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27-HYDROXYCHOLESTEROL CONTRIBUTES TO DISRUPTIVE EFFECTS ON LEARNING AND MEMORY BY MODULATING CHOLESTEROL METABOLISM IN THE RAT BRAIN

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Abstract—Cholesterol metabolism is important for neuronal function in the central nervous system (CNS). The oxysterol 27-hydroxycholesterol (27-OHC) is a cholesterol metabolite that crosses the blood–brain barrier (BBB) and may be a useful substitutive marker for neurodegenerative diseases. However, the effects of 27-OHC on learning and memory and the underlying mechanisms are unclear. To determine this mechanism, we investigated learning and memory and cholesterol metabolism in rat brain following the injection of various doses of 27-OHC into the caudal vein. We found that 27-OHC increased cholesterol levels and upregulated the expression of liver X receptor-α (LXR-α) and adenosine triphosphate (ATP)-binding cassette transporter protein family member A1 (ABCA1). In addition, 27-OHC decreased the expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CR) and low-density lipoprotein receptor (LDLR) in rat brain tissues. These findings suggest that 27-OHC may negatively modulate cognitive effects and cholesterol metabolism in the brain. © **2015 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: 27-hydroxycholesterol, cognitive, cholesterol metabolism, HMG-CR, LXR-α, ABCA1.

INTRODUCTION

Dysregulation of cholesterol homeostasis is a well-established risk factor for Alzheimer's disease (AD) pathophysiology and has been shown to affect the progression of cognitive impairment and neural degeneration (Jansen et al., 2012; Park et al., 2013). Abnormal cholesterol metabolism states in the brain are likely to include aberrant neuronal function, interrupted cell–cell interactions, myelin breakdown, and neuronal loss (Bjorkhem, 2006). Due to the impermeability of the blood–brain barrier (BBB), cholesterol homeostasis in the brain is regulated through *de novo* synthesis, with little or no cholesterol derived from the peripheral circulation (Lange et al., 1999). The side-chain oxidized oxysterols 24-hydroxycholesterol (24-OHC) and 27-hydroxycholesterol (27-OHC) cross the BBB into and out of the brain and continuously influx or flux between the circulation and the brain (Heverin et al., 2005). Moreover, high levels of oxysterols and an abnormal 24-OHC/27-OHC ratio in the brain can trigger cholesterol imbalance and neurodegeneration (Marwarha, 2014). 27-OHC levels are markedly increased in the brains of AD patients, and this increase has been associated with an increased production of soluble amyloid precursor protein (sAPP) forms in the cerebral spinal fluid (CSF) (Shafaati et al., 2011; Popp et al., 2012). Excessive 27-OHC also occurs following hypercholesterolemia and neuronal dysfunction; thus, it is important to understand the mechanism by which 27-OHC affects cholesterol metabolism in the brain (Jansen et al., 2012). A cholesterol-enriched diet significantly affects spatial learning memory and synaptic function, and 27-OHC may be a negative mediator of the effect of dietary cholesterol on cognition in mice (Thirumangalakudi et al., 2008; Heverin et al., 2014). Another study has suggested that high levels of circulating cholesterol increase the entry of 27-OHC into the brain, which may induce learning and memory impairment (Marwarha, 2014). Based on this evidence, 27-OHC may be associated with neurodegenerative processes and interrupted cholesterol homeostasis in the brain. However, the mechanism by which 27-OHC causes cholesterol dysfunction in the brain and contributes to the pathogenesis of AD remains unclear.

Cholesterol metabolism in the central nervous system (CNS) and in the peripheral circulation exhibits several important features. The main effector molecules in cholesterol biosynthesis, metabolism and efflux include cholesterol, the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CR), low-density lipoprotein receptor (LDLR), liver X receptors (LXR), adenosine triphosphate (ATP)-binding cassette (ABC) transporter protein A1 (ABCA1), 24-hydroxylase (CYP46A1), and the oxysterols 24-OHC and 27-OHC, to which cholesterol is converted in the brain and circulation (Benarroch, 2008). Cholesterol ester transfer protein (CETP) mediates

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Abbreviations: AD, Alzheimer's disease; BBB, blood–brain barrier; CE, cholesterol esterase; FC, free cholesterol; HMG-CR, 3-hydroxy-3-methylglutaryl-CoA reductase; LXR-α, liver X receptor-α; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; 27-OHC, oxysterol 27-hydroxycholesterol; PBS, phosphate-buffered saline.

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cholesterol transport and can affect other enzymes that are involved in the regulation of cholesterol metabolism, *e.g.*, HMG-CR and microsomal triglyceride transfer protein (MTTP) (Bruce et al., 1998; Agarwal-Mawal et al., 2007).

