Stimulation of LH secretion in sheep by central administration of corticotrophin-releasing hormone

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Corticotrophin-releasing hormone (CRH) has been proposed as a mediator of the anti-reproductive effects of stress through an action within the hypothalamus to inhibit GnRH secretion. This hypothesis was tested in sheep by studying the responses to central administration of CRH in both sexes and in both seasons. Sexually mature, Ile-de-France ewes and Romanov rams that had been gonadectomized and implanted with a permanent guide cannula into the third cerebral ventricle were used. Ewes were studied in the presence and absence of exogenous oestradiol plus progesterone, in both the breeding and anoestrous seasons. All rams were treated with testosterone and were studied only during the breeding season. Each observation involved serial samples (every 10 min) of jugular blood for 5 h before (control) and 5 h after an intracerebroventricular (icv) injection of either saline (vehicle) or 5 nmoles CRH in 20 µl vehicle. The saline injections did not affect any of the endocrine variables measured; however, CRH always increased cortisol concentrations in jugular plasma. In the absence of treatment with replacement sex steroids, icv injection of CRH had no effect on pulsatile LH secretion in females either during the breeding season or during anoestrus. However, LH pulse frequency and mean LH concentrations increased significantly on every occasion on which animals were treated with sex steroids. Treatment with CRH also increased LH secretion in the testosterone-treated rams. It is concluded that, contrary to the hypothesized role of CRH as an inhibitor of reproductive activity, this neuropeptide stimulates pulsatile LH (and thus GnRH) secretion, at least in this species. The fact that gonadal steroids seem to be obligatory for the expression of this effect suggests that the protocols used in past studies need to be reassessed.

Introduction

Under stressful conditions, the neuropeptide, corticotrophin-releasing hormone (CRH) is released into the hypophyseal portal system to activate the synthesis and release of ACTH from the pituitary gland (Rivier and Plotsky, 1986). In addition, CRH appears to act within the central nervous system of the rat to mediate the anti-reproductive effects of stress. For example, intracerebroventricular (icv) administration of CRH inhibits the secretion of GnRH into hypophyseal portal blood and decreases LH secretion (Rivier and Vale, 1984; Petraglia *et al.*, 1987). The inhibition of LH secretion by footshock is also known to be associated with a large release of CRH into portal blood and can be reversed by icv administration of either CRH antagonist or antiserum directed against CRH (Rivier *et al.*, 1986). There may be differences between species in the action of CRH, as inhibition of LH secretion in gonadectomized monkeys caused by hypoglycaemic stress does not appear to be mediated by hypothalamic CRH (Van Vugt *et al.*, 1997).

Immunohistochemical studies in the rat have lent anatomical support to the postulate of a central action of CRH on GnRH neurones. Synaptic contacts between CRH axon terminals and GnRH dendrites have been found in the medial preoptic area (MacLusky *et al.*, 1988), a site where direct infusion of CRH inhibits GnRH release into the median eminence (Rivest *et al.*, 1993).

The relationship between CRH and GnRH activities is not so clear in sheep. Stress is known to inhibit pulsatile LH secretion in gonadectomized and intact ewes (Rasmussen and Malven, 1983; Adams *et al.*, 1993) and to suppress the preovulatory surge of LH in the intact ewe (Martin *et al.*, 1980; Przekop *et al.*, 1984). These effects are usually associated with increases in the circulating concentrations of cortisol. Under various stress conditions, a large increase in the secretion of CRH into hypophyseal portal blood has been demonstrated (Caraty *et al.*, 1988a, 1990; Engler *et al.*, 1989). Moreover, it has been reported that stress-induced CRH release into portal blood is correlated with a decrease in GnRH secretion in testosterone-treated gonadectomized rams (Papinot *et al.*, 1989). However, peripheral administration of large quantities of CRH appears to have no effect on LH secretion, according to Donald *et al.*

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(1983) who worked with ewes for which neither ovarian status nor season were stated, and to Parrott *et al.* (1988) who worked with castrated rams. Clarke *et al.* (1990) observed no effect on LH secretion with icv administration of CRH, whereas Naylor *et al.* (1990) reported a stimulatory effect. Both of these studies used gonadectomized ewes that had not been treated with exogenous sex steroids. Taken together, these observations suggest that the sex steroid status of the animals needs to be adequately controlled in studies of interactions between the adrenal and gonadal axes.

