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Cystathionine beta synthase-hydrogen sulfide system in paraventricular nucleus reduced high fatty diet induced obesity and insulin resistance by brain-adipose axis

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ABSTRACT

Hydrogen sulfide (H2S) is an essential neuromodulator, generates by cystathionine β synthase (CBS) or 3-mercaptopyruvate sulfurtransferase (3MST) in the brain. H2S can mediate paraventricular nucleus (PVN) neuron activity, and regulate neuroendocrine hormones secretion. On the other hand, CBS deficiency caused metabolic disorder and body weight reduction. However, whether CBS/H2S of PVN regulates neuroendocrine hormones to mediate energy metabolism is unknown. Here, we first identified the CBS co-localization with thyrotropin-releasing hormone (TRH) and corticotropin releasing hormone (CRH) positive neurons. In HFD induced obese rats, CBS protein of hypothalamus decreased. By contrast, overexpression CBS in PVN via lentivirus, lowered food uptake, body weight and fat mass, and reduced blood glucose, lipid disorders and insulin resistance. Intriguingly, CBS overexpression increased the pre-TRH expression, slightly elevated plasma thyroxine and thyrotropin level, but decreased the plasma ACTH and corticosterone level. Then, we found that mTOR activation contributed to pre-TRH up-regulation by CBS/H2S system. In db/db obese mice, hypothalamus CBS/H2S system also down-regulated association with reduction pre-TRH expression; in contrast, CBS overexpression in PVN slightly elevated plasma leptin. Next, leptin stimulated FOXO3a nuclear translocation, increased FOXO3a binding activity to two binding sites of CBS promoter, and then enhanced CBS protein expression. In conclusion, leptin activates neuron CBS-H2S system by FOXO3a, regulates neuroendocrine hormones to modulate the energy homeostasis, thus highlights a new brain-adipose feedback axis in energy metabolism.

1. Introduction

Hydrogen sulfide (H2S) is an endogenous gastrotransmitter in mammalian after nitric oxide and carbon monoxide. Biogenesis of endogenous H2S mainly dependents on three enzymes: cystathioninase (CSE), cystathionine beta synthase (CBS) and 3-mecaptopyruvate sulfurtransferase (3MST) [1]. In the brain, endogenous H2S acts as an essential neuromodulator in neuron activity, astrocyte function, and neuroprotectant in ischemic stroke, Alzheimer's disease, Parkinson's disease, recurrent febrile seizure; which dependent on CBS and 3MST [2, 3]. In adult rat, CBS major localized Purkinje cell layer and hippocampus [4], thus CBS-dependent H2S stimulated NMDA receptor and facilitated LTP -a synaptic model of learning and memory [5]. 3MST mainly distributed in glia cell but not neuron [6]. CBS-dependent H2S in the brain induced a rapid effect on blood pressure, whereas the effect of 3MST-dependent H2S was more durable [7]. These studies suggested that biological effects of H2S were dependent on CBS and 3MST distribution in the brain. More interestingly, H2S inhibited potassium chloride stimulated corticotropin-releasing hormone release and CBS inhibiter reversed this effect [8]. Recent work found microinjection H2S

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donor into paraventricular nucleus (PVN) increased renal sympathetic nerve activity and cardiac sympathetic afferent reflex [9]. H2S donor also directly induced depolarizing of paraventricular neuron [10]. These works highlight that H2S acts as an important neuromodulator in hypothalamus linked with neuroendocrine function.

Hypothalamus function tightly associated with energy metabolism and obesity [11, 12]. Many neuroendocrine hormones such as leptin, alpha-melanocyte stimulating hormone (α-MSH, a proteolytic product of proopiomelanocortin (POMC)), glucagon-like peptide 1 (GLP-1), ghrelin etc. mediated body energy homeostasis and associated with diabetes and obesity [12]. Leptin is an adipocyte-derived hormone and function as an essential regulator in controlling adipose mass [13]. Leptin can across the blood-brain barrier, and then activate leptin receptor in neuron of hypothalamus to mediate neuroendocrine hormones secretion (such as thyrotropin-releasing hormone, TRH) and food intake to maintain energy homeostasis [11, 14]. In PVN neuron, leptin activated STAT-3 signal to increase pre-pro-TRH transcript. Leptin also inhibited NYP/AgRP and stimulated POMC, in turn cleavage into α-MSH to increase TRH secretion. Over TRH from PVN regulates thyrotropin (TSH) releasing from pituitary and then mediates thyroid hormone (TH, an essential hormone for body metabolic process regulation) releasing or secretion. Leptin-TRH interaction is an important pathway of PVN to regulate body energy storage or expenditure balance [15].

CBS deficiency induced body weight lower in four inbred strain mice [16]. By contrast, H2S donor reduced high fat diet (HFD) induced insulin resistance and lipid metabolism disorder by activation peroxisome proliferator-activated receptor gamma [17, 18]. These studies indicated that CBS-H2S system positively linked with obesity and insulin resistance. More intriguingly, increasing endogenous H2S production by CBS agonist inhibited potassium chloride induced corticotropin-releasing hormone (CRH) releasing [8], which raised us make a hypothesis, whether hypothalamus CBS-H2S might mediate neuroendocrine hormone to regulate body energy homeostasis. In present study, we investigated that CBS-H2S in PVN neuron role in HFD-induced obesity and insulin resistance, and its possible molecular mechanism.

