Control of GnRH secretion

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Introduction

During a discussion at the 1st International Symposium on Reproduction in Domestic Ruminants following Dr Gerald Lincoln’s presentation, it became apparent that we needed a method to monitor gonadotrophin-releasing hormone (GnRH) secretion in sheep. Within the 6 years that have elapsed, three models have been developed for this purpose and it is now possible to monitor accurately the secretion of GnRH from the median eminence and to relate this to LH and FSH release. We are therefore able to conduct meaningful experiments to ascertain the roles of the various neural systems and feedback effects that might regulate GnRH secretion.

This paper will review the progress that has been made in measuring the secretion of GnRH, particularly in sheep, and consider steroidal feedback effects. Finally, brief consideration will be given to some of the various neural systems that might be involved in regulating GnRH secretion.

Models for the measurement of GnRH secretion

Two systems have been developed for the direct sampling of hypophysial portal blood in conscious sheep. One involves the accessing of the anterior face of the pituitary gland by unilateral transnasal transphenoidal surgery (Clarke & Cummins, 1982). An artificial ethmoid sinus is created anterior to the gland and two 12-gauge needles are implanted through the non-operative side to enter the artificial sinus. One needle is directed at the portal vessels that course down the anterior face of the pituitary gland in this species, and this is later used as a guide needle for a stillette that punctures the vessels. The other needle is used for sample collection.

After surgery, sheep are allowed to recover for up to 3 days before they are heparinized and their portal vessels are punctured and sampled. This method has many advantages.

1. It allows the direct sampling of portal blood from conscious animals whilst not totally compromising pituitary function; thus GnRH and LH secretion may be monitored contemporaneously.
2. The surgical preparation time is less than 2 h so that 3–4 sheep can be prepared early in a week then sampled during the remainder of the week.
3. The surgical operation does not disturb the brain or the pituitary gland.
4. The portal blood collection rate is generally 0.2–0.4 ml/min, providing adequate volumes of blood in 5–10-min intervals for GnRH assay.
5. Samples can be collected for up to 10 h, without significant discomfort to the animal.

A major disadvantage of this procedure is that an animal may be used only once. Another is that, due to variations in the vascular arrangements, identical lesions cannot be made in a series of sheep and this limits interpretations of differences in GnRH pulse amplitude between animals.

The push–pull perfusion system, which was originally developed by Levine & Ramirez (1980) in rats, has also been adapted for use in sheep (Levine et al., 1982). This method requires the intro-
duction of concentric needles through the brain and into the median eminence. The surgical technique requires X-ray equipment to guide the cannulae into position. Animals are allowed to recover and GnRH secretion is monitored in perfusates of the median eminence that are pushed through the inner needle and pulled through the external needle. Accurate calibration of the ‘push’ and ‘pull’ pumps is essential to prevent tissue damage. Like the method of Clarke & Cummins (1982), this ‘push–pull’ perfusion may be performed on conscious animals with minimal trauma and GnRH secretion and LH secretion may be monitored simultaneously. Drawbacks with the ‘push–pull’ system are that X-ray equipment is necessary for cannula placement and tissue damage occurs at the time of sampling. Other disadvantages are that GnRH is not always measurable in the perfusates and, since one is not measuring GnRH in portal blood, one cannot be absolutely certain that the ‘pulses’ seen in the perfusates also appear in portal blood. Finally, this technique generally requires some histological examination of the median eminence to identify the site of cannula tip placement. Caraty et al. (1982) have also described a ‘push–pull’ perfusion system for sheep but in their model portal blood was sampled; it was not made clear whether the samples also contained cerebrospinal fluid.

In conscious monkeys, it is possible to monitor the pulsatile release of GnRH by the ‘push–pull’ perfusion (Levine et al., 1985) or by the withdrawal of cerebrospinal fluid from the third ventricle (Van Vugt et al., 1986). The latter system has the considerable advantage of enabling samples to be taken from animals on more than one occasion, without perturbation of the hypothalamo–pituitary anatomy. One problem with this procedure is that GnRH and LH pulses do not always appear synchronously; the appearance of some LH pulses without concomitant GnRH pulses suggests that GnRH pulses that are presumably present in portal blood do not occur in the cerebrospinal fluid. In sheep, the measurement of GnRH in cerebrospinal fluid has not provided satisfactory results (R. S. Carson, personal communication).

