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Physiological and pharmacological properties of inhibitory postsynaptic currents mediated by α5β1γ2, α5β2γ2 and α5β3γ2 $GABA_A$ receptors

Xiumin Chen, Angelo Keramidas, Joseph W. Lynch*

Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia

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ABSTRACT

α5-containing $GABA_ARs$ are potential therapeutic targets for clinical conditions including age-related dementia, stroke, schizophrenia, Down syndrome, anaesthetic-induced amnesia, anxiety and pain. α5-containing $GABA_ARs$ are expressed in layer 5 cortical neurons and hippocampal pyramidal neurons where they mediate both tonic currents and slow inhibitory postsynaptic currents (IPSCs). A range of drugs has been developed to specifically modulate these receptors. The main α5-containing $GABA_ARs$ that are likely to exist *in vivo* are the α5β1γ2, α5β2γ2 and α5β3γ2 isoforms. We currently have few clues as to how these isoforms are distributed between synaptic and extrasynaptic compartments or their relative roles in controlling neuronal excitability. Accordingly, the aim of this study was to define the basic biophysical and pharmacological properties of IPSCs mediated by the three isoforms in a hippocampal neuron-HEK293 cell co-culture assay. The IPSC decay time constants were slow (α5β1γ2L: 45 ms; α5β1γ2L: 80 ms; α5β3γ2L: 184 ms) and were largely dominated by the intrinsic channel deactivation rates. By comparing IPSC rise times, we inferred that α5β1γ2L $GABA_ARs$ are located postsynaptically whereas the other two are predominantly perisynaptic. α5β3γ2L $GABA_ARs$ alone mediated tonic currents. We quantified the effects of four α5-specific inverse agonists (TB-21007, MRK-016, α5IA and L-655708) on IPSCs mediated by the three isoforms. All compounds selectively inhibited IPSC amplitudes and accelerated IPSC decay rates, albeit with distinct isoform specificities. MRK-016 also significantly accelerated IPSC rise times. These results provide a reference for future studies seeking to identify and characterize the properties of IPSCs mediated by α5-containing $GABA_AR$ isoforms in neurons.

1. Introduction

$GABA_A$ receptors ($GABA_ARs$) are ligand-gated Cl^- channels that mediate synaptic and extrasynaptic inhibition in the brain. As $GABA_ARs$ are pentameric oligomers constructed from a family of 19 different subunits (α1-6, β1-3, γ1-3, δ, ε, π, θ and ρ1-3), the number of possible stoichiometries is enormous. As with many other types of ion channel, their biophysical and pharmacological properties and their cellular and subcellular distribution patterns vary according to their subunit stoichiometry (D'Hulst et al., 2009; Mele et al., 2016; Sieghart and Sperk, 2002; Vithlani et al., 2011). α5-containing $GABA_ARs$ are found mainly in layer 5 cortical neurons and hippocampal pyramidal neurons where they are located both extrasynaptically and synaptically (Caraiscos et al., 2004; Collinson et al., 2002; Sieghart, 1995; Sieghart and Sperk, 2002; Yamada et al., 2007). Because extrasynaptic α5-containing $GABA_ARs$ can be activated by the low ambient GABA concentrations that exist outside of the synapse, they generate a persistent Cl^- 'leak' current that tonically inhibits neurons (Farrant and Nusser, 2005; Lee and Maguire, 2014). Synaptic α5-containing $GABA_ARs$ contribute a slow component to the GABAergic inhibitory postsynaptic currents (IPSCs) (Caraiscos et al., 2004; Collinson et al., 2002; Glykys et al., 2008; Hausrat et al., 2015; Prenosil et al., 2006; Vargas-Caballero et al., 2010; Zarnowska et al., 2009). α5-containing $GABA_ARs$ are

Abbreviations: $GABA_AR$, GABA type-A receptor; IPSC, inhibitory postsynaptic current; DMEM-FBS, Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum.

* Corresponding author. Queensland Brain Institute, Building 79, University of Queensland, St Lucia, QLD 4072, Australia.
E-mail address: j.lynch@uq.edu.au (J.W. Lynch).

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considered promising therapeutic targets for a range of clinical conditions including age-related dementia, stroke, schizophrenia, Down syndrome, anaesthetic-induced amnesia, anxiety and pain (Botta et al., 2015; Brickley and Mody, 2012; Clayton et al., 2015; Perez-Sanchez et al., 2017; Rudolph and Mohler, 2014; Soh and Lynch, 2015), and indeed a wide range of drugs has been developed to specifically modulate them (Clayton et al., 2015; Soh and Lynch, 2015).

The main α5-containing $GABA_A$Rs that are likely to exist *in vivo* are the α5β1γ2, α5β2γ2 and α5β3γ2 isoforms (Olsen and Sieghart, 2009). We currently have few clues as to how these isoforms are distributed between synaptic and extrasynaptic compartments or their relative contributions to controlling neuronal excitability. A first step towards addressing this would be to define the basic biophysical and pharmacological properties of IPSCs mediated by individual α5-containing $GABA_A$R isoforms. To date, the pharmacological profiles of α5-specific drugs have been evaluated under equilibrium conditions, whereby the drug and a low (e.g., EC_{20}) concentration of GABA have been co-applied for an extended period (typically several seconds) (Ballard et al., 2009; Chambers et al., 2002, 2003; Quirk et al., 1996; Sternfeld et al., 2004). These conditions are relevant to quantifying drug effects on tonically-activated $GABA_A$Rs. However, they are not relevant to IPSCs because the kinetics of the synaptic agonist transient plays a crucial role in determining the susceptibility of IPSCs to pharmacological modulation (Barberis et al., 2011).