In this study, we evaluated the hypothesis that impaired memory function is caused by aberrant cholesterol metabolism *via* increased levels of 27-OHC in the circulation and an increased flux of 27-OH into the brain. Following high doses of 27-OHC, which were injected into the caudal vein of rats, we investigated changes in learning and memory and determined cholesterol levels and the expression of HMG-CR, LDLR, LXR-α and ABCA1 in brain tissues. Additionally, enzyme-linked immunosorbent assays (ELISAs) were used to determine the levels of HMG-CR, low-density lipoprotein (LDL), CETP and MTTP.

EXPERIMENTAL PROCEDURES

Drugs and animals

Forty-eight adult male SD rats (SPF class, weighing 250–300 g) were provided by the Academy of Military Medical Science. The animal studies adhered to the guidelines established by the Chinese Committee on Experimental Animal Supervision. The study was conducted in four cohorts of a total of 48 animals. The rats were randomly assigned to four groups as follows: (1) the Control group (Control); (2) the Low group (Low); (3) the Middle group (Mid); and (4) the High group (High). Circulating 27-OHC levels are typically 0.15–0.73 μM in humans but can reach millimolar concentrations in pathological situations (Brown and Jessup, 1999). In our study, 27-OHC was administered *via* an intravenous injection at injected doses sufficient to achieve concentrations of 7, 21 and 70 μM in the whole blood of the rats in the Low, Mid and High groups, respectively. 27-OHC (purchased from Santa Cruz Biotechnology, USA) was dissolved in ethanol and physiological saline at a concentration of 1000 μM and stored at −80 °C until use. The rats were subjected to the Morris water maze and light–dark exploration on the 21st day, and the brains were collected from rats sacrificed on the 28th day following caudal vein injection and stored at −80 °C.

Morris water maze

The Morris water maze was used to evaluate learning and memory of rats (Gao et al., 2011). Briefly, a pool was filled with water and opaque dyes; the rat's body was made prominent using ink to improve the ability of the tracking software to detect the rats. Water was maintained at 21 ± 1 °C. Animals were released for swimming at random positions facing the wall, and the time taken to reach the escape platform and the swimming distance were measured in each trial. Each rat was trained for four consecutive days to identify the hidden platform, and the entire process was recorded. The escape latency was recorded each day, and the mean swim speed and total distance taken were recorded in the final test, which occurred on the fifth day.

Light–dark exploration

The light–dark box apparatus comprised two equal rectangular compartments connected by a door. One compartment was white and illuminated, and the other compartment was black and dark. The rats were gently placed in the center of the light area and allowed to explore the arena. For the following 5 min on three consecutive days, the latency to the first entry of the dark area and the number of false entries were recorded.

Amplex Red cholesterol assay

Brain cholesterol content was determined using the Amplex Red Cholesterol Assay Kit (Invitrogen, USA) according to the manufacturer's instructions. Briefly, brain tissue proteins were suspended in 1× cholesterol reaction buffer (0.1 M potassium phosphate, pH 7.4, 0.05 M NaCl, 5 mM cholic acid, and 0.1% Triton X-100). Subsequently, 50 μL of 150 μM Amplex Red reagent (1 U/ mL horseradish peroxidase, 1 U/ mL cholesterol oxidase, and 1 U/ mL cholesterol esterase (CE)) was added to 50-μL samples in 96-well plates. After incubation for 60 min at 37 °C in the dark, sample fluorescence was measured using a microplate reader (Tecan, Switzerland) at 530 nm (excitation) and 590 nm (emission). The total cholesterol (TC) content was determined by measuring the cholesterol concentration following digestion with CE. To measure free cholesterol (FC), CE was omitted from the assay. Each sample was analyzed in triplicate, and at least three independent experiments were performed. Values were obtained from a cholesterol standard curve and then normalized.

ELISA assay

Cholesterol metabolism-related proteins (*i.e.*, HMG-CR, LDL, MTTP and CETP) were measured in rat brain tissue using ELISA kits (Cloud-Clone Corp., China) according to the manufacturer's instructions. Briefly, the brain tissues were minced in ice-cold PBS (phosphate-buffered saline; 0.01 mol/L, pH 7.0–7.2), and the homogenates were centrifuged for 20 min at 5000×*g*. One hundred microliters of the resultant suspension was added to a 96-well plate coated with the appropriate purified antigen and incubated for 2 h at 37 °C. The absorption was subsequently measured at 450 nm using a microplate reader (Tecan, Switzerland). The mean and standard errors of the repeated (at least three) measurements were calculated for each tested sample.

Real-time polymerase chain reaction (real-time PCR)

Total mRNA was purified from the rat brains using the SV Total RNA Isolation System (Promega Corporation, USA). mRNA expression levels of HMG-CR, LDLR, CYP46A1, LXR-α, LXR-β, and ABCA1 and 18S rRNA (invariant control) were analyzed using real-time PCR. Reverse transcription (RT) was performed using a Reverse Transcription System (Promega Corporation, USA). Briefly, double-stranded DNA was synthesized from 1 μg of total RNA and used as a template for the real-time polymerase chain reaction. The forward and

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