Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Dysfunction in diurnal synaptic responses and social behavior abnormalities in cathepsin S-deficient mice

Fumiko Takayama [a, 1], Xinwen Zhang [b, 1], Yoshinori Hayashi [a], Zhou Wu [a], Hiroshi Nakanishi [a, *]

[a] Department of Aging Science and Pharmacology, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan
[b] Center of Implant Dentistry, School of Somatology, China Medical University, China

ARTICLE INFO

Article history:
Received 1 June 2017
Accepted 13 June 2017
Available online xxx

Keywords:
Cathepsin S
Social behavior abnormality
Microglia
Synaptic activity

ABSTRACT

The expression of cathepsin S (CatS), a microglia-specific lysosomal cysteine protease in the brain, is regulated by the intrinsic microglial circadian clock. We herein report that the diurnal variation of evoked synaptic responses of cortical neurons disappeared in cathepsin S-deficient ($CatS^{-/-}$) mice. The dendritic spine density of the cortical neurons was significantly reduced by incubation with a recombinant CatS. Furthermore, $CatS^{-/-}$ mice exhibited impaired social interaction and social novelty recognition in the three-chamber test. These findings indicate that the circadian clock-regulated secretion of CatS from microglia is involved in the diurnal variation of synaptic responses and dendritic spine density through the proteolytic modification of perisynaptic ECM molecules. Therefore, a dysfunction of the diurnal synaptic responses due to CatS deficiency may lead to social behavior abnormalities.

1. Introduction

Cathepsin S (CatS; EC 3.4.22.27) is a member of the cysteine cathepsin protease family. In contrast to other cysteine cathepsin family members, CatS has a uniquely restricted tissue expression and is relatively stable at a neutral pH, which enables CatS to act on extracellular substrates in aspects other than being involved in the endosomal-lysosomal pathway. CatS is preferentially expressed in cells of mononuclear phagocytic origin, including dendritic cells, macrophages and microglia. CatS has been implicated in several disease processes, including autoimmune diseases [1], allergic inflammation and asthma [2,3], diabetes and obesity [4], cardiovascular disease [5], cancer [6,7] and chronic pain [8,9]. We recently provided the first evidence that CatS deficiency is associated with sleep disorders [10]. Furthermore, diurnal variations in the dendritic spine density and the frequency of miniature postsynaptic currents of cortical neurons, which were found in wild-type mice, disappeared in $CatS^{-/-}$ mice.

Dendritic spines undergo morphological changes in response to stimuli that modulate neuronal activity. Such remodeling supports the formation and long-term storage of information in the brain, whereas alterations in spine remodeling accompany neurodegenerative and neuropsychiatric diseases. It was recently reported that neuronal activity-dependent fusion of lysosomes with the plasma membrane releases active CatB [11]. After fusion, the lysosome releases CatB, which stimulates the activity of matrix metalloproteinase-9 by cleaving its endogenous inhibitor tissue inhibitor of metalloproteinase-1, leading to dendritic spine growth. This suggests that CatB/matrix metalloproteinase-9 regulate the structural plasticity of dendritic spines through the remodeling of perineuronal extracellular matrix (ECM) molecules. Fenale $CatB^{-/-}$ mice were found to exhibit an increase in depression-like behavior [12]. These observations prompted us to further examine the possible association of CatS deficiency with synaptic dysfunctions and behavioral abnormalities.

2. Materials and methods

2.1. Animals

$CatS^{-/-}$ mice on a DBA/2 background were used. Under light-

Abbreviations: ACSF, artificial cerebrospinal fluid; ANOVA, one-way analysis of variance; CatS, cathepsin S; CysC, cystatin C; ECM, extracellular matrix; EPSCs, excitatory postsynaptic currents; LPS, lipopolysaccharide; NMDA, *N*-methyl-D-aspartate; PPR, paired pulse ratio; SEM, the standard error of the mean; ZT, Zeitgeber time.

* Corresponding author.
E-mail address: nakan@dent.kyushu-u.ac.jp (H. Nakanishi).

[1] F.T. and X.Z. contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2017.06.061
0006-291X/

dark conditions, Zeitgeber time 0 (ZT0) was designated as lights on, and ZT12 was designated as lights off. All animal experiments were conducted in accordance with the guidelines contained in the Act on Welfare and Management of Animals (Ministry of Environment of Japan) and Regulation of Laboratory Animals (Kyushu University) and under the protocols approved by the Institutional Animal Care and Use committee review panels at Kyushu University.

2.2. Patch clamp recordings

Cortical slices (200 μm thick) were cut from the somatosensory cortex of wild-type and *CatS*$^{-/-}$ mice using a vibrating microtome (VT1000S; Leica, Wetzlar, Germany) at ZT2 and ZT14. Slice patch analyses were performed as described previously [10]. All recordings were made at a holding potential of 270 mV. Excitatory postsynaptic currents (EPSCs) were evoked in layer 2/3 cortical neurons by the stimulation of layer 4 in the presence of 10 μM bicuculline methiodide (Sigma-Aldrich Japan, Tokyo, Japan), a competitive $GABA_A$ receptor antagonist. Stimulation was increased in 20-μA intervals up to 100 μA. Each response was normalized by the maximum response evoked by 100 μA.

