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Activation of $GABA_{B2}$ subunits alleviates chronic cerebral hypoperfusion-induced anxiety-like behaviours: A role for BDNF signalling and Kir3 channels

Yun Lu [a, 1], Chang-jun Li [b, 1], Cheng Chen [a], Pan Luo [a], Mei Zhou [a], Cai Li [a], Xu-lin Xu [a, c], Qing Lu [a, c], Zhi He [d, *], Lian-jun Guo [a, c, **]

[a] Department of Pharmacology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[b] Neurology Department, Tongji Medical College, Huazhong University of Science and Technology, The Central Hospital of Wuhan, Wuhan 430030, China
[c] Institute of Brain Research, Huazhong University of Science and Technology, Wuhan 430030, China
[d] Department of Neuropsychopharmacology, Medical School of China Three Gorges University, Yichang 443002, China

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ABSTRACT

Anxiety is an affective disorder that is commonly observed after irreversible brain damage induced by cerebral ischemia and can delay the physical and cognitive recovery, which affects the quality of life of both the patient and family members. However, anxiety after ischemia has received less attention, and mechanisms underlying anxiety-like behaviours induced by chronic cerebral ischemia are under-investigated. In the present study, the chronic cerebral hypoperfusion model was established by the permanent occlusion of the bilateral common carotid arteries (two-vessel occlusion, 2VO) in rats, and anxiety-related behaviours were evaluated. Results indicated that 2VO induced obvious anxiety-like behaviours; the surface expressions of $GABA_{B2}$ subunits were down-regulated; Brain derived neurotrophic factor (BDNF), tyrosine kinase B (TrkB) and neural cell adhesion molecule (NCAM) were reduced; Meanwhile, the surface expressions of G protein-activated inwardly rectifying potassium (GIRK, Kir3) channels were up-regulated in hippocampal CA1 in 2VO rats. Baclofen, a $GABA_B$ receptor agonist, significantly ameliorated the anxiety-like behaviours. It also improved the down-regulation of $GABA_{B2}$ surface expressions, restored the levels of BDNF, TrkB and NCAM, and reversed the increased surface expressions of Kir3 in hippocampal CA1 in 2VO rats. However, the effects of baclofen were absent in shRNA-$GABA_{B2}$ infected 2VO rats. These results suggested that activation of $GABA_{B2}$ subunits could improve BDNF signalling and reverse Kir3 channel surface expressions in hippocampal CA1, which may alleviate the anxiety-like behaviours in rats with chronic cerebral hypoperfusion.

1. Introduction

Cerebral ischemia often causes irreversible brain damage with neuronal injury and death (Cao et al., 2015; Wang et al., 2013). Therefore, several negative physiological outcomes are commonly observed in cerebral ischemic patients (Boussi-Gross et al., 2015); however, post-ischemia affective disorder is an often-neglected aspect. Prevalence rates suggest 25% or more stroke survivor cohorts have post-stroke anxiety (Campbell Burton et al., 2013; Menlove et al., 2015). Anxiety can delay physical and cognitive recovery with high morbidity and mortality (Chemerinski and Levine, 2006; Seignourel et al., 2008), which affects the quality of life for both the patient and family members (Mannarini and Boffo, 2015; Nepon et al., 2010). However, the biological mechanisms implicated

Abbreviations: 2VO, two-vessel occlusion; $GABA_B$, γ-aminobutyric acid type B; CA1, Cornu ammonis 1; MWM, Morris water maze test; NOR, novel object recognition test; OFT, open field test; SBI, social behaviour interaction; LTP, long-term potentiation; fEPSP, field excitatory postsynaptic potential; I/O curves, input/output curves; Kir3, G protein-activated inwardly rectifying potassium, GIRK; BDNF, brain derived neurotrophic factor; TrkB, Tyrosine kinase B; NCAM, neural cell adhesion molecule; SNX27, Sorting nexin 27; ZO-2, zonula occludens-2.

