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Short communication

Ameliorative effect of membrane-associated estrogen receptor G protein coupled receptor 30 activation on object recognition memory in mouse models of Alzheimer's disease

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ABSTRACT

Membrane-associated estrogen receptor "G protein-coupled receptor 30" (GPR30) has been implicated in spatial recognition memory and protection against neuronal death. The present study investigated the role of GPR30 in object recognition memory in an Alzheimer's disease (AD) mouse model (5XFAD) by using novel object recognition (NOR) test. Impairment of long-term (24 h) recognition memory was observed in both male and female 5XFAD mice. Selective GPR30 agonist, G-1, ameliorated this impairment in female 5XFAD mice, but not in male mice. Our study demonstrated the ameliorative role of GPR30 in NOR memory impaired by AD pathology in female mice.

A novel membrane-associated estrogen receptor GPR30 mediates the rapid effects of estradiol (1) probably via intracellular signaling pathways linked to growth factor receptors, followed by extracellular signal-regulated kinase (ERK)1/2 activation (2) and acetylcholine release (3). Selective agonist for GPR30, G-1 can differentiate the functions of GPR30 from the classical estrogen receptors, establishing GPR30's role in exerting an antidepressant effect and protection against neuronal death in the hippocampus (2,4). The enhancing effect of G-1 on spatial recognition memory has been demonstrated recently in the Y-maze task in ovariectomized rat (5). The results of the study suggested that the activation of GPR30 besides classical estrogen receptors is sufficient to enhance spatial memory. Previous studies demonstrated the ameliorative effects of GPR30 activation on memory function and neurodegeneration in an ovariectomized model or ischemic model. However, the effect of brain GPR30 activation on memory function in mouse models of Alzheimer's disease (AD), wherein the major symptom is the impairment of learning and memory, remains to be elucidated.

The present study investigates the effect of GPR30 activation on object recognition memory by using novel object recognition (NOR) test in AD mouse models. For studying the effect of GPR30 activation, it is important to avoid exposing the animals to stress to prevent unexpected facilitation of estrogen production. NOR test is carried out under conditions that do not cause intensive stress, which would be inevitable in the other tests involving water exposure or fasting. NOR test mainly involves the prefrontal cortex and hippocampus, which express GPR30 abundantly. This study was designed to determine the role of GPR30 in short- and long-term object recognition memory by using 5XFAD mice as a model of AD. Most of the transgenic mice with familial AD mutations often fail to exhibit pronounced neuron loss although they have succeeded in mimicking the extensive amyloid plaque pathology seen in clinical AD. 5XFAD mouse is unique in displaying obvious neurodegeneration along with deposition of amyloid plaque (6) and therefore, is a useful model for studying memory dysfunctions associated with neurodegeneration in AD. We provide the first evidence for the ameliorative effect of GPR30 signaling on cognitive impairment in AD mouse models, which show Aβ-related neuron loss.

Eight-to ten-month-old male and female B6SJL-Tg (APPSwFILon, PSEN1*M146L*L286V) 6799Vas/J (5XFAD) mice (The Jackson Laboratory, Bar Harbor, USA) and age-matched non-transgenic wild type (WT) littermates were used in this study. Mice were housed four per cage at the maximum, and were maintained in a temperature- and light-controlled environment with a 12-h light/dark cycle (lights on at 8:00 a.m.) with free access to food and water. All

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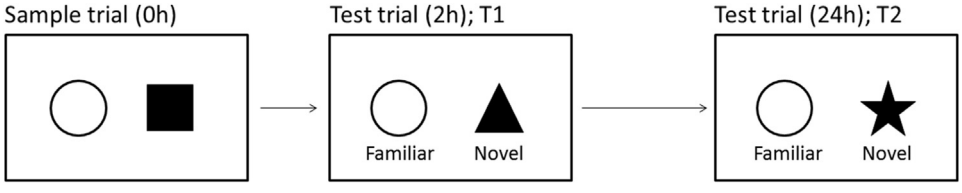
procedures were carried out according to the guidelines established by the Institutional Animal Investigation Committee at the Tokushima Bunri University. Selective GPR30 agonist G-1 (Tocris Bioscience, Bristol, UK) was diluted to 1.0 mM with 10% dimethyl sulfoxide (DMSO)-containing saline. Selective GPR30 antagonist G-15 (Tocris Bioscience) was diluted to 10 mM with 10% DMSO-containing saline. Both G-1 and G-15 solutions (0.1 ml per mouse) were administered intraperitoneally 30 min before the onset of each trial. The doses of G-1 and G-15 were selected based on the previous study reported by Dennis et al (4). In the NOR test, vehicle-treated non-transgenic littermate mice were used as a control group. The NOR test was carried out as described in Fig. 1. The ratio of time required to explore novel object versus that required for familiar object was measured. Total RNA from the prefrontal cortex (PFC) and hippocampus was isolated using the Get pureRNA kit (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. RT-PCR was performed using Super-Script III First-Strand Synthesis SuperMix (Invitrogen, California, USA) and specific primers for GPR30 and GAPDH. Statistical significance for NOR was determined by two-way ANOVA followed by Tukey–Kramer post hoc test.

