HYPOPHYSIAL AND LOCAL MEDIATORS OF
ADRENOCORTICAL GROWTH AND FUNCTION BEFORE BIRTH

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SUMMARY

In the sheep fetus, adrenocortical growth and increases in steroidogenesis are essential for the maturation of a range of organ systems including the lungs, gut and brain, vital to successful adaptation to extra-uterine life; for the appropriate hormonal responses to acute and chronic stress in utero and for the timing and process of parturition. The precise mechanisms that coordinate growth and functional development of the ovine fetal adrenal gland during the last two weeks of gestation (term=150 days of gestation) are unknown. This thesis examines the relative roles of pituitary hormones, glucocorticoids, tissue growth factors and fetal stress, in modulating the increase in adrenocortical growth and steroid synthesis in late-gestation. Chapter 1 reviews the literature regarding adrenal growth and the synthesis of steroid hormones within the adrenal gland of the sheep fetus. The relative roles of the fetal pituitary gland and cortisol in modulating the growth and functional activation of the adrenal cortex are also reviewed. The potential role of peptides arising from the amino (N)-terminal region of the adrenocorticotrophin (ACTH) precursor, pro-opiomelanocortin (POMC), is specifically discussed. Finally, the role of the insulin-like growth factors (IGFs) and fetal stress are each considered in the regulation of the growth and functional development of the ovine fetal adrenal gland prior to birth.

Chapter 2 examines the changes in adrenal growth throughout gestation, and the messenger ribonucleic acid (mRNA) abundance of the adrenal steroid-synthesising enzymes during the two weeks prior to birth in the fetal sheep. The input of the hypothalamus in the regulation of fetal adrenal growth and function is also studied, by surgical disconnection of the fetal hypothalamus and pituitary (hypothalamo-pituitary disconnection; HPD) at approximately 110 days (d) of gestation. Maintenance of fetal cortisol from 135-140 d of gestation, following fetal HPD, is also included to compare the separate effects of HPD and cortisol on adrenal development in late-gestation. This study demonstrates that there is a differential temporal regulation of mRNA expression of the steroid-synthesising enzymes within the fetal adrenal during the two weeks prior to delivery. While there is no change in the adrenal mRNA abundance of IGF-II during the two weeks prior to delivery, the adrenal expression of mRNA for IGF binding protein-2 (IGFBP-2), the major fetal IGF-II binding protein, decreases during late-gestation, suggesting that bioactivity of the IGF axis may be modulated through altered abundance and activity of the IGF binding proteins during this
time. Fetal HPD at 110 d of gestation abolishes the late-gestation increase in adrenal growth and steroidogenic enzyme mRNA abundance. Replacement of cortisol following fetal HPD, however, stimulates adrenal growth in the absence of any effects on adrenal levels of IGF-II or steroidogenic enzyme mRNA. Cortisol may, therefore, act directly at the fetal adrenal to modulate the activity of locally produced growth factors, in part through altered abundance of the IGF binding proteins, or indirectly at the surgically disconnected pituitary to stimulate adrenal growth.

Chapter 3 examines the effects of a premature elevation of fetal plasma cortisol levels on adrenal growth and steroidogenesis in fetuses with an intact hypothalamo-pituitary-adrenal (HPA) axis, prior to the onset of the ontogenic rise in fetal circulating levels of adrenocorticotropic hormone (ACTH) and endogenous cortisol. While exposure to elevated fetal circulating levels of cortisol during the period 109-116 d of gestation does not stimulate adrenal growth or steroidogenic enzyme gene expression, the adrenal expression of mRNA for IGFBP-2 and the steroid-metabolising enzyme \( 11\beta \) hydroxysteroid dehydrogenase (11\( \beta \)HSD-2) is decreased. It is possible that a decrease in adrenal IGFBP-2 and 11\( \beta \)HSD-2 expression and increased intra-adrenal glucocorticoids may enhance adrenal growth and steroidogenic responsiveness only when the fetal adrenal is simultaneously exposed to the high ACTH concentrations which prevail in the week before birth, or during chronic intra-uterine stress.

Studies in the fetal sheep have demonstrated that pro-ACTH and N-terminal POMC(1-77) are present in 20-50 fold higher concentrations than is ACTH(1-39) in fetal sheep circulation. While the N-POMC peptides have potent mitogenic and steroidogenic effects on adult rat adrenocortical cells \textit{in vivo} and \textit{in vitro}, no studies have examined their effect on fetal adrenal development \textit{in utero}. Chapter 4 examines the role of peptides derived from the N-terminal region of POMC in the regulation of fetal adrenal growth and development. N-POMC(1-77) and N-POMC(1-49) were extracted and purified from adult bovine pituitaries and infused into the circulation of fetal sheep during late-gestation. Intra-fetal infusion of N-POMC(1-77), but not N-POMC(1-49), results in an increase in adrenal weight and in the expression of mRNA for the adrenal steroid-synthesising enzyme P450c17 (CYP17). These data indicate a possible novel role for the N-terminal POMC peptide in adrenal growth and steroidogenesis before birth.

IGFs are potent mitogenic factors and are important in the regulation of many aspects of fetal growth. Previous studies have demonstrated the presence of IGF-I and IGF-II mRNA
and peptide and IGF receptors in the adrenal glands of the developing fetus. Thus, IGFs potentially play an important role in the growth and development of the fetal adrenal. In Chapter 5, the effect of intra-fetal infusion of IGF-I on adrenal maturation has been investigated. Consistent with in vitro studies, a 10 d intra-fetal infusion of recombinant human IGF-I results in a marked increase in adrenal growth, in the absence of an effect on the expression of mRNA for the steroidogenic enzymes. These results demonstrate for the first time that IGF-I has a substantial growth promoting effect on the adrenal gland of the ovine fetus in vivo.

Finally, in Chapter 6, the response of the fetal adrenal gland to chronic fetal growth restriction has been examined in an experimental model wherein restriction of placental growth is secondary to the surgical removal of most of the sites of placental development (the maternal caruncles) prior to mating. A quantitative relationship has been demonstrated between adrenal growth and the degree of fetal growth restriction, regardless of the cause of growth restriction. Thus, there is a continuum among fetuses in which growth restriction is induced experimentally by carunclectomy and in fetuses where growth restriction occurs spontaneously, i.e. those control singleton and twin fetuses which were <3.5 kg. Placentally restricted fetal sheep have a higher ratio of adrenal : fetal body weight, and an enhanced adrenal expression of mRNA for the steroid-synthesising enzyme P450sec (CYP11A1), the rate-limiting enzyme in steroid synthesis, in addition to higher fetal circulating levels of cortisol. While adrenal growth is promoted in the placentally restricted group, the adrenal expression of mRNA for IGF-II is suppressed, in the absence of any changes in IGFBP-2 mRNA expression.

In conclusion, this dissertation describes the interactions among pituitary-derived peptides, intra-adrenal exposure to glucocorticoids and the local adrenal and endocrine IGF axes in the growth and functional activation of the ovine fetal adrenal gland before birth. The involvement of these systems in the fetal response to chronic stress and intra-uterine growth restriction is also considered. Throughout this thesis, several conceptual models of the control of adrenal growth and function in late-gestation are proposed and developed.