Neuropharmacology 125 (2017) 117–128

Contents lists available at ScienceDirect

Neuropharmacology

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

Overexpression of the 18 kDa translocator protein (TSPO) in the hippocampal dentate gyrus produced anxiolytic and antidepressant-like behavioural effects

Lei Li [a, b, c, e, 1], Wei Wang [d, 1], Li-Ming Zhang [a], Xiang-Yun Jiang [a, b, c], Shu-Zheng Sun [e], Li-Jun Sun [a], Ying Guo [b], Jie Gong [b], You-Zhi Zhang [a], Heng-Lin Wang [b, *], Yun-Feng Li [a, **]

[a] State Key Laboratory of Toxicology and Medical Countermeasures, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China
[b] Department of Anesthesiology, Hospital NO.309 of Chinese People's Liberation Army, Beijing 10091, China
[c] Department of Postgraduate, Hebei North University, Zhangjiakou 075000, China
[d] Department of Anesthesiology, The General Hospital of the PLA Rocket Force, Beijing 100088, China
[e] Department of Anesthesiology, Beijing Chuiyangliu Hospital, Beijing 10022, China

ARTICLE INFO

Article history:
Received 1 December 2016
Received in revised form
18 June 2017
Accepted 22 June 2017
Available online 24 June 2017

Keywords:
Dentate gyrus
18 kDa translocator protein
Allopregnanolone
Anxiety and depression

ABSTRACT

The 18 kDa translocator protein (TSPO) is a five transmembrane domain protein that plays a crucial role in neurosteroid (e.g., allopregnanolone) synthesis by promoting the transport of cholesterol to the inner mitochondrial membrane. This protein is predominantly expressed in steroid-synthesizing tissues, including the central and peripheral nervous system, affecting stress-related disorders such as anxiety and depression. Recent studies have focused on the hippocampal dentate gyrus, which is very important for involvement of anxiety and depression. However, the exact role that TSPO plays in the pathophysiology of anxiety and depression and the involvement of the hippocampal dentate gyrus in regulating these behavioural effects remain elusive. This study used the lentiviral vectors mediating TPSO overexpression to assess the effects of TPSO overexpression in the hippocampal dentate gyrus on anxiolytic and antidepressant-like behavioural effects in mice. The expression of TSPO and the concentration of allopregnanolone in hippocampus tissues (3 mm in diameter around the injection site on both sides) were measured by Western blot and ELISA, respectively. The results indicated that microinjection of the LV-TSPO resulted in a significant increase in TSPO expression and allopregnanolone concentration in the hippocampus. Moreover, TSPO overexpression of the mouse hippocampal dentate gyrus generated significant anxiolytic and antidepressant-like behavioural effects in a series of behavioural models. These effects were completely blocked by the TSPO antagonist PK11195 (3 mg/kg, intraperitoneally) and the 5α-reductase inhibitor finasteride (5 mg/kg,intraperitoneally). Meanwhile, the increased allopregnanolone was also reversed by PK11195 and finasteride. In addition, neither PK11195 nor finasteride had an effect on the expression of TSPO. Overall, our results are the first to suggest that the overexpression of TSPO in the hippocampal dentate gyrus produced anxiolytic and antidepressant-like behavioural effects that are partially mediated by downstream allopregnanolone biosynthesis. Our results suggest that TSPO would be a potential anxiolytic and antidepressant therapeutic target.

1. Introduction

Anxiety and depressive disorders are serious and burdensome psychiatric illnesses that have high rates of comorbidity (Schoepf et al., 2014; Silove et al., 2015). Currently, the underlying pathophysiological mechanisms of anxiety and depression remain unclear. The first line treatment for both depression and anxiety disorders is selective serotonin reuptake inhibitors (SSRIs) (Mula, 2013; Zonda et al., 2011). However, SSRIs have drawbacks,

Abbreviations: TSPO, 18 kDa translocator protein; CSF, plasma and cerebrospinal fluid; AP, anteroposterior; ML, mediolateral; DV, dorsoventral; i.p, intraperitoneally.