Vehicle-treated WT (n = 9) and 5XFAD male mice (n = 5) had similar exploratory ratio (a chance level) in the sample trial. G-1-treated group (WT: n = 4, 5XFAD: n = 6) and G-1 + G-15-treated group (n = 4) also showed similar exploration ratio (Fig. 2A). In T1 (Fig. 2B), both vehicle-treated WT mice and 5XFAD mice spent longer time in exploring the novel object, showing an increased exploratory ratio (WT: 70.8 ± 2.1%, 5XFAD: 64.0 ± 2.8%), and G-1-treated group and G-1 + G-15-treated group also showed similar exploration ratios (64.0 ± 5.0% and 58.1 ± 4.5%, respectively). There was no significant difference among these groups. In T2 (Fig. 2C), vehicle-treated 5XFAD mice failed to identify the novel objects, spending equal amount of time exploring both novel and familiar objects, whereas vehicle-treated WT mice retained the object memory (WT: 61.3 ± 3.9%, 5XFAD: 48.0 ± 3.0%). Vehicle-treated WT (n = 5) and 5XFAD female mice (n = 6) had similar exploratory ratio in the sample trial as shown in Fig. 2D. G-1-treated WT mice (n = 4), 5XFAD mice (n = 8), and G-1 and G-15 co-treated 5XFAD mice (n = 4) also showed similar exploration ratio and there was no significant difference among these groups (Fig. 2D). In T1 (Fig. 2E), all groups of mice spent more time exploring the novel object than familiar object (WT: vehicle 69.8 ± 2.7%, G-1 69.4 ± 3.8%, 5XFAD: vehicle 61.6 ± 3.8%, G-1 68.2 ± 2.3%, G-1 + G-15 70.9 ± 4.6%). There was no significant difference among these groups. Thus, female 5XFAD mice retained short-term object recognition memory, and G-1 and G-15 had no effect. In T2 (Fig. 2F), vehicle-treated 5XFAD mice failed to discriminate the novel objects, and showed significantly lower exploratory ratio than the vehicle-treated WT mice (49.5 ± 3.9, $p < 0.05$). Since the exploratory ratio was about 50%, a chance level, female 5XFAD mice might have impaired object recognition memory as compared with WT mice. Decrement of exploratory ratio was ameliorated by treatment with G-1 (61.4 ± 3.5, $p < 0.05$ vs. vehicle-treated 5XFAD mice) and this effect was antagonized by co-administration of G-1 and G-15 (48.6 ± 6.0, $p < 0.05$ vs. G-1-treated 5XFAD mice), thus indicating that GPR30 is involved in this function. G-1 did not have any effect on exploratory ratio in WT mice (vehicle 61.4 ± 2.7, G-1: 60.1 ± 2.6). No sex-specific difference was observed in GPR30 mRNA expression in the PFC or hippocampus, either in WT or in 5XFAD mice (Fig. 3).

Since both male and female 5XFAD mice showed a small but not significantly lower exploratory ratio than WT mice in T1, 5XFAD mice may have short-term retention of object recognition memory during these ages. In contrast, long-term retention of object recognition memory was impaired in both male and female 5XFAD mice. In concordance with this data, decrease of exploratory ratio with twenty four hours retention interval has been reported using another line of AD mouse model, which expresses excessive Aβ deposition (7). In 5XFAD mice, synaptic degeneration begins at four months of age and obvious neurodegeneration was observed at nine months of age (6). Deposition of Aβ plaques was also observed from the age of four months (6). Although a sex-specific difference was not observed in the expression of GPR30 in 5XFAD mice, the ameliorative effect of G-1 on the long-term retention of recognition memory was observed only in female 5XFAD mice. Epidemiological studies have reported that estrogen replacement therapy has a protective effect on cognitive function, and reduces the risk of AD in postmenopausal females (8). However, similar findings have not been reported in males. Abrahám et al. (9) reported that estrogen increases the phosphorylation of cyclic AMP response element-binding protein (CREB) via a non-genomic pathway in gonadotropin-releasing hormone (GnRH)-positive neurons in female but not male mice. Ramsden et al. (10) demonstrated that treatment of orchidectomized male rats with E2, failed to prevent Aβ accumulation in the brain, whereas treatment with androgen was found to be effective. In male AD mouse models, upregulated endogenous testosterone was found to prevent the development of AD-related cognitive decline, as assessed by hole-board test (11). Taken together, androgens rather than estrogens can serve as an important parameter in preventing AD related symptoms in male.

5XFAD female mice showed significantly lower level of long-term object recognition memory than the control mice; this was ameliorated by treatment with G-1. These results suggest that impairment of long-term retention of object recognition memory observed in 5XFAD mice was due to the disturbance of GPR30



$$\text{Exploratory ratio (\%)} = \frac{\text{time spent exploring novel object (sec)}}{\text{time spent exploring familiar and novel object (sec)}} \times 100$$

Fig. 1. Experimental schedule for the novel object recognition test. The study consisted of three trials: a sample (familiarization) trial, and two test trials carried out at two hours (T1) and twenty-four hours (T2) after the sample trial. In the NOR task, mice were placed in the home cage with two objects placed on either side of the cage, and were allowed to explore the two objects for 10 min. Object exploration was defined as sniffing or touching the object with either the nose or forepaw but not sitting or standing on the objects. After a 2 h and 24 h delay, mice were allowed to explore two objects, one identical to the familiarization object in the sample trial and a novel object, for 10 min.