Reproduction Supplement **61**, 299–310

Sexually differentiated regulation of GnRH release by gonadal steroid hormones in sheep

J. E. Robinson[1]*, R. A. Birch[2], J. A. E. Grindrod[1], J. A. Taylor[1] and W. P. Unsworth[1]

[1] *Laboratory of Neuroendocrinology, The Babraham Institute, Babraham, Cambridge CB2 4AT, UK; and* [2] *Department of Reproductive Science and Medicine, Imperial College School of Medicine, Hammersmith Hospital, London W12 0HS, UK*

Exposure of the sheep fetus to testosterone from day 30 to day 90 of a 147 day gestation causes the neurones that control GnRH secretion, the GnRH neuronal network, to become organized in a sex-specific manner. After androgen exposure *in utero*, GnRH neurones are activated in a sexually differentiated pattern by gonadal steroid hormones. Specifically, follicular phase concentrations of oestrogen trigger a GnRH 'surge' in ewes, but not in rams or females treated with androgen during fetal life. Furthermore, progesterone is a less potent inhibitor of GnRH release in rams or females treated with androgen during fetal life. The reasons for the sexual differentiation of these steroid feedback mechanisms probably reside in a dimorphism in steroid-sensitive neural inputs to GnRH neurones. The density of neurones containing oestrogen receptor α is sexually differentiated in areas of the ovine brain that are known to be involved in the steroidal regulation of GnRH. Furthermore, neurones in these regions are activated in a gender-specific pattern. A determination of the neural phenotype of these steroid-sensitive cells will form a basis for understanding the mechanisms by which the GnRH neuronal network is organized and activated in a sexually differentiated manner.

Introduction

The reproductive neuroendocrine system of many mammals functions in a sexually differentiated manner during specific phases of the life of the animal. In sheep, for example, a sex-specific pattern of gonadotrophin release from the pituitary gland can be demonstrated before birth (Matwijiw and Faiman, 1989), at puberty (Claypool and Foster, 1990) and in adulthood (Foster and Karsch, 1975). The development and activity of the gonads are governed largely by this sexually differentiated pattern of gonadotrophin secretion and results in an earlier onset of puberty in the male compared with the female lamb and in cyclic versus non-cyclic gonadal activity in adult ewes and rams, respectively (Wood and Foster, 1998).

*Current address: Department of Preclinical Veterinary Studies, Division of Physiology and Pharmacology, The University of Glasgow, Bearsden Rd, Glasgow G61 1QH
Email: jane.robinson@vet.gla.ac.uk

Central to the control of the reproductive axis is a population of approximately 2000 specialized neurones that are located in the pre-optic and hypothalamic areas of the brain and that synthesize and release a decapeptide known as GnRH (Caldani *et al.*, 1988). The activity of these neurones is modified by many factors in the internal and external environment of the animal, including the gonadal steroid hormones testosterone, oestrogen and progesterone. This review is focused on the sex-specific regulation of GnRH release by these steroid hormones at different stages in the reproductive life of sheep.

Sexual differentiation of the GnRH system in the sheep fetus

It is well established that LH secretion is sexually differentiated in the sheep fetus in mid- and late gestation (Sklar *et al.*, 1981; Matwijiw and Faiman, 1989). Furthermore, studies using a specific GnRH antagonist have shown that tonic LH secretion is dependent on GnRH release from at least day 104 of gestation (Matwijiw and Faiman, 1987), although there is potential for limited hypothalamic control from about day 60, when GnRH fibres can be seen in the developing median eminence (Caldani *et al.*, 1995) and the portal vessels have developed (Levidiotis *et al.*, 1989). It follows, thus, that ovine GnRH neurones have the potential to function in a sex-specific manner from a very early stage of development. As is the case with other mammals, GnRH neurones and the network of other neural elements that control GnRH release become organized in a sex-specific manner under the influence of steroid hormones that are released from the developing male gonads (Goy and McEwan, 1980; MacLusky and Naftolin, 1981). In sheep, the developmental window during which androgens exert this organizing influence was delimited by studies carried out by Short (1974) and Clarke *et al.* (1976). Timed exposure of the female fetus to exogenous testosterone was used to show that the 'critical period' for the sexual differentiation of the reproductive neuroendocrine system occurs between day 30 and day 90 of gestation (term 147 days). It is intriguing to note that, during the earlier part of this period, the ovine GnRH neurones are migrating from their origin in the nose into their adult location in the developing hypothalamus (Caldani *et al.*, 1995) and raises questions about the mechanisms by which steroids might exert their organizational effects on migrating neurones. GnRH neurones in the adult do not appear to contain receptors for testosterone (Herbison *et al.*, 1996), progesterone (Skinner *et al.*, 2001) or oestrogen receptor α (Herbison *et al.*, 1993). Therefore, in adult sheep, one major mechanism by which steroids regulate GnRH release must be via intermediate neurones. Whether fetal GnRH neurones transiently express steroid receptors and, therefore, the potential for direct steroid hormone actions during development is unknown. However, as fetal exposure to androgen does not alter the migratory route or gross morphology of GnRH neurones (Wood *et al.*, 1992), it would appear more likely that androgens from the gonad of the male fetus act on other types of cell within the developing hypothalamus to differentiate sexually the environment into which the GnRH cells migrate and, thereby, alter their synaptic connections.

