*Neuroscience* 298 (2015) 137–144

# $GABA_A$ RECEPTOR α2 SUBTYPE ACTIVATION SUPPRESSES RETINAL SPREADING DEPRESSION

**M. WANG,** [a,b]* **Y. LI** [a] **AND Y. LIN** [c]

[a] *Centre for Neuroscience, Xi'an Jiaotong-Liverpool University (XJTLU), Suzhou 215123, China*

[b] *Department of Biological Sciences, XJTLU, Suzhou 215123, China*

[c] *Department of Applied Chemistry, XJTLU, Suzhou 215123, China*

**Abstract—Cortical spreading depression (SD) is a transient propagating neuronal excitation followed by depression, which is generally accepted as the underlying cause of migraine. The inhibitory γ-aminobutyric acid type A ($GABA_A$) receptor activation not only reduces cortical SD frequency and propagation, but also relieves migraine headache. This study aims to determine the role of major α subtypes of $GABA_A$ receptor in mediating SD genesis and propagation using an efficient *in vitro* chick retinal model. We firstly demonstrated that abundant α2, and to a lesser extent, α5 of $GABA_A$ receptor expression in the chick retina, enabled the tissue useful for studying $GABA_A$ receptor pharmacology and SD. Marked suppression of SD by SL651498 and TPA023 was observed at 10 μmol $L^{-1}$ and 50 μmol $L^{-1}$, respectively, suggesting a critical role of $GABA_A$ receptor α subtypes, in particular α2, in modulating retinal SD elicitation and propagation. The negative data on NS11394 at 3 μmol $L^{-1}$ on SD and the little positive selectivity of TPA023 for α5 did not support that α5 subtype is involved in SD genesis and propagation. Our data provide strong evidence that α2, but not α5 is involved in the early stage of migraine, indicating that α2 subtype is a possible drug target related to migraine with aura.** 

**Key words: $GABA_A$ receptor, α2, TPA023, migraine, cortical spreading depression, chick retina.**

## INTRODUCTION

Spreading depression (SD) is a transient neuronal excitation followed by depression that can propagate slowly across the cerebral cortex, sub-cortical regions and retina. Cortical SD is generally regarded as the underlying mechanisms of migraine (Lauritzen, 1994, 2001; Ayata, 2010; Bhaskar et al., 2013; Karatas et al., 2013) and it was evidenced that cortical SD wave propagation coincides with the visual scotoma progression in migraine patients with aura (Hadjikhani et al., 2001). Further, chronic daily administration of migraine prophylactic drugs dose-dependently suppressed the frequency of cortical SD (Ayata et al., 2006) and cortical SD may lead to migraine headache involving the activation of a gap junction protein, PANX1 (Karatas et al., 2013). Inhibition of cortical SD could therefore form a preventative strategy against migraine.

Evidence showed that topiramate that has function to positively modulate the inhibitory γ-aminobutyric acid type A ($GABA_A$) receptors (Kawasaki et al., 1998; Braga et al., 2009) elevates cortical SD threshold (Green et al., 2013) and reduces cortical SD frequency and propagation (Unekawa et al., 2012). The important function of $GABA_A$ receptor in SD was supported by the fact that $GABA_A$ receptor inhibition by bicuculline prevented the reduction of cortical SD amplitudes by tumor necrosis factor (Richter et al., 2014). Furthermore, topiramate was reported to relieve migraine headache (Braga et al., 2009) and inflammatory or neuropathic pain (Munro et al., 2009, 2013). The fact that the potentiated $GABA_A$ receptor current modulates neuronal instability during cortical SD that is associated with migraine, suggests $GABA_A$ receptor as a promising target for prophylactic treatment of migraine.

$GABA_A$ receptor is a pentameric ligand-gated ion channel composed of α1–6, β1–3, γ1–3, ρ1–3, ε, π, δ or θ subtypes while the most common combination is two α, two β and two γ subtypes (Hevers and Luddens, 1998; Sieghart, 2006). Variations in the gene expression of different $GABA_A$ receptor subtype isoforms were found in migraine patients, suggesting that specific subtypes of the receptor may play a key role in migraine pathology (Plummer et al., 2011). Furthermore, $GABA_A$ receptor function related to neuropathic pain, anxiety and epilepsy was reported to involve α subtypes and drugs targeting some of these receptor subtypes have been developed (Griebel et al., 2003; Atack et al., 2006; Mirza et al., 2008; Munro et al., 2008). At this point, whether α subtypes of $GABA_A$ receptor activation contribute to suppression of SD remains unknown.

Dissecting out how $GABA_A$ receptor α subtypes that do not work differ mechanistically from those that do will provide avenues through which one can develop new

*Correspondence to: M. Wang, SA451, Department of Biological Sciences, Xi'an Jiaotong-Liverpool University, 111 Ren Ai Road, Suzhou 215123, China. Tel: +86-512-8816-1662.
E-mail addresses: minyan.wang@xjtlu.edu.cn (M. Wang), yanli.li0820@gmail.com (Y. Li), yi.lin@xjtlu.edu.cn (Y. Lin).

