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GPR30 activation improves memory and facilitates DHPG-induced LTD in the hippocampal CA3 of middle-aged mice

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ABSTRACT

Reduced estrogen levels and decreased expression of related receptors are typical cerebral features of aging. The G protein-coupled estrogen receptor 1 (GPER1, also known as GPR30) is considered a novel therapeutic target for neurodegenerative diseases. In this study, we demonstrated that hippocampal GPR30 expression was reduced in middle-aged mice compared with young adult mice. GPR30 agonist G1 improved both fear and spatial memory in both male and female middle-aged mice, but not in young adult mice, which were blocked by the GPR30 antagonist G15. Interestingly, a group I metabotropic glutamate receptor (mGluR) agonist, 3,5-dihydroxyphenylglycine (DHPG)-induced long-term depression (LTD) in mossy fiber-cornu ammonis 3 (MF-CA3) synapses but not Schaffer collateral-CA1 (SC-CA1) synapses was facilitated in brain slices from G1-treated middle-aged mice. Long-term potentiation (LTP) in SC-CA1 synapses was not affected in slices from G1-treated mice. The effects of GPR30 activation on memory and DHPG-LTD in MF-CA3 synapses were further confirmed by viral expression of GPR30 in the CA3. The regulation of hippocampal synaptic plasticity by G1 treatment might be related to brain-derived neurotrophic factor (BDNF)-tropomyosin receptor kinase B (TrkB) signaling, as G15 also blocked G1-induced activation of the BDNF-TrkB pathway. Moreover, we found that DHPG triggered GluA internalization in slices from G1-treated mice but not control mice. Pharmacological experiments showed that G1-mediated facilitation of DHPG-induced LTD in MF-CA3 synapses was dependent on protein kinase B (Akt), mammalian target of rapamycin (mTor), and TrkB signaling. In conclusion, our results indicate that GPR30 activation improves memory in middle-aged mice, likely through facilitating synaptic plasticity in the CA3. This study provides novel evidence that GPR30 activation can improve memory in middle-aged animals.

1. Introduction

It is estimated that over one-third of the population will be older than 60 years in 2050 (Chatterji et al., 2008). Aging-related disorders including cardiovascular disease, diabetes, and neurodegenerative conditions seriously affect patient quality of life and dramatically increase medical costs. Aging is the primary risk factor for Alzheimer disease (AD) (Qiu, Kivipelto, & von Strauss, 2009). Although memory impairment in aged populations is not the same as AD, age-related memory impairment likely aggravates AD symptoms and leads to dementia (Arlt, 2013). Therefore, it is important to identify novel approaches to combat aging-related memory impairment.

A variety of pathological factors contribute to aging (Tosato, Zamboni, Ferrini, & Cesari, 2007). Lower levels of estrogen and its receptors are important features in the brains of aged animals (Brooks & Garratt, 2017). Two major types of estrogen receptors are found in the brain: classical estrogen receptor α/β and G protein-coupled estrogen receptor 30 (GPER1, also known as GPR30). The effects of estrogen receptor α/β on memory have been reported previously (Frick, 2015; Frick, Kim, Tuscher, & Fortress, 2015; Newhouse & Dumas, 2015). Estrogen replacement improves memory in aged female animals via its effects on estrogen receptor α/β (Han et al., 2013). However, the function through classic estrogen receptors is likely associated with tumorigenesis, as estrogen receptor α/β predominately exert genomic actions. GPR30 is a membrane-associated estrogen receptor (Funakoshi, Yanai, Shinoda, Kawano, & Mizukami, 2006). Different from the classic nuclear function of estrogen receptor α/β, GPR30 mainly functions through non-genomic estrogen signaling events including the second messengers Ca^{2+}, cyclic adenosine monophosphate, and nitric oxide, as well as activation of receptor tyrosine kinases (Cheng, Graeber, Quinn,

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& Filardo, 2011). GPR30 has also been identified as an important therapeutic target for neurodegenerative diseases (Bessa et al., 2015; Bourque, Morissette, Cote, Soulet, & Di Paolo, 2013; Kubota, Matsumoto, & Kirino, 2016). Especially, GPR30 activation can also enhance social learning (Ervin, Mulvale et al., 2015), and the effect is both rapid and long lasting (Ervin, Lymer et al., 2015). GPR30 activation was also reported to repair recognition memory in AD mice (Kubota et al., 2016) and ovariectomized rats (Hawley, Grissom, Moody, Dohanich, & Vasudevan, 2014). Inerestingly, the aging process was also accompanied with GPR30 reduction (Lindsey, da Silva, Silva, & Chappell, 2013).

Schaffer collateral-CA1 (SC-CA1) and mossy fiber-cornu ammonis 3 (MF-CA3) pathways are the most studied hippocampal circuits. Long-term potentiation (LTP) and long-term depression (LTD) are two opposing regulation of synaptic transmission. Both types of synaptic plasticity correlate with memory (Li, Yang, & Zhu, 2017; Zhu, Liu, Wang, Bi, & Baudry, 2015). GPR30 activation was reported to mediate 3,5-dihydroxyphenylglycine (DHPG)-induced LTD in the CA3 (Briz, Liu, Zhu, Bi, & Baudry, 2015). In this study, we aimed to assess the effects of GPR30 activation on memory and hippocampal synaptic plasticity in middle-aged mice. Our results provide novel evidence implicating that G1 can improve memory function specifically in middle-aged mice.

