論　文　要　約

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C-type natriuretic peptide modulates permeability of the blood-brain barrier

（血液脳関門の透過性に対する
C 型ナトリウム利尿ペプチドの関与）

【序論および目的】

Blood-brain barrier (BBB) functions for transport of molecules into/out of brain and is contributed by its tight junction proteins Zonula Occludens-1 (ZO-1), Claudin-5 and Occludin-1. C-type natriuretic peptide (CNP) belongs to the natriuretic peptide family, together with atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). CNP potently stimulates guanylate cyclase activity by binding to guanylate cyclase-B (GC-B) receptor which enhances the production of cyclic GMP (cGMP). Despite the abundance of CNP in brain and regulatory function of CNP on vascular cells, the effect of CNP on BBB permeability has not been clarified yet, which led us to undertake this study.

【材料および方法】

Three systems were used to evaluate the BBB permeability: i) in-vitro BBB constructed by co-culture of primary bovine brain microvascular endothelial cells (BBMEC) and primary astrocytes, ii) commercially available in-vitro BBB kit^{TM} composed of endothelial cells, astrocytes and pericytes, and iii) *in-vivo* permeability using mice. Measurement of transendothelial electrical resistance (TEER), western blotting, RT-PCR, immunocytochemical analyses and siRNA transfection were performed to check the effect of CNP on BBB and the underlying mechanism. Fluorimetric analysis using sodium fluorescin and FITC-dextran was performed in mice to evaluate change of *in-vivo* BBB permeability. All procedures were approved by the Institutional Animal Care and Use Committee of Kagoshima University (ID: MD11037) and were in accordance with its guidelines.

【結　果】

Transendothelial electrical resistance (TEER) of in-vitro BBB model, composed of bovine brain microvascular endothelial cells and astrocytes, was significantly dose-dependently decreased by CNP (1, 10 and 100 nM). CNP treatment reduced both the mRNA and protein expressions of tight junction (TJ) protein zonula occludens-1 (ZO-1). The effects on TEER, mRNA and protein expressions of ZO-1 were mimicked by cGMP analogue 8-bromo-cGMP (1 μM) and reversed by protein kinase G (PKG) inhibitor Rp-8-CPT-cGMPS (100 μM), thus implying the role of PKG and cGMP signaling in BBB function. Transcription factor JunD knockdown by small interfering RNA resulted in no change of permeability by CNP. *In-vivo* study of mouse brain by fluorimetric analysis with intravenous (i.v.) administration of sodium fluorescein (40 mg/kg) also showed a significant increase in BBB permeability by CNP (10 nmol/kg, i.v.).

【結論及び考察】

Our findings in this study represent that CNP can effectively enhance the permeability of BBB both *in-vitro* and *in-vivo* via cGMP/PKG and JunD dependent mechanism. The significant enhancement of BBB permeability to sodium fluorescein can be considered to be of functional significance as it mimics the permeation of various small molecular weight drugs across BBB. Indeed, further experiments should be required to refine the system for delivery of therapeutic compounds into the CNS. Nevertheless, the results we presented demonstrate the pivotal role of CNP in modulating BBB permeability by altering ZO-1 expression and might form a basis for developing a new drug-delivery system into brain.