

## Neuroactive Steroids: Regulators of Normal Brain Development with Potential for Preventing Pre- and Perinatal Brain Damage?

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The neurosteroid allopregnanolone (AP) is a GABAergic agonist that suppresses CNS activity in the adult brain, and by reducing excitotoxicity is considered to be neuroprotective. A role for neurosteroids in the developing brain, particularly in late pregnancy, is still debated. AP synthesis is increased rapidly in the fetal brain following *in utero* asphyxia induced by umbilical cord occlusion (UCO) due to increased 5 $\alpha$ -reductase type II (5 $\alpha$ R-II) expression (1,2). Therefore, we hypothesized that Finasteride, a specific inhibitor of 5 $\alpha$ R-II activity, would potentiate asphyxia-induced brain injury in fetal sheep.

**Objectives:** To determine the effect suppressing neurosteroid synthesis using finasteride on the extent of fetal brain injury caused by brief (5 min) UCO.

**Methods:** An inflatable cuff was implanted around the umbilical cord in fetal sheep at ~125 days gestation, and catheters were inserted into right and left brachial arteries to administer drugs and obtain blood samples. At 3-4 days post-surgery, fetuses received a 2 h infusion of either finasteride (40mg/kg/h; n=5), or vehicle (40% hydroxypropyl- $\beta$ -cyclodextrin, n=5) and at 30 min after starting the infusion, the UCO (or sham) procedure was performed. Other fetuses received either finasteride or vehicle alone (n=5, each group). Brains were obtained at 24 h after the real or sham UCO procedure to determine cell death (apoptotic or necrotic) in the hippocampus and cerebellum, areas known to be susceptible to excitotoxic damage.

**Results:** UCO for 5 min caused marked fetal hypoxia ( $PO_2$  4.20  $\pm$  0.48 mmHg), acidemia (pH 7.04  $\pm$  0.05), and hypercapnia ( $PCO_2$  93.0  $\pm$  11.2 mmHg). Finasteride treatment alone significantly increased apoptosis (caspase-3 expression) in the CA3 and CA1 regions of the hippocampus, the granular and molecular layer of the cerebellum compared to fetuses receiving vehicle. Finasteride treatment alone increased the number of dead (pyknotic) cells in the hippocampus and cerebellum (Purkinje cell layer) compared to controls. In finasteride-treated fetuses subjected to UCO, caspase-3 immunoreactivity (IR) in the hippocampal CA3 was significantly greater (57.3  $\pm$  1.6% IR per field of view) compared to vehicle + UCO treated fetuses (39.2  $\pm$  3.6%); double-label immunohistochemistry showed the majority of these cells were neurons. In the Purkinje cell layer, finasteride treatment caused significantly greater increase in pyknosis after UCO (13.6  $\pm$  0.5 cells/mm), compared to vehicle + UCO treated fetuses (10.3  $\pm$  1.1 cells/mm).

**Conclusion:** Neurosteroids such as AP appear to have a protective role in limiting cell death following acute asphyxia *in utero*. The evidence also suggests that AP modulates the normal [constitutive] rate of cell death in some areas of the fetal brain. Perturbation to normal levels of neurosteroids in the fetal brain, which can occur due to preterm birth or after administration of synthetic steroids to the mother, could therefore adversely influence fetal brain development and increase its vulnerability to injury.

1. Nguyen PN et al, *Pediatr Res.*, **53**: 956-964, 2003.

2. Nguyen PN et al *J Physiol.*, **560**: 593-602, 2004.