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Hypothalamic AMPK-induced autophagy ameliorates hypercatabolism in septic rats by regulating POMC expression

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ABSTRACT

Hypercatabolism plays a critical role in the pathogenesis of post-critical care debility in critical patients. Central nervous system may exerte a critical role in the regulation of hypercatabolism. However, little is known about the exact mechanisms of the central role. Here, we reported that actived hypothalamic AMP-activated protein kinase (AMPK)-induced autophagy modulated the expression of POMC to ameliorate hypercatabolism in septic rats. Firstly, rats were i.c.v. injected with the lentiviral vector containing shRNA against POMC. Two weeks after injections, rats were intraperitoneally injected with LPS or saline. Twenty-four hours later, blood, skeletal muscle and hypothalamus tissues were obtained. Hypercatabolism markers and neuropeptides expression were detected. Then, rats were injected with AICAR or saline into third ventricle and promptly intraperitoneally injected with LPS or saline. Twenty-four hours after infection, blood, skeletal muscle and hypothalamus tissues were obtained. Hypercatabolism, hypothalamic AMPK-induced autophagy markers and neuropeptides expression were also detected. Results showed that sepsis would decrease the level of hypothalamic autophagy accompany with the alterations of POMC expression and hypercatabolism. Knocking out hypothalamus POMC expression could significantly ameliorate hypercatabolism. Moreover, Central activation of AMPK-induced autophagy pathway via third ventricle injection of AICAR, an AMPK activator, could efficiently ameliorate hypercatabolism as well as attenuate the elevated POMC expression rather than other neuropeptides. Taken together, these results suggested that hypothalamic AMPK-autophagy pathway as a regulatory pathway for POMC expression was essential for hypercatabolism during sepsis. And hypothalamic AMPK-autophagy activation could attenuate the POMC expression to ameliorate hypercatabolism. Pharmaceuticals with the ability of activating hypothalamic AMPK-autophagy pathway may be a therapeutic potential for hypercatabolism in septic patients.

1. Introduction

In septic patients, sustained hypercatabolism is prevalent and contributes to serious complications [1]. Two large clinical prospective trials, EPaNIC and EDEN trials, employed different nutrition strategies to counteract the hypercatabolism in critical patients, showing little effects on metabolic changes regardless of early parental feeding or full enteral feeding [2,3]. Recent researches showed acute skeletal muscle wasting, an important hypermetabolic change in the early stage of sepsis, was a key factor affecting the survival rates of septic patients [4,5]. However, several researches including intensive insulin therapy, protein C, hydrocortison and estrogen showed little effects on the hypercatabolism during sepsis [6–10], thus questioning the exact mechanism of hypercatabolism during sepsis.

Hypothalamus is the regulatory centre of energy metabolism and feeding behavior [11,12]. Two major neuronal populations in the arcuate nucleus (ARC) of the hypothalamus, POMC and AgRP neurons, play an important role in food intake. One of them co-expresses the anorexigenic POMC and CARTPT/CART, which could be pyrolysed into α-mela-nocytestimulating hormone (α-MSH), binding to MC3-R and MC4-R to derive anorectic effects [13]. Whereas the other neuron co-expresses the orexigenic peptides NPY and AgRP and promotes food intake [14,15]. The hypothalamic

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melanocortin system exerted a critical role in mediating food intake and cachexia in both acute and chronic illness [16]. And we also found the expression of hypothalamic neuropeptides exerted a critical role in the regulation of the hypercatabolism during sepsis [8–10,17].

Recent studies showed that hypothalamus autophagy was involved in the regulation of neuropeptide and food intake [18–20]. Autophagy is a self-degradation process in which cytosolic components are degraded and recycled through lysosomes to maintain cellular homeostasis [21]. Actived autophagy in brain could maintain the needed levels of ATP production and protein synthesis to protect neurons during nutrient deprivation [22,23]. Inhibition of autophagy by siRNAs or a protease inhibitor decreased Npy mRNA expression and increased POMC mRNA expression in normal conditions [24]. The specific loss of Atg7 (autophagy related 7) or Atg12 in hypothalamic POMC showed decreasing levels of α-MSH and elevated adiposity, which was consistent with increased food intake [25,26]. Using the model of sepsis, we recently observed the inactivated autophagy during sepsis and mild hypothermia reversed the level of hypothalamic autophagy, accompanied with the alleviated muscle wasting [27]. Emerging evidence suggested that Hypothalamic AMP-activated protein kinase (AMPK) activity was closely involved in the induction of autophagy and the regulation of energy balance [24]. Mice with AMPK knockdown in the ARC showed decreased autophagy, food intake and body weight [28]. During endotoxemia, the inactivation of hypothalamic AMPK led to hypoglycaemia. And intracerebroventricular (i.c.v.) administration of AICAR, an AMPK activator could prevent it [29].

We therefore hypothesized that hypothalamus AMPK-induced autophagy was a possible mechanism of down-regulation of POMC expression and ameliorated hypercatabolism during sepsis, and tested whether activating hypothalamus AMPK-induced autophagy or knocking out POMC could mitigate the detrimental process.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (median weight 283 g; range 257–307 g) were obtained from the animal center of Jinling Hospital. All the rats were kept in an animal house under regular lighting conditions (light cycle 6:00–18:00) at 25 °C and were provided ad libitum access to water and standard rat pellet chow. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Nanjing University and Jinling Hospital.