We, therefore, reinvestigated the role of CRH in the control of reproduction in sheep by studying the responses to central administration of the neuropeptide. Gonadectomized animals were used to ensure control over the amount of sex steroids. The responses of females were studied during both sexual seasons; in the presence and absence of exogenous oestrogen or progesterone during anoestrus; and in the presence and absence of a combination of the sex steroids during the breeding season. Males were studied only in the breeding season in the presence of physiological doses of exogenous testosterone.

Materials and Methods

Animals

Sexually mature, Ile-de-France ewes and Romanov rams were housed indoors in separate, group pens, under natural photoperiod, at the INRA Station de la Reproduction des Mammifères Domestiques (Nouzilly). They were fed concentrated pellets once a day, and hay and water were available *ad libitum*. Experimental animals were always maintained in contact with other sheep, even during the sampling periods, to prevent the stress of social isolation (Rasmussen and Malven, 1983).

Surgical procedures

Guide-cannulae were constructed from blunted stainless-steel luer-lock needles (o.d. 1.20 mm; i.d. 0.86 mm), cut to a length of 40 mm and stereotactically implanted into the third cerebral ventricle according to a previously described procedure (Skinner *et al.*, 1995). This operation was carried out under halothane anaesthesia in a stereotaxic frame with the aid of frontal and sagittal X-rays. The tip of the cannula was placed 1.5 mm behind the vertical tangent of the massa intermedia and 1.5 mm below the horizontal line which was drawn through the middle of the foramen of Monro. When the tip of the cannula was in the third ventricle, cerebrospinal (CSF) flowed freely back up the tube. The cannula was then closed with a stainless steel stylette with a plastic stopper and fixed to the skull with acrylic dental cement. The whole device was protected by a threaded cylindrical Teflon cap which was anchored to the skull with four stainless steel screws and acrylic cement. After surgery, animals were injected daily with antibiotics (5 ml chloram-frecortyl; Vetoquinol, Lure, France) for 5 days and diuretics (3 ml Diurizone; Vetoquinol) for 3 days. Chloram-frecortyl contains 0.22% prednisolone but this is cleared within 8 h and the animals were not used until a month after surgery.

Blood sampling and injection procedure

The day before each sampling period, a jugular cannula was inserted into each animal and kept patent with heparinized saline (50 units heparin ml⁻¹ in saline). The next day, 5 ml samples of blood were taken from each animal every 10 min for 10 h, starting at 09.00 h. After an initial 5 h control period, the animals were given an icv injection of either CRH (5 nmoles in 20 µl; Bachem, Marina del Rey, CA) or sterile saline (the vehicle), after which sampling continued for another 5 h. The injections were aided by the insertion of a 20 g needle connected to 10 cm piece of polyethylene tubing (o.d.: 1.09 mm; i.d.: 0.38 mm) containing the injection solution. The needle was lowered to the required depth (extending 2 mm from the tip of the guide tube) and the injection was delivered under gravity.

Experimental designs

Experiment 1: treatment of gonadectomized ewes with CRH and sex steroids during the anoestrous season. Five Ile-de-France ewes that had been gonadectomized and implanted with permanent guide-cannulae into their third cerebral ventricles two months earlier were subjected to serial blood samples and icv injections on four occasions, one week apart, during the anoestrous season. The animals were injected with saline on the first occasion and with CRH on the others. The day after the first CRH injection, a 10 mm s.c. implant made from Silastic tubing (Dow Corning, Midland, MI) and filled with oestradiol, was inserted. These implants provide luteal phase amounts of oestradiol in the circulation (3 pg ml⁻¹; Martin *et al.*, 1988). The second CRH injection and serial blood sampling were carried out one week later. The oestradiol implant was then removed and animals were immediately given 3 cm intra-vaginal progesterone-releasing pessaries ('Plasthyd', Sanofi Research, Montpellier). This size and type of pessary has been shown to maintain circulating concentrations of progesterone at about 0.5–0.7 ng ml⁻¹ for 12 days. These concentrations are lower than those normally observed during the luteal phase in intact ewes in our laboratory, but avoid a total blockade of LH pulsatile secretion, and thus allow detection of possible inhibitory effects of CRH administration. The final CRH injection and serial blood sampling were performed one week later.