Feedback control of GnRH secretion by ovarian steroids

To adopt a systematic approach to the study of steroidal effects on GnRH secretion we have defined three types of feedback effect. These are best defined in ovariectomized animals and in terms of the changes that occur after oestrogen treatment of ovariectomized sheep (Clarke et al., 1982). The first and immediate effect is a fall in plasma LH concentrations; this may be called a short-term negative feedback effect. Following this, plasma LH concentrations rise well above preinjection values (Clarke et al., 1982) by what may be called a positive feedback mechanism. Thereafter, and with continued oestrogen treatment, plasma LH concentrations are held below those of untreated ovariectomized animals by a long-term (tonic) negative feedback effect (Dieckman & Malven, 1973). The positive feedback effect may also be obtained in seasonally anoestrous ewes (Goding et al., 1969).

This classification may be applied to normal cyclic events. The short-term negative feedback effect might operate in the late follicular phase of the sheep oestrous cycle as suggested by Thomas (1983) and by the data of Scaramuzzi & Radford (1983). The positive feedback mechanism that is seen in oestrogen-treated ovariectomized ewes, and in anoestrous ewes, has been considered similar to that which generates the normal preovulatory LH surge although this may not be the case (see below). Finally, long-term tonic negative feedback is pertinent in terms of the patterns of gonadotrophin secretion that are seen during the luteal phase of the cycle and during anoestrous periods.

Short-term negative feedback

Sakar & Fink (1980) found that an intravenous injection of 1 μg oestradiol caused an acute reduction in GnRH secretion in ovariectomized rats but in another study (Sherwood & Fink, 1980), the subcutaneous injection of 50 μg oestradiol benzoate had no acute effect. In both studies
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The doses used may be regarded as high. The intravenous injection of 1 μg oestradiol had no immediate effect on portal GnRH concentration in ovariectomized monkeys (Carmel et al., 1976).

In sheep, it was found that the secretion of GnRH continued after a 50 μg intramuscular injection of oestradiol benzoate (Clarke & Cummins, 1985); this could occur when the secretion of LH ceased. Schillo et al. (1985), using similarly treated ewes but sampling by 'push–pull' perfusion of the median eminence, also found that secretion of GnRH could continue during the 'pre-surge' period, whilst LH secretion ceased. This led to the conclusion that short-term negative feedback is the result of a pituitary action of oestrogen and support for this notion was gained by studies in ovariectomized ewes with hypothalamo–pituitary disconnection given oestrogen during pulsatile GnRH treatment. It was clearly seen that the initial effect of oestrogen was virtually to eliminate pituitary responses to GnRH (Clarke & Cummins, 1984).

Thus, with the exception of one study in rats, the evidence clearly indicates that there is no short-term negative feedback effect of oestrogen at the hypothalamic level to limit GnRH secretion, but that the effect observed in hypothalamo–pituitary intact ewes (Clarke et al., 1982) is the result of pituitary action of oestrogen.

Positive feedback

The preovulatory surge in LH secretion has fascinated neuroendocrinologists since it first became apparent that a signal emanating from the brain was responsible for ovulation and that this signal was triggered by coitus (in reflex ovulators) or by ovarian steroids. By virtue of this mechanism, the pituitary gland in sheep may lose up to 90% of its stores of LH within a few hours (Roche et al., 1970).

It is clearly established that the responsiveness of the pituitary gland to GnRH is increased at the time of the normal cyclic preovulatory LH surge (Reeves et al., 1971) and at the time of an oestrogen-induced surge in ovariectomized ewes (Coppings & Malven, 1976). This increased responsiveness accounts for a 2–3-fold rise in plasma LH concentrations (Clarke & Cummins, 1984). To achieve the much higher levels that are seen during the preovulatory LH surge, an additional factor is required. This is most likely to be a rise in the level of GnRH secretion or an alteration in the pattern of its secretion. Studies of rats (Sarkar et al., 1976), women (Miyake et al., 1980) and monkeys (Neill et al., 1977) indicate that the LH surge is associated with a rise in GnRH secretion. In sheep the primary signal for the generation of a surge in LH secretion appears to be a rise in ovarian oestrogen secretion (Goding et al., 1969). In some species, e.g. humans (Liu & Yen, 1983), the positive feedback trigger may involve a combination of oestrogen and progesterone effects. This does not appear to be the case in sheep since there is no rise in plasma progesterone at this time (Baird & Scaramuzzi, 1976). If, in sheep, oestrogen alone is the trigger, how is the rise in GnRH secretion manifest and is there an increase in GnRH pulse frequency, amplitude or both?

