Betamethasone: A neuroactive steroid deficit and adverse effects in the brain?

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DECLARATION: STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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I hereby certify that the work embodied in this thesis contains a published paper of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication.

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STATEMENT OF ENDORSEMENT OF AUTHORSHIP BY SUPERVISORS

We attest that Research Higher Degree candidate Amy McKendry contributed to:

a) the conception and design of the research,
b) collection, analysis and interpretation of research data,
c) drafting and revision of significant parts of the work to contribute to the interpretation

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ABSTRACT

There are ongoing concerns that antenatal corticosteroids, which are administered to women at high risk of delivering preterm to reduce the incidence of respiratory distress syndrome, have adverse effects on fetal brain development and subsequent effects on behaviour and learning, when administered as repeated courses. There is evidence that neuroactive steroids have a role in brain development and could be altered by antenatal corticosteroids. 5α-reductase is the key rate limiting enzyme in the synthetic pathway of the potent neuroactive steroid, allopregnanolone. The objective of this study was to examine if repeated betamethasone treatment alters 5α-reductase expression, alters plasma and brain allopregnanolone concentrations, affects brain development and whether this effect is potentiated in growth restricted fetuses. To investigate this, pregnant guinea pigs carrying either control (sham surgery) or growth-restricted fetuses were treated with vehicle or betamethasone (1mg/kg/day) for 4 days prior to sacrifice (65GA). Placental insufficiency was induced by the ablation of uterine artery branches supplying each placenta at mid gestation, resulting in fetal growth restriction characterised by 'brain sparing'. Real time RT-PCR was used to determine relative 5α-reductase type 1 and 2 mRNA expression in the placenta, adrenal glands and brain. Radioimmunoassay was used to measure allopregnanolone concentrations in the plasma and brain. Immunohistochemistry was used to examine GFAP (marker of astrocytes) and MBP (marker of myelination) expression in the subcortical white matter, CA1 and dentate (GFAP only) regions of the hippocampus. 5α-reductase type 2 mRNA expression in the brain was markedly reduced by betamethasone treatment in male fetuses compared with vehicle treated controls but not in female fetuses. In addition, 5α-reductase type 1 expression in the brain was increased by growth restriction and/or

betamethasone treatment in female fetuses but expression in male fetuses did not increase. 5α-reductase type 2 expression in the placenta was markedly reduced by betamethasone treatment compared with vehicle treated control. Plasma allopregnanolone concentration was reduced by betamethasone and IUGR. Brain allopregnanolone concentration was not significantly altered but trended towards a reduction in males with IUGR and betamethasone and an increase in females with IUGR/betamethasone. IUGR and betamethasone treatment reduced GFAP expression in the CA1 region of the hippocampus in the brains of male but not female fetuses. There were no significant changes in MBP expression in male or female fetuses. These data indicate that betamethasone treatment suppresses placental enzyme expression, plasma allopregnanolone and has sexually dimorphic effects on expression of neuroactive steroid synthetic enzymes and allopregnanolone in the brain. These actions may lead to adverse effects on the developing brain, particularly in male fetuses, such as the observed effects on GFAP expression.

ABBREVIATIONS

3α-HSR	3α-Hyroxysteroid Oxidoreductase
3β-HSD	3β-Hyroxysteroid Dehydrogenase
5α-DHP	5α-Dihydroprogesterone
5αR1	5α-Reductase type 1
5αR2	5α Reductase type 2
11βHSD2	11β-Hydroxysteroid Dehydrogenase 2
Beta	Betamethasone
CNS	Central Nervous System
DHEA	Dehydroepiandosterone
EPSP	Excitatory Post Synaptic Potential
GA	Gestational Age
GABA	γ-aminobutyric Acid
$GABA_A$ receptor	γ-aminobutyric Acid Receptor Type A
GFAP	Glial Fibrillary Acidic Protein
IPSP	Inhibitory Post Synaptic Potential
IUGR	Intrauterine Growth Restriction
MBP	Myelin Basic Protein
NIH	National Institute of Health
P450scc	Cytochrome P450 Side Chain Cleavage Enzyme
PBS	Phosphate Buffered Saline
RDS	Respiratory Distress Syndrome
SGA	Small for Gestational Age