Experiment 2: treatment of gonadectomized ewes with CRH and sex steroids during the breeding season. Ten Ile-de-France ewes were gonadectomized and implanted with cerebral guide-cannulae during the breeding season. Two months later, they were assigned to two groups of five animals. One group was treated with both oestradiol (1 cm s.c. implant) and progesterone (3 cm intravaginal pessary) using the same implants as described for Expt 1. The other group did not receive any sex-steroid replacement treatment. After 15 days, two or three ewes from each group were injected (icv) with saline while the remainder were injected with CRH. The day after these injections, the steroid and icv treatments were crossed over and a second series of samples was taken 15 days later, effectively providing five replicates for each of the four treatments.

Fig. 1. Plasma concentrations of cortisol in gonadectomized ewes given intracerebroventricular injections (arrow) of saline (●) or corticotrophin-releasing hormone (CRH) (○) in the absence of exogenous sex steroids, or given CRH in the presence of oestrogen (□) or progesterone (△). All values are mean $\pm$ SEM ($n = 5$).

Experiment 3: treatment of testosterone-implanted gonadectomized rams with CRH during the breeding season. During the breeding season, four Romanov rams were implanted with permanent guide-cannulae into the third cerebral ventricle. One month later, animals were gonadectomized and given testosterone implants (s.c.) (Roussel UCLAF, Romainville) that have been shown to maintain circulating concentrations of testosterone at about 1 ng ml^{-1} for one month. Eight days later, serial blood samples were taken and the animals were injected icv with either saline or CRH. One week later, the icv injection treatments were reversed during a second series of blood samples, effectively providing four replicates per treatment.

Hormone assays

Plasma collected from centrifuged blood samples was either assayed immediately or stored at -20°C before assay. All samples from any given experiment were measured in one assay to avoid interassay variation. Plasma LH concentrations were measured in duplicate aliquots by radioimmunoassay using the method described by Pelletier *et al.* (1968). The sensitivity of the assay was 0.1 ng ml^{-1} . The standard preparation (CY1051; kindly donated by Y. Combarous, INRA, Nouzilly) has an activity of $2.5 \times \text{NIH-LH-S1}$. The intra-assay coefficient of variation was 8%. Testosterone concentrations were measured in duplicate aliquots by the radioimmunoassay using the method described by Garnier *et al.* (1978). The sensitivity of the assay was $0.1\text{--}0.2 \text{ ng ml}^{-1}$ and the intra-assay coefficient of variation was 10%. Progesterone concentrations were measured in duplicate aliquots by radioimmunoassay using the method described by Saumande *et al.* (1985). The sensitivity of the assay was 0.05 ng ml^{-1} and the intra-assay coefficient of variation was 10%. Cortisol concentrations were measured in duplicate aliquots of every third sample (i.e., every 30 min) taken in Expt 1, using the radioimmunoassay described by Brunet and Sebastian (1991). The sensitivity of the assay was 0.2 ng ml^{-1} and the intra-assay coefficient of variation was 7.9%.

Statistical analyses

The LH pulse profiles were analysed with a modified version of the 'Pulsar' algorithm modified for the Apple Macintosh computer ('Munro', Zaristow Software, West Morham, Haddington, East Lothian EH4 4PD, UK), as described by Martin *et al.* (1987). The baseline was calculated as a moving average over a 120 min window (60 min each side of the sample being tested). The individual peaks were tested with a threshold, where a pulse was accepted if the concentration at the peak exceeded the concentration at the previous nadir by more than one standard deviation and the interval to the previous pulse was more than 25 min. The Baxter parameters, which describe the parabolic relationship between the mean and the standard deviation of the LH concentration, were derived from the quality controls for each assay. The G-parameters (the number of standard deviations by which a peak must exceed the baseline in order to be accepted) were set at 3.0, 2.5, 1.9, 1.2, and 0.9 standard deviations for pulses containing one to five samples above baseline concentration, respectively.

