Glial-Cell Derived Neurotrophic Factor is a Potent Inducer of Human Endoneurial Endothelial Cell Tight Junction Specialization Via Tyrosine Kinase Dependent Signaling Pathways

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INTRODUCTION

Glial cell-derived neurotrophic factor (GDNF) promotes survival of central and peripheral neurons during development.

GDNF signals via a multicomponent receptor system consisting of "rearranged during transfection" (RET) tyrosine kinase and glycosyl-phosphatidylinositol-anchored co-receptor (GFRα-1)

GDNF and several cytokines, growth factors and hormones have been implicated in tight junction formation at the mammalian blood-brain barrier.

Factors that induce tight junction formation at the human blood-nerve barrier are unknown.

Regulation of barrier formation may be important in the restoration of endoneurial homeostasis following ischemic, toxic or traumatic injury to peripheral nerves.

OBJECTIVE

To determine the molecular inducers and signaling pathways responsible for human blood-nerve barrier tight junction specialization *ex vivo*.

METHODS

Primary human endoneurial endothelial cells (pHEndECs) were cultured on rat tail collagen-coated, 6.5 mm diameter, 3.0 µm pore Corning polyester transwell inserts at 80,000 cells/ insert to form the human *in vitro* blood-nerve barrier (IVBNB) model.

IVBNB treated with **GDNF** (0.1-10 ng/mL), basic fibroblast growth factor (**b-FGF**; 1-500 ng/mL), transforming growth factor-β1 (**TGF-β1**; 1-250 ng/mL) and **hydrocortisone** (100-5000 nM) on day 5 (luminal surface).

Transendothelial electrical resistance (TEER) was measured with EVOM voltohmmeter using STX2 electrodes 48 hours later (day 7).

Most effective doses of each factor were combined to determine if these factors had synergistic effects on IVBNB TJ barrier properties.

The effect of exogenous cyclic adenosine monophosphate (cAMP) on TEER was determined by treating the human IVBNB model with a cell permeable cAMP analog and a phosphodiesterase (PDE) inhibitor.

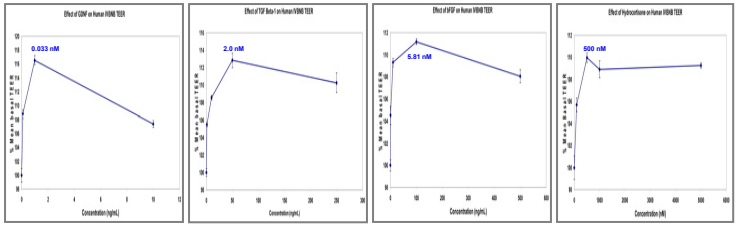
The 2nd messenger signaling pathways involved TJ function were also studied using specific inhibitors against tyrosine kinase and protein kinase A.

Solute permeability to 70 KDa dextran-FITC was measured under basal conditions and following treatment with GDNF.

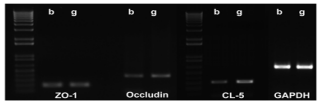
Polymerase chain reaction and western blot assays were performed to determine effect of GDNF on adherens and tight junction associated mRNA and protein expression.

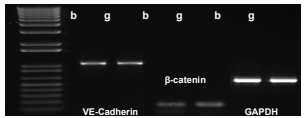
Immunocytochemistry was performed on pHEndECs cultured on glutaraldehyde cross-linked rat tail collagen-coated glass coverslips to determine effect of GDNF on endothelial cell cytoskeletal organization and development of adherens and tight junctions following contact inhibition *ex vivo*.

Effect of extrinsic modulators on IVBNB TEER (Basal TEER ~120 Ω.cm²)



Human IVBNB Adherens and Tight Junction associated mRNA and protein expression





Gene	Untreated (HBMEC, mean C_T value)	GDNF-treated (mean C_T value)	Fold change ($2^{-\Delta\Delta C_T}$)
Zona occludens-1 (ZO-1)	30.69	30.28	1.04
Occludin	32.71	32.23	1.07
Claudin-5 (CL-5)	22.46	22.03	1.05
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	26.01	25.82	N/A

