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## Neuroendocrinology and Pituitary

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### *Neuromedin B Receptor Antagonist Suppresses ACTH Secretion and Cell Proliferation in Human and Mouse Corticotroph Adenoma*

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**Objective:** We previously reported that Neuromedin B (NMB) is expressed in murine pituitary corticotrophs under adrenal insufficiency (1). Because NMB is also expressed in several cancer cells and stimulates ACTH secretion, we

hypothesized that NMB is related to corticotroph adenoma cell proliferation and hormone secretion. To examine this hypothesis, we investigated the effects of a NMB receptor (NMBR) antagonist on AtT-20 cells, a tumor xenograft model and patient-derived corticotroph adenoma cells. **Methods:** 1. NMB and NMBR expression in human pituitary adenoma: We performed real-time qPCR and immunostaining on human pathological specimens of corticotroph and non-functioning pituitary adenomas to investigate NMB and NMBR expression. 2. Experiments in AtT-20 cells: We extracted RNAs, proteins and mediums from AtT-20 cells after incubation with NMBR antagonist PD168368, and performed real-time qPCR, western blotting and ELISA analyses. We also performed WST-1 assay to investigate cell proliferation. 3. Experiments in a tumor xenograft model: AtT-20 cells in Matrigel were injected subcutaneously into BALB/c-nu mice. A week after inoculation, we administered 1.2 mg/kg PD168368 intraperitoneally once daily for 14 days. Upon completion of treatment, tumors were measured and cardiac blood was collected. 4. Experiments in patient-derived corticotroph adenoma cells: We isolated surgically resected human corticotroph adenoma cells from patients who underwent trans-sphenoidal surgery and investigated mRNA expression and medium ACTH secretion after incubation with PD168368. Statistical analysis: Comparisons between two groups were made by unpaired Student t test. Multiple groups were compared using one-way ANOVA followed by Dunnett's test for comparison with control group. Statistical significance was defined as $p < 0.05$. **Results:** 1. NMB and NMBR expression levels were significantly higher in human corticotroph adenomas (13 and 33 times higher, respectively) than in non-functioning adenomas in the qPCR analyses. Immunostaining confirmed higher expression of NMB and NMBR in corticotroph adenoma. 2. Treatment with 100 nM PD168368 significantly suppressed Pomc mRNA and protein expression in AtT-20 cells by 22% and 25% respectively compared control group. Medium ACTH secretion, mRNA and protein expression of cyclin E1 and cell proliferation were also suppressed by PD168368. 3. Mice treated with PD168368 had significantly lower tumor growth rate, plasma ACTH and corticosterone than control group (70 vs 161%, 97 vs 180 pg/ml, 813 vs 1045 ng/ml, respectively). 4. Treatment with PD168368 significantly suppressed POMC mRNA expression (12-31%) in 4 out of 6 patient-derived corticotroph adenoma cells. Medium ACTH secretion was also suppressed (14-53%) in 3 out of 4 cases which could be evaluated. Cyclin E mRNA expression were also suppressed in 3 out of 4 cases in which POMC mRNA expression were suppressed. **Conclusions:** NMBR antagonist may represent a potential treatment for Cushing disease, which effect may be mediated by decreased cyclin E expression. Reference: (1) Kameda H et al., Endocrinology 2014;155(7): 2492–9.

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