For each experiment, LH pulse frequencies observed before and after the icv infusions were compared using the Wilcoxon test for paired series. The other variables provided by the pulse analysis (mean concentration, interpulse nadir, pulse amplitude, pulse area) and the cortisol data were normally distributed, so the values before and after icv injection were compared using the Student's paired *t* test. Control (pretreatment) values were tested where appropriate (for example, for responses to exogenous sex steroids) by one-way analysis of variance.

Results

Amongst all of the measures of LH secretion resulting from pulse analysis, only the pulse frequencies and the mean concentrations were affected by treatment. In the interests of brevity, these are the main data presented for this hormone.

Experiment 1: treatment of gonadectomized ewes with CRH and sex steroids during the anoestrous season

Cortisol concentrations in jugular samples were not affected by icv administration of saline (Fig. 1), mean concentrations

before and after treatment were 6.3 ± 2.5 versus 7.6 ± 1.7 ng ml⁻¹, respectively. Injection of CRH increased ($P < 0.05$) cortisol concentrations, and the responses were similar in the absence of steroid replacement (increasing from 8.6 ± 2.4 ng ml⁻¹ to 17.6 ± 1.5 ng ml⁻¹), in the presence of oestradiol (6.8 ± 1.6 ng ml⁻¹ to 15.5 ± 1.3 ng ml⁻¹), and in the presence of progesterone (7.7 ± 1.2 ng ml⁻¹ to 19.2 ± 2.4 ng ml⁻¹). The responses were thus similar in amplitude and duration in all groups (Fig. 1).

Saline injected into the third ventricle did not alter any of the characteristics of LH secretion in gonadectomized ewes that were not treated with oestradiol (Fig. 2). Similarly, there were no significant responses to CRH in ewes not treated with oestradiol (Fig. 2). When gonadectomized ewes were treated with oestradiol, LH secretion was suppressed – only one animal of the five showed a pulse during the 5 h before the injection. The pulse profiles observed in this situation suggest that icv CRH injections increase LH secretion in the presence of exogenous oestradiol (Fig. 3a), in contrast to the profiles observed in the absence of exogenous oestradiol (Fig. 3b). This result was supported by the statistical analysis for both LH pulse frequency ($P < 0.05$) and mean LH concentrations ($P < 0.01$) in animals treated with oestrogen (Fig. 2).

The progesterone pessaries increased plasma progesterone concentrations from 0.09 ± 0.01 ng ml⁻¹ to 0.69 ± 0.18 ng ml⁻¹ ($P < 0.05$), but LH pulse frequency and mean concentrations did not differ from those observed in untreated, gonadectomized ewes (Fig. 2). Administration of CRH into the third ventricle of progesterone-treated ewes increased the mean LH concentrations ($P < 0.001$) but the increases in LH pulse frequency (Fig. 2), pulse amplitude (6.1 ± 0.5 ng ml⁻¹ to 7.0 ± 0.4 ng ml⁻¹), and nadir concentrations (4.0 ± 0.3 ng ml⁻¹ to 4.8 ± 0.5 ng ml⁻¹) were not significant.

Experiment 2: treatment of gonadectomized ewes with CRH and sex steroids during the breeding season

As in the anoestrous season, icv injection of saline or CRH in gonadectomized ewes during the breeding season had no effect on LH secretion (Fig. 4). When gonadectomized animals were treated with a combination of oestrogen and progesterone, there was a significant decrease in LH secretion ($P < 0.01$). Again, the pulse profiles suggested that icv CRH injections increased LH secretion in the presence of exogenous sex steroids (Fig. 3c), in contrast to the profiles observed in their absence (Fig. 3d). The statistical analysis showed that both LH pulse frequency and mean LH concentrations were increased ($P < 0.05$) by icv CRH in ewes treated with oestradiol plus progesterone (Fig. 4).

