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European Journal of Pharmacology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Propofol selectively alters GluA1 AMPA receptor phosphorylation in the hippocampus but not prefrontal cortex in young and aged mice

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A R T I C L E I N F O

Article history:
Received 9 January 2014
Received in revised form
27 May 2014
Accepted 28 May 2014

Q3 *Keywords:*
Q4 Glutamate
GluA1
S831
S845
Anesthesia
Postoperative cognitive dysfunction
Righting reflex
Hippocampus
Prefrontal cortex

A B S T R A C T

Propofol is a commonly used general anesthetic agent which has been previously shown to enhance the inhibitory GABAergic transmission in the central nervous system. In addition to the GABAergic element, the excitatory transmission may be another central molecular site impacted by propofol. Increasing evidence implies that the *alpha*-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor represents an excitatory amino acid receptor subtype subjected to the regulation by propofol. Indeed, in this study, we found that a single injection of propofol at an anesthetic dose increased AMPA receptor GluA1 subunit phosphorylation in young (2–3 months old) and aged (20–21 months old) mice in vivo. Propofol caused an increase in GluA1 phosphorylation in the hippocampus but not in the prefrontal cortex. The propofol effect was also site-selective as the drug elevated GluA1 phosphorylation at serine 831 (S831) but not serine 845. Interestingly, while propofol induced a moderate and transient increase in S831 phosphorylation in young mice, the drug caused a substantial and sustained S831 phosphorylation in aged animals. Total GluA1 abundance remained stable in the hippocampus and prefrontal cortex in both young and aged mice in response to propofol. These results provide evidence supporting the sensitivity of GluA1 AMPA receptors to propofol. A single dose of propofol was able to upregulate GluA1 phosphorylation in the confined hippocampus in an age-dependent manner.

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1. Introduction

The neurotransmitter glutamate interacts with postsynaptic glutamate receptors to regulate cellular and synaptic activities. A major ionotropic glutamate receptor subtype is the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor which is broadly expressed in the mammalian brain and mediates fast synaptic transmission (Traynelis et al., 2010). AMPA receptors form functional channels by assembling four subunits (GluA1-4 or formerly GluR1-4) into homo- or heterotetramers. Like many other synaptic proteins, AMPA receptors are regulated by posttranslational phosphorylation (reviewed in Mao et al., 2011; Wang et al., 2014). GluA1 is a major subunit subjected to phosphorylation. In relatively long intracellular C-terminal (CT) tails of this subunit, phosphorylation occurs at two serine residues: serine 831 (S831) and serine 845 (S845) (Roche et al., 1996; Barria et al., 1997; Mammen et al., 1997; Serulle et al., 2007). While S831 is phosphorylated by protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), S845 is phosphorylated by protein kinase A (PKA) and cGMP-dependent protein kinase II (cGKII). By changing phosphorylation levels at these sites, the responsible protein kinases modulate neurochemical and physiological properties of GluA1 AMPA receptors (Mao et al., 2011; Wang et al., 2014).

Anesthetic agents are believed to impact specific molecular substrates in the central nervous system to induce general anesthesia or other effects. Many anesthetic agents target the inhibitory gamma-aminobutyric acid $(GABA)_A$ ion channel to enhance its activity, thereby inducing anesthesia (Hales and Lambert, 1991; Sonner et al., 2003; Irifune et al., 1999, 2003; Jurd et al., 2003). In addition to inhibitory ion channels, excitatory ion channels are among central targets sensitive to anesthetics (Harris et al., 1995). To this end, excitatory AMPA receptor ion channels draw particular attention. Available data show that propofol, a general anesthetic which is widely used for induction and maintenance of general anesthesia and sedation, altered homomeric GluA1 receptor- or heteromeric GluA1/GluA2 receptor-mediated currents in transfected

Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisozazole-4-propionic acid; AUC, area under the curve; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; cGKII, cGMP-dependent protein kinase II; EGTA, ethylene glycol tetraacetic acid; GABA, gamma-aminobutyric acid; HEPES, hydroxyethyl piperazineethanesulfonic acid; LORR, loss of righting reflex; PFC, prefrontal cortex; PKA, protein kinase A; PKC, protein kinase C; POCD, postoperative cognitive dysfunction; SDS, sodium dodecyl sulfate; S.E.M., standard error of the mean

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http://dx.doi.org/10.1016/j.ejphar.2014.05.053
0014-2999/© 2014 Published by Elsevier B.V.

Please cite this article as: Mao, L.-M., et al., Propofol selectively alters GluA1 AMPA receptor phosphorylation in the hippocampus but not prefrontal cortex in young and aged mice. Eur J Pharmacol (2014), http://dx.doi.org/10.1016/j.ejphar.2014.05.053

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heterologous cells (Yamakura et al., 1995; Krampfl et al., 2005). Propofol also increased GluA1 S845 phosphorylation in cultured rat striatal and cortical neurons (Haines et al., 2008). However, little is known about the effect of propofol on AMPA receptor phosphorylation in rodent brains in vivo. Moreover, it is unclear whether propofol has a different influence on AMPA receptor phosphorylation in young versus aged animals.