2. Materials and methods

2.1. Animals

All animal procedures complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (document No. 55, 2001) and the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, updated 2011). The care and use of laboratory animals was approved by the Laboratory Animal Ethics Committee of Peking University. Sprague-Dawley (SD) rats (200–220 g) were supplied by the Animal Center, Peking University Health Science Center.

SD rats were bilaterally microinjected (0.05 μL) with lenti-CBS-GFP (5×10^9) into the PVN (B: −1.6 mm; L: 1.41 mm; D: 7.4 mm; n = 10). Control rats were also bilaterally microinjected Lenti-GFP 6.3×10^9 into the same area [19]. The rats were fed high fat diet (HFD, 60% energy from fat) for 60 days. Rats were fasted for 16-h before OGTT. For OGTT, blood (tail vein) glucose levels were measured at baseline and 15, 30, 60, 90, and 120 min after gavage (gastric tube, outer diameter 1.2 mm) glucose (150 mg) by using an Accu CheK Active glucometer (Roche Diagnostics). After 1 week for OGTT assay, the rats were fasted for 12 h, and blood were collected by abdominal artery. Fasting blood glucose was determined by glucose oxidase method. Fasting serum insulin was determined radioimmunoassay using a rabbit anti-mouse insulin antibody, ^{125}I-labeled bovine insulin as tracer, and mouse insulin as standard. HOMA index was counted by (fasting glucose × fasting insulin)/22.5. TSH (CBS-E05115r), T4 (CBS-E05082r), free T4 (CBS-E05079r), T3 (CBS-E05085r), free T3 (CBS-E05076r), ACTH (CBS-E06875r), corticosterone (CBS-E07014r), C-peptide (CBS-E05067r) and leptin (CBS-E07433r) were measured by commercial ELISA kit (CUSBIO, Wuhan).

2.2. Primary neonatal rat thalamus neuron culture

Primary neonatal rat thalamus neuron was prepared with Lolait SJ's method [20]. Thalamus from 1-day old neonatal rat was isolated and dissociated with 1 mm^2 size. The tissues were digested with 0.125% trypsin, and then added fetal bovine serum, centrifuged at 250*g* RT. Supernatant were removed and cells were collected and cultured with neuronal medium (ScienCell, USA) for 24–48 h. Observed the synapse growth from the neuron cell, the immunofluorescence stain or western blot was performed.

2.3. Western blot assay

Thalamus tissue concussion slice (400 μm) were prepared and cultured in Krebs-Henseleit buffer containing 1% BSA and continuously bubbled with O2% and 5% CO2 gas. Tissues were treated by GYY4137 (1 mM, ab142145, abcam), hydroxylamine (500 μM, 431,362, Sigma), leptin (1 μg/mL, 003–12, Phoenix biotech (Beijing) CO. LTD) or α-MSH (1 μg/mL, 043–01, Phoenix biotech (Beijing) CO. LTD) for 12 h. Then the thalamus slice was collected and lysed. The protein content of lysates was determined by the BCA assay. Equal amounts of protein were denatured, then loaded and separated by SDS-PAGE. Proteins transferred on membranes were recognized with use of primary antibodies [pre-TRH (ac171958), mTOR and its substance antibody kit (#9862. Cell Signal Technology), phosphor-FOXO3a (#9466, CST) or FOXO3a (#12829, CST)], and horseradish peroxidase-conjugated secondary antibodies. The bands were developed by use of ECL reagents. If required, membranes were stripped by a commercial stripping buffer, and blots were re-probed with other antibodies.

2.4. Chromatin immunoprecipitation (ChIP) assay

293-HEK cells were transfected FOXO3a (CH844427, Vigene Biosciences, shandong) or blank plasmid. HepG2 cell or FOXO3a overexpression 293-HEK Cell were cross-linked with formaldehyde, and sonicated to shear DNA (200–300 bp). Immunoprecipitations were carried out using 3 μg of FOXO3a antibodies or 3 μg IgG, and then the binding DNA were extracted. The eluted DNA and input control were analyzed by PCR with two specific primers (DNA binding domain 1(DBM1): F: 5′- TCA TCT CTG CCG CCA TCT-3′; R: 5′-GAG ACT GAG CGA GAC TGT-3′. DBM2: F: 5′- CTC ATC AGT AAA GGT TCC T-3′; R: 5′-GTC TTC TAC CTG GTG TTC-3′. The end PCR product were also separated on a 1.2% agarose gel (DBM1 product: 141 bp; DBM2 product: 81 bp) [18].

2.5. Electrophoretic mobility shift assay (EMSA)

The 5′-end biotin-labeled DBM1 (5′-CTC TCT TCT TAT AAA GAC TGG-3′) and DBM2 (5′-GGT TCC TTA AAT TCC CGA AGG-3′) of FOXO3a in CBS promoter double-stranded oligonucleotide probes were synthesized by Thermo Fisher Scientific. Unlabeled probe was a cold competitive probe. Nuclear protein (10 μg) extracted from FOXO3a overexpression 293-HEK cell or HepG2 cell by use of a kit (NE-PER nuclear and cytoplasmic extraction reagent, Pierce Biotechnology, Rockford, IL). EMSA involved the LightShift Chemiluminescent EMSA Kit (Pierce Biotechnology) [21].

2.6. Luciferase reporter assay

Reporter assays were performed as described previously. Briefly, 293-HEK cells were transfected with empty pGL3-basic vector or with pGL3 vector containing the full CBS promoter, 279–1258 or 1906–1258