Experiment 3: treatment of testosterone-implanted gonadectomized rams with CRH during the breeding season

Plasma testosterone concentrations were 1.47 ± 0.24 ng ml⁻¹ during the first sampling session and 1.36 ± 0.57 ng ml⁻¹ during the second sampling session. The difference was not significant. As with the ewes, saline injection into the third

Fig. 2 (a) LH pulse frequencies and (b) mean LH concentrations in plasma in gonadectomized ewes over 5 h, before treatment (□) and after intracerebroventricular injections of saline (▨) or corticotrophin-releasing hormone (CRH) (■). The responses were observed with and without pretreatment with oestradiol (E₂) or progesterone (P₄) during the anoestrous season. Differences between values before and after injections (▤) were subjected to a paired *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. All values are mean $\pm$ SEM ($n = 5$).

cerebral ventricle had no effect on LH secretion in the rams. However, CRH administration increased the frequency of LH pulses (Fig. 5), as shown by a significant change in mean LH concentrations ($P < 0.05$) and a marginal change in pulse frequency ($P = 0.067$).

Fig. 3. Representative LH profiles showing responses of gonadectomized ewes to a single injection of corticotrophin-releasing hormone (CRH) into the third cerebral ventricle. Ewes were treated with (a) exogenous oestradiol or (b) received no steroid treatment during the anoestrous season, or with (c) exogenous oestradiol plus progesterone or (d) did not receive steroid treatment during the breeding season. (·) in the profiles indicates significant pulses and the broken vertical lines indicate the time of administration of CRH.

Discussion

The results of the three experiments reported here lead us to reject the hypothesis that hypothalamic CRH exerts an inhibitory effect on GnRH secretion in sheep. On the contrary, CRH administration induced clear and repeatable increases in LH secretion, an effect that appears to depend on the presence of sex steroids, at least in females. These observations differ markedly from those reported for rats (Petraglia *et al.*, 1987) or monkeys (Olster and Ferin, 1987) and, together with other studies using sheep (Clarke *et al.*, 1990, Naylor *et al.*, 1990), they suggest a major species difference in the interactions at the hypothalamus between the corticotrophic and gonadotrophic axes.

The amount of CRH administered into the third ventricle was similar to that used by Naylor *et al.* (1990), but the physiological relevance of such a high dose needs to be considered, if only because the CSF concentrations achieved must be well above those usually observed in this compartment in sheep (50–100 pg ml⁻¹; P. Poindron, personal communication). Rivier and Vale (1984) and Almeida *et al.* (1988) injected the same mass of CRH (thus giving a dose that is arguably higher on a body mass basis) into rats, and observed opposite effects on LH secretion to those observed in the present study in sheep. This difference is difficult to understand if the response is simply a pharmacological side-effect rather than a reflection of a normal action of CRH. Moreover, higher icv doses of CRH (50 µg) have been used to induce normal maternal behaviour in the female sheep (Keverne and Kendrick, 1991). Doses of this order are probably necessary to achieve

CRH concentrations typically found at the synaptic cleft because the peptide is rapidly cleared from the CSF, yet needs to diffuse from the ventricle to the target tissue.

Since the stimulatory effect of CRH was manifest as an increase in LH pulse frequency, it is very likely that it was due to activation of hypothalamic GnRH neurones (Clarke and Cummins, 1982, Caraty and Locatelli, 1988). The fact that, in females at least, the stimulatory effect of CRH was only observed in the presence of sex steroids, and then only when the LH pulse frequency was intrinsically low, raises the possibility that the response depends on the initial status of the GnRH pulse generator. This hypothesis is supported by data from ewes treated only with a low dose of progesterone during the anoestrous season, a treatment that did not significantly reduce LH pulse frequency. In this situation, CRH had no effect on LH pulse frequency and elicited only a small increase in LH concentrations. This finding contrasts with the situation in oestradiol-treated ewes during anoestrus, where LH pulses were almost completely blocked, as expected (see review by Martin, 1984). In this situation, CRH was able to induce very clear increases in pulsatile LH secretion. Similarly, LH secretion was stimulated by CRH during the breeding season in ovariectomized ewes treated with a combination of oestradiol and progesterone. In contrast, no changes were observed in gonadectomized ewes, during the breeding or anoestrous seasons, in the absence of ovarian steroids. This result agrees with a previous report by Clarke *et al.* (1990), although Naylor *et al.* (1990) reported a small, transitory increase in LH secretion with the same dose of CRH in the absence of sex steroids. This response was not affected by naloxone treatment, but this is