* Corresponding author.
** Corresponding author. Department of Pharmacology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China.
E-mail addresses: hezhi2003@126.com.cn (Z. He), ljguo@hust.edu.cn (L.-j. Guo).
[1] Yun Lu and Chang-jun Li contributed equally to this work.

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in cerebral ischemia induced anxiety are not fully clear. Previous studies have reported that both transient and repeated ischemia can induce anxiety-like behaviour, and the hippocampus, especially the CA1 area is sensitive to ischemia and also involved in affective disorders (Sarkaki et al., 2015; Soares et al., 2013). Our previous studies have reported that 2VO results in cognitive impairments accompanied by the down-regulation of $GABA_B$ receptors in hippocampal CA1 (Li et al., 2014). However, whether chronic cerebral hypoperfusion could cause affective disorders, such as anxiety-like behaviour, and whether the underlying mechanism is associated with $GABA_B$ receptors in the CA1 area remain largely unknown.

GABA is the primary inhibitory neurotransmitter in the CNS, which acts on three classes of receptors: $GABA_A$, $GABA_B$, and $GABA_C$. The $GABA_A$ and $GABA_C$ receptors are ligand-gated chloride channels that mediate a fast GABA response, whereas $GABA_B$ receptors are G-protein-coupled receptors (GPCRs) that mediate a slow GABA response. The dysregulation of $GABA_B$ receptor expression and function has been implicated in various CNS disorders (Benke, 2013; Kumar et al., 2013). Interestingly, some reports indicate activation of $GABA_B$ receptor can ameliorate anxiety-like behaviours (Frankowska et al., 2007; Knapp et al., 2007; Mombereau et al., 2005), while other reports suggest blockade of the $GABA_B$ receptor can ameliorate anxiety-like behaviours (Partyka et al., 2007). Although the crucial role of $GABA_B$ receptors in anxiety have been reported for many years, which $GABA_B$ receptor subunit plays the dominant role in anxiety, especially in cerebral ischemia induced anxiety remains unclear. $GABA_B$ receptors are composed of $GABA_{B1}$ and $GABA_{B2}$ subunit. Interestingly, $GABA_{B2}$ but not $GABA_{B1}$ subunit is essential for G-protein coupling, the surface expression of $GABA_B$ receptors and the mediation of downstream signalling (Pooler et al., 2009; Robbins et al., 2001), which indicates that the function of $GABA_{B2}$ subunit is quite crucial. Another study demonstrated that $GABA_{B2}$ subunits are dramatically reduced during oxygen–glucose deprivation and NMDA-induced excitotoxicity in cultured hippocampal slices (Cimarosti et al., 2009), which further highlights the role of $GABA_{B2}$ subunit in cerebral ischemia. Based on the above information, we inferred that the function of $GABA_{B2}$ subunit may be notable in anxiety-like behaviours caused by chronic cerebral hypoperfusion. To address this possibility, we used $GABA_{B2}$ lentiviral shRNA and pharmacological manipulations to explore the underlying mechanisms of anxiety-like behaviours caused by chronic cerebral hypoperfusion.

It has been reported that $GABA_B$ receptor activation triggers the BDNF release and promotes the functional maturation of GABAergic synapses in hippocampus (Fiorentino et al., 2009). BDNF is present throughout the CNS which regulates the synaptic plasticity (Ma et al., 2013; Panja et al., 2014) and neuronal survival (Yao et al., 2012). BDNF levels appear to be reduced in anxiety patients (Suliman et al., 2013) and animal models (Varani et al., 2015), which has been regarded as a potential biomarker for anxiety and other neuropsychiatric disorders. However, less is known regarding whether and how BDNF could be regulated by $GABA_{B2}$ subunits, especially under chronic cerebral hypoperfusion induced anxiety.