As stated earlier, fetal exposure to androgen does not affect the pathway by which GnRH neurones reach the hypothalamus because by the end of the 'critical period', male and female GnRH neurones are similar in number, distribution and gross morphology (Wood *et al.*, 1992). Recently, we have determined that there is a substantial increase in the cellular content of GnRH mRNA during the critical period (Fig. 1). However, GnRH gene expression is not sexually differentiated at either day 55 or day 70 of gestation and similar increases occur in both male and female fetuses (Forsdike and Robinson, 1998). The increase in GnRH mRNA at this time may be of particular importance for stimulating the development of the pituitary gonadotrophs. These specialized cells can first be identified at the base of the anterior pituitary

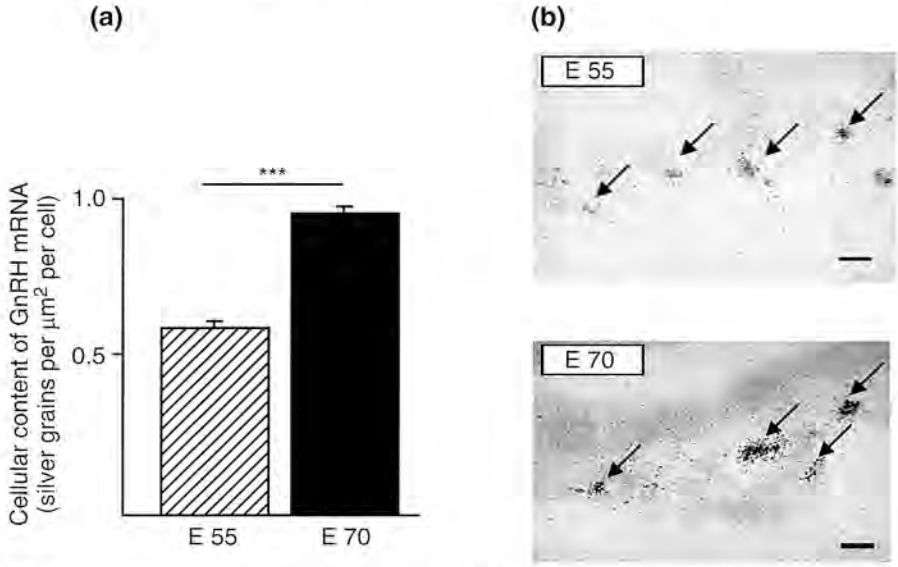

Fig. 1. GnRH mRNA in the sheep fetus. A 39mer oligonucleotide probe that has been used to identify and quantify cellular GnRH mRNA content in neurones of adult sheep was used (Harris *et al.*, 1998). (a) The cellular content of GnRH mRNA increases significantly (***$P < 0.001$) in the 15 day period between embryonic day 55 (E 55) and E 70 in both male and female fetuses. (b) Silver grains can be seen clustered over cells in the rostral areas of the brain. GnRH expressing neurones in an (E 55) male fetus can be seen on their migratory route into the developing hypothalamus area (top panel) and in the medial preoptic area of a (E 70) female fetus (bottom panel). Scale bars represent 10 μm.

gland at about day 65 of gestation (Sheng *et al.*, 1998). At this fetal age neither the number of gonadotrophs (identified as LHβ-immunopositive cells) nor the percentage containing oestrogen receptors are sexually differentiated, perhaps reflecting similar stimulation of the synthesis of LH and FSH subunits by GnRH in both the male and the female (Sheng *et al.*, 1998). However, it is interesting to note that GnRH gene expression has become sexually differentiated by the end of fetal life when LH secretion has also assumed a sex-specific pattern (Taylor *et al.*, 2001).

Sex differences in the GnRH system between birth and puberty

As excellent comprehensive reviews have been published (Wood and Foster, 1998; Wood *et al.*, 2000) the reader is referred to these for details of the striking sex differences in GnRH secretion before puberty in sheep, and how release is regulated in the postnatal period by both gonadal steroids and photoperiod.

Sex differences in the gonadal steroid control of GnRH release in adulthood

The organizational actions exerted by testosterone on the developing neurones that control GnRH release have profound consequences for the functions of this neural network in adult sheep. This is demonstrated most markedly in the response to gonadal steroid feedback. The actions of specific steroids on GnRH release are best studied in gonadectomized animals

in which steroid concentrations have been 'clamped' by constant-release implants of the hormone. Such experiments have shown that *in utero* androgen exposure alters oestrogen positive feedback (Herbosa *et al.*, 1996) as well as progesterone negative feedback (Robinson *et al.*, 2002). These sexually differentiated responses are detailed in the next two sections.

Sexually differentiated responses to oestrogen

In the ovariectomized ewe, in which low physiological concentrations of oestrogen are maintained by s.c. implantation of a Silastic capsule of steroid, the female responds to an acute increase in oestrogen concentrations with a massive release of GnRH, termed the GnRH surge. This hormonal response is sexually differentiated, as oestrogen will not trigger a GnRH surge in males or in ewes that have been treated *in utero* with testosterone from day 30 to day 90 of gestation. It is not simply that the animals exposed to androgen are less responsive to oestrogen because even supra-physiological concentrations of the hormone still fail to elicit a surge release of GnRH (R. A. Birch and J. E. Robinson, unpublished). It appears that the stimulatory neural circuits by which oestrogen normally activates the GnRH neurone in female sheep may have been prevented from developing by a short period of exposure to testosterone during fetal life. Alternatively, inhibitory mechanisms may have formed under the influence of testosterone to block oestrogen positive feedback in postnatal life. Extensive and elegant studies, carried out in the laboratory of D. Foster at The University of Michigan, have established that sexual differentiation of the surge generating mechanism by fetal androgen exposure is dependent on the precise timing of androgen administration (Wood and Foster, 1998), the concentration of androgen achieved (Kosut *et al.*, 1997) and whether it can be aromatized to oestrogen (Masek *et al.*, 1999).

