# 論　文　内　容　要　旨

Medial prefrontal area reductions, altered expressions of cholecystokinin, parvalbumin, and activating transcription factor 4 in the corticolimbic system, and altered emotional behavior in a progressive rat model of type 2 diabetes

（進行性2型糖尿病モデルラットにおける内側前頭前皮質面積減少、皮質辺縁系のコレシストキニン、パルブアルブミン、転写因子 ATF4 の発現変化と情動行動変化）

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Background

Metabolic disorder, such as diabetes, can be accompanied with psychiatric disorders. We have reported that 20-week-old Otsuka Long-Evans Tokushima fatty (OLETF) rats, a model of progressive type 2 diabetes with age, exhibit increased anxiety-like behavior with brain area reductions and increased cholecystokinin (CCK)-positive neurons in the corticolimbic system (Ochi et al., *J Physiol Sci*, 70(1):42, 2020). Parvalbumin (PV)-positive neurons in the corticolimbic system are also involved in anxiety. However, these alterations in different stages of type 2 diabetes remain unclear. In addition, increased expressions of apoptosis-related factors, such as activating transcription factor 4 (ATF4) and caspase-3, are found in some of diabetic animals. However, these apoptosis-related properties in OLETF rats at different ages remain unclear. Our purpose was to investigate emotional behaviors and its possible mechanisms in OLETF rats at different diabetic stages, 8 and 30 weeks of age.

Methods

OLETF rats were used, and Long Evans Tokushima Otsuka (LETO) rats were served as non-diabetic controls. At 8 or 30 weeks of age, an oral glucose tolerance test was performed to analyze the glucose tolerance of rats. After the oral glucose tolerance test at each age, an open field test was performed to examine the emotional states. After the open field test at each age, the brain samples were collected, brain regional areas in the corticolimbic system were evaluated, and immunohistochemistry for CCK and PV was carried out to examine possible mechanisms of altered emotional behaviors in OLETF rats. In addition, immunohistochemistry for caspase-3 and ATF4 was conducted to examine possible mechanisms of brain area reductions and decreased numbers of neurons.

Results

OLETF rats showed mild hyperglycemia with normal insulin level and severe hyperglycemia with a mixture of hyper- or hypo-insulinemia at 8 and 30 weeks of age, respectively. In the open field test, locomotion in the center zone was less and latency to leave the center zone was longer in OLETF rats than in LETO rats at 8 and 30 weeks of age, respectively. The area of the medial prefrontal cortex was smaller in OLETF rats than in LETO rats at both ages. The densities of CCK-positive neurons were higher in OLETF rats than in LETO rats in the anterior cingulate and infralimbic cortices and hippocampal CA3 at both ages, and in the lateral and basolateral amygdala at only 8 weeks of age. The densities of PV-positive neurons were lower in OLETF rats than in LETO rats in the prelimbic and infralimbic cortices at both ages, and in the hippocampal CA2 at only 8 weeks of age. No

caspase-3-positive reaction was found in both strains regardless of age, whereas the percentage of ATF4 co-expression in CCK- and PV-positive neurons was higher in OLETF rats than in LETO rats at both ages in the anterior cingulate cortex and basolateral amygdala, respectively.

Discussion

According to the properties in the oral glucose tolerance test, OLETF rats were in the prediabetic stage and progressive stage of diabetes at 8 and 30 weeks of age, respectively. OLETF rats showed altered emotional behaviors in the anxiogenic situations (i.e., center zone in the open field test), medial prefrontal area reduction, and altered numbers of CCK- and PV-positive neurons in the corticolimbic system, which were already found at the prediabetic stage and were maintained regardless of the progression of diabetes. These results suggest that altered emotional behaviors and neurobiological changes in the corticolimbic system are already found at the prediabetic stage. In addition, we investigated whether apoptosis is involved in the reductions of the medial prefrontal area and PV-positive neurons in the corticolimbic system in OLETF rats. Although no caspase-3-posistive reaction was observed, the ATF4 co-expressions in CCK- and PV-positive neurons in the corticolimbic system were increased in OLETF rats from the prediabetic stage, which indicates that the expression of the specific apoptosis-related factor could be increased at the prediabetic stage.