To investigate this issue, the GnRH concentrations in portal blood were monitored in oestrogen-treated ovariectomized ewes before and during the LH surge (Clarke & Cummins, 1985). At the time of these studies we were attempting to sample the upper reaches of the portal network and, on some occasions this resulted in blood being sampled from the median eminence, leading to artefactually high GnRH readings in some of the controls and treated animals. In spite of this, an increase in GnRH pulse frequency was detected at the time of the LH surge; mean (± s.e.m.) inter-pulse intervals were 26.8 ± 9.8 min during the LH surge compared to 53.5 ± 8.7 min in controls. A striking example of this increase in the secretion of GnRH at the time of the LH surge is seen in Fig. 1(a). Schillo et al. (1985) found variable patterns of GnRH secretion associated with oestrogen-induced surges of LH secretion in ovariectomized ewes. GnRH was undetectable in the perfusates of 5 of 11 sheep sampled during the surge and in those with detectable GnRH, 3 patterns of secretion were seen. In some ewes a large GnRH pulse was detected before the surge and a sustained rise was seen during the surge; in others there was a gradual rise in GnRH output and in some there was little change during the surge. Figure 1(b) shows results from one ewe in this study,
Fig. 1. GnRH secretion during the LH surge induced by oestrogen injection (50 μg, i.m.) in ovariectomized ewes. (a) Hypophysial portal plasma GnRH shown as pg/min (■) or pg/ml (○) and jugular venous LH (●) after the start of the LH surge (vertical broken line). GnRH pulses (▲) and LH pulses (▲) are indicated. Taken from Clarke & Cummins (1985), with permission. (b) GnRH (●) in ‘push–pull’ perfusates of the median eminence and jugular venous LH (○). Taken from Schillo et al. (1985) with permission.

illustrating the large GnRH pulse that was sometimes seen at the onset of the LH surge. In ovariectomized monkeys and again using the ‘push–pull’ technique, Levine et al. (1985) also found variable patterns of GnRH secretion associated with the oestrogen-induced LH surge. Is this between animal variation in GnRH secretion at the time of the LH surge an artefact of the collection technique, or can the surge in LH secretion be generated by more than one pattern of GnRH secretion? Preliminary data from cyclic ewes support the latter, for reasons discussed below.

The long-term ovariectomized animal may not be an appropriate model for the study of GnRH secretion during the LH surge because of the chronic deprivation of steroids and the inherently high pulse frequency. A much better model may be the anoestrous ewe, in which both of these problems are overcome, and in which an LH surge may be induced by oestrogen (Goding et al., 1969). Accordingly, GnRH secretion was monitored in 6 anoestrous Corriedale ewes after injections of 50 μg oestradiol benzoate. In 4/6 ewes the LH surge was clearly associated with elevated GnRH secretion. In the remaining animals a large GnRH pulse was seen at the beginning of the LH surge (data not shown). At this point, it was apparent that oestrogen provoked a rise in GnRH secretion but the pattern of response differed in the 2 experimental ovariectomized and anoestrous ewes; in the former there was a rise in GnRH pulse frequency and in the latter a more pronounced ‘surge’ of secretion was evident. Which of these is more likely to represent the situation in the cyclic ewe?

To monitor GnRH secretion during cyclic LH surges preovulatory events were precipitated by injecting prostaglandin F-2α and causing luteal regression. GnRH secretion during the LH surge
has been measured in 6 ewes (data to be published). In one ewe there was a large GnRH pulse at the start of the LH surge and very little activity thereafter, this being reminiscent of the results obtained in some of the animals studied by Schillo et al. (1985) and in one of our oestrogen-treated anoestrous ewes. In 2 other ewes the GnRH concentrations and pulse frequencies were similar to those of the follicular phase. In 3 ewes GnRH concentrations during the LH surge were clearly elevated above those in the follicular phase of the cycle. Even in ewes in which there was a clear elevation in GnRH secretion, the values were comparatively modest (up to 30 pg/ml) and well below those seen in 4/6 oestrogen-treated anoestrous ewes (> 100 pg/ml, data not shown). This may mean that the latter is inappropriate as a model for the cyclic LH surge.

Based on these studies of sheep and monkeys and using two different techniques and in 3 laboratories, it is apparent that the pattern of secretion of GnRH that is associated with the positive feedback surge in LH secretion may vary between animals. It is therefore extremely difficult to generalize about the neural mechanisms involved in the positive feedback phenomenon.

