Brain Research 1684 (2018) 21–29

Contents lists available at ScienceDirect

Brain Research

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

Research report

Activation of AMP-activated protein kinase (AMPK) aggravated postoperative cognitive dysfunction and pathogenesis in aged rats

Mengya Cao [a,1], Jiakai Fang [a,1], Xueqin Wang [b], Yi Wang [a], Kaiming Duan [a], Feng Ye [a,b], Wen Ouyang [a], Jianbin Tong [a,b,*]

[a] Department of Anesthesiology, The Third Xiangya Hospital, Central South University, Changsha, Hunan, PR China
[b] Center for Experimental Medicine, The Third Xiangya Hospital, Central South University, Changsha, Hunan, PR China

ARTICLE INFO

Article history:
Received 1 November 2017
Received in revised form 18 January 2018
Accepted 21 January 2018
Available online 3 February 2018

Keywords:
Postoperative cognitive dysfunction
AMP-activated protein kinase
N-methyl-D-aspartic acid receptor
Neuroinflammation
Rat

ABSTRACT

The upstream signal molecule modulating neuro-inflammation and synaptic changes during the pathogenesis of postoperative cognitive dysfunction (POCD) is still elusive. Here, we examined the effects and mechanisms of energy sensor AMP-activated protein kinase (AMPK) in the pathogenesis of POCD. Our data showed that surgery significantly increased the expression of p-AMPK in aged rats ($p < 0.05$), but not in adult rats ($p > 0.05$). Moreover, inhibiting AMPK activation via compound C during operation significantly improved surgery-induced impairment of the learning and memory of aged rats in water maze ($p < 0.05$). Further mechanism studies showed that corresponding to the impairment of learning and memory after surgery, surgery significantly increased the activation of microglia, decreased the expressions of NR2B and p-NR2B, and increased the expressions of Tau and p-Tau, which also were obviously restored by inhibiting AMPK during operation. In contrast, Inhibiting AMPK activation during operation didn't change ATP level in the hippocampus of aged rats after surgery. These data suggest that surgery induced activation of AMPK in hippocampus in an age-dependent manner. AMPK plays important roles in POCD of aged rats via multiple mechanisms, and is a possible molecular target for the prevention and treatment of POCD.

1. Introduction

Postoperative cognitive dysfunction (POCD) is a common complication among aged patients after surgery, which is characterized by the impairment of memory, information processing ability, and mental flexibility (Chen et al., 2013; Hovens et al., 2012; Pandharipande et al., 2013; Ramaiah and Lam, 2009). During the pathogenesis of POCD, peripheral surgery or anesthesia alone both can induce obvious neuroinflammation, oxidative stress, Tau's abnormality and synaptic impairments in the brain(Degos et al., 2013; Dong et al., 2016; Hovens et al., 2014; Le et al., 2014; Li et al., 2017; Lin et al., 2014; Terrando et al., 2010; Terrando et al., 2011; Vacas et al., 2013; Vacas et al., 2014; Xu et al., 2014; Zhang et al., 2016). And the pathological changes of brain are modulated by peripheral inflammatory factors and immune cells (He et al., 2012; Terrando et al., 2010; Terrando et al., 2011; Terrando et al., 2016; Vacas et al., 2013, 2014). However, whether there is a critical upstream signal molecule initiating the brain's pathogenesis during POCD is still elusive.

AMP-activated kinase (AMPK) is a ubiquitous serine/ threonine protein kinase, and comprises a catalytic α subunit, regulatory β and γ subunits (Thornton et al., 2016). AMPK is an energy sensor activated by the increase of AMP/ADP: ATP ratio. AMPK activation requires phosphorylation of Thr172 at the activation loop of the catalytic α subunit (Cheng et al., 2016). Under the physiological condition, AMPK maintains the cellular energy homeostasis via up-regulating catabolic processes or down–regulating energy-consuming processes (Burkewitz et al., 2015). It also modulates the protein synthesis, especially the synthesis of long-term memory related proteins (Costa-Mattioli et al., 2009; Klann and Dever, 2004). Moreover, AMPK is closely involved in ischemic diseases (Choi et al., 2013; Li et al., 2007; Nam et al., 2013; Rousset et al., 2015), neurodegenerative diseases (Cai et al., 2012; Du et al., 2015; Liu et al., 2015; Ma et al., 2014; Vingtdeux et al., 2011), and pain (Bullon et al., 2016; Song et al., 2015). For example, AMPK expression was up-regulated in the post-ischemic hippocampus, and the impairment of hippocampus was significantly alleviated by inhibiting the AMPK activation through reducing ATP depletion, lactate accumulation, and

