ISCHEMIC PRECONDITIONING DOES NOT DISPLAY NEUROPROTECTION IN HIGH-FAT DIET-INDUCED OBESE GERBILS BUT RATHER ELICITS NEURONAL DEATH THROUGH INCREASE OF ABNORMAL mTOR ACTIVATION-MEDIATED OXIDATIVE STRESS AND NEUROINFLAMMATION

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Introduction

Transient ischemia (TI) in the whole brain following a complete interruption of blood flow can lead to irreversible neuronal damage/death in vulnerable brain areas. The CA1 field (CA1) in the hippocampus is known to be especially sensitive to TI. Extensive loss of pyramidal neurons in the CA1 occurs typically over several days after TI. Clinically, a brief TI in brains can trigger some neuroprotection against subsequent severe episode of TI by inducing ischemic tolerance known as ischemic preconditioning (IPC). In this regard, researchers have demonstrated that a brief duration (1-3 min) of TI can prevent neuronal death in CA1 against a subsequent longer TI that can result in massive loss of CA1 pyramidal neurons in rats, mice, and gerbils. Many factors affect the pathophysiology in ischemic brains. Among these factors, high fat diet (HFD)-induced obesity is a significant and serious public health problem. It is a global epidemic according to the World Health Organization. Obesity is known to increase the morbidity and mortality following a variety of diseases including cardio-cerebrovascular disease. It is considered a major factor that can lead to worse outcome after ischemic insults. Recently, preclinical studies have shown that prolonged diet-induced obesity can exacerbate TI-induced brain injury in rats, mice, and gerbils. However, underlying mechanisms of obesity-enhanced ischemic brain injury remain unclear.

Experimental purpose

Although researchers have focused on deleterious effects of HFD-induced obesity on neuronal damage/death in ischemic brains, the impact of a brief episode of TI that can confer protection (tolerance) against a subsequent longer or severer TI in normal animals has not yet been reported in animal models of HFD-induced obesity. Therefore, the objective of this study was to investigate effects of a brief episode (2 min) of TI on neuronal damage/death in the hippocampus of HFD-induced obese gerbils and related mechanisms. HFD-induced obese gerbil is chosen in this study because it is a well-established animal model for studying TI-induced neuronal damage/death and its related mechanisms.

Materials and Methods

Experimental animals

Mongolian gerbil (male, 6 months of age)

Immunohistochemistry

Mouse anti - NeuN, GFAP, 4-HNE

Rabbit anti - Iba-1, IL-1 β , TNF- α , (p)-mTOR

Tissue staining

Fluoro-Jade B (F-J B)

Cresyl violet stining (CV)

Dihydroethidium (DHE)

Tissue processing for histology

Experimental design

T1 operation

Results

Fig. 1. CV staining in the hippocampus of the ND 5-min TI group (left column) and ND 2-min TI/5-min TI group (right column) at sham (A1, A2), 2 days (A3, A4) and 5 days (A5, A6) post-ischemia. In the ND 5-min TI group, very pale CV+ cells (A5, arrows) are shown in the stratum pyramidale of the CA1 at 5 days post-ischemia, whereas CV+ cells (A6, asterisk) in the ND 2-min TI/5-min TI group are not damaged. CA, cornus ammonis; DG, dentate gyrus. scale bar = 400 μ m.

Fig. 3. (a) Representative gerbils in the ND-fed group (left), HFD/Vehicle-fed group (middle), and HFD/RAPA-fed group (right) depicting gross images of whole body (top) and epididymal fat (bottom). (b-g) Physiological parameters, such as body weight (b), epididymal fat weight (c), blood glucose (d), serum total cholesterol (e), triglyceride (f), and leptin levels (g). Note that no significant differences are shown between the HFD/Vehicle-fed and HFD/RAPA-fed groups. The bars indicate the means $\pm$ SEM. n = 14/group. ***P < 0.001 vs. ND-fed group

Fig. 2. (a) CV staining in the hippocampus of the ND-fed group (left column) and HFD-fed group (right column) at sham (A1, A2), 2 days (A3, A4) and 5 days (A5, A6) after 2-min TI. In the HFD 2-min TI group, CV+ cells are very pale in the stratum pyramidale (A6, asterisk) of the CA1 at 5 days post-ischemia. CA, cornus ammonis; DG, dentate gyrus. scale bar = 400 μ m.