Sexually differentiated responses to progesterone

Progesterone is the gonadal steroid hormone that plays the key role in timing and coordinating hormonal and behavioural events in the ovine oestrous cycle (Goodman, 1994). Unlike the situation in female rodents in which progesterone can have stimulatory or inhibitory actions on gonadotrophin release, progesterone has an exclusively suppressive action on GnRH release in ewes. Thus, progesterone inhibits episodic GnRH release during the luteal phase of the cycle (Karsch *et al.*, 1987) and can block the oestrogen-stimulated GnRH surge if present during the follicular phase (Kasa-Vubu *et al.*, 1992). It is now clear that the inhibitory action of this steroid is also sexually differentiated, as males and androgenized females are less responsive to progesterone negative feedback than are normal ewes (Fig. 2). This finding was true whether the gonadectomized animals under study were exposed to exogenous progesterone in the presence or absence of low physiological concentrations of oestrogen, or in the anoestrous or breeding season (Robinson *et al.*, 1999). Unlike the situation with oestrogen, for which positive feedback cannot be demonstrated even with supra-physiological concentrations of steroid, high physiological concentrations of progesterone can exert a suppressive action on LH release in the androgenized female and male, although it is notably less potent than in the female (R. A. Birch and J. E. Robinson, unpublished). It is clear that exposure to testosterone from day 30 to day 90 of fetal life severely disrupts the neural mechanisms by which the ovarian hormones that normally regulate the activity of the GnRH neurone develop. The following sections begin to explore the neural mechanisms that might underlie the sexually differentiated control of GnRH release by steroids.

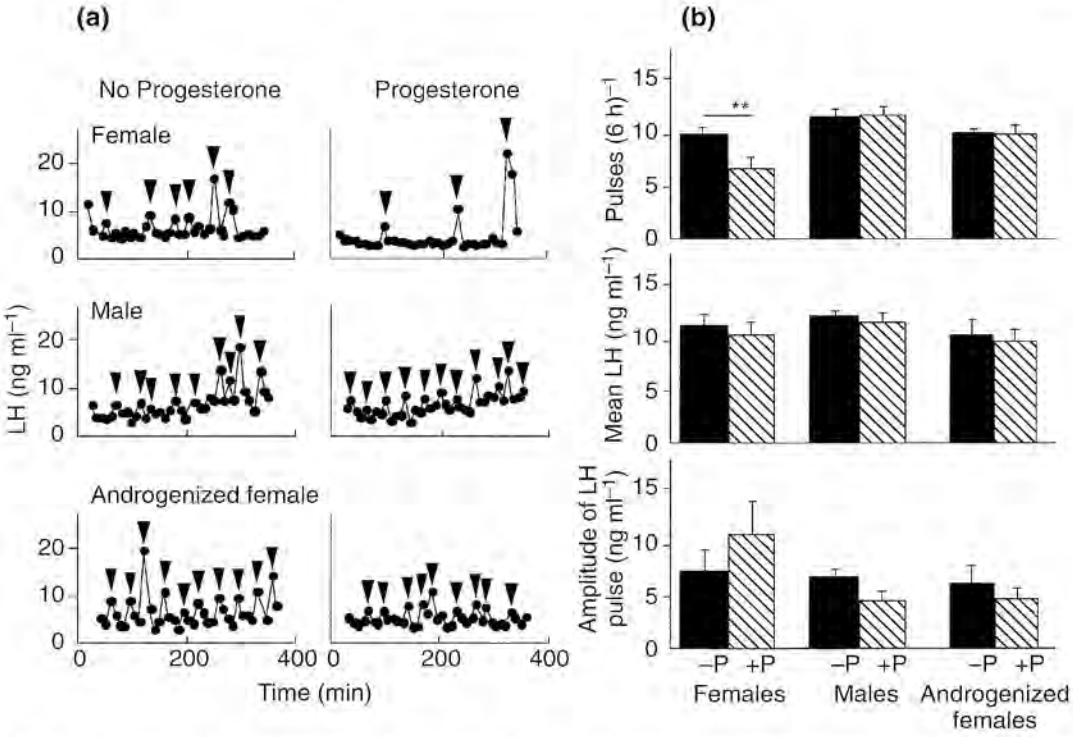

Fig. 2. The sexually differentiated inhibition of tonic LH release by progesterone in sheep. (a) The pulsatile pattern of LH release in a representative gonadectomized female and male sheep, and in an androgenized female sheep in the absence of progesterone (no progesterone) and after implantation of a progesterone controlled internal drug releasing device (CIDR). Statistically identified pulses of LH are indicated by arrowheads. (b) The effects of administration of progesterone (–P, no progesterone; +P, progesterone) during the breeding season on the characteristics of episodic LH secretion: pulse frequency, mean LH concentrations and LH pulse amplitude in gonadectomized female and male sheep, and in androgenized female sheep. Note that progesterone inhibits LH pulse frequency in the female only. $^{**}P < 0.01$. (Adapted from Robinson *et al.*, 1999).

