（第３号様式）

学　位　論　文　要　旨

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論　文　名　アンジオテンシン変換酵素２の欠損によりマウス認知機能は低下する

学位論文要旨

Background: The classical renin–angiotensin system (RAS) known as the angiotensin (Ang) converting enzyme (ACE)/Ang II/Ang II type 1 (AT1) receptor axis has a role not only in the cardiovascular system but also in the central nervous system. On the other hand, recent experimental studies have also demonstrated the existence of novel pathways beyond the classical actions of RAS. A new axis of RAS, the ACE2/Ang-(1-7)/Mas receptor axis has been highlighted as exerting antagonistic actions against the classical RAS axis in the cardiovascular system. Ang-(1-7) is finally produced from Ang I or Ang II by the catalytic activity of ACE2, and the discovery that Ang-(1-7) opposes the pressor, proliferative, fibrotic, and thrombotic actions mediated by Ang II via the AT1 receptor has contributed to the realization that RAS is composed of two opposing arms. Recently, RAS has been frequently reported to be associated with dementia and cognitive function. However, the roles of the ACE2/Ang-(1-7)/Mas axis in cognitive function are largely unknown and remain to be elucidated in more detail. In this study, we focused on the effects of deficiency of ACE2 on cognitive function, employing ACE2-deficient mice.

Methods: Male 10-week-old C57BL6 (wild-type: WT) mice and ACE2 knockout (KO) mice were subjected to the Morris water maze task and Y maze test to evaluate cognitive function. In the Morris water maze test, spatial learning was evaluated and escape latency was considered as cognitive function, while in Y maze test, the alternation behavior was considered as cognitive function. Cerebral blood flow (CBF) was measured by laser speckle flowmetry. Histological analysis was measured by hematoxylin-eosin staining using paraffin sections. Quantitative Real-time PCR and Western blotting were used to determine the mRNA level and protein expression. Superoxide anion was measured by dihydroethidium staining. Telmisartan, one kind of AT1 receptor blocker, was administrated in drinking water for two weeks at the concentration 1mg/kg/day. Ang-(1-7) was administrated intraperitoneally using osmotic mini-pump at the concentration 0.5 mg/kg/day for two weeks. Systolic blood pressure was monitored in conscious mice by the tail-cuff method.

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Results: No significant differences in body weight, brain weight, and brain weight/body weight ratio and blood pressure was found between 10-week-old ACE2KO mice and WT mice. Heart rate in ACE2KO mice increased significantly compared with that in WT mice. ACE2KO mice exhibited significant prolonged escape latency compared with WT mice in Morris water maze test, and in Y maze test ACE2KO mice showed lower alternation behavior compared with WT mice, indicated impaired cognitive function in ACE2KO mice. ACE2KO mice and WT mice showed no significant difference in cerebral blood flow and morphological changes in the hippocampus including cell numbers in the central area of CA1 and dentate gyrus of the hippocampus. Next, we investigated the expression of AT1, AT2 and Mas receptor in hippocampus and cortex of these mice. AT1 receptor mRNA level in hippocampus was higher in ACE2KO mice compared with WT mice. In contrast, AT2 receptor mRNA level did not differ between the two strains. Mas receptor mRNA was highly expressed in the hippocampus compared with the cortex, with no significant difference between ACE2KO and WT mice. Superoxide anion production increased in ACE2KO mice, with increased mRNA levels of NADPH oxidase subunit, $p22^{phox}$, $p40^{phox}$, $p67^{phox}$, and $gp91^{phox}$ in the hippocampus of ACE2KO mice compared with WT mice. Protein level of SOD3 decreased in ACE2KO mice compared with WT mice. Superoxide anion production was increased in ACE2KO mice compared with WT mice. The mRNA levels of inflammatory cytokines, such as TNFα and MCP-1 in the hippocampus showed no significant difference between ACE2KO and WT mice. Brain-derived neurotrophic factor (BDNF) mRNA and protein level were lower in the hippocampus in ACE2KO mice compared with WT mice. Administration of Ang-(1-7) intraperitoneally with no effect on blood pressure attenuated the cognitive decline in ACE2KO mice. Administration of telmisartan also showed no influence on blood pressure, but also improved impaired cognitive function in ACE2KO mice.

Conclusion: In conclusion, this study showed that ACE2 deficiency in the mouse resulted in impaired cognitive function, and the mechanisms of the cognitive decline may be associated at least in part with increased oxidative stress as well as decreased level of BDNF in the hippocampus in ACE2KO mice. Administration Ang-(1-7) and blocked AT1 receptor attenuated the cognitive decline in ACE2KO mice. It can be assumed that the ACE2/Ang-(1-7)/Mas axis as well as the AT2 receptor is a putative target in the treatment of neurological diseases that involve cognitive impairment.

キーワード（３～５）	Renin-Angiotensin System, Angiotensin converting enzyme 2, Cognitive function,　Oxidative stress, Brain-derived neurotrophic factor (BDNF)