* Corresponding author at: Center for Experimental Medicine, The Third Xiangya Hospital of Central South University, Changsha, Hunan, PR China.
E-mail address: jianbintong@csu.edu.cn (J. Tong).
[1] Contribute to the work equally.

https://doi.org/10.1016/j.brainres.2018.01.027
0006-8993/

oxidative stress (Li et al., 2007; McCullough et al., 2005; Nam et al., 2013). The expression and activation of AMPK also increased in the brains of patients with Alzheimer's Disease, and inhibiting AMPK's activation improved the impairment of synaptic plasticity of hippocampus via decreasing amyloid-β protein (Aβ) 's generation and modulating eukaryotic elongation factor 2 (eEF2) and its kinase eEF2K (Cai et al., 2012; Ma et al., 2014). In addition, activated AMPK modulated the production of inflammatory cells through down-regulating phosphatidylinositol 3-kinase/p38 mitogen-activated protein kinase (PI3K/p38), mitogen-activated protein kinase (MAPK), nuclear factor kappa B (NF-κB) and Nod-like receptor protein 3 (NLRP3) (Bullon et al., 2016; McCullough et al., 2005; Song et al., 2015; Zhu et al., 2015). The above studies have shown that AMPK can modulate inflammation response, Aβ's neurotoxicity, oxidative stress and synaptic plasticity in acute and chronic brain injuries. Interestingly, neuroinflammation, Aβ's neurotoxicity, oxidative stress, and synaptic changes all are important pathological mechanisms of POCD (Degos et al., 2013; Lin et al., 2014; Terrando et al., 2010; Terrando et al., 2011; Vacas et al., 2013, 2014; Xu et al., 2014). Therefore, we proposed that AMPK could be a 'switch' molecule modulating the pathogenesis of POCD. Our data showed that laparotomy induced AMPK's activation in the hippocampus of aged rats, but not in the adult rats. Inhibiting AMPK activation during operation limited microglia activation, NR2B down-regulation, and Tau and p-Tau up-regulation in the hippocampus of aged rats with surgery, and resulting in an overall improvement of POCD in aged rats.

2. Results

2.1. Laparotomy induced AMPK activation in hippocampus of aged, but not adult, rats

Phospho-AMPK (p-AMPK) is the active type of AMPK. AMPK was mainly expressed in neurons (Fig. 1A). Compared to the control group of aged rats, p-AMPK levels in the hippocampus in the surgery group were significantly increased [$F_{(3,12)} = 6.509$, $p = 0.007$], but not the AMPK levels (Fig. 1B). The activation level of AMPK (p-AMPK/AMPK ratio) was increased significantly at 6 h, 1 day and 3 days after laparotomy ($p = 0.016$, 0.023, and 0.030, respectively). In contrast, the expression levels of AMPK and p-AMPK in young adult hippocampus were similar under normal condition and after laparotomy [$F_{(3,12)} = 0.890$, $p = 0.474$] (Fig. 1C). These data suggested that AMPK activation after surgery was affected by the age of rats.

2.2. Inhibiting AMPK activation during laparotomy improved POCD in aged rats

In order to clarify the effects of AMPK activation on POCD in aged rats, we detected the learning and memory functions of the aged rats by Morris water maze among the CTL group (control group), the SD (surgery + DMSO) group and the SC (surgery + AM PK inhibitor compound C) group.