Fig. 4 (a) LH pulse frequencies and (b) mean LH concentrations in plasma in gonadectomized ewes before treatment (□) and after intracerebroventricular injection of saline (▨) or corticotrophin-releasing hormone (CRH) (■). The responses were observed with and without pretreatment with oestradiol and progesterone (E₂+P₄) during the breeding season. Differences between values before and after injections (▨) were subjected to a paired *t* test: **P* < 0.05. All values are mean ± SEM (*n* = 5).

perhaps not surprising since opioidergic control of gonadotrophin secretion in sheep depends largely on the presence of sex steroids (Brooks *et al.*, 1986). The reasons for the differences between these studies are unclear and could be due to differences in the breed used, or differences in time after castration.

Several hypotheses can be advanced to explain the stimulatory effect of CRH on GnRH secretion in the presence of sex steroids. First, CRH is an excitatory peptide (Siggins *et al.*, 1985) and may thus stimulate pathways that have positive

effects on GnRH secretion. For example, it has been shown that LH secretion is increased by icv administration of enkephalins in long-term gonadectomized rats (Motta and Martini, 1982) and by morphine in intact male rats (Piva *et al.*, 1986), and CRH itself is able to stimulate the release of β -endorphin, dynorphin and enkephalin from hypothalamic tissue (Nikolarakis *et al.*, 1986). The connections between the CRH and GnRH neurones might be altered by sex steroids; it is well known that progesterone and oestradiol affect the morphology of the central nervous system (Raisman and Field, 1973) and recent studies have shown that gonadal steroids modulate the effect of endogenous CRH on the gonadal axis. CRH administration inhibits LH secretion in gonadectomized monkeys and rats through a mechanism that depends partially on opioid tone (Gindoff and Ferin, 1987; Olster and Ferin, 1987; Almeida *et al.*, 1988). However, a similar stimulus is totally ineffective in ovary-intact monkeys, in the follicular or luteal phases (Norman, 1994). Long-term gonadectomized rats do not show a reduction in serum LH concentrations in response to an opioid agonist (Almeida *et al.*, 1987) or CRH (Nikolarakis *et al.*, 1988). Thus, it appears that sex steroids raise the opioid tone in the hypothalamus and, therefore, allow full expression of CRH effects on GnRH secretion. In gonadectomized female monkeys, CRH is itself able to stimulate the release of β -endorphin (Nikolarakis *et al.*, 1986), yet LH secretion is inhibited by an interleukin-1-induced CRH release, and pretreatment with oestradiol leads to stimulation of both LH and corticotrophin secretion (Ferin, 1995). Such observations show that the effects of stress on the reproductive axis are more complex than have been imagined, and they also suggest that care is needed when interpreting studies in animals deprived of sex steroids. Moreover, CRH may be associated with changes in GnRH secretion through more than one pathway, only one of which (perhaps inhibitory) is related to stress.

Central administration of CRH also released cortisol into the general circulation. This release was not observed with the saline injection, so it was not due to any stress that might have been associated with the technique. Naylor *et al.* (1990) used one tenth of the dose of CRH used in the present study, and also observed an increase in cortisol secretion. The most likely explanation is sequestration of the hormone from the CSF by the tanycytes of the basement membrane of the third cerebral ventricle (Ben-Jonathan *et al.*, 1974; Oldfield *et al.*, 1985), followed by transport to the anterior pituitary gland, from where ACTH is released. Given that ACTH and cortisol have an inhibitory effect on LH secretion (Dobson *et al.*, 1988; Porter *et al.*, 1990), and that no differences between the sex steroid treatments in the cortisol response to CRH were found, it is very unlikely that CRH transported to the pituitary gland can account for the increases in LH secretion observed in the steroid-treated animals.

