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Stress and reproduction in farm animals

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Transport of post-partum cows or sheep before an oestradiol-induced LH surge delayed gonadotrophin secretion possibly by affecting hypothalamic activity but not via an opioid mediated mechanism as the effect could not be reversed by naloxone. In addition, reduced LH responses to GnRH were observed in cattle during transport. In sheep, adrenocorticotrophic hormone (ACTH) also diminished the LH response to GnRH, but only when GnRH was administered 3 h after ACTH, not after 0.5 h. This finding suggests that very early suppression of LH secretion by stressors is not mediated by ACTH action at the pituitary but that immediate activation of the sympathetic nervous system may be involved. In ewes during the breeding season, repeated exposure to GnRH at intervals of 2 h during transport resulted in lower LH responses to the second and third injections. When anoestrous ewes were treated with oestradiol and GnRH while being restrained and isolated, the onset of the LH surge was delayed. The effects of hypothalamus–pituitary–adrenal hyperactivity on LH release may involve suppression of GnRH receptor activity, a reduction in releasable LH, or both factors. Studies *in vitro* with perifused ovine pituitaries showed that ACTH or corticotrophin releasing hormone markedly suppressed LH secretion in response to the second of two exposures to GnRH. This occurred with pituitaries obtained from anoestrous ewes irrespective of prior treatment with oestradiol, suggesting that compounds from the hypothalamus–pituitary–adrenal do not exert effects on the oestradiol-sensitizing mechanisms on the pituitary. In conclusion, stressors affect reproductive function via actions at the hypothalamus as well as impairing pituitary LH release induced by GnRH.

Introduction

There is little doubt that stress has a deleterious effect on reproductive efficiency in farm animals; however, much of the evidence is at best circumstantial, at worst in the realms of old wives' tales. Clearly, attempts to establish the mechanisms involved in the influence of increased hypothalamus–pituitary–adrenal activity on reproductive function must be accompanied by definitive proof of the effect of stressors on specific reproductive parameters.

In dairy cows, the chronic stressors of periparturient diseases or lameness increase the interval from calving to conception and the number of inseminations required per conception (Borsberry and Dobson, 1989; Collick *et al.*, 1989). Frequent wetting of sheep during oestrus or transport of cows during a superovulation programme result in a significant decrease in ovulation rate (Doney *et al.*, 1976; Edwards *et al.*, 1987), and intermittent foot-shocks or administration of *Escherichia coli* toxin are associated with the presence of cystic follicles (Przekop *et al.*, 1984; Peter *et al.*, 1990).

Administration of adrenocorticotrophic hormone (ACTH) during the follicular phase of the oestrous cycle in heifers results in the formation of persistent follicles (cysts) and emergence of other follicles is suppressed for up to 10 to 15 days (Fig. 1; A.Y. Ribadu, R.F. Smith and H. Dobson, unpublished).

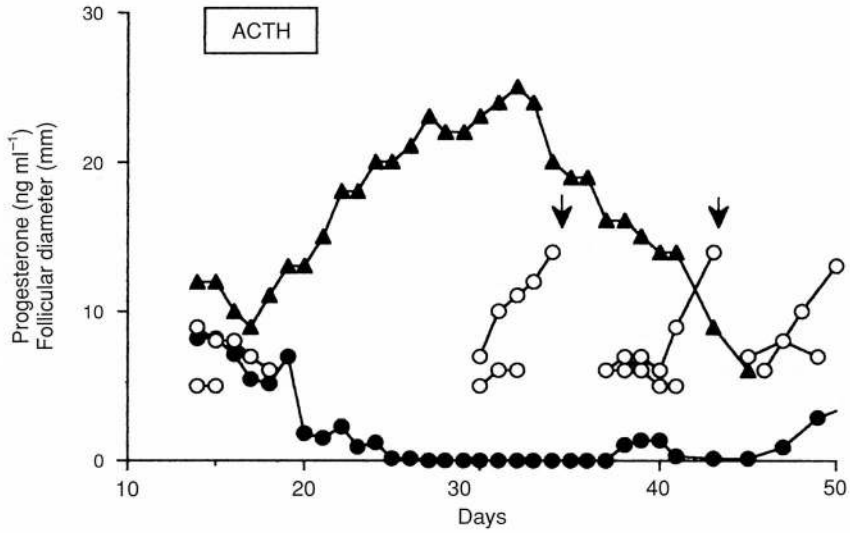

Fig. 1. Peripheral concentration of progesterone (●) and the diameter of a persistent follicle (▲) and all other ovarian follicles > 5 mm (○) as determined by ultrasound in a representative heifer treated on day 15 of the oestrous cycle with 100 iu ACTH every 12 h for 7 days as shown by the horizontal bar. The arrows denote ovulation of the appropriate follicles.

There is no detailed information available concerning the effect of stressors on fertility per se in male ruminants.

Responses to Stressors

Types of stressors include *physical stressors*, e.g. transport or mechanical injury, *psychological stressors*, e.g. isolation in sheep or the approach of strangers, and *physiological stressors*, e.g. insulin-induced hypoglycaemia or changes in blood pressure. Different stressors evoke different activation mechanisms within the hypothalamus–pituitary–adrenal axis. For example, the ratio of the principal corticotrophin releasing factors, corticotrophin releasing hormone (CRH) to arginine vasopressin (AVP), in hypophysial–portal blood, and that of ACTH to β-endorphin in peripheral blood differ after exposure to a barking dog or insulin-induced hypoglycaemia (Engler *et al.*, 1988, 1989). Nevertheless, all stimuli result in increased corticosteroid secretion from the adrenal glands. The significance of the different ratios of the hypothalamus–pituitary–adrenal hormones is not clear; however, investigations into mechanisms by which different stressors influence the hypothalamus–pituitary–gonad axis need to take these different ratios into account.