In this study, we initiated an effort to investigate the impact of propofol on AMPA receptor phosphorylation in mouse brains with an emphasis to compare the response of AMPA receptors between young and aged animals in vivo. We carried out a time-course study to monitor time-dependent changes in GluA1 phosphorylation at S831 and S845 sites following a systemic injection of propofol at an anesthetic dose that induced reliable loss of righting reflex (LORR). Two forebrain regions that are intimately implicated in cognitive and memory functions, i.e., the prefrontal cortex (PFC) and hippocampus, were analyzed in parallel to define the effect of propofol on GluA1 phosphorylation.

2. Materials and methods

2.1. Animals

C57BL/6 mice were obtained from Charles River (New York, NY). Young (2–3 months old weighing 21 to 26 g) and aged (20–21 months old weighting 40 to 49 g) mice were used. Given that the mean lifespan of C57BL/6 mice is approximately 27 months (Turturro et al., 1999) as compared to 80 years known for human lifespan, a mouse with 20 months of age corresponds to approximately 60 years of a human age. Animals were individually housed at 23 °C and humidity of 50 ± 10% with food and water available ad libitum. The animal room was on a 12/12 h light/dark cycle with lights on at 0700. All animal use procedures were in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee.

2.2. Anesthesia

Propofol (2,6-diisopropylphenol) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and was freshly prepared on the day of the experiment. Propofol was prepared to a 25 mg/ml solution in intralipid (Sigma). To induce general anesthesia, mice were injected intraperitoneally (i.p.) with propofol at a single dose of 250 mg/kg. Anesthesia was assessed by measuring LORR, which usually occurred between 2 and 4 min after drug injection (as tested every 20 s or as needed). The selection of the anesthetic dose of 250 mg/kg was based on the ED_{50} value of 140 mg/kg (i.p.) in mice for inducing LORR (Irifune et al., 1999) and the fact that propofol at 250 mg/kg (i.p.) caused LORR in all mice without death (Snyder et al., 2007) and that propofol at this dose (i.p.) increased Tau phosphorylation in the mouse hippocampus (Whittington et al., 2011). Mice that received injection of an equivalent volume of intralipid served as a vehicle control. These control mice were returned to their home cages at room temperature after injection. Mice treated with propofol were initially returned to home cages. Once they lost their righting reflex, they were placed in a heating device maintaining an environmental temperature at 37 °C. Body temperature of mice was monitored with a rectal probe (TCAT-2 controller, Harvard Apparatus, Holliston, MA).

2.3. Brain protein extraction

Mice were killed by cervical dislocation at the time indicated. Brains were immediately removed. The PFC and hippocampus were quickly dissected on ice. Brain tissue was homogenized in an ice-cold, isotonic homogenization buffer containing 0.32 M sucrose, 10 mM HEPES, pH 7.4, 2 mM EDTA, a protease inhibitor cocktail (Thermo Scientific, Rochester, NY), and a phosphatase inhibitor cocktail (Thermo Scientific). Homogenates were then centrifuged at 760 g for 10 min. The supernatant was centrifuged again at 10,000 g for 30 min. The pellet 2 (P2) containing crude synaptosomal plasma membranes was washed and centrifuged at 10,000 g (30 min). The washed P2 was solubilized in the homogenization buffer containing 0.5% Triton X-100 and 1% SDS. Protein concentrations were determined with a Pierce BCA assay kit. Samples were stored at −80 °C until use.

2.4. Western blot analysis

Western blots were performed as described previously (Guo et al., 2010; Jin et al., 2013). Briefly, proteins were separated on SDS NuPAGE Bis-Tris 4–12% gels (Invitrogen, Carlsbad, CA). They were then transferred to polyvinylidene fluoride membranes. Membranes were incubated with primary antibodies overnight at 4 °C. This was followed by an incubation of a secondary antibody (1:2000). Immunoblots were developed with the enhanced chemiluminescence reagent (GE Healthcare Life Sciences, Piscataway, NJ). MagicMark XP Western protein standards (Invitrogen) were used for protein size determination. Immunoblots were measured using NIH gel analysis software. The values reflect relative density of the bands normalized to actin. Primary antibodies used in this study include the rabbit polyclonal antibodies against GluA1 with phosphorylated S831 (pS831) (PhosphoSolutions, Aurora, CO), GluA1 pS845 (PhosphoSolutions), GluA1 (Millipore, Billerica, MA), or actin (Millipore).

2.5. Behavioral assessment

We monitored righting reflex in mice after propofol administration to assess the state of anesthesia and to compare the anesthetic effect of propofol between young and aged mice. Righting reflex was scored according to the rating scale described previously (Irifune et al., 2003). In this score system, a score of 0 reflected a normal righting reflex; +1 indicated that animals righted themselves within 2 s on all three trials (slightly impaired righting reflex); +2 specified a righting response with a latency period of >2 s, but <10 s in three trials (i.e., moderately or severely impaired righting reflex); and +3 corresponded to the loss of righting reflex (no righting responses within 10 s on all three trials).

2.6. Statistics

The results are presented as means ± S.E.M. The righting reflex behavioral data were analyzed by calculating area under the curve (AUC) for the rating values plotted against time, followed by Student's *t*-test for group comparison. The Western blot data were evaluated using Student's *t*-test or a one-way analysis of variance, as appropriate, followed by a Bonferroni (Dunn) comparison of groups using least-squares-adjusted means. Probability levels of <0.05 were considered statistically significant.

3. Results

3.1. Normal levels of pS831 and pS845 proteins in the PFC and hippocampus

The PFC and hippocampus are key forebrain structures implicated in the cognitive regulation (Arushanyan and Beier, 2008;

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