O-3 GnRH is Expressed in GnRH-Deficient Hypogonadal (Hpg) Mice after GnRH Gene Transfer with Herpes Simplex Virus (HSV) Amplicon Vector

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Background & Objectives: The challenge of gene therapy is to develop non-toxic vectors that can achieve stable transgene expression in target cells. The HSV amplicon vector is superior to other vectors in several aspects: (i) it has essentially no toxicity due to the use of a helper virus-free packaging system; (ii) it has a large transgene capacity (130+kb); (iii) it has a high affinity for neurons; and (iv) it has the potential to integrate the transgene into a specific site in the genome as opposed to random integration. hpg mice are genetically deficient in GnRH production in the hypothalamus, resulting in many deficits in the reproductive system including sexual infantilism, hypogonadotropic hypogonadism, arrested germ cell development, and infertility. Our goal was to determine neuronal expression of GnRH and the recovery of gonadal function after CNS delivery of an HSV amplicon vector containing the GnRH gene using hpg mice.

Method: Thirteen adult female hpg mice were studied. Ten mice were injected with the HSV amplicon vector in the POA and three mice were injected with vehicle only as control. The vector (3.4×105 transducing units in 2 ml) was injected into the POA of hpg mice under anesthesia using a stereotaxic apparatus. Mice were sacrificed 10 days (n=5) or 50 days (n=5) after injection. GnRH production was analyzed by IHC in hypothalamic sections. GFP expression was detected by fluorescence microscopy. LH and FSH production were analyzed by IHC in pituitary tissues. The weights of the ovary, uterus and oviduct were compared between the study and control groups. Vaginal cytology was also checked each day to evaluate estrous cyclicity.

Results: GnRH was detected in hypothalamic neurons in the POA near the injection site in hpg mice, as determined by IHC, 10 days and 50 days following HSV amplicon vector injection. Pituitary FSH and LH content, as determined by IHC, was higher in hpg mice 10 days and 50 days following HSV amplicon vector injection than in control vehicle-injected hpg mice. There was a significant increase in the combined ovarian and uterine weight in hpg mice 50 days after HSV amplicon vector injection compared to that in control vehicle-injected hpg mice. Vaginal cytology demonstrated changes from the small cells of diestrus to the cornified cells of estrus.

Conclusions: Hypothalamic injection of the HSV amplicon vector into the POA is capable of directing GnRH gene expression and GnRH protein synthesis in select cells in the expected distribution and pattern of GnRH neurons. This gene therapy approach is able to restore at least some aspects of reproductive

function, suggesting that GnRH secretion is appropriately and coordinately regulated (i.e., in a pulsatile fashion). To our knowledge, this is the first example of the correct targeting of a gene with its cognate promoter to neurons with resulting selective expression to direct synthesis of immunologically and biologically active peptide.

O-4 Roles of ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 Genes in Mouse Uterus During Periimplantation Period

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Background & Objectives: ADAMs are a unique family of protein members consisting of a prodomain, metalloprotease, disintegrin-like and cysteine-rich domains, and, in most cases, epidermal growth factor-like, transmembrane, and cytoplasmic domain. The presence of a disintegrin domain implies their possible role in cell-cell and cell-matrix interactions, and the presence of a metalloprotease domain suggests their involvement in the proteolytic processing of the extracellular domain of transmembrane proteins and matrices. The role of ADAMs in tissue remodeling of mammalian uterus particularly around the time of implantation is poorly understood. In the present study, whether genes of ADAM-8, -9, -10, -12, -15, -17 and ADAMTS-1 might play a role during early pregnancy in mouse uterus has been investigated.

Method: Gene and protein expression of ADAMs were examined in mouse uterus during periimpamtation period by RT-PCR, immunoblotting and immunohistochemistry techniques. To determine the potential role of ADAMs at the time of implantation of mice, effects of intrauterine antibody injection experiments were performed in vivo.

Results: RT-PCR analyses showed that mRNA expression of ADAM-8, -12 and -15 genes decreased from day 1 to day 5 of pregnancy while the expression of other ADAM genes did not undergo significant change during the same period. From day 6 to day 8 of pregnancy period, the mRNA level of all ADAM genes in uterine tissues was significantly higher at the implantation site than that at the interimplantation site. Western blot analyses demonstrated that proteins of all ADAM genes consistently appeared throughout day 1 today 8 but with a distinct variation depending on the species of ADAM genes, the progression of pregnancy and the site of the uterus. Immunohistochemical analyses indicated that ADAM proteins were mostly localized in the luminal and/or glandular epithelial layers with a varying degree of intensity depending on the species of ADAM and the progression of pregnancy. The results also showed that ADAM proteins, particularly ADAM-8, -12 and -15, were predominantly located in the implantation site of the uterine tissues whereas little protein was observed in the interimplantation site. Finally, injection of anti-ADAM antibodies resulted in a significantly reduced number of embryonic implants when any antibody was injected into the