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Sodium butyrate triggers a functional elongation of microglial process via Akt-small RhoGTPase activation and HDACs inhibition

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ABSTRACT

Microglia, a type of immune cell in the brain, are in a ramified status with branched processes in normal conditions. Upon pathological stimulation, microglia retract their processes and become activated. Searching methods to make the activated microglia return to ramified status would help cope with injuries induced by neuroinflammation. Here, we investigated the influence of sodium butyrate (SB), a sodium salt form of butyrate produced by fermentation of dietary fibers in the gut on microglial process. Results showed that SB induced reversible elongations of microglial process in both normal and inflammatory conditions, and these elongations were accompanied with significant changes in markers reflecting the pro-inflammatory and anti-inflammatory status of microglia. The protein kinase B (Akt)-RhoGTPase signal was considered to mediate the effect of SB on microglial process, as: i) SB activated the small RhoGTPases Rac1 and Cdc42; ii) SB promotes Akt phosphorylation; iii) Rac1, Cdc42, and Akt inhibition abrogated the pro-elongation effect of SB on microglial process. Further analysis showed that incubation of microglia with two other histone deacetylases (HDACs) inhibitors trichostatin A (TSA) and valproic acid (VPA) also promoted microglial process elongation and Akt phosphorylation, suggesting that the SB-triggered microglial process elongation may be mediated by HDACs inhibition. Furthermore, Akt inhibition prevented the anti-inflammatory effect of SB in primary cultured microglia, and abrogated the inhibitory effects of SB on microglial process retraction and behavioral abnormalities induced by lipopolysaccharide (LPS). These results for the first time identify an anti-inflammatory role of SB from the aspect of microglial process elongation.

1. Introduction

Microglia are the unique immune cells in the central nervous system (CNS). One of the major functions of microglia is to protect the brain against the environmental challenges induced by different pathophysiological stimuli such as neurodegeneration and aging (Barrientos et al., 2015; Moehle and West, 2015). The normal microglia are in a ramified status with branched processes, whose formation need the rearrangement of actin filament and microtubule cytoskeleton induced by small GTPases (Rac1, Cdc42) and their upstream kinases such as phosphatidylinositol-3-kinase (PI3K) and protein kinase B (Akt) (Benseddik et al., 2013; Bouchet et al., 2016; Park et al., 2014). During cellular rearrangement, two structures lamellipodia and filopodia are essential for cell mobility, neuronal outgrowth, and dendritic spine development. Lamellipodia are projections at the edge of mobile cells forming a meshwork, while the filopodia are long and transient processes (Caesar et al., 2015; Du et al., 2016). With the help of lamellipodia and filopodia, the microglia become ramified and regulate synaptic remodeling (Chetta et al., 2015; Kim et al., 2006; Sahasrabudhe et al., 2016) and cellular polarization (Huang et al., 2016b; Huang et al., 2016a; Neubrand et al., 2014). However, upon being overactivated the microglia are transformed into a status with amoeboid morphologies, which are characterized by the retraction of microglial process and the overproduction of pro-inflammatory cytokines (Fu et al., 2015; Parrott et al., 2016). Skewing the amoeboid microglia return to their branched status would prevent neuroinflammation.

Butyrate, a short-chain fatty acid, is a functional molecule produced in the colon by fermentation of non-digestible fibers by bacteria, and is

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enriched in butter and dairy products (Bourassa et al., 2016; Steckert et al., 2015). Increasing studies show that butyrate exhibits promising therapeutic potentials in various disorders, such as diabetes, energy metabolism, and colorectal cancer, through mechanisms ranging from metabolic effects to receptor signaling and histone deacetylases (HDACs) inhibition (Chang et al., 2014; Priyadarshini et al., 2016; Stoldt et al., 2016; Thangaraju et al., 2009; Zhang et al., 2016). The gut-derived butyrate can reach the brain easily via penetration of the blood brain barrier (Achanta and Rae, 2017), and according to this characteristic some neuroprotective effects of butyrate and its sodium salt form sodium butyrate (SB) have been revealed in recent years (Butchbach et al., 2016; Naia et al., 2017). For example, SB has been shown to improve motor behaviors in Huntington's disease (HD) mice (Naia et al., 2017). Butyrate-based compounds are found to protect the mice against spinal muscular atrophy (Butchbach et al., 2016). Inhibition of microglial overactivation is also considered to mediate the neuroprotective effect of SB, as SB can protect the neurons against ischemic stimuli in neonatal and adult animals via conversion of microglial polarization from M1 to M2 phenotype (Jaworska et al., 2017; Park and Sohrabji, 2016; Patnala et al., 2017). How SB regulates microglia remains largely unknown. Since neuroinflammation is actively involved in the pathogenesis of neurodegenerative and psychiatric disorders, and the host immune system depends on butyrate as a potent regulator (Garden and Campbell, 2016; Lee et al., 2016; Schmidt et al., 2016), investigation of this issue would help understand the mode of action of SB in disorders associated with neuroinflammation.