*Abbreviations:* AOI, area of interest; cDNA, complementary deoxyribonucleic acid; DMSO, dimethyl sulfoxide; $GABA_A$, γ-aminobutyric acid type A; RNA, ribonucleic acid; RT-PCR, real-time polymerase chain reaction; SD, spreading depression; TBST, Tris-Buffered Saline with Tween-20.

http://dx.doi.org/10.1016/j.neuroscience.2015.04.016
0306-4522/

treatments to treat patients with migraine. The primary aim of the present study was to identify the role of major α subtypes: α2-, α3- and α5-containing $GABA_A$ receptors in SD, by investigating the effects of α subtype-selective and positive modulators in an effective *in vitro* chick retinal model (Wang et al., 2012). To this end, three drugs with a specific subtype selectivity were chosen: (i) SL651498, a selective agonist at α2/α3 subtypes of $GABA_A$ receptors and a partial agonist at α1- and α5-containing $GABA_A$ receptor subtypes (Sans et al., 2000; Griebel et al., 2003; de Haas et al., 2009). (ii) TPA023, a novel α2/α3-selective containing $GABA_A$ receptor agonist (Atack et al., 2006; Munro et al., 2009); and (iii) NS11394 (a positive α5/α3 preferring $GABA_A$ receptor allosteric modulator with a notably higher α5- and α3-containing $GABA_A$ receptor efficacy (Mirza et al., 2008). Expression of corresponding $GABA_A$ receptor subtypes in the chick retina was also thoroughly confirmed and the model was also validated with topiramate.

## EXPERIMENTAL PROCEDURES

All animals were housed in an animal unit of Soochow University with food and water available *ad libitum*. Male Hyline Brown chicks ($n = 37$, purchased at 1-day old, Wuxi poultry Ltd, Jiangsu, China) were housed for at least 1 week before use (aged 8–28 days). Adult, male Sprague–Dawley rats ($n = 3$, Shanghai SLAC Laboratory Animal Corporation Ltd, China) were housed to 300–400 g. All animal procedures were approved by the Ethics Review Panels of Xi'an Jiaotong-Liverpool University (XJTLU) and Soochow University and performed in accordance with the relevant national guidelines.

### Real-time polymerase chain reaction (RT-PCR)

RT-PCR was performed to detect α2, α3, α5 messenger ribonucleic acid (mRNA) transcripts using chick gene-specific primers together with SYBR Green (TaKaRa Biotechnology Co. Ltd., Dalian, China). The primer sequences of α2, α3 and α5 are shown in Table 1.

Following rapid dissection, vitreous was taken away, chick retina peeled away from the eye cup and immediately frozen in liquid nitrogen, stored at −80 °C until further use. Chick retina was homogenized and total RNA was extracted using UNIQ-10 column trizol total RNA isolation kit (SK1321, Sangon Biotech, Shanghai, China). Complementary deoxyribonucleic acids (cDNA) were prepared using AMV First Strand cDNA Synthesis Kit (SK2445, Sangon Biotech, Shanghai, China). The resulting cDNA samples ($n = 3$) were analyzed and quantitative PCR was performed using a RT-PCR Detection System (LightCycler480, Roche Applied Science, Indianapolis, IN, USA) following the manufacturer's instruction. Threshold cycle was automatically calculated by the instrument. All mRNA expression data were expressed as a normalized target, β-actin, ratio in experimental samples.

### Western blot

Western blot was used to examine whether $GABA_A$ receptor α2, α3, α5 proteins are expressed in chick retina. Membrane protein was extracted from the homogenized chick retina using membrane protein extraction kit (Beyotime Institute of Biotechnology, Shanghai, China) by following the manufacturer's instruction with minor modifications. Protein samples (200 μg) were separated by 10% sodium dodecyl sulfate–polyacrylamide loading buffer (NuPAGE® LDS Sample Buffer 4X, Invitrogen, Carlsbad, CA, USA), transferred onto nitrocellulose membranes. Non-specific binding was blocked with 5% milk in Tris-Buffered Saline with Tween-20 (TBST, 20 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 7.6). The membranes were then exposed to rabbit polyclonal anti-$GABA_A$ receptor α2 (ab72445), α3 (ab72446), α5 (ab83003) antibodies (Abcam, Cambridge, UK) respectively and incubated overnight at 4 °C. Once excess primary antibodies were washed by TBST, membranes were incubated with goat anti-rabbit horseradish peroxidase-labeled secondary antibody (Sangon Biotech, Shanghai, China). Protein bands were detected by incubating the membrane with Western Bright enhanced chemiluminescence working solution (Advansta, Menlo Park, CA, USA) and using Kodak medical X-ray film (Kodak XBT-1, Carestream, Xiamen, China). The film was scanned and analyzed with Bio-rad Gel Doc $XR^+$ with Image Lab 2.0 Software (BIO-RAD, Shanghai, China). The same procedure was applied to rat cortex samples and results were used as positive controls. Both rat cortex and retinal samples were run in the same gel for comparison. Detection of membrane NR2A using rabbit polyclonal anti-NR2A receptor (ab106957, Abcam) was also analyzed as positive controls and the 2nd antibody only was used as a negative control.

**Table 1.** Summary of specific primer sequences for real-time PCR

| $GABA_A$R gene | NCBI ref. (*Gallus gallus*) | Primer sequence (5′–3′) |
|---|---|---|
| β-Actin | L08165.1 (Gene bank) | F: AGTGTCTTTTTGTATCTTCCGCC<br>R: CCACATACTGGCACTTTACTCCTA |
| α2 | XM_001233849.1 | F: CAGAACCCAACAAGGCAAAA<br>R: GCCAAGTAAGACAGGCTCCC |
| α3 | XM_420268.2 | F: TCTGTCGCTTTCTCCTGTCTTT<br>R: TTCCCAACTCTACTCTTACTACTTCTG |
| α5 | XM_416880.3 | F: CATCAGTACCAGTACAGGCGAATA<br>R: TGTCACTCCAAAGACAGTCCG |