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[Supplement]

[Materials and Methods]

Materials

Antibodies for brain-derived neurotrophic factor (BDNF), cAMP response element binding protein (CREB), p-CREB, p-p65, p65, and β-actin were purchased from Cell Signaling Technology (Beverly, MA, U.S.A.). A enzyme-linked immunosorbent assay (ELISA) kit for corticosterone (E-EL-M0349) was purchased from Elabscience (Hebei, China). ELISA kits for interleukin (IL)-6, tumor necrosis factor (TNF)-α were purchased from Invitrogen (Carlsbad, CA).

Methods

Culture of SH-SY5Y cells

SH-SY5Y cells were cultured according to the method of Lee et al. [1]. Cultured SH-SY5Y cells were stimulated with corticosterone (300 μM) with or without gut bacteria for 24 h, BDNF expression was assayed by immunoblotting.

Behavioral tasks

Elevated plus maze (EPM) task was carried out in the plus-maze apparatus, , which consisted of two open [30 × 7 cm] and two enclosed arms [30 × 7 cm] with 20-cm-high walls extending from a central platform [7 × 7 cm] on a single central support to a height of 60 cm above the floor) for 5 min, according to the method of Jang et al. [2]. Tail suspension test (TST) was carried out according to the method of Jang et al. [2]. Mice were suspended on the edge of table 30 cm above the floor by taping 1 cm from the tail tip. Immobility time was measured for 5 min.

When mice did not move and passively hang, mice were judged to be immobile. Forced swimming test (FST) was carried out according to the method of Jang et al. [2]. FST was performed in a round transparent plastic jar (20 × 40 cm) containing fresh water (25°C) of 25 cm height according to the method of Dunn and Swiergiel [2]. Immobility time was measured during 5 min. When mice remain floating in the water without movement, mice were judged to be immobile,.

Immunobloting and ELISA Assay

Hippocampus and colon tissues were lysed with ice-cold lysis RIPA buffer containing 1% phosphatase inhibitor cocktail and 1% protease inhibitor cocktail and centrifuged (10,000 *g*, 10 min, 4°C) [3]. For the immunoblotting analysis, supernatants were electrophoresed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a nylon membrane, blocked with 5% non-fat dried-milk proteins, probed with the corresponding antibodies, washed with PBS containing tween 20, incubated with horseradish peroxidase-conjugated secondary antibodies, and visualized with an enhanced chemiluminescence detection kit.

For the ELISA analysis, the supernatants of hippocampus and colon tissues and bloods, which was centrifuged (3,000 *g*, 5 min, 4°C), were transferred to 96-well plate. Their corticosterone, IL-6, and TNF-a levels were determined using ELISA kits [2].

Immunofluorescence staining

The immunofluorescence staining were carried out according to the method of Jang et al. [3]. Mice were trans-cardiacally perfused with paraformaldehyde (4%). Brains and colons were removed, post-fixed, cytoprotected, freezed, and cryosectioned. Sectioned tissue slices were treated with Iba1 antibody for microglia and CD11b and CD45 antibodies for dendritic cells (DCs)/macrophages and incubated with secondary antibodies conjugated with Alexa Fluor 488

or Alexa Fluor 594. Cell nuclei were stained with 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI). Immunostained slices were observed by using a confocal laser microscope.

Myeloperoxidase activity assay

Colon tissues (second and sixth segments) were homogenized in 10 mM potassium phosphate buffer (pH 7.0) containing 0.5% hexadecyl trimethyl ammonium bromide, and centrifuged for 10 min (20,000 ×*g* at 4°C) [2]. The supernatant (50 μL) was incubated with 0.75 mL of the pre-incubated reaction mixture containing 0.1 mM hydrogen peroxide and 1.6 mM TMB at 37°C for 5 min and periodically monitored for its absorbance at a wavelength of 650 nm.

