrotoxin (100 μM; Sigma–Aldrich, USA) was added to aCSF for all experiments. The recordings were made from PCs in lobules IV–VI, which were visually identified based on their location using a model BX50WI upright microscope (Olympus, Japan) with Nomarski optics and a 40 × water-immersion objective. Recording pipettes (GC150T-7.5; Harvard Apparatus, Canada) were fabricated by pulling glass capillaries on a model PP-830 microelectrode puller (Narishige Scientific Instruments, Japan). Patch pipettes had resistances of 3–5 MΩ. All experiments were performed in PCs voltage clamped at −70 mV in whole-cell configuration. The standard internal solution was a K-based solution containing (in mM) 140 K-gluconate, 10HEPES, 0.1 EGTA, 4 KCl, and 5 Mg-ATP (pH adjusted to 7.3 with KOH). Experiments were performed using an EPC-10 amplifier; stimulation and data acquisition were controlled using PatchMaster software (HEKA Elektronik, Germany). Signals were filtered at 3 kHz and digitized at 10 kHz. LTD was studied by monitoring PF-EPSCs every 15 s. After obtaining PF-EPSCs that were stable for 10 min, a pairing stimulation was applied for LTD induction. Pairing stimulation was accomplished by coupling stimulation of the PFs in the molecular layer (two pulses separated by 60 ms, delivered every second) with depolarization of the PC (to about 0 mV for 120 ms); total protocol duration was 2 min [17]. Recordings were discarded when input resistance values changed by >20% or the holding current values exceeded −650 pA. Our control data were recorded in the presence of dimethyl sulfoxide (DMSO; Sigma–Aldrich, USA). Propofol (Sigma–Aldrich, USA) was dissolved in DMSO to make a 250 mM stock solution, which was diluted into the external solution to the desired propofol concentration. To record metabotropic glutamate receptor 1 (mGluR1) mediated slow current, PF stimulation consisted of 10 pulses at 100 Hz in the presence of the AMPA/kainate receptor blocker, NBQX (10 μM; Tocris, USA). Focal puff application of the group I metabotropic glutamate receptor agonist (RS)-3,5-dihydroxyphenylglycine (DHPG, 100 μM; Tocris, USA) was achieved by pressure application (100 ms

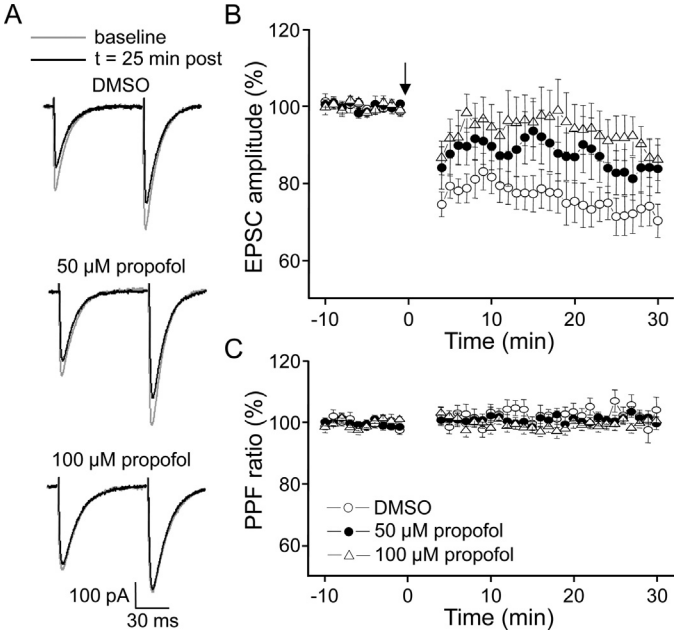

Fig. 1. Propofol impairs long-term depression (LTD) at PF–PC synapses. (A) Superimposed traces of PF–EPSCs measured before and after pairing protocol (25 min). (B) Averaged time course of normalized EPSC amplitude in 0.04% DMSO (open circles, $n = 11$), 50 μM propofol (closed circles, $n = 12$), and 100 μM propofol (open triangles, $n = 9$). Data were normalized to a baseline (−10 to 0 min) before LTD induction. LTD was observed in DMSO, whereas LTD was altered in propofol (50, 100 μM). The arrow denotes pairing protocol. (C) The paired-pulse ratio was normalized and unchanged by pairing protocol. Data shown represent the mean ± SEM.