**Long-term negative feedback**

It is well accepted that a combination of oestrogen and progesterone exerts a tonic negative feedback effect on gonadotrophin secretion during the luteal phase of the oestrous cycle. Using ovariectomized Suffolk ewes, Goodman & Karsch (1980) dissected the separate effects of these two steroids and showed that oestrogen reduced LH pulse amplitude and progesterone reduced pulse frequency. In a subsequent study, Goodman et al. (1981) used a lower dose of progesterone which, when given alone, had no effect on LH secretion, but in combination with oestrogen was able to reduce pulse frequency. Treatment of ovariectomized Merino sheep with lower dosages of oestrogen and progesterone than those used by Goodman & Karsch (1980) had no effect on LH secretion during the breeding season unless the steroids were given in combination (Martin et al., 1983).

When ovariectomized Corriedale ewes were treated with oestrogen or progesterone in the doses used previously by Goodman & Karsch (1980), either steroid, given either during the anoestrous or breeding season, completely abolished GnRH secretion (Karsch et al., 1987). Two major conclusions were drawn from this. Firstly, both steroids are able to exert powerful negative feedback effects on GnRH secretion. Secondly, since these treatments abolished GnRH/LH secretion in Corriedale ewes but did not eliminate LH secretion in Suffolk ewes (Goodman & Karsch, 1980), the former breed appears more responsive to lower amounts of steroid than the latter. To identify alterations of GnRH frequency and amplitude that might result from either oestrogen or progesterone feedback, it will be necessary to use lower doses in our Corriedale sheep.

**Neuromodulation of GnRH secretion**

The generation of GnRH pulses depends upon central aminergic systems which have not been as exhaustively studied in the sheep as the rat. Jackson (1975, 1977) has shown that α-adrenergic and dopaminergic systems are involved, but since the aminergic systems have not been mapped in the sheep brain, we are unable to pinpoint the centres involved. In other species (e.g. rat) the aminergic afferent inputs to the hypothalamus arise from the brain stem nuclei and it is highly likely that this is also the case for the sheep.

There is strong evidence to suggest that opioid systems may interact with the aminergic elements that regulate GnRH secretion. It is significant that some endorphin- and dynorphin-containing neurones may concentrate oestrogen in the rat hypothalamus (Morrell et al., 1985), and that opiate receptors are found in areas such as the locus coeruleus (origin of the A6 ascending fibres; Pert et al., 1976), and the medial preoptic area (Hammer, 1985), the site where GnRH cell bodies are found (Lehman et al., 1986).
Fig. 2. GnRH secretion (pg/min) into hypophysial portal blood and jugular venous plasma concentrations of LH in a ewe given naloxone (40 mg/h) during the mid-luteal phase of the oestrous cycle. ▼ Pulses. (R. Horton & I. J. Clarke, unpublished data.)

In male (Schanbacher, 1982; Ebling & Lincoln, 1985) and female (Brooks et al., 1986) sheep, opiate agonists can suppress LH secretion and opiate antagonists increase LH secretion. In females the effect of the antagonist naloxone is most marked during the luteal phase of the oestrous cycle (Brooks et al., 1986), whereas in males the effect depends upon the presence of testosterone (Schanbacher, 1982). Studies in a variety of species (see Van Vugt, 1985, for references) have suggested that steroidal feedback on GnRH involves opioid systems. In the female, this seems most pertinent to the luteal phase of the ovarian cycle (Ferin et al., 1984). There are, however, some problems with this hypothesis. Firstly, treatment with naloxone is not always followed by an LH response (Van Vugt et al., 1983, 1984; Brooks et al., 1986). Secondly, after continued treatment with agonist or antagonist the effect on LH is temporary (Ebling & Lincoln, 1985; Brooks et al., 1986). This may mean that the opioid system is facilitatory rather than obligatory for feedback regulation.

Using the portal access model we have monitored the GnRH secretion that results from the treatment of mid-luteal phase ewes with naloxone. An example of the responses obtained is shown in Fig. 2. It is clear that the naloxone-induced GnRH pulse is of greater amplitude than that normally seen, and that the effect is short-lived. Further studies are now required to determine which sub-class(es) of opiate receptors is/are responsible for this response, and which neural centres are involved.

Conclusions

The development of methods for the measurement of GnRH secretion in conscious sheep has permitted definition of the feedback effects of ovarian steroids at the central level. In particular, the patterns of secretion associated with the preovulatory LH surge have been demonstrated. Further studies are required to determine the exact nature (change in frequency and/or amplitude) of the long-term negative feedback action of steroids. Feedback effects at the level of the pituitary gland have also been characterized using the hypothalamo–pituitary disconnected ewe (see Clarke, 1987). Having defined the sites of the various feedback effects and the resultant changes in GnRH and gonadotrophin secretion, we are now in a position to study the central mechanisms that mediate these feedback effects in sheep.
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