Fig. 4. (a) NeuN immunofluorescence (A1-A9) and F-J B histofluorescence staining (B1-B9) in the CA1 of the ND-fed group (left column), HFD/Vehicle-fed group (middle column), and HFD/RAPA-fed group (right column) at sham (A1-A3, B1-B3), 2 days (A4-A6, B4-B6), and 5 days (A7-A9, B7-B9) after 2-min TI. In the HFD/Vehicle 2-min TI group, a few NeuN+ CA1 pyramidal cells (A8, arrows) and many F-J B+ CA1 pyramidal cells (B8, asterisk) are observed 5 days post-ischemia. However, at this time, abundant NeuN+ CA1 pyramidal cells (A9, asterisk) and a few F-J B+ CA1 pyramidal cells (B9, arrows) are observed in the HFD/RAPA 2-min TI group. SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bar = 60 μ m. (b and c) The mean numbers of NeuN+ (b) and F-J B+ (c) CA1 pyramidal cells (n = 7/group). The bars indicate the means $\pm$ SEM. ***P < 0.001 vs. each sham-group, ####P < 0.001 vs. HFD/Vehicle 2-min TI group.

Fig. 5. DHE immunofluorescence staining (a) and HNE immunohistochemistry (b) in the CA1 of the ND-fed group (left columns), HFD/Vehicle-fed group (middle columns), and HFD/RAPA-fed group (right columns) at sham (A1-A3, B1-B3), 2 days (A4-A6, B4-B6), and 5 days (A7-A9, B7-B9) after 2-min TI. In the HFD/RAPA sham group, DHE immunofluorescence (A3) and HNE immunoreactivity (B3) in CA1 pyramidal cells (asterisks) are significantly decreased compared to the HFD/Vehicle sham group. In the HFD/RAPA 2-min TI groups at 2 days post-ischemia, DHE immunofluorescence (A6) and HNE immunoreactivity (B6) are significantly lower than the HFD/Vehicle 2-min TI group. Note that DHE immunofluorescence (A8) and HNE immunoreactivity (B8) in the HFD/Vehicle 2-min TI group at 5 days post-ischemia are very low due to death of CA1 pyramidal cells. Scale bar = 60 μ m. (c and d) Quantitative analyses of DHE immunofluorescence (c) and HNE immunoreactivity (d) in CA1 pyramidal cells (n = 7/group). Relative ratio was calibrated as %, with the ND sham group designated as 100%. The bars indicate the means $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001 vs. each sham group, #P < 0.05, ##P < 0.01, ###P < 0.001 vs. ND-fed group, †P < 0.05, ††P < 0.01, †††P < 0.001 vs. HFD/Vehicle-fed group.

Fig. 6. (a) Iba-1 immunohistochemistry in the CA1 of the ND-fed group (left column), HFD/Vehicle-fed group (middle column), and HFD/RAPA-fed group (right column) at sham (A1-A3), 2 days (A4-A6), and 5 days (A7-A9) after 2-min TI. In the HFD/Vehicle-fed group, Iba-1+ microglia are activated after 2-min TI, and the activated Iba-1+ microglia are aggregated in the stratum pyramidale (SP) (A8, asterisk) at 5 days post-ischemia. However, the activation and aggregation of Iba-1+ microglia are markedly reduced in the HFD/RAPA-fed group after 2-min TI. High magnification images are shown in white boxes. Scale bar = 60 μ m. (b) Quantitative analysis of Iba-1+ structure in the CA1 (n = 7/group). ROD was calibrated as %, with the ND sham group designated as 100%. The bars indicate the means $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001 vs. each sham group, #P < 0.05, ##P < 0.01, ###P < 0.001 vs. ND-fed group, †P < 0.05, ††P < 0.001 vs. HFD/Vehicle-fed group.