2.2. Study protocol

Experiment 1: effects of knockdown of hypothalamic POMC expression on hypothalamic AMPK-induced autophagy, neuropeptides and muscle wasting in septic rats.

We used lentivirus to knockout hypothalamic POMC expression. The sequences of shRNA against rat POMC was CUCUUCAAGAACGCCAUCA (5′–3′) (GenPharma Co., Ltd Shanghai). The lentivirus (1×10^9, 5 μl) or vehicle (5 μl) were i.c.v. injected. Two weeks after injections, rats were intraperitoneally injected with LPS (5 mg/kg, *E. coli*, 055:B5, Sigma) or saline. LPS infection was usually used to study sepsis-induced muscle wasting due to the controllability, reproducibility and representativeness. Rats were then returned to the animal facility. Twenty-four hours after infection, body weight change and food-intake were measured. Then rats were anaesthetized with 2% pentobarbital sodium (0.2 ml/100 g) and transcardially perfused with 200 ml of saline containing heparin (50 i.u./L). The hypothalamus of brain and extensor digitorum longus (EDL) were rapidly dissected and frozen in −80 °C.

Experiment 2: effects of hypothalamic AMPK activation on hypothalamic AMPK-induced autophagy, neuropeptides and muscle wasting in septic rats.

Rats were anaesthetized with 2% pentobarbital sodium and underwent surgical implantation of a 26-gauge stainless steel cannula into the third cerebral ventricle proved by Angiotensin II experiment. After one week recovery period, rats were injected with AICAR (30 μg/5 μl) or saline (5 μl) into third ventricle and promptly intraperitoneally injected with LPS or saline. Twenty-four hours after infection, rats were anaesthetized and tissues were harvested and stored as described in experiment 1.

2.3. Western-blotting

Proteins extracted from the hypothalamus and EDL were dissolved in a lysis buffer and separated by SDS/PAGE and then were transferred onto polyvinylidene difluoride membranes. Primary antibodies included AMP-activated protein kinase (AMPK) (Cell Signaling Technology #2603), phosphorylated AMPK (*p*-AMPK) (CST #2537), LC3 (CST #13118), ULK1 (CST #6439), *p*-ULK1 (CST #12753), POMC (abcam #210605–100), MuRF-1 (abcam #172479), MAFBx (abcam #168372) and β-Actin (AP0060). The densitometric analyses of Western blotting images were performed using Image-Pro Plus software (Media Cybernetics).

2.4. Real-time polymerase chain reaction

Total RNA was extracted from hypothalamus and gastrocnemius muscle using TRIzol reagent (Invitrogen 15596–026). Once isolated, 5 μg of total RNA was reverse transcribed to yield cDNA. Real-time PCR was performed with a SYBR Green PCR kit (TOYOBO QPR-201) in a qPCR machine (ABI Step one plus Real time-PCR system) for 40 cycles using the following primers: POMC: 5′-GACCTCACCACGGAAAGCAAC-3′ (forward) and 5′-GGGCTGTTCATCTCCGTTGC-3′ (reverse); CART: 5′-AAGAAGTACGGCCAAGTCCC-3′ (forward) and 5′-CAGTCACACAGCTTCCCGAT-3′ (reverse); AgRP: 5′-TGTTGCTGAGCTGTGTCCTGC-3′ (forward) and 5′-ATCTTCTGCTCGGTCTGCTGC-3′ (reverse); NPY: 5′-GTGGACTGACCCTCGCTCTAT-3′ (forward) and 5′-GGGCATTTTCTGTGCTTTCTC-3′ (reverse); MuRF-1: 5′–CCTTGAGGGCCATCGACTTT-3′ (forward) and 5′-CAGAGGGCGTCTCACTCATC-3′ (reverse); MAFBx: 5′-AGCTTGTGCGATGTTACCCA-3′ (forward) and 5′-GGTGAAAGTGAGACGGAGCA-3′ (reverse); GAPDH: 5′-GGCCTTCCGTGTTCCTACC-3′ (forward) and 5′-CGCCTGCTTCACCACCTTC-3′ (reverse). Expression levels of each gene were normalized to an internal control gene (GAPDH mRNA).

2.5. Hypothalamic immunostaining

Rats were anaesthetized and transcardially perfused with 200 ml of saline, followed by 200 ml of 4% paraformaldehyde.The brain was fixed for 1 h, placed in phosphate-buffered saline containing 30% sucrose and stored at 4 °C. Brain sections of 6 μm thickness were made using a cryostat at −20 °C. Fixed brain sections were blocked with serum of the appropriate species, penetrated with 0.2% TritonX-100, and treated with primary rabbit anti-POMC antibodies, and subsequently reacted with FITC-labeled Goat Anti-Rabbit secondary antibody (Invitrogen). The nucleus was stained by DAPI (4,6-diamidino-2-phenylindole). Images were captured under a FW1000 confocal microscope.

2.6. Blood glucose, serum insulin, leptin and corticosterone

Blood glucose was measured by blood glucose meter (Roche). Serum insulin and leptin were measured by elisa (ELR-Insulin-1

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