The Effect of Vascular Endothelial Growth Factor (VEGF) and Corticotrophin Releasing Hormone (CRH) on the Production of Nitric Oxide in Trophoblasts.

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Background:
Nitric Oxide (NO) functions as a paracrine/autocrine agent in the placenta. NO has been implicated in maintenance of vascular tone in the human fetal placenta circulation in vitro, and has been demonstrated to attenuate the action of vasoconstrictors in the placental circulation. NO is produced by vascular endothelium in placental vessels and by syncytiotrophoblasts. The anti-platelet adhesion/aggregation properties of NO may permit syncytiotrophoblasts to prevent platelet aggregation in the intervillous space of the placenta. L-arginine and molecular oxygen combine to yield citrulline and NO in a reaction catalyzed by the enzyme nitric oxide synthase (NOS). Three isoforms of NOS have been cloned and sequenced. Type III, endothelial NOS (eNOS) has been immunohistochemically localized in the trophoblast.
VEGF and CRH are regulatory peptides produced in the placenta. VEGF has been found to promote angiogenesis in in vivo models. The FIt-1 receptor for VEGF has been identified in trophoblasts. CRH is produced in large amounts during the second and third trimesters of pregnancy and has been demonstrated to cause vasodilation in the human-fetal placental circulation. Receptors for CRH have been identified in the placenta. Reports indicate that while CRH stimulates NO production, NO itself exhibits negative feedback activity on CRH production in the perfused placenta. Hence there may be interactions between peptide hormones and NO.

Hypothesis:
VEGF and CRH regulate the production of NO in trophoblasts.

Methods:
The BeWo trophoblast cell line was used to evaluate the effects of the agonists (VEGF and CRH) on NO production. Initially a dose response relationship between dose of agonist and production of NO by the trophoblast was examined. Three concentrations of VEGF (10?°M - 10?8M) and four concentrations of CRH (10?°M -10?7M) were applied to isolates of BeWo cells previously grown to confluence, for a period of 2 hours at 370C. The medium was collected and analyzed for NO content using a NO electrode (World Precision Instruments). A second component of the investigation examined the time course of NO production in the BeWo cultures when exposed to an optimum concentration of agonist (1x10-9M VEGF and 1x10-9M CRH). Cells were incubated with or without the agonists at 37°C and samples were collected at 5,10,15,30,60 and 120 minutes. The medium collected was assayed for NO production by chemiluminescence (Sievers).

Results:
The initial nested ANOVA analysis of the dose response relationship between VEGF or CRH and NO showed a significant concentration dependent relationship. Using the Tukey test for pairwise comparisons, the amount of NO produced at 10-9M VEGF and the amount of NO produced at 10-10M CRH were both statistically different than the amount of NO produced

in the absence of their respective agonists, with p values <.05.
Based on preliminary data concentrations of 1 x1 0-9M VEGF and CRH were chosen for the time course experiments. With both peptides the peak of NO production was at 10 minutes. While the response at 10 minutes was clearly the largest for both VEGF and CRH, VEGF production at 5 and 30 minutes were statistically greater from the production at the zero time point, as was the CRH production at 5 minutes.

Conclusions:
The results from these experiments indicate that VEGF and CRH may regulate the production of NO in trophoblasts. This experiment suggests that the optimum concentration of VEGF is 1 x10-9M, while the optimum concentration of CRH is unclear. Since the time course experiment suggested that the peak NO production occurred at 10 minutes, the initial dose response experiment should be repeated with a 10 minute incubation period, rather than 2 hours.