論文審査の要旨

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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)

Blood-brain barrier (BBB) comprises of tight junction proteins (TJPs); claudins, occludins and zonula occludens (ZO). C-type natriuretic peptide (CNP) belongs to the natriuretic peptide family and elicits cGMP production by binding to guanylate cyclase-B receptor. CNP is abundant in brain and is reported to exert autocrine function in vascular cells, but its effect on BBB permeability has not been clarified yet. Here, the applicant examined this effect.

The applicant constructed *in-vitro* BBB model composed of primary bovine brain microvascular endothelial cells (BBMEC) and astrocytes and then evaluated the effect of CNP on the BBB permeability with measurement of transendothelial electrical resistance (TEER) and expressions of TJPs (by western blotting, RT-PCR and immunocytochemistry).

TEER of *in-vitro* BBB model was significantly decreased by CNP on dose-dependent (1, 10 and 100 nM) and time-dependent (3-24 h) basis. CNP (100 nM) treatment reduced both the mRNA, protein and immunocytochemical protein expressions of ZO-1 but not other TJPs. 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 cGMP and PKG signaling in BBB function. CNP and 8-bromo-cGMP both increased the protein expression of transcription factor JunD, which is reported to suppress ZO-1, while Rp-8-CPT-cGMPS reversed this effect of CNP. CNP treatment also reproduced similar results using a commercially available BBB kit (PharmaCo-Cell Co. Ltd.). *In vivo* study of mouse brain by fluorimetric analysis with intravenous (i.v.) sodium fluorescein (376Da) injection (40 mg/kg) also revealed that BBB permeability was significantly increased by CNP (10 nmol/kg, i.v.). But, the permeability was not changed for FITC-dextran (10KDa) by CNP. These findings suggest that CNP, via cGMP/PKG- and JunD-dependent mechanism, modulates the BBB permeability, which might form a basis to develop a new drug delivery system into brain.

以上のように学位申請者は、CNP が ZO-1 の発現抑制を通して脳血液関門の透過性を亢進することを明らかにした。本研究成果は CNP の脳血液関門に対する調節機構の解明に寄与するのみならず、今後、種々の脳疾患の病態解明や脳に対する drug delivery の手法の開発に大きく寄与するものと思われる。よって本研究は学位論文として十分な価値を有するものと判定した。