GLIAL CELL-DERIVED NEUROTROPHIC FACTOR IS A POTENT INDUCER OF TIGHT JUNCTION FORMATION AT THE BLOOD-NERVE BARRIER *EX VIVO* .

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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 (TJ) formation at the mammalian blood-brain barrier.

Factors that induce TJ formation at the human blood-nerve barrier (BNB) 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 molecular inducers of TJ formation at the human BNB *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** (0-10 ng/mL), basic fibroblast growth factor (**b-FGF**; 0-500 ng/mL), transforming growth factor-β1 (**TGF-β1**, 0-250 ng/mL) and **hydrocortisone** (0-5000 nM) on transwell inserts (luminal surface) on day 5.

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 using a cell permeable 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.

Basal IVBNB resistance was **~120 Ω.cm²** on day 7.

RESULTS

Figure 1. Effect of extrinsic modulators on IVBNB TEER

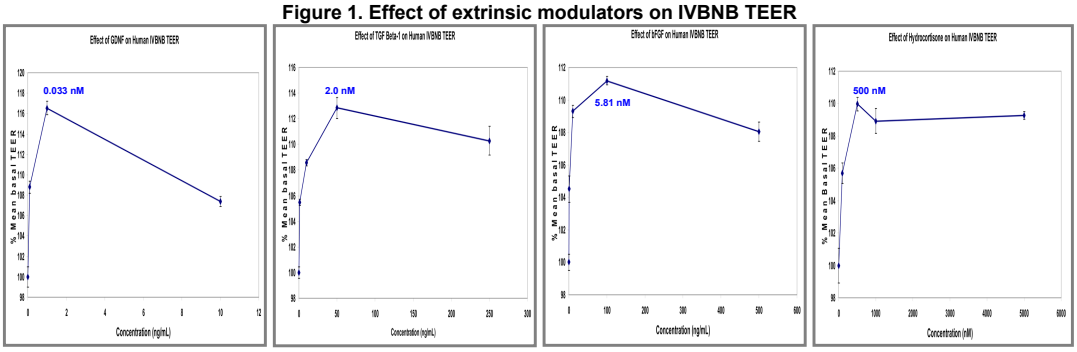


Figure 2. Synergistic effect of extrinsic modulators on IVBVB TEER

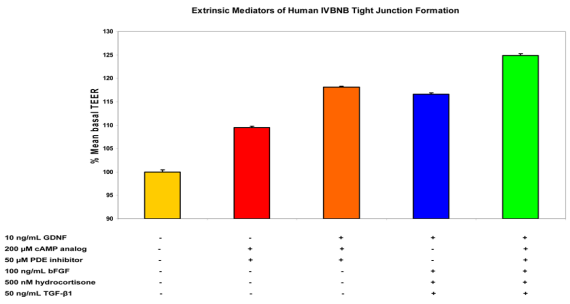


Figure 4. Human IVBNB Formation Pathways

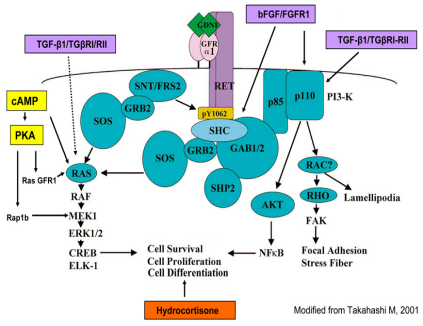
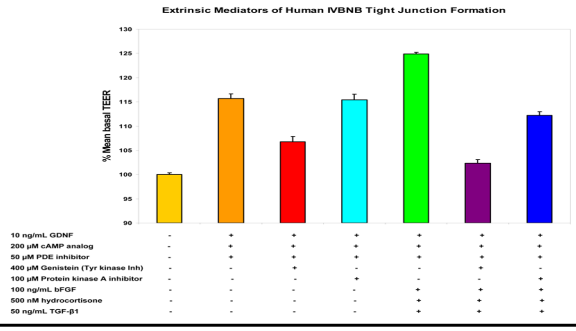


Figure 3. 2nd messenger pathways for IVBNB TJ formation



CONCLUSIONS

pHEndECs that form the human blood-nerve barrier have an intrinsic ability to form intercellular TJ *in vitro*.

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

GDNF, most likely secreted by Schwann cells in peripheral nerves, may play a critical role in endoneurial endothelial TJ formation necessary for normal peripheral nerve function.

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.

Preliminary studies suggest these extrinsic mediators induce cytoskeletal changes in pHEndECs that result in more continuous cell-to-cell contacts *in vitro*.

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

Autocrine and paracrine factors may be necessary for TJ formation at the BNB *in vivo*.

These mediators could promote restoration of BNB function following peripheral nerve injury.

Further Reading:

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