An earlier study with gonadectomized rams showed no effect of peripheral CRH administration on LH secretion (Parrot *et al.*, 1988), perhaps because the route of peptide administration was inappropriate or because, in the absence of testosterone, the GnRH pulse generator was already operating near maximum frequency thus preventing detection of further increases. In any case, there is little real evidence that hypothalamic CRH inhibits GnRH secretion in male sheep. The observation that hypoglycaemia simultaneously increases CRH

Fig. 5. LH pulse frequencies and plasma LH concentrations in testosterone-implanted gonadectomized rams, before treatment (□) and after intracerebroventricular injections of (a) saline (□) or (b) corticotrophin-releasing hormone (CRH) (■) during the breeding season. Values before and after injections were subjected to a paired *t* test: **P* < 0.05 for concentration, *P* = 0.067 for frequency (▨). All values are mean ± SEM (*n* = 4). The LH profiles shown in (c, d) are from two rams treated with CRH. (·) : significant pulses.

and decreases GnRH secretion into the hypophyseal portal bloodstream in testosterone-treated, gonadectomized rams (Papinot *et al.*, 1989) is countered by the fact that haemorrhage increases CRH secretion but has no effect on GnRH secretion in gonadectomized rams (Caraty *et al.*, 1988b). Both of these experiments were carried out in the same laboratory as the present studies showing that CRH stimulates GnRH release, so it seems most unlikely that the suppression of GnRH secretion by hypoglycaemia is due to a direct action of the CRH on GnRH neuronal activity. The same seems to apply to female sheep and monkeys, in which activation of the hypothalamo-pituitary-adrenocortical axis did not appear to be responsible for the decrease in LH secretion following insulin-induced hypoglycaemia (Clarke *et al.*, 1990; Van Vugt *et al.*, 1997). Moreover, as the response to central CRH administration was not altered by naloxone (Naylor *et al.*, 1990), the enhancement of the response to CRH by testosterone is not likely to be due to a steroid-dependent opioidergic pathway, such as that which seems to mediate the inhibitory effect of CRH in rats (Nikolarakis *et al.*, 1988). It is thus probable that the inhibition of GnRH secretion by hypoglycaemia (Papinot *et al.*, 1989) was due to other factors released by the stress (such as vasopressin and noradrenaline) that overrode, or were even independent of, the pathways through which CRH stimulates LH release.

It is clear that CRH is a neurotransmitter of the stress-responsive pathway in sheep (Caraty *et al.*, 1988; 1990; Engler *et al.*, 1989) and this role of CRH makes it difficult to explain why icv CRH stimulates LH secretion in this species. However, it should be considered that, in many species, stress itself can be both a stimulatory and an inhibitory influence on the gonadotrophic axis, depending on the novelty, duration and intensity of the stimulus. Chronic stress usually inhibits LH secretion (Gray *et al.*, 1978; Rasmussen and Malven, 1983) but acute stress appears to be stimulatory (Armario *et al.*, 1987; Hayashi and Moberg, 1987). It is possible, therefore, that the CRH-induced LH release observed in the present experiments results from the activation of such stress-responsive stimulatory pathway on LH secretion. Moreover, many 'chronic' stressors appear to become acute through habituation and 'rebound' effects (Adams *et al.*, 1993; Martin 1995). This property has been used in the past, perhaps unwittingly, to induce reproductive function in farm animals. For example, the stress of transport can induce ovulation in anoestrous ewes (Braden and Moule, 1964) and puberty in prepubertal sows (Du Mesnil du Buisson and Signoret, 1962), and the stress of blood sampling can induce multiple ovulation in cyclic ewes (Adams *et al.*, 1993). A very important consideration here is the need to distinguish between the effects of central CRH on GnRH

secretion and the effects of stress on GnRH and CRH secretion into the pituitary portal circulation, because these responses may not be linked.

In summary, the results presented here show that central CRH administration into gonadectomized sheep can induce a pronounced increase in LH secretion, particularly when the gonadal steroids have been replaced. The lack of an inhibitory effect suggests that CRH is not involved in any inhibition of reproduction by stressors. Further experimentation is now required to test whether hypothalamic CRH plays an important role in the pathways that control the gonadotrophic axis.

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