Sexually dimorphic distribution of oestrogen receptor α-immunoreactive cells

The mechanisms by which fetal androgen exposure brings about these radical alterations in the steroidal control of the activity of the adult GnRH neurone are unclear. However, because of the lack of evidence for oestrogen or progesterone receptors in ovine GnRH neurones (Herbison *et al.*, 1993; Skinner *et al.*, 2001), an important route by which these hormones are thought to influence GnRH release is via steroid receptive intermediate neurones. Identification of sex differences in steroid receptive inputs to GnRH neurones may prove a useful approach in determining mechanisms by which oestrogen and progesterone normally modulate gonadotrophin secretion. Recently, we have sought to determine whether the distribution of neurones that contain oestrogen receptor α is sexually dimorphic in areas of the brain that have been implicated in oestrogen feedback mechanisms. These studies focused on hypothalamic sites in which microimplants of oestrogen have been shown to exert inhibitory (preoptic area; POA) or stimulatory (ventromedial nucleus; VMN) actions on GnRH release (Caraty *et al.*, 1998). The results showed that there was a greater density of immunoreactive oestrogen receptive (irERα) cells in the VMN of female compared with male sheep (Taylor

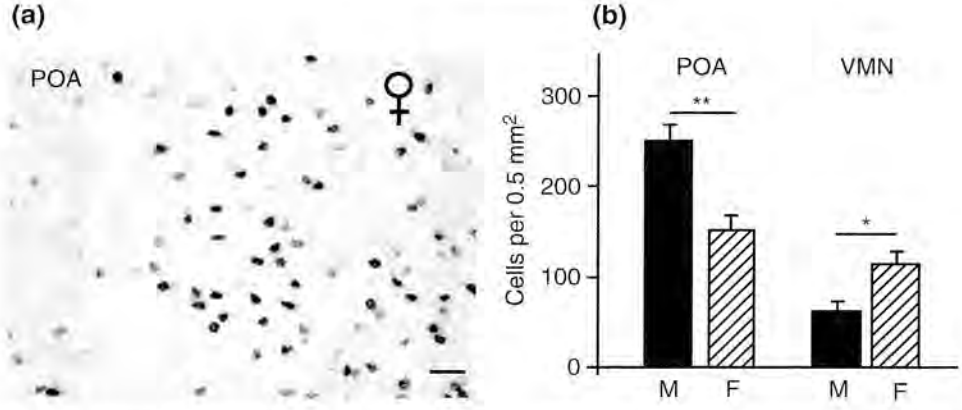

Fig. 3. Sexual differentiation of immunoreactive oestrogen receptor α (irERα) in the preoptic area (POA) and ventromedial nucleus (VMN) of sheep. (a) Immunoreactive oestrogen receptor α (monoclonal mouse antibody; ID5, DAKO, Copenhagen) in the POA of the sheep brain at the level of the organum vasculosum of the lamina terminalis. Scale bar represents 50 μm. (b) The number of cells containing immunoreactive receptors was determined in 4 × 0.5 mm^2 grids for each region in all sheep. The mean number of cells per grid is shown for brains of male (M; $n = 6$) and female (F; $n = 6$) sheep. Note that there is a significantly greater density of irER in the POA of the male than the female sheep. However, there are fewer cells in the male VMN. $^*P < 0.05$; $^{**}P < 0.01$.

et al., 2002) (Fig. 3). These additional oestrogen receptive cells may be critical for the oestrogen stimulated surge in females and absent from males in which oestrogen will not stimulate a surge. These data support findings of Scott *et al.* (2000) in which *in situ* hybridization histochemistry was used to describe sex differences in mRNA for both oestrogen receptor α and β in the VMN: a higher density of ER mRNA and a greater concentration of mRNA per cell was found in females compared with males. In our immunocytochemical studies we also noted a sex difference in the density of ERα in the POA. However, in this area males had a higher density of cells than females (Taylor *et al.*, 2002) (Fig. 3). In contrast to these results, Scott *et al.* (2000) did not report a sex difference in either ERα or β mRNA in the POA. It is now imperative to focus on determining the phenotype of these oestrogen receptive cells before we can determine the role that these sexually dimorphic regions play in the sex-specific regulation of GnRH secretion.

Sex differences in the neural input to ovine GnRH neurones

It is known in both rats (Chen *et al.*, 1990) and sheep (Kim *et al.*, 1996) that GnRH cell bodies in the adult male receive about half the number of synaptic inputs compared with GnRH cell bodies in females. In sheep, this sex difference is under the influence of prenatal androgens (Kim *et al.*, 1996). It seems reasonable to hypothesize that a proportion of these additional inputs in the female convey information about the steroidal status of the individual. Identifying the phenotype of synapses on individual GnRH neurones is a technically challenging and labour intensive undertaking. Less onerous, if not so definitive, is a determination of the phenotype of neurones that are steroid sensitive, sexually differentiated, project to GnRH rich regions and are activated during periods of steroid feedback on gonadotrophin release. We have recently identified two populations of interest: one population synthesizes a tachykinin known as neurokinin B (NKB) and the other population synthesizes somatostatin. Although

their precise role in the steroid modulation of GnRH release is unclear at present they will serve as a focus of future work.

