［課程－２］

審査の結果の要旨

氏名　アイヌルー トルデイ
阿依努尔・吐尔地
AYINUER TUERDI

Exercise (Ex), protein restriction (PR), and caloric restriction (CR) are robust interventions that reduce oxidative stress and have positive effects on individual organs. However, it remains unknown whether such interventions have similar effects on the olfactory system. I investigated whether 10 months of voluntary Ex, PR, or CR affects cell dynamics in the olfactory epithelium (OE) in male C57BL/6 mice. I first tested the effectiveness of our Ex, PR, and CR regimes to generate such changes. Then I sought to explore the histological and functional effects of long-term Ex, PR, and CR on cell dynamics in the OE.

1. Ex, PR, and CR induce a reduction in the number of OSNs in the DM region of the OE

I compared coronal sections of the OE from 12-month-old control mice and from 2-month-old mice that had not undergone any experimental regime. The results indicate that 10 mo of control diet does not induce any histological changes in the OE.

Next, I examined whether 10 mo of Ex, PR, or CR affects the number of OSNs in the OE. The number of OSNs and OMP-positive cells in the DM region was significantly decreased in Ex, PR, and CR mice compared with age-matched controls. In other regions did not observe significant differences between the experimental and control groups. These results indicate that Ex, PR, and CR induce histological changes selectively in the DM region of the OE.

I examined whether the DM region of the OE that showed histological change corresponds to the dorsal zone. The number of NQO1-positive cells in the dorsal zone decreased in Ex, CR, and PR mice compared with age-matched control mice. Furthermore, I found OMP staining was significantly lower in the NQO1-positive square than in the NQO1-negative square in Ex, PR, and CR mice. These results indicate that 10 mo of Ex, PR, or CR can induce damage in the dorsal zone of the OE.

I measured the size of the OMP-stained area within individual glomeruli of the OB in Ex, PR, and CR

mice. Results suggest that the experimental interventions resulted in the projection of fewer axons from OSNs in the dorsal zone.

To examine whether dorsal-zone-specific injury to the OE functionally disrupts stable sensory inputs to the OB, I measured the expression of the neural activity marker c-fos in NQO1-positive and -negative areas of the OB in Ex, PR, and CR mice after odor stimulation. I observed a significant reduction in the number of c-fos-positive cells in the NQO1-positive area of the OB in Ex, PR, and CR mice compared with age-matched control mice. I did not detect significant differences in the NQO1-negative area of the OB in Ex, PR, or CR mice relative to their age-matched controls. These results suggest that the decrease in dorsal-zone OMP-positive cells and OSNs and their axonal projections to the glomeruli is associated with a decrease in the glomerular response to odorants.

2. Long-term Ex, PR, or CR is required for dorsal-zone-specific injury

I assessed histological changes in the OE after 4 mo of Ex, PR, or CR. I did not observe significant differences in the number of OSNs or OMP-positive cells in any of the four areas of the OE in Ex, PR, or CR mice compared with age-matched control mice. The results indicate that a period longer than 4 mo is required to induce histological changes in the dorsal zone of the OE.

3. Ex, PR, and CR increase proliferation and apoptosis in OSNs

To examine the mechanism underlying the reduction in OSNs in the dorsal zone of the OE following Ex, PR, or CR, I examined the number of OE cells that were positive for Ki67 and caspase-3. The number of dorsal-zone Ki67-positive cells and caspase-3 was significantly higher in Ex, PR and CR mice than in age-matched controls. However, there were no significant increases in proliferation and caspase-3 staining in the ventral zone. These results indicate that Ex, PR, and CR increase the mitosis of progenitor basal cells but that the recovery of cell numbers is incomplete because of an increase in apoptotic OSNs in the dorsal zone.

4. Oxidative stress in dorsal-zone OSNs is strongly linked to NQO1 activity

I hypothesized that increased ROS generation, mediated by NQO1 activity, induced oxidative stress in this study, resulting in increased apoptosis in the dorsal zone of the OE. I examined the expression of 8-OHdG in Ex, PR, and CR mice as a marker of oxidative stress. I observed a significant increase in the number of 8-OHdG-positive cells in the dorsal zone in Ex, PR, and CR mice compared with age-matched controls, but no such increase in the ventral zone. Furthermore, I co-labeled OSNs with anti-NQO1 and

anti-8-OHdG to examine the role of oxidative stress in NQO1 expression. A significant number of 8-OHdG-positive cells were co-labeled with NQO1. These results indicate that increased oxidative stress is present within NQO1-positive cells. I co-labeled OSNs with anti-caspase-3 and anti-8-OHdG. I found that a significant number of 8-OHdG-positive cells were co-labeled with anti-caspase-3. These results indicate that increased oxidative stress induced apoptosis of the OSNs in the dorsal zone in Ex, PR, and CR mice.

I measured the intensity of anti-MnSOD immunostaining to quantify MnSOD activity. Result indicated that enhanced oxidative stress in Ex, PR, and CR mice is involved in the bioactivation of NQO1 under enhanced MnSOD activity, leading to prominent cell death of OSNs in the dorsal zone.

The present findings show that Ex, PR and CR induce negative structural and functional effects in the dorsal zone of the OE and that these changes are associated with NQO1 bioactivation. NQO1 protects cells against quinone toxicity; however, NQO1 bioactivation within metabolic pathways involving multiple enzymes is not simple. In particular, NQO1 could interact with MnSOD activities through the reaction of unstable hydroquinones with oxygen, leading to oxidative stress. I hypothesize that the physiological properties of NQO1 under enhanced MnSOD activity may cause OSNs to become highly susceptible to damage from environmental quinone agents or endogenous neurotoxins. MnSOD does have antioxidant enzymatic properties; however, an imbalance between ROS generation and antioxidant defenses within the dorsal zone might induce continuous oxidative damage. This would result in structural and functional damage to dorsal-zone OSNs despite the generation of new neurons.

Other possible mechanisms of dorsal-zone-specific damage could include alterations in internal hormonal signaling, because nutritional status dynamically and profoundly impacts olfactory sensitivity. There may be a decrease in systemic insulin/IGF1 signaling under Ex, PR, and CR conditions in this study that affects the regulation of cell dynamics in the OE. I hypothesize that the high susceptibility of dorsal-zone OSNs to damage is facilitated by a decrease in insulin/IGF1 signaling.

The present study firstly reports that following 10 mo of Ex, PR, and CR induce a negative structural and functional effect, which was opposite effects compared to auditory system, in the dorsal zone of the OE and that these changes are associated with NQO1 bioactivation. This dissertation is worthwhile to grant the degree. Exercise (Ex), protein restriction (PR), and caloric restriction (CR) are robust interventions that reduce oxidative stress and have positive effects on individual organs. However, it remains unknown

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