qPCR for gut microbiota analysis

qPCR was performed with total DNA (100 ng) isolated from the mouse feces with SYBR premix in a Takara thermal cycler according to the method of Lim et al. [4]. The thermal cycling conditions were as follows: 95°C for 30 s, followed by 42 cycles of denaturation and amplification at 95°C for 5 s and 63°C for 30 s, respectively. Gene expression levels were calculated relative to bacterial 16S rRNA, using Microsoft Excel. Primers were used were as follows: 16s rRNA forward 5'-TCGTCGGCAGCGTCA GATGTGTATAAGAGACAGGTGCCAGCMGCC GCGGTA A-3' and reverse 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGG ACTACHVGGGTWTCTAAT-3'; Firmicutes forward 5'-GGAGYATGTGGTTTAATTCGA AGCA-3' and reverse 5'-AGCTGACGACAACCATGCAC-3'; Bacteroidetes forward 5'-AAC GCGAAAAACCTTACCTACC-3' and reverse 5'-TGCCCTTTCGTAGCAACTAGTG-3'; δ/γ-Proteobacteria forward 5'-GCTAACGCATTAAGTRYCCCG-3' and reverse 5'-GCC ATGCRGCACCTGTCT-3'.

Results

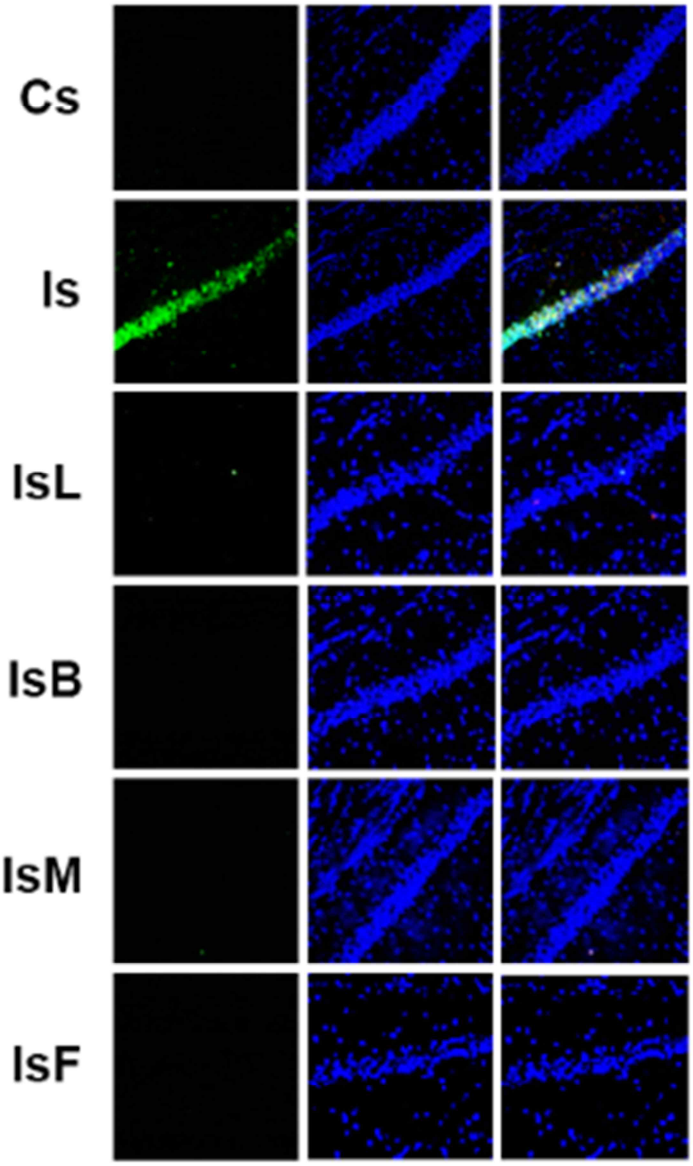

Figure S1. Effects on the infiltration of Iba1^{+} cells into the CA1 region of hippocampus. Mice except control group (Cs) were exposed to IS and test agents (Cs, vehicle [1% dextrose]; Is, vehicle; IsL, 1×10^{9} CFU/mouse/day of NK41; IsB, 1×10^{9} CFU/mouse/day of NK46; IsM, 1×10^{9} CFU/mouse/day of the (1:1) mixture of NK41 and NK46]; and IsF, 12 mg/kg/day of fluoxetine) were gavaged orally once a day for 5 days. Iba1^{+} cells were observed by a confocal microscope.

References

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