Sexual differentiation of somatostatin neurones

Studies in which microimplants of oestrogen were placed into the brains of ovariectomized ewes have identified the mediobasal hypothalamus, in the region of the ventromedial nucleus, as a key site for oestrogen feedback on GnRH release (Caraty *et al.*, 1998). Both anterograde (Goubillon *et al.*, 2002) and retrograde (Tillet *et al.*, 1993; Goubillon *et al.*, 1999) tracing studies have provided evidence for a trans-synaptic pathway from the VMN to the preoptic–diagonal band region (POA–dBB). Goubillon *et al.* (2002) estimate that about 50% of GnRH neurones ipsilateral to the injection site are in close contact with VMN projections, and may, therefore, receive direct inputs from neurones in this site. Furthermore, in females, between 20 and 40% of VMN neurones that project to GnRH-rich areas contain oestrogen receptor α. Although we have demonstrated that oestrogen receptive neurones in this region are sexually differentiated, the identity of these neurones has only recently been explored. In ewes, the majority of oestrogen receptive neurones in the VMN (approximately 70%) synthesize somatostatin (Herbison, 1995). Therefore, we sought to determine whether this neural phenotype is sexually differentiated. A dense plexus of somatostatin fibres and terminals was identified in the medial portion of the VMN with a substantial population of cell bodies located in the ventrolateral aspects of the nucleus (vlVMN). Somatostatin fibres were also found in close association with approximately 80% of GnRH neurones in the medial POA (Unsworth and Robinson, 2002). However, this population of somatostatin neurones does not appear to be sexually differentiated (Fig. 4b). Specifically, we did not identify a sex difference in either the number of somatostatin neurones in the vlVMN or the number that co-localize oestrogen receptors (W. P. Unsworth, J. A. Taylor and J. E. Robinson, unpublished). These studies have been extended to determine whether somatostatin neurones are activated in a sex-specific manner during oestrogen negative feedback. Using the protein product of the immediate early gene c-*fos* as a marker of neuronal activation we first determined whether there was a sex difference in the number of activated neurones in the VMN after implantation of Silastic capsules of oestrogen under the skin of gonadectomized male, female and androgenized female sheep (Fig. 4c). The concentration of oestrogen produced by these implants was in the high physiological range and was sufficient to trigger a GnRH surge in the control females (determined in an earlier experiment) but not in the males and androgenized sheep. Animals were killed after 6 h of oestrogen treatment, which is during the negative feedback phase that precedes the surge. Ewes that were treated with oestrogen had significantly more fos positive nuclei in the vlVMN than those that were not given oestrogen implants (Fig. 4). A similar increase in fos-positive nuclei in the vlVMN was noted during the surge release of GnRH (Moenter *et al.*, 1993) although not after a short exposure (1 h) to oestrogen (Clarke *et al.*, 2001). These findings are of interest as they indicate that the VMN contains activated neurones during at least two important phases of the oestrogen induced surge: one phase when the oestrogen receptive neurones (but not the GnRH neurones) are being activated (Evans *et al.*, 1997) and the other when the GnRH neurones themselves are secreting the decapeptide. A key additional finding, in our study, was that males and ewes androgenized *in utero* had significantly fewer fos-positive cells in the VMN than did oestrogen-treated control ewes. It was clear, from double labelling studies, that a population of these sexually differentiated, steroid-activated neurones also synthesize somatostatin (Fig. 4d). These data support a role for somatostatin neurones that are located in the vlVMN in the regulation of GnRH release by oestrogen. It will be important to determine whether somatostatin, which is

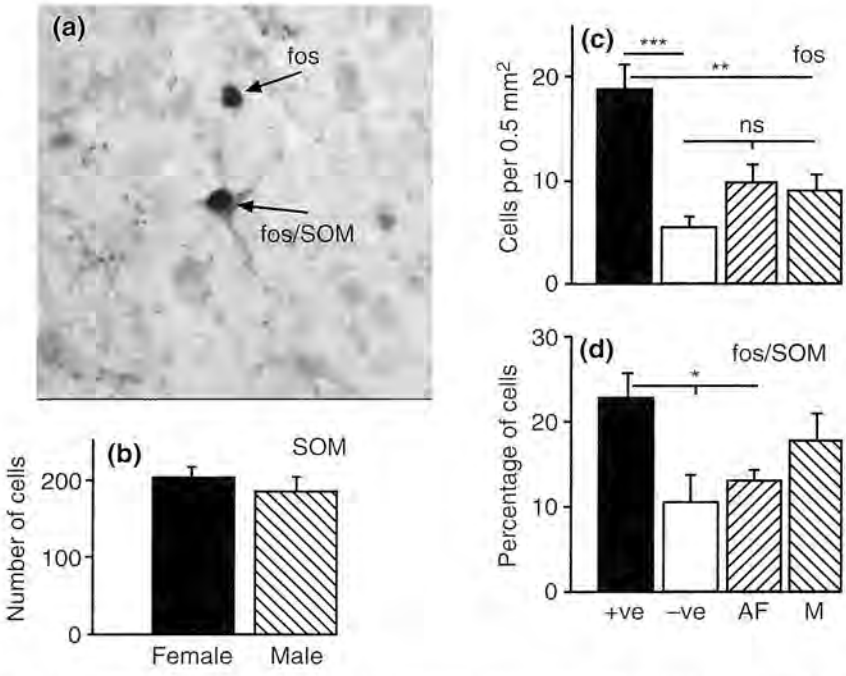

Fig. 4. Somatostatin neurones in the ventro–lateral aspects of the ventromedial nucleus (vlVMN) of sheep. (a) High powered view of the vlVMN showing double labelling immunocytochemistry for somatostatin (SOM; brown cytoplasmic stain) and fos (black nuclear stain). (b) There is no significant difference between the number of somatostatin cells in the vlVMN of the adult female and male sheep. (c) Exposure to oestrogen for 6 h leads to a significantly ($***P < 0.001$) greater number of fos-positive neurones in the vlVMN in the female (+ve) than the untreated female sheep (–ve), or the oestrogen-treated androgenized female (AF) ($**P < 0.01$) and male (M) sheep ($**P < 0.01$). (d) The number of double-labelled fos/SOM neurones after oestrogen treatment ($*P < 0.05$). Note that a proportion of the cells activated by oestrogen in a sex-specific manner are somatostatin neurones.

normally thought of as an inhibitory neurotransmitter, is important for the suppressive actions of oestrogen that precede the GnRH surge. In this regard somatostatin may be one of the first populations of neurones in a pathway by which the GnRH surge is stimulated by follicular phase concentrations of oestrogen. A failure to activate this population would block the chain of events in the male and the androgenized ewe. Whether somatostatin may also have a role a reproductive behaviour, as it appears to in rodents (Herbison, 1994; Dufourny and Warembourg, 1999), is also of interest as the androgenized ewe fails to exhibit normal sexual behaviour (Fabre-Nys and Venier, 1991).