2.3. Intracellular labeling of cortical neurons in the fixed-slice preparations

Wild-type mice were deeply anesthetized with an overdose of sodium pentobarbital (120 mg/kg, i.p.) and perfused transcardially with phosphate-buffered saline (pH 7.4) followed by 4% paraformaldehyde at ZT2. The cell bodies of cortical neurons were visualized by incubation with YOYO-1 (1:10,000; Thermo Fischer Scientific Inc., Waltham, MA, USA) for 3 min. In some experiments, the cortical slices (200 μm thick) were incubated with artificial cerebrospinal fluid (ACSF) or 50 nM human recombinant CatS (R&D Systems, Minneapolis, MN, USA) for 3 h at 37 °C, and then fixed with 4% paraformaldehyde for 48 h at 4 °C. The cells were injected with 4% Lucifer yellow (Sigma-Aldrich Japan) through a glass pipette similar to the method previously described [13,14]. The sections were then incubated with 4% paraformaldehyde for 24 h at 4 °C, after which they were further stained with an anti-Lucifer yellow antibody (1:50,000; Thermo Fischer Scientific Inc.) for 5 days at 4 °C and visualized with Cy3-conjugated donkey anti-rabbit IgG (1:400; Jackson ImmunoResearch, West Groves, PA, USA) for 3 h at 4 °C.

2.4. Quantitative analyses of dendritic spines

All of the images were processed using the ImageJ 1.47 software program (National Institutes of Health). Images of dendritic spines and filopodia were obtained with 100 × 1.4 NA oil-immersion lenses and a stack of 70–90 serial optical sections using a confocal laser scanning microscope (CLSM; C2si, Nikon, Tokyo, Japan). The mean density of dendritic spines and filopodia was determined by manual identification using three-dimensional images to measure the dendrite length with the Simple Neurite Tracer plug-in bundled in the FIJI software program (freely downloadable from http://fiji.sc/Fiji). Dendritic spines and filopodia were classified as described previously [15,16] with some modifications. Dendritic spines were defined as protrusions <2 μm in length that had distinct mushroom-shaped heads, and filopodia were defined as headless filamentous protrusions longer than 2 μm.

2.5. Immunoblot analyses

The MG6 cells were cultured on 100-mm Petri dishes at a density of 5×10^5 cells/ml. For the detection of CatS and cystatin C (CysC), which is an endogenous cysteine protease inhibitors, MG6 microglial cells were treated with 100 ng/ml lipopolysaccharide (LPS; Sigma-Aldrich Japan) and 5 mM ATP (Sigma-Aldrich Japan) for 1 h following replacement of the control ACSF. Each cell lysate and culture medium were collected and subjected to electrophoresis using a 12% SDS-polyacrylamide gel. Proteins on the SDS gels were transferred electrophoretically to nitrocellulose membranes. The membranes were washed with phosphate-buffered saline and incubated at 4 °C overnight under gentle agitation with each primary antibody: anti-CatS (1:1000; Santa Cruz), anti-CysC (1:500; R&D Systems) and anti-β-actin (1:5000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). After washing, the membranes were incubated with horseradish peroxidase-labeled anti-goat (1:1000; R&D Systems) or anti-rabbit (1:1000; GE Healthcare, Princeton, NJ, USA) antibody for 1 h at room temperature. Subsequently, the membrane-bound horseradish peroxidase-labeled antibodies were detected using an enhanced chemiluminescence detection system (ECK lit; GE Healthcare) with an image analyzer (LAS-1000; Fuji Photo Film, Tokyo, Japan).

2.6. Sociability and preference for social novelty

The three-chamber social interaction test was conducted in according with the method as previously described [17]. The test mouse was placed in the three-chambered apparatus and habituated for 10 min. For the social interaction test, the test mouse was placed in the center chamber, and a stranger mouse was placed in the left compartment (Stranger). The right compartment was empty at first (Object). For the social novelty recognition test, the test mouse was placed in the center chamber again, and a familiar mouse was placed in the left compartment (Familiar). A new unfamiliar mouse was placed in the right compartment (Stranger) that had been empty during the previous session. Each session lasted 10 min. The location of the Stranger alternated between the left and right sides of the social test box across subjects. After 10 min, the wild-type or *CatS*$^{-/-}$ mice was gently guided to the center chamber, and the two entrances to the center chamber were closed while a stranger mouse was placed in one of the side chambers. The two entrances were then opened to allow the mouse in the center to explore the new environment freely for 10 min. Exploration was defined as the time in which the test mouse tried to sniff each wire cage or oriented its nose towards and closed to each wire cage. The time spent in each chamber was measured by a video-tracking system (ANY-maze; Stoelting Co., Wood Dale, IL, USA). In addition to exploration, the preference index, which represents the numerical difference between the times spent sniffing the targets (Stranger/Object) divided by the total time spent sniffing both targets, was calculated as described previously [18].

2.7. Olfactory function test

An olfactory function test was performed in accordance with the method previously described [19]. To generate social cues, Kimwipes soaked with 20 μl water or non-social cues (banana and coffee) were introduced to a stranger mouse cage for 1day before the experiment. The wipes were retrieved from the stranger mouse cage right before the experiment and used for social cues. The non-social and social cues were contained in small, round shaped petri dishes with holes. Both wild-type and *CatS*$^{-/-}$ mice were placed in the test cage for 30 min before the experiment. Social and non-social cues were introduced sequentially to the test cage for 6 min each. The cues were retrieved from the test cage after each session, and the inter-session interval was 1 min. The sniffing behavior toward the cue-containing petri dishes was measured in a blinded manner.

Please cite this article in press as: F. Takayama, et al., Dysfunction in diurnal synaptic responses and social behavior abnormalities in cathepsin S-deficient mice, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.06.061