In this study, we showed that SB induced obvious and reversible elongations of microglial process in vitro and in vivo. These elongations were mediated by Akt -small RhoGTPase activation and were associated with the anti-inflammatory effect of SB. Targeting HDACs may be a critical mechanism for the effect of SB on microglial process elongation, as inhibition of HDACs by two other classical HDACs inhibitors trichostatin A (TSA) and valproic acid (VPA) (Huynh et al., 2016; Lopes-Borges et al., 2015) also triggered Akt phosphorylation and microglial process elongation. These findings for the first time identify a neuroprotective mechanism of SB from the aspect of microglial process elongation, and promote the understanding about the role of butyrate in neuroinflammation-associated disorders. Furthermore, given that elongation of macrophages directly by physical stimuli can increase the expression of M2 markers (McWhorter et al., 2013), and Akt inhibition simultaneously suppressed the microglial process elongation as well as the acquirement of an anti-inflammatory status of microglia after SB treatment, we hypothesize that the microglial process elongation may be tightly associated with the anti-inflammatory effect of SB.

2. Materials and methods

2.1. Reagent

Lipopolysaccharide (LPS, *E. coli* 055:B5, catalog #sc-221,855), poly-L-lysine (catalog #sc-221,855) and Hoechst 33,258 (catalog #sc-394,039) were the products of Santa Cruz Biotechnology (Santa Cruz, CA, USA). SB was purchased from MedChem Express (Princeton, NJ, USA, catalog #HY-B0350A). SB and LPS were prepared in PBS and administered intraperitoneally (i.p.) in a volume of 10 mL/kg. Antibodies against Akt (catalog #2920), phospho-Akt (catalog #4060), Cdc42 (catalog #2462), and glyceraldehydes-3-phosphate dehydrogenase (GAPDH, catalog #3683) were purchased from Cell Signaling Technology (Beverly, MA, USA). The antibodies against Rac1 (catalog #ab33186) and Iba-1 (catalog #ab178847) were the products of Abcam (Cambridge, MA, USA). LY294002 (catalog #440206) and VIII (catalog #124018) were purchased from Millipore (Billerica, MA, USA). W56 (catalog #2221) and ML 141 (catalog #4266) were from the products of Tocris (Avonmouth, Bristol, UK). Dulbecco's Modified Eagle's Medium DMEM/F12 was obtained from Biotium and Gibco Invitrogen Corporation. PAK-PBD affinity beads were purchased from Cytoskeleton (Denver, CO, USA, catalog #PAK02-A). Other general agents were purchased from commercial suppliers. All the drugs were prepared as stock solutions. All stock solutions were stored at − 20 °C. These stock solutions were diluted to the final concentration with the extracellular solution before application. The final concentration of dimethyl sulphoxide (DMSO) was < 0.05%. No detectable effect of DMSO was found in the experiments. The Akt inhibitor LY294002 was intracerebroventricularly (i.c.v.) infused.

2.2. Animals

Male C57BL/6J mice (8–10 weeks) were housed 5 per cage under the condition of 12-h light/dark cycle, lights on from 07:00 to 19:00, 23 ± 1 °C environmental temperature and 55 ± 10% humidity for 1 week with free access to food and water. Behavioral experiments were carried out during the light phase. Animal experiments were conducted in accordance with internationally accepted guidelines for the use of animals in toxicology as adopted by the Society of Toxicology in 1999 and were approved by the University Animal Ethics Committee of Nantong University (Permit Number: 2,110,836).

2.3. Cell preparation

For this experiment, newborn (day 0–1) C57BL/6J mice were decapitated, and the removed prefrontal cortexes were digested with 0.125% trypsin for 15 min at 37 °C. Followed by trituration and centrifugation at 118*g* for 5 min, the total cells were re-suspended and plated on poly-L-lysine (1 mg/mL)-coated culture flasks. For preparation of primary cultured microglia, the individual cell suspensions were cultured in DMEM/F12 supplements with 10% FBS and 1% penicillin-streptomycin (100 U/mL, Bi Yuntian Biological Technology Institution, Shanghai, China, catalog #C0222), and the medium was replaced every 3 days. After 10 days, mixed cells were shaken gently 2 h at 37 °C and the supernatants were collected and plated on poly-L-lysine-coated culture dishes. All cells were maintained in a 37 °C incubator containing 95% air and 5% CO_2. The purity (> 99%) of microglia was identified by a marker of microglia Iba-1 using immunofluorescence technique. The use of new-born mice was approved by the University Animal Ethics Committee of Nantong University (Permit Number: 2110836).

2.4. Cell viability

Cell viability was measured using MTT Cell Proliferation and Cytotoxicity Assay Kit (Bi Yuntian Biological Technology Institution, catalog #C0009). Briefly, methylthiazolyldiphenyl-tetrazolium bromide (5 mg/mL) was dissolved in prepared MTT-dissolved solutions and kept at − 20 °C. After washing with PBS, the microglia in 96-well culture plates were added 20 μL of MTT solutions and kept at 37 °C for 4 h. The blue crystals were dissolved in formazan-dissolved solutions. The absorbance was read at 570 nm.

2.5. Cytokine detection

The IL-10 (catalog #555252) and TNF-α (catalog #560478) protein level were determined using cytokine specific BD OptEIA enzyme-linked immunosorbent assay kits (BD Biosciences Pharmingen, San Diego, CA).

2.6. Immunofluorescence

The immunofluorescence experiment was performed according to previous studies with some modifications (Huang et al., 2016b; Huang et al., 2016a; Huang et al., 2010). The cultured microglia were fixed with 4% paraformaldehyde in 0.01 M PBS (pH 7.4) for 30 min and rinsed 3 times with PBS for 10 min each. The primary cultured microglia and pre-fixed cortexes were permeabilized with a PBS containing

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