Sexual differentiation of neurokinin B neurones

Another population of neurones, located in the caudal portion of the arcuate nucleus (cARC) and synthesizing NKB, is also sexually dimorphic (Fig. 5b) and this is determined by exposure to testosterone during fetal life (Goubillon *et al.*, 2000). A role for this tachykinin in the steroidal control of GnRH release in sheep is supported by the observation that about 97% of NKB neurones co-localize oestrogen receptors (Goubillon *et al.*, 2000). Furthermore, a substantial number of oestrogen-receptive neurones from the ARC project into the preoptic

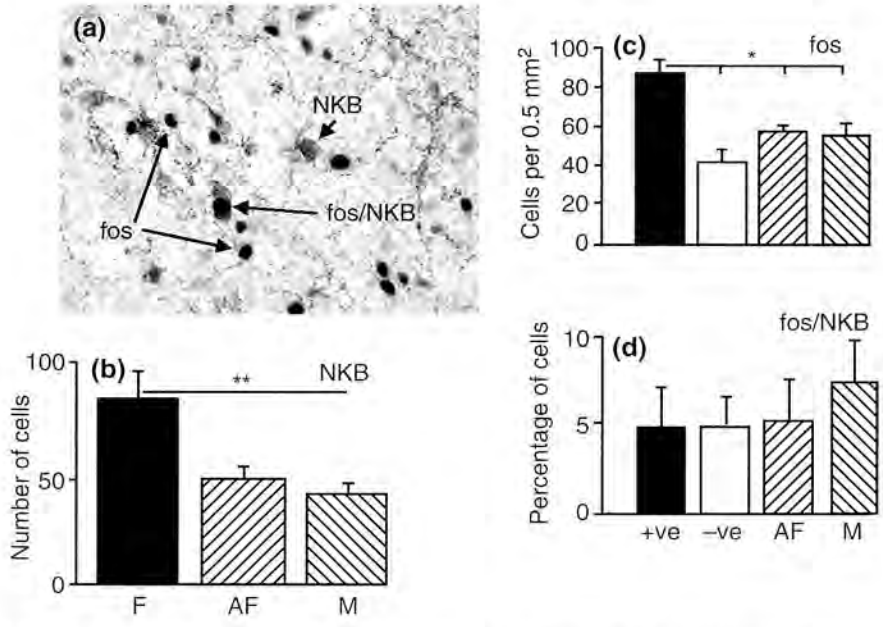

Fig. 5. Neurokinin B (NKB) neurones in the caudal arcuate nucleus (cARC) of sheep. (a) High powered view of the cARC showing double labelling immunocytochemistry for NKB (brown cytoplasmic stain) and fos (black nuclear stain). (b) There is a significant (**$P < 0.01$) difference between the number of NKB cells in the cARC of the adult female (F) and male (M) sheep. Furthermore, this difference is brought about by exposure of the female sheep fetus to androgens (AF). (c) Exposure to oestrogen for 6 h leads to a significantly (*$P < 0.01$) greater number of fos-positive neurones in the cARC of the female (+ve) than the untreated female (–ve), or the oestrogen treated androgenized female (AF) and male (M) sheep. (d) The number of double labelled fos/NKB neurones after oestrogen treatment. Note that fewer than 6% of NKB neurones contain fos-positive nuclei and that this is not sexually differentiated.

area (Goubillon *et al.*, 1999). Using paraformaldehyde fixed tissue from the same animals that were described in the earlier section on somatostatin, we found a sexually differentiated pattern of fos-positive neurones in the ARC after oestrogen administration (Grindrod *et al.*, 2002). Specifically, the ARC of the oestrogen-treated ewes contained significantly more fos-positive nuclei than did the ARC of non-steroid treated ewes or of the androgenized females or males that had been exposed to oestrogen for 6 h (Fig. 5c). A similar difference in the cellular activation of neurones in the ARC was noted between ewes treated with oestrogen for 1 h and untreated ewes (Clarke *et al.*, 2001). Could these be NKB neurones? Our recent double-label fos–NKB immunocytochemical studies indicate that they are not. Specifically, a low percentage (<6%) of neurones co-localized both peptides after the 6 h exposure to high follicular phase concentrations of oestrogen (Grindrod *et al.*, 2002). Furthermore, this was not sexually differentiated (Fig. 5d). Whether these neurones may be involved in a different phase of the pathway by which oestrogen stimulates the surge of GnRH awaits further study. Alternatively, these neurones may become inhibited at this time point and, thus, not show a change in fos immunoreactivity. In addition, future experiments should concentrate on identifying the phenotype of the sexually dimorphic population of fos-positive neurones in this region of the brain. Steroid receptive candidates include neurones that synthesize ACTH, neuropeptide Y,

dopamine, β-endorphin and dynorphin. Whether these might be activated by oestrogen and progesterone in a sex-specific manner awaits investigation.

Conclusions

Although it is well known that exposure of the developing brain to male hormones during a window of development causes it to become organized along masculine lines, the mechanisms by which this occurs are currently obscure. Key to this is an understanding of where and how testosterone influences the synaptic contacts that fetal GnRH neurones make with other cells in the brain. It is now clear that the neural circuits by which the steroid hormones oestrogen and progesterone regulate GnRH secretion in adulthood are permanently disrupted by exposure of the female to androgens *in utero*. However, we have yet to identify more than a handful of steroid receptive neural phenotypes in the ovine hypothalamus and have virtually no information about whether they function in a sexually differentiated manner. Comparison of steroid-sensitive populations of neurones between normal ewes and those that have been exposed to testosterone as a fetus should begin to identify the neurones that may be involved in both oestrogen positive and progesterone negative feedback on GnRH release.