BDNF activates its primary receptor TrkB to regulate physiological functions in the brain. Rogalski et al. have reported that BDNF activates TrkB to inhibit the Kir3 channel (Rogalski et al., 2000). One of the major function of Kir3 channel is controlling neuronal excitability (Luscher and Slesinger, 2010). Neuronal Kir3 channels are formed by homo- and hetero-assembly between Kir3.1, 3.2 and 3.3 subunits (Lujan et al., 2014), which have been implicated in psychiatric disorders, such as anxiety behaviours (Kobayashi et al., 2010; Pravetoni and Wickman, 2008). Amount of reports are available on the current of the channel. Surprisingly, few reports have focused on the alterations of Kir3 levels under chronic cerebral ischemia up till now. It is also unknown whether Kir3 channels were implicated in anxiety-like behaviours induced by chronic cerebral hypoperfusion.

The aim of the present study was to explore whether $GABA_{B2}$ subunits play a role in regulating the anxiety-like behaviours induced by chronic cerebral hypoperfusion and investigate the underlying mechanism.

2. Materials and methods

2.1. Animals

Adult male (250–280 g) Sprague-Dawley rats (approval number SCXK(E) 2008-005) were obtained from the Tongji Medical College animal centre. The rats were housed with free access to water and food. The housing room was maintained at 22 ± 2 °C with a 12 h light-dark cycle. Animals were adapted to these conditions for 7 days before experiments. All experiments were approved by the Review Committee for the Care and Use of Laboratory Animals of Tongji Medical College, Huazhong University of Science and Technology. All efforts were made to minimize both the suffering and number of animals used.

2.2. Experimental procedures

The experimental design is detailed in Fig. 1A. 2VO was performed to induce chronic cerebral hypoperfusion as previously described (Ni et al., 1994). Briefly, rats were anesthetized with chloral hydrate (350 mg/kg, i.p.), and the bilateral common carotid arteries were gently separated and double ligated with a 6-0 silk suture and cut between the double ligations. Sham rats only received the vessels separation without ligation. 17 days after 2VO, the repeated baclofen (Meryer Chemical Technology Co., Ltd, Shanghai, China) treatment was performed once daily at 20:00–21:00 during the last 23 days. Based on previous studies (Babcock et al., 2002; Lal et al., 1995), 50 mg/kg baclofen has neuropretective effects on ischemic gerbil hippocampus. According to the formula of Meeh-Rubner equation, we calculated the dose for rats to be about 25–35 mg/kg. In our previous research (Li et al., 2014), we found that 25 mg/kg baclofen could improve the cognitive impairments induced by 2VO. Therefore, 25 mg/kg baclofen was further used in this study which was dissolved in saline at concentration of 2.5 mg/ml and was administered by i.p. injection in a volume of 10 ml/kg. Normal saline was used as vehicle control in the same volume. 34 days after induction of hypoperfusion, cognitive functions were tested to ensure that the 2VO model induces cognitive impairment and all the behavioural tests were carried out in a double-blind manner.

2.3. Stereotaxic injection

The lentivirus-mediated shRNA for silencing $GABA_{B2}$ subunits (sh-$GABA_{B2}$: GGACAAAGACTTGGAAGAA) containing green fluorescence protein (GFP) and a nontargeting sequence as the negative control shRNA (sh-Con) were constructed by Genechem (Shanghai, China). Stereotaxic surgery was performed under anaesthesia with chloral hydrate (350 mg/kg, i.p.). With a 28-gauge stainless steel micro-injection cannula, the rats were injected bilaterally with lentiviral sh-$GABA_{B2}$ (1μl/10min) into the dorsal hippocampal CA1 region (−3.8 mm anterior posterior, ±3.0 mm medial lateral, −2.4 mm dorsal ventral from dura). After completion of the infusion, the injector was left in place for 10 min before withdrawal, and the rats were returned to their home cage. We randomly chose half of the sh-$GABA_{B2}$ infected rats to test the site of injection and the effects of sh-$GABA_{B2}$ on $GABA_{B2}$ subunit expression which showed the efficiency of the sh-$GABA_{B2}$ was stable.