The aim of this study was to characterize the physiological and pharmacological properties of IPSCs mediated by the α5β1γ2L, α5β2γ2L and α5β3γ2L $GABA_A$Rs to improve our understanding of their individual roles in controlling neuronal excitability and the mechanisms by which α5-modulating drugs influence neuronal activity. When taken in conjunction with human clinical, animal behavioural and cell biological studies, this information should help provide new insights into which isoform should be preferentially targeted (and how it should be targeted) when seeking to treat the various disorders for which α5-containing $GABA_A$R modulation has been implicated.

The study of individual $GABA_A$R isoforms in neurons is confounded by the large number of $GABA_A$R isoforms present and the unknown or poor selectivity profiles of available pharmacological blockers. This problem can be circumvented via the use of a neuron-HEK293 cell co-culture system, whereby functional 'heterosynapses' can be induced to form between neuronal presynaptic terminals and HEK293 cells that recombinantly express the $GABA_A$R isoform of interest (Brown et al., 2014; Dixon et al., 2015; Dong et al., 2007). Using such a system, we investigated the biophysical properties of IPSCs mediated by recombinant α5β1γ2L, α5β2γ2L and α5β3γ2L $GABA_A$Rs and characterized their sensitivity to four widely-used α5-specific inverse agonists: TB-21007, MRK-016, α5IA and L-655708.

2. Methods

2.1. Drugs

TB-21007 and MRK-016 were obtained from Tocris Bioscience. Bicuculline methiodide, α5IA, L-655708, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), AP5, tetrodotoxin (TTX) and GABA were obtained from Sigma-Aldrich. TB-21007, MRK-016, bicuculline methiodide, α5IA and L-655708 were dissolved in DMSO as 10 mM stock solutions. CNQX and AP5 stocks were dissolved in DMSO at 1 mM and 20 mM, respectively. The TTX and GABA stocks were dissolved in H_2O at 1 and 500 mM, respectively. All stocks were stored at −20 °C until use.

2.2. Primary culture of cortical and hippocampal neurons

Cortical and hippocampal neurons were prepared using methods as recently described (Dixon et al., 2015). Briefly, E18 timed-pregnant rats were euthanized via CO_2 inhalation in accordance with procedures approved by the University of Queensland Animal Ethics Committee. The cortices and hippocampi were rapidly removed, triturated and plated onto poly-D-lysine-coated coverslips in a 4-well plate at a density of $8–10 \times 10^4$ cells/well, and cultured for 3–4 w until spontaneous IPSCs could be detected. The cells were initially cultured in Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum (DMEM-FBS). After 24 h the entire DMEM-FBS medium was replaced with Neurobasal medium that included 2% B27 and 1% GlutaMAX supplements. A second (and final) feed 1 w later replaced half of this medium with fresh Neurobasal medium. Neurons were used in co-culture experiments between 3 and 5 w later.

2.3. HEK 293 cell culture, transfection and heterosynapse formation

HEK293 cells were cultured in DMEM-FBS until approximately 90% confluent. One day prior to transfection, they were trypsinized and plated onto glass coverslips in 35 mm culture dishes at a density of 5×10^3 cells/dish.

This study employed the following plasmid DNAs: human $GABA_A$R α5 (pcDNA3.1Zeo), human $GABA_A$R β1 (pcDNA3.1Zeo), human $GABA_A$R β2 (pcDNA3.1Zeo), human $GABA_A$R β3 (pcDNA Zeo), human $GABA_A$R γ2L (pcDNA3.1), mouse neuroligin 2A (pNice) and GFP (pEGFP). $GABA_A$R clones were obtained from Prof. Neil Harrison (Columbia University), whereas neuroligin 2A was obtained from Addgene (plasmid 15259) and pEGFP from Clontech. Each 35 mm dish of HEK293 cells was transfected with GFP, α5, β and γ2L $GABA_A$R plasmid DNAs in a ratio of 1:1:1:5. Neuroligin 2A (100 ng) was also transfected, with the total plasmid DNA amount totalling around 0.5 μg per dish. Transfection was performed via a Ca^{2+} phosphate-DNA co-precipitation method for 5–20 h in a 3% CO_2 incubator and terminated by washing cells twice with divalent cation-free phosphate buffered saline. Cells were trypsinized the next day, centrifuged and re-suspended in Neurobasal medium (including 2% B27 and 1% GlutaMAX supplements) then seeded onto the neurons. One 35 mm dish of HEK293 cells was typically sufficient to seed four coverslips of neurons. Once seeded with HEK293 cells, the co-cultures were returned to the incubator overnight to allow synapses to form. Cultures were used for patch clamp recording over the following 2–3 days.

2.4. Electrophysiology

Patch clamp recordings were performed at room temperature (22 ± 1 °C) at a clamped potential of −70 mV, in whole-cell or outside-out configurations. All recordings were performed using a Multiclamp 700B amplifier and pClamp10 software (Molecular Devices), filtered at 4 kHz and sampled at 10 kHz. Patch pipettes (4–8 MΩ resistance) were fabricated from borosilicate glass (GC150F-7.5, Harvard Apparatus) and filled with an internal solution comprising (in mM): 145 CsCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES and 10 EGTA, adjusted to pH 7.4 with CsOH. The extracellular solution comprised (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 D-glucose, adjusted to pH 7.4 with NaOH.

For macropatch recordings, pipettes were fire-polished to a resistance of ~10 MΩ and filled with the same internal solution. Outside-out patches pulled from transfected HEK293 cells were activated by brief (<1 ms) exposure to agonists using a piezoelectric translator (Siskiyou). The speed of the solution exchange system was regularly calibrated by rapidly switching the solution