The authors would like to thank Wellbeing and the Royal College of Obstetricians and Gynaecologists and the BBSRC for their financial support for this work. Grateful thanks are also extended to A. Dady, J. Jones, M. Russel, T. Tilly and M. White for unstinting help over many years. Without them these studies would not have been possible.

References

Caldani M, Batailler M, Thiery J-C and Dubois M (1988) LHRH-immunoreactive structures in the sheep brain *Histochemistry* **89** 129–139

Caldani M, Antoine M, Batailler M and Duittoz A (1995) Ontogeny of GnRH systems *Journal of Reproduction and Fertility Supplement* **49** 147–162

Caraty A, Fabre-Nys CJ, Delaleu B, Locatelli A, Bruneau G, Karsch FJ and Herbison AE (1998) Evidence that the mediobasal hypothalamus is the primary site of action of estradiol in inducing the preovulatory gonadotropin releasing hormone surge in the ewe *Endocrinology* **139** 1752–1760

Chen W-P, Witkin JW and Silverman AJ (1990) Sexual dimorphism in the synaptic input to gonadotropin releasing hormone neurones *Endocrinology* **126** 695–702

Clarke IJ, Scaramuzzi RJ and Short RV (1976) Effects of testosterone implants in pregnant ewes on their female offspring *Journal of Embryology and Experimental Morphology* **36** 87–99

Clarke IJ, Pompolo S, Scott CJ, Rawson JA, Caddy D, Jakubowska AE and Pereira AM (2001) Cells of the arcuate nucleus and ventromedial nucleus of the ovariectomised ewe that responds to oestrogen: a study using fos immunohistochemistry *Journal of Neuroendocrinology* **13** 934–941

Claypool LE and Foster DL (1990) Sexual differentiation of the mechanism controlling pulsatile secretion of luteinising hormone contributes to sexual differences in the timing of puberty in the sheep *Endocrinology* **126** 1206–1215

Dufourny L and Warembourg M (1999) Estradiol modulation of neuropeptides: somatostatin, neurotensin and substance P in the ventrolateral and arcuate nuclei of the female guinea pig *Neuroscience Research* **33** 223–228

Evans NP, Dahl GE, Padmanabhan V, Thrun LA and Karsch FJ (1997) Estradiol requirements for induction and maintenance of gonadotropin-releasing hormone surge: implications for neuroendocrine processing of the estradiol signal *Endocrinology* **138** 5408–5414

Fabre-Nys C and Venier G (1991) Sexual differentiation of sexual behaviour and preovulatory LH surge in ewes *Psychoneuroendocrinology* **16** 383–396

Forsdike RA and Robinson JE (1998) Gonadotropin releasing hormone (GnRH) mRNA expression is similar in male and female fetal sheep during the 'critical period' for sexual differentiation *Society of Neuroscience* **24** (Abstract 515.5)

Foster DL and Karsch FJ (1975) Development of the mechanisms regulating the preovulatory surge of luteinising hormone in sheep *Endocrinology* **97** 1205–1209

Goodman RL (1994) Neuroendocrine control of the ovine estrous cycle. In *The Physiology of Reproduction* Vol. 2 pp 659–710 Eds E Knobil and JD Neill. Raven Press, New York

Goubillon M-L, Delaleu B, Tillet Y, Caraty A and Herbison AE (1999) Localization of estrogen-receptive neurons projecting to the GnRH neuron-containing rostral preoptic area of the ewe *Neuroendocrinology* **70** 228–236

Goubillon M-L, Forsdike RA, Robinson JE, Ciofi P, Caraty A and Herbison AE (2000) Identification of neurokinin B-expressing neurones as a highly estrogen-receptive, sexually dimorphic cell group in the ovine arcute nucleus *Endocrinology* **141** 4218–4225

Goubillon M-L, Caraty A and Herbison AE (2002) Evidence in favour of a direct input from the ventromedial nucleus to gonadotrophin-releasing hormone neurones in the ewe. An anterograde tracing study *Journal of Neuroendocrinology* **14** 95–100

Goy RW and McEwan BS (1980) *Sexual Differentiation of the Brain* MIT Press, Cambridge, MA

Grindrod JAE, Unsworth WP, Taylor JA and Robinson JE (2002) Sexually differentiated GnRH secretion: a role for neurokinin B? *5th International Congress of Neuroendocrinology*

Harris TG, Robinson JE, Evans NP, Skinner DC and Herbison AE (1998) Gonadotropin-releasing hormone messenger ribonucleic acid expression changes before the onset of the estradiol-induced luteinizing hormone surge in the ewe *Endocrinology* **139** 57–64

Herbison AE (1994) Somatostatin-immunoreactive neurones in the hypothalamic ventromedial nucleus possess oestrogen receptors in the male and female rat *Journal of Neuroendocrinology* **6** 323–328

Herbison AE (1995) Neurochemical identity of neurones expressing oestrogen and androgen receptors in sheep hypothalamus *Journal of Reproduction and Fertility Supplement* **49** 271–283

Herbison AE, Robinson JE and Skinner DC (1993) Distribution of oestrogen receptor-immunoreactive cells in the preoptic area of the ewe: co-localization with glutamic acid decarboxylase but not luteinising hormone releasing hormone *Neuroendocrinology* **57** 751–759

Herbison AE, Skinner DC, Robinson JE and King I (1996) Androgen receptor immunoreactive cells in the ram hypothalamus; distribution and co-localization patterns with gonadotrophin releasing hormone, somatostatin and tyrosine hydroxylase *Neuroendocrinology* **63** 120–131