* Corresponding author.
** Corresponding author.
E-mail addresses: whlin309@163.com (H.-L. Wang), lyf619@aliyun.com (Y.-F. Li).

[1] Lei Li and Wei Wang are co-first author.

http://dx.doi.org/10.1016/j.neuropharm.2017.06.023
0028-3908/

including a delayed onset of action, a partial response with residual symptoms, a lack of response and the induction of sexual dysfunction/suicidal tendencies (Sheeler et al., 2012; Nelson and Philbrick, 2012) Accordingly, considerable effort has been invested in the discovery of non-monoamine-based antidepressants for the treatment of depression and anxiety through in-depth research of the pathogenesis of these disorders and the development of novel therapeutic strategies (Berton and Nestler, 2006).

The 18 kDa translocator protein (TSPO) is a five transmembrane domain protein that plays a crucial role in neurosteroid synthesis by promoting the transport of cholesterol to the inner mitochondrial membrane. This protein is predominantly expressed in steroid-synthesizing tissues, including the central and peripheral nervous system. TSPO is the rate-limiting enzyme involved in the synthesis of neurosteroids such as pregnenolone and allopregnanolone, which are positive allosteric γ-aminobutyric acid receptor (GABAA) receptor modulators with anxiolytic properties (Papadopoulos et al., 2006; Papadopoulos and Lecanu, 2009). The roles of TSPO in many processes, such as apoptosis, inflammation, cancer, mitophagy, the transport of porphyrin and the stress response, have received more attention in the pathophysiology of stress-related disorders (Batarseh A et al., 2010; Wendler et al., 2003; Decaudin et al., 2002; Kreisl et al., 2013; Gerhard et al., 2006). TSPO plays a crucial role in cholesterol transport and neurosteroidogenesis, which are associated with the pathophysiology of anxiety and depression (Scarf et al., 2009; Nothdurfter et al., 2012; Frye, 2009).

Interestingly, TSPO may represent a new potential therapeutic target for neurological and psychiatric disorders (Rupprecht et al., 2010). For example, TSPO levels are reduced in the peripheral blood cells of anxious subjects. Neurosteroids have been shown to be synthesized in the brain and act as endogenous modulators of GABAA receptors, which play significant roles in the treatment of anxiety and depression. Studied have not defined the precise regions upon which neurosteroids act (Eser et al., 2008). The anxiolytic and antidepressant-like effects are mediated by TSPO and associated with allopregnanolone biosynthesis. In addition, TSPO expression is reduced in platelets from depressed patients compared with healthy patients. Moreover, the administration of antidepressants such as paroxetine may increase TSPO expression and block or reverse the synaptic deficits (Da Pozzo et al., 2012; Dennis et al., 1995; Qiu et al., 2013). As shown in experimental models, the level of allopregnanolone, a potent positive allosteric modulator of the activity of γ-aminobutyric acid (GABA) at GABAA receptors, is markedly altered in association with the development of anxious and depressive behaviours (Shirayama et al., 2011; Pinna and Rasmusson, 2012). In clinical studies, allopregnanolone levels were decreased in serum, plasma and cerebrospinal fluid (CSF) of patients suffering from anxiety and depression but were elevated by the administration of antidepressants (Hellgren et al., 2014; Uzunova et al., 2004). Based on this evidence, allopregnanolone plays a significant role in the treatment of anxiety and depression.