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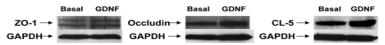
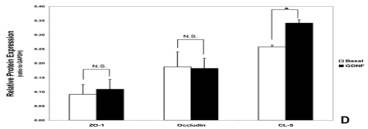
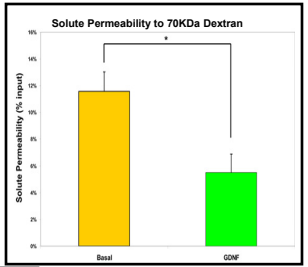
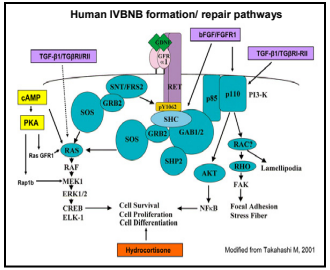




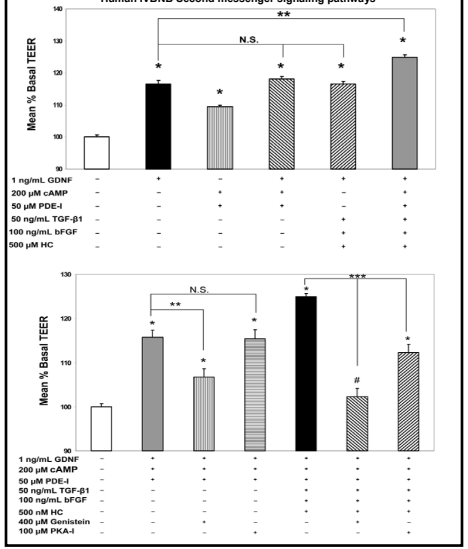
Solute Permeability to 70KDa Dextran



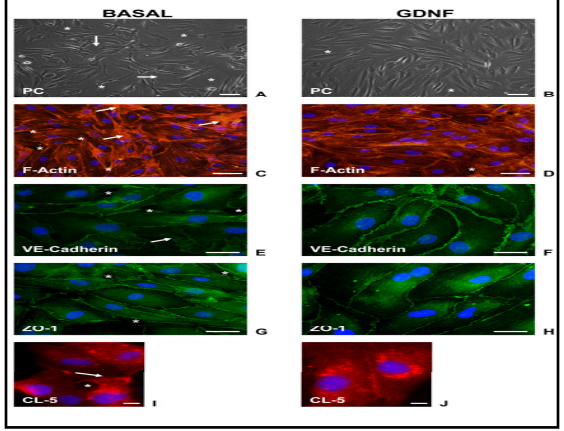
Human IVBNB formation/ repair pathways



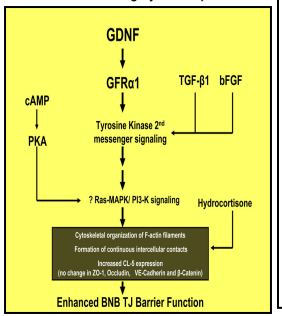
Human IVBNB Second messenger signaling pathways

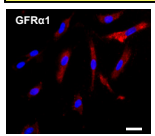


Effect of GDNF on IVBNB cytoskeletal organization and intercellular junctions



GDNF-induced IVBNB tight junction specialization





CONCLUSIONS

pHEndECs that form the human blood-nerve barrier develop intercellular adherens and tight junctions *in vitro*.

Proliferating cells do not express VE-Cadherin and Claudin-5.

GDNF via RET-tyrosine kinase pathways is the most potent molar inducer of tight junction properties at the human IVBNB.

Mild additive effect with b-FGF, TGF-β1 and hydrocortisone only in the presence of cAMP may imply downstream effects (e.g. hyperactivated mitogen-activated protein kinase [MAPP] pathways) on resistance.

There is no significant increase in ZO-1, occludin, β-catenin, VE-Cadherin and Claudin-5 mRNA following GDNF treatment.

There is a small increase in Claudin-5 protein expression, implying GDNF-mediated post-transcriptional effects.

GDNF induces cytoskeletal changes in pHEndECs that result in more continuous intercellular adherens and tight junction contacts, and fewer intercellular gaps *in vitro*.

GDNF, most likely secreted by Schwann cells in peripheral nerves, may play a critical role in blood-nerve barrier formation/ specialization required for normal peripheral nerve function.

Redundant effects of these extrinsic mediators on IVBNB resistance may be necessary *in vivo*.

GDNF could promote restoration of blood-nerve barrier tight junction function following peripheral nerve injury.

Further Reading:

Yosef N, Xia RH, Ubogu EE. Development and characterization of a novel human *in vitro* blood-nerve barrier model using primary endoneurial endothelial cells. J Neuropathol Exp Neurol 2010; 69:82-97.

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