Herbosa CG, Dahl GE, Evans NP, Pelt J, Wood RI and Foster DL (1996) Sexual differentiation of the surge mode of gonadotropin secretion: prenatal androgens abolish the gonadotropin-releasing hormone surge in the sheep *Journal of Neuroendocrinology* **8** 627–633

Karsch FJ, Cummins JT, Thomas GB and Clarke IJ (1987) Steroid feedback inhibition of pulsatile secretion of gonadotropin-releasing hormone in the ewe *Biology of Reproduction* **36** 1207–1218

Kasa-Vubu JZ, Dahl GE, Evans NP, Thrun LA, Moenter SM and Padmanabhan V (1992) Progesterone blocks the estration-induced gonadotropin discharge in the ewe by inhibiting the surge of gonadotropin-releasing hormone *Endocrinology* **131** 208–221

Kim S-J, Foster DL and Wood RI (1996) Sexual differentiation of synaptic input to GnRH neurons in the sheep: role of prenatal testosterone *Society of Neuroscience* **22** 1588 (Abstract 624.9)

Kosut SS, Wood RI, Herbosa-Encarnacion C and Foster DL (1997) Precocious puberty in the female: relationship between dose of prenatal androgens and timing of the pubertal LH rise in sheep *Endocrinology* **138** 1072–1077

Levidiotis ML, Wintour EM, McKinley MJ and Oldfield BJ (1989) Hypothalamic–hypophysial vascular connections in the fetal sheep *Neuroendocrinology* **49** 47–50

MacLusky NJ and Naftolin F (1981) Sexual differentiation of the central nervous system *Science* **211** 1294–1302

Masek KS, Wood RI and Foster DL (1999) Prenatal dihydrotestosterone differentially masculinizes tonic and surge modes of luteinising hormone secretion in sheep *Endocrinology* **140** 3459–3466

Matwijiw I and Faiman C (1987) Control of gonadotropin secretion in the ovine fetus: the effects of a specific gonadotropin-releasing hormone antagonist on pulsatile luteinizing hormone secretion *Endocrinology* **121** 347–351

Matwijiw I and Faiman C (1989) Control of gonadotropin secretion in the ovine fetus. II. A sex difference in pulsatile luteinizing hormone secretion after castration *Endocrinology* **124** 1352–1358

Moenter SM, Karsch FJ and Lehman MN (1993) Fos expression during the estradiol-induced gonadotropin-releasing hormone (GnRH) surge of the ewe: induction in GnRH and other neurons *Endocrinology* **133** 896–903

Robinson JE, Forsdike RA and Taylor JA (1999) *In utero* exposure of female lambs to testosterone reduced the sensitivity of the GnRH neuronal network to inhibition by progesterone *Endocrinology* **140** 5797–5805

Robinson JE, Birch RA, Foster DL and Padmanabhan V (2002) Prenatal exposure of the ovine fetus to androgens sexually differentiates the steroid feedback mechanisms that control gonadotropin secretion and disrupts ovarian cycles *Archives of Sexual Behavior* **31** 29–35

Scott CJ, Tilbrook AJ, Simmons DM, Rawson JA, Chu S, Fuller PJ, Ing NH and Clarke IJ (2000) The distribution of cells containing estrogen receptor-α (ERα) and ERβ messenger ribonucleic acid in the preoptic area and hypothalamus of the sheep: comparison

of males and females *Endocrinology* **141** 2951–2962

Sheng C, McNeilly AS and Brooks AN (1998) Immunohistochemical distribution of oestrogen receptor and luteinizing hormone β subunit in the ovine pituitary gland during foetal development *Journal of Neuroendocrinology* **10** 713–718

Short RV (1974) Sexual differentiation of the brain of the sheep *INSERM* **32** 121–142

Skinner DC, Caraty A and Allingham R (2001) Unmasking the progesterone receptor in the preoptic area and hypothalamus of the ewe: no co-localisation with gonadotropin-releasing neurones *Endocrinology* **142** 573–579

Sklar CA, Mueller PL, Gluckman PD, Kaplan SL, Rudolph AM and Grumbach MM (1981) Hormone ontogeny in the ovine fetus. VII. Circulating luteinizing hormone and follicle stimulating hormone in mid- and late gestation *Endocrinology* **108** 874–880

Taylor JA, Birch RA and Robinson JE (2001) GnRH gene expression is sexually dimorphic in the foetal sheep in late, but not early, gestation *British Neuroendocrine Association Meeting* (Abstract 48.02)

Taylor JA, Grindrod J and Robinson JE (2002) Sex differences in the distribution of oestrogen receptor α (ERα) in the adult sheep hypothalamus *5th International Congress on Neuroendocrinology* Abstract p 293

Tillet Y, Batailler M and Thibault J (1993) Neuronal projections to the medial preoptic area of the sheep, with special reference to monoaminergic afferents: immunohistochemical and retrograde track tracing study *Journal of Comparative Neurology* **330** 195–220

Unsworth WP and Robinson JE (2002) Role of the VMN in the oestrogen-induced GnRH/LH surge in the ewe; potential involvement of somatostatin? *5th International Congress on Neuroendocrinology* Abstract, p 294

Wood RI and Foster DL (1998) Sexual differentiation of reproductive neuroendocrine function in sheep *Reviews of Reproduction* **3** 130–140

Wood RI, Newman SW, Lehman MN and Foster DL (1992) GnRH neurons in the fetal lamb hypothalamus are similar in males and females *Neuroendocrinology* **55** 427–433

Wood RI, Robinson JE, Forsdike RA, Padmanabhan V and Foster DL (2000) Sexual differentiation of the timing of sexual maturation in sheep. In *The Proceedings of the 5th International Conference on the Control of the Onset of Puberty* pp 269–287 Eds J-P Bourginon and TM Plant, Elsevier