The hippocampus has important roles in processing emotions, fear conditioning, and memory processing and has been implicated in the pathophysiology of anxiety and depression and their associated cognitive impairments (Bambico and Belzung, 2013; DeCarolis and Eisch, 2010; Santarelli et al., 2003). Activated neural progenitor cells (NPCs) that produce neurons (neurogenesis) have been identified in the hippocampal dentate gyrus, which appear to spontaneously divide at a slow rate throughout life (Ernst et al., 2014). Depression is associated with neuronal atrophy, volumetric changes, neuronal death, decreased neurogenesis and other structural changes in the hippocampus (Boldrini et al., 2013; Stockmeier et al., 2004). Antidepressants prevent the deterioration of neuronal cells in the hippocampal dentate gyrus and regulate synaptic plasticity, neurogenesis, and emotional behaviours (DeCarolis and Eisch, 2010; Thuret et al., 2009). Currently, no evidence is available to clarify the relationship between hippocampal TSPO expression and anxious or depressive-like behaviour. Therefore, we assessed the behavioural effects of TSPO overexpression in the mouse DG using lentiviral transduction.

Translocator protein (TSPO) is expressed in different organs, including the adrenal cortex, luteal cells, the testis, ovarian granulosa cells, the placenta and glial cells in the brain. Specifically, we overexpressed TSPO in the bilateral hippocampus using a lentivirus to study the important role of TSPO in the hippocampal dentate gyrus. We produced a lentivirus containing the TSPO cDNA to overexpress TSPO. The anxiolytic and antidepressant effects of TSPO overexpression were assessed using the elevated plus maze test, staircase test, hole board test and two behavioural despair models, the tail suspension test and the forced swimming test. Additional pharmacological tools were used, such as PK11195, a TSPO antagonist, and finasteride, an inhibitor of steroidogenic enzymes that blocks the conversion of progesterone to allopregnanolone. The change in hippocampal allopregnanolone levels was also assessed.

2. Materials and methods

2.1. Animals and housing

Adult male ICR mice (18–20 g) were purchased from the Beijing SPF Animal Technology Company (China). All mice were housed under SPF-grade conditions of temperature (24 ± 1 °C) and relative humidity (45%) in a 12-h light/dark cycle (light on, 8:00AM-8:00PM) controlled room. The mice had free access to water and food. All experiments were conducted according to current laws and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 86–23, revised 1996). All efforts were made to minimise the suffering and to reduce the number of animals used for experiments.

2.2. Drugs and reagents

PK11195 was purchased from Sigma-Aldrich (St. Louis, MO, USA) (Kita et al., 2004). Finasteride was purchased from TCI Co. Ltd (Shanghai, China) (Liu et al., 2014; Zhang et al., 2016a,b). PK11195 and finasteride were used at doses that were previously shown to partially antagonise TSPO activity and inhibit neurosteroid synthesis (Dhir and Rogawski, 2012; Qiu et al., 2016; Hatty et al., 2014; Batarseh and Papadopoulos, 2010; Wang et al., 2016).

2.3. Lentivirus production and stereotaxic injection

The coding sequence of the TSPO (NM-009775) gene was amplified and integrated into the GV287 vector (Ubi-MCS-3FLAG-SV40-EGFP) by AgeI. The GV287 vector and lentivirus were purchased from Shanghai GeneChem Co. Ltd. All the lentivirus vectors contained EGFP as a marker to track TSPO gene expression by fluorescence microscopy. The mice were placed in a stereotaxic holder (Kopf Instruments, Tujunga, CA, USA) after anesthetisation with 4% chloral hydrate. The hippocampal dentate gyrus (AP, −1.7 mm from Bregma; ML, ±1.8 mm from the midline; DV, −2.0 mm from the dura) was injected bilaterally via a 33-gauge Hamilton microsyringe (Lesnikov and Tsvetkova, 1985). Lentiviral vectors harbouring either Lenti-TSPO-cDNA (LV-TSPO) or the negative control sequence (LV-control) were microinfused into the hippocampal dentate gyrus at the rate of 0.2 μl/min (2×10^{10} TU/ml, 1 μl/side) using a UMP3 microsyringe injector and a Micro4 controller (World Precision Instruments, Sarasota, FL, USA). After