The latency to the platform during the training of water maze was analyzed by two-way repeated measures ANOVA with the treatment as between-subjects factor and measure time as within subjects' factor. The latency to the platform was obviouslyaffected by the treatments [$F_{(2,27)} = 4.005$, $p = 0.030$], measure time [$F_{(3,25)} = 25.783$, $p < 0.001$], but no significant effect on the interaction of treatment × measure time [$F_{(6,52)} = 1.479$, $p = 0.207$]. Further analysis showed that the latency to the platform of SD group was significantly longer than that of CTL or SC group ($p = 0.018$, $p = 0.024$, respectively) (Fig. 2A). These data showed that the learning function in the SC group was better than that of the SD group.

During the probe trial of water maze, latency for the first time to the area of the hidden escape platform, the numbers of crossings through the platform and the time percent spent in the target quadrant of the SD group all were significantly different from that of the CTL and the SC groups ($p < 0.05$, respectively). Latency for the first time to the area of the hidden escape platform of the SD group was longer than that of the SC group ($p = 0.006$) (Fig. 2B). And the numbers of crossings through the platform, the time percent spent in the target quadrant of the SD group both were less than that of the SC groups ($p = 0.026$ for crossing times; $p < 0.001$ for% time in the target quadrant) (Fig. 2C and D). These data showed that the memory function in the SD group were worse than that of the SC group.

Consistent with the improvement of learning and memory functions in the SC group, the p-AMPK expression of the SC group was significantly less than that of the SD group ($p < 0.01$, respectively) (Fig. 2F), although the total AMPK expression was comparable in both groups ($p > 0.05$, respectively) (Fig. 2E). These results suggested that inhibiting AMPK activation during operation improved POCD of the aged rats.

2.3. Inhibiting AMPK activation during laparotomy limited microglia's activation in aged rats

In order to investigate the underlying mechanisms by which inhibiting AMPK activation improved POCD, we first detected the activation of the microglia, a mark of neuroinflammation. In the young adult rats, surgery significantly increased the percentage of the activated microglia in the dentate genus (DG) 6 h after operation [$F_{(3,12)} = 3.527$, $p = 0.049$], but not in CA1 region during the first three days after operation [$F_{(3,12)} = 1.694$, $p = 0.221$] (Supl. Fig. 1). In the aged rats, compared to the other two groups, the percentage of the activated microglia in the DG and the CA1 region in the SD group increased significantly at 6 h [$F_{(2,9)} = 83.413$, $p < 0.001$ for DG; $F_{(2,9)} = 73.00$, $p < 0.001$ for CA1] and 1 day [$F_{(2,9)} = 93.256$, $p < 0.001$ for DG; $F_{(2,9)} = 117.380$, $p < 0.001$ for CA1] after surgery (Fig. 3). These data showed that inhibiting AMPK activation during operation limited microglia's activation in the aged rats.

2.4. Inhibiting AMPK activation during laparotomy limited NR2B down-regulation

N-methyl-D-aspartic acid receptor (NMDAR) is closely involved in synaptic plasticity and memory (Kandel, 2012). NR1, NR2A and NR2B are the main subunits. Previous studies have shown that AMPK and NMDAR can reciprocally regulate the activation and the expression via calcium ion and ATP-sensitive K (+) (K-ATP) current (Seixas da Silva et al., 2017; Shen et al., 2014). Therefore, we detected the expression levels of these subunits. In young adult rats, surgery didn't change the expressions of NR1, NR2A and NR2B in hippocampus during the first three days after operation ($p > 0.05$, respectively) (Supl. Fig. 2). In the aged rats, compared to the control group, NR2B expression of the SD group decreased on the 1st day after surgery [$F_{(2,9)} = 8.323$, $p = 0.012$] (Fig. 4C). The NR2B down regulation in SD group was restored after inhibiting AMPK activation [$F_{(2,9)} = 8.323$, $p = 0.035$] (Fig. 4C). In contrast to the obvious changes of NR2B, there was no difference of NR1 and NR2A expressions among the control group, the SD group and the SC group ($p > 0.05$, respectively) (Fig. 4A and B).

2.5. Inhibiting AMPK activation during laparotomylimited the expressions of Tau and p-Tau

Tau and p-Tau are closely associated with the stability of microtubules and cognition. And AMPK directly phosphorylates Tau (Thornton et al., 2011). So, we also detected the expressions of