2. Materials and methods

2.1. Animals and treatments

Male and female C57BL/6 mice (12 months and 2 months) were purchased from Shanghai SLAC (Shanghai, China). G1 and G15 were purchased from Tocris (Bristol, UK). Mice were housed in a room maintained at a temperature of 23 ± 2 °C, a relative humidity of 45–65% and experienced a 12 h light/dark cycle. Animals had *ad libitum* access to food and water. The behavioral experiments were periformed in light phase (8 a.m.–8p.m.). All animal studies were supervised by the Ethics Committee of Anhui University of Traditional Chinese Medicine. Efforts were made to minimize animal suffering and reduce the number of mice used.

Mice were randomly allocated into a control group, low-dose G1 group, high-dose G1 group, and G15 with high-dose G1 group through the randomization table method (N = 9 in each group). Mice in the G1 groups received G1 (1 or 5 μg/kg, subcutaneously [sc]) for 15 days following a report showing the effects of G1 on depression-like symptoms (Dennis, Burai, Ramesh, Petrie, Alcon, Nayak, Bologa, Leitao, Brailoiu, Deliu, Dun, Sklar, Hathaway, Arterburn, Oprea, and Prossnitz, 2009), while mice in control group received the same volume (sc) of saline for 15 days. In the G15 with high-dose G1 group, 10 nmol/mice G15 (sc) was given with G1 at the same time to block the effect of G1 as previously described (Dennis et al., 2009). Experimenters were blinded for the groups. Two hours after last drug treatment, the behavioral tests were conducted.

2.2. Morris water maze

Spatial memory was tested after drug treatments in the Morris water maze. The navigation experiment was carried out for five consecutive days (twice a day). The mice were randomly placed in each quadrant, and latency to find the platform was recorded. If the platform was not found in 90 s, the mouse was led to the platform, and the latency was counted as 90 s. The spatial exploration experiment was carried out to test memory on day 6. The mouse was put in the contralateral quadrant, and the time spent in the platform quadrant was recorded when the platform was withdrawn.

2.3. Fear conditioning

After adaptation, mice were placed in the fear-conditioning chamber located in the center of a sound-attenuating cubicle. The conditioning chamber was cleaned with 10% ethanol to remove any odor. After a 2-min exploration period, one or three tone-footshock pairings separated by 1-min intervals were delivered (85-dB 2-kHz tone lasted for 30 s, 0.75-mA footshocks lasted 2 s). Mice remained in the training chamber for another 30 s before being returned to their home cages. On the third day, contextual memory was tested using the similar context as the training day, while cue memory was tested on day 4 by delivering the similar tone protocol. Time spent freezing was calculated and normalized to the total recording time. In the subsequent experiments, male middle-aged mice were included to investigate the potential mechanisms.

2.4. Hippocampal slice preparation

The second day after behavioral experiments, the male middle-aged mice were anesthetized with isoflurane, and the brains were quickly removed following decapitation and transferred to oxygenated, ice-cold cutting medium including 124 mM NaCl, 26 mM $NaHCO_3$, 10 mM D-glucose, 3 mM KCl, 1.25 mM KH_2PO_4, 5 mM $MgSO_4$, and 1.5 mM $CaCl_2$. Transverse hippocampal slices (350-μm thick) were prepared using a vibratome (Leica, Wetzlar, Germany) and transferred to an interface recording chamber and exposed to a warm, humidified atmosphere with 95% O_2 and 5% CO_2 and continuously perfused with oxygenated and preheated (32 ± 0.5 °C) artificial cerebrospinal fluid (aCSF; in mM: 110 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.5 $MgSO_4$, 1.24 KH_2PO_4, 10 D-glucose, 27.4 $NaHCO_3$) at a flow speed of 1.6 mL/min.

After incubation for 1 h, the slices were used in electrophysiological experiments, western blotting, and immunohistochemistry (IHC). The slices were treated with DHPG (100 μM) for 10 min, and the CA3 was dissected out for western blotting. IHC staining was applied to detect GluA expression.

2.5. Electrophysiological experiments

After incubation for 1 h in a recording chamber, a single glass pipette filled with 2 M NaCl was used to record field excitatory postsynaptic potentials (fEPSPs) elicited by stimulation of the Schaffer collateral pathway, or by stimulation of the mossy fiber pathway with twisted nichrome wires (single bare wire diameter, 50 μm) placed in the CA1 or CA3 stratum radiatum. Responses were recorded by a differential amplifier (EXT-20F, npi electronic GmbH, Tamm, Germany) using 3-kHz high-pass and 0.1-Hz low-pass filters. LTD was induced by application of the mGluR1/5 agonist DHPG (100 μM, 10 min). LTP was induced by strong theta-burst stimulation (TBS; 10 bursts, 4 pulses at 50-ms interval) or weak TBS (5 bursts, 3 pulses at 50-ms interval). Data were collected and digitized by Clampex, and the fEPSP slope was analyzed. LTD level was normalized to the baseline.

2.6. Biochemical experiments

The CA3 was isolated and lysed. Protein concentrations were determined using the bicinchoninic acid protein assay kit (Thermo Fisher, Waltham, MA, USA). Equivalent amounts of proteins were processed for sodium dodecyl sulphate-polyacrylamide gel electrophoresis and western blot as previously described (Li et al., 2017). The primary antibodies used in this experiment were anti-BDNF (1:1000, Millipore, Billerica, MA, USA), anti-p-TrkB (1:1000, Cell Signaling Technology, Danvers, MA, USA), anti-TrkB (1:1000, Cell Signaling Technology, Danvers, MA, USA), anti-GluA (1:1000, Cell Signaling Technology, Danvers, MA, USA), anti-GPR30 (1:1000, Cell Signaling Technology, Danvers, MA, USA) and anti-actin (1:10,000, Abcam, Cambridge, UK).

2.7. Immunochemical staining

After fixation in 4% paraformaldehyde for 1 h, hippocampi were