Fig. 7. (a) Western blotting for IL-1 β and TNF- α in the CA1 of the ND-fed, HFD/Vehicle-fed, and HFD/RAPA-fed groups at sham, 2 days, and 5 days after 2-min TI. (b and c) Semi-quantification of band intensities of IL-1 β (b) and TNF- α (c) in the CA1 (n = 7/group). The bars indicate the means $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001 vs. each sham group, #P < 0.05, ##P < 0.01, ###P < 0.001 vs. ND-fed group, †††P < 0.001 vs. HFD/Vehicle-fed group. (d and e) Immunohistochemistry for IL-1 β (d) and TNF- α (e) in the CA1 of the ND-fed group (left columns), HFD/Vehicle-fed group (middle columns), and HFD/RAPA-fed group (right columns) at sham (A1-A3, B1-B3), 2 days (A4-A6, B4-B6), and 5 days (A7-A9, B7-B9) after 2-min TI. IL-1 β and TNF- α immunoreactivity in the HFD/Vehicle-fed group is significantly increased in CA1 pyramidal cells (asterisks) at sham (A2, B2) and 2 days (A5, B5) post-ischemia. Note that IL-1 β and TNF- α immunoreactivity (asterisks) in the HFD/RAPA-fed group (A3, A6, B3, B6) is significantly lower compared to the HFD/Vehicle-fed group. Scale bar = 60 μ m. (f and g) Quantitative analyses of IL-1 β (f) and TNF- α (g) immunoreactivity in CA1 pyramidal cells (n = 7/group). RI was calibrated as %, with the ND sham group designated as 100%. The bars indicate the means $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001 vs. each sham group, #P < 0.05, ##P < 0.01, ###P < 0.001 vs. ND-fed group, †††P < 0.001 vs. HFD/Vehicle-fed group.

Figure 8. (a) Western blotting of mTOR and p-mTOR in the CA1 of the ND-fed, HFD/Vehicle-fed, and HFD/RAPA-fed groups at sham, 2 days, and 5 days after 2-min TI. (b and c) Semi-quantification of band intensities of mTOR (b) and p-mTOR (c) in the CA1 (n = 7/group). The bars indicate the means $\pm$ SEM. *P < 0.05, ***P < 0.001 vs. each sham group, ##P < 0.01, ###P < 0.001 vs. ND-fed group, †††P < 0.001 vs. HFD/Vehicle-fed group. (d and e) Immunohistochemistry for mTOR (d) and p-mTOR (e) in the CA1 of the ND-fed group (left columns), HFD/Vehicle-fed group (middle columns), and HFD/RAPA-fed group (right columns) at sham (A1-A3, B1-B3), 2 days (A4-A6, B4-B6), and 5 days (A7-A9, B7-B9) after 2-min TI. mTOR and p-mTOR immunoreactivity in the HFD/Vehicle-fed group is significantly higher in CA1 pyramidal cells (asterisks) than the ND-fed group at sham (A2, B2) and 2 days (A5, B5) after 2-min TI. In the HFD/RAPA-fed group, mTOR and p-mTOR immunoreactivity (asterisks) is significantly lower (A3, A6, B3, B6) compared to the HFD/Vehicle-fed group. Scale bar = 60 μ m. (f and g) Quantitative analyses of mTOR (f) and p-mTOR (g) immunoreactivity in the CA1 pyramidal cells (n = 7/group). RI was calibrated as %, with the ND sham group designated as 100%. The bars indicate the means $\pm$ SEM. *P < 0.05, ***P < 0.001 vs. each sham group, #P < 0.05, ##P < 0.01, ###P < 0.001 vs. ND-fed group, †††P < 0.001 vs. HFD/Vehicle-fed group.

Conclusion

In conclusion, results of this study clearly showed that 2 min of TI could induce IPC-mediated neuroprotection in ND-fed non-obese gerbils and provoke death of CA1 pyramidal neurons in HFD-induced obese gerbil. This might be closely associated with abnormal increase of mTOR activation-mediated severe oxidative stress and neuroinflammation in the hippocampal CA1 in pre- and/or post-ischemic phases under obesity condition. Taken together, our findings strongly suggest that a brief period of TI known to confer ischemic tolerance to a subsequent ischemic insult in normal brains could lead to neuronal damage/death in obese patients.