In addition, it is known that there are negative feedback mechanisms that are necessary to protect the animal against overactivation of the hypothalamus–pituitary–adrenal axis, and such negative feedback mechanisms vary between stressors (Munck *et al.*, 1984; Canny *et al.*, 1989, 1990). For example, within 45 min, peripheral cortisol concentrations begin to decline, reaching the baseline value by the end of 6 h restraint and isolation in sheep, in spite of continued behavioural signs of agitation (C. Guy, R. F. Smith and H. Dobson, unpublished), whereas values do not decrease to baseline values during first transportation (Smart *et al.*, 1994a). It may be differences in negative feedback within the hypothalamus–pituitary–adrenal axis that influence reproductive hormone secretion rather than the initial activation of the hypothalamus–pituitary–adrenal axis. This aspect has received no attention in the literature.

Furthermore, it must be remembered that habituation to stressors does occur, an extremely important fact in experimental design. For example, Rasmussen and Malven (1983) showed a complete reversal of the suppressive effects on LH frequency and amplitude by the third day of exposure to

restraint on three consecutive days. However, when sheep were isolated at intervals of 3 days, hypothalamus–pituitary–adrenal responses were similar (Niezgoda *et al.*, 1987). Mechanisms associated with habituation, such as the downregulation of CRH receptors (Canny *et al.*, 1990), may be involved in differential effects of chronic or acute stressors on reproductive hormone secretion. This aspect has also received no attention in ruminants.

A word of caution must be given when considering stress and reproduction studies in rats. First, the unstressed rat depends on adrenal progesterone to facilitate the onset of the preovulatory LH surge at an exact time on the day of oestrus (Brann and Mahesh, 1991). Second, with such a short 4 or 5 day oestrous cycle, very precisely timed events may be overlooked or masked when many animals have to be used to obtain sufficient volumes of samples to measure certain hormones with the relatively insensitive assay systems currently available. Third, anaesthesia has been used in many experiments when obtaining appropriate samples for hormone measurement from specific regions of the hypothalamus or from hypophysial portal vessels, and this process interferes with spontaneous responses to applied stressors.

Hypothesis to Explain the Influence of Stress on Reproduction

On the basis of our own work and that of others, our hypothesis is that there are two main mechanisms by which activation of the hypothalamus–pituitary–adrenal axis reduces the efficiency of the hypothalamus–pituitary–gonad axis. The first mechanism involves interference with correctly timed GnRH secretion controlled by neurotransmitters, and the other is the deleterious influence of hypothalamus–pituitary–adrenal hormones (especially ACTH) on the action of GnRH at the pituitary.

Most evidence suggests that, although stressors can cause fetal losses in mid-to-late pregnancy, the highest percentage of stress-induced reproductive losses occurs as a result of interference with correct hypothalamus–pituitary function; early embryonic losses result from inappropriate exposure of the ovum to gonadotrophins within the follicle (Staigmiller and Moor, 1984).

The following review is not intended to include every publication on stressors and reproduction in farm animals, but rather to present evidence for a coherent hypothesis for further testing. For brevity we have omitted some observations but we are not aware of any of significance that would make the hypothesis untenable. References to heat as a stressor have been excluded to avoid confusion with the consequent effects of altered rates of metabolism rather than activation of the hypothalamus–pituitary–adrenals *per se*. There is little evidence in domestic ruminants so, when appropriate, reference is made to primates and rats.

Effects of Common Stressors on Reproduction

Transport

Transport delays the onset of the LH surge in both cows and sheep (Nanda *et al.*, 1989; Smart *et al.*, 1994b; Table 1). This stressor must be initiated within hours of the onset of the LH surge (Fig. 2), and the delay is more marked if transport is imposed early in the postpartum period rather than in the middle of the breeding season (Nanda *et al.*, 1990b; Dobson and Nanda, 1992; Table 1). In control animals, morphine acts at the hypothalamus to block the LH surge in cattle, and the effects are reversed by the opioid antagonist, naloxone (Nanda *et al.*, 1990a). However, stressor suppression of LH secretion in cows and sheep could not be reversed by prior treatment with naloxone, contrary to similar experiments in rats (Malven, 1987; Nanda *et al.*, 1989; Table 1). At the pituitary, the response to GnRH (to give LH concentrations similar to the spontaneous pulse amplitude; Rahe *et al.*, 1980) was reduced within 15 min of the start of transport in cattle (Fig. 3; Dobson, 1987). Furthermore, in ewes during the breeding season, repeated exposure to GnRH at intervals of 2 h during transport resulted in markedly lower LH responses to the second and third GnRH injections (Fig. 4; J. Phogat, R. F. Smith and H. Dobson, unpublished).

Table 1. Time (± SEM) to the onset of the LH surge after 50 μg oestradiol benzoate (injected at time 0) in ewes of different reproductive and hypothalamus–pituitary–adrenal status, with or without additional injections of 500 ng GnRH

Reproductive status	Time (h)	Number of ewes
Day 14 postpartum		
Oestradiol alone	14.7 ± 0.5*	8
Transport from + 10 h to + 14 h	17.5 ± 0.8	5
Transport plus naloxone (3×1 mg kg^{-1} $(2\ h)^{-1}$ from + 10 h)	18.0 ± 0.4	8
0.8 mg ACTH at 0 h and + 10 h	18.0 ± 0.7	8
Mid-anoestrus		
GnRH at + 10.5 h and + 13 h	15.1 ± 1.7	5
Restraint and isolation from + 10 h to + 16 h	16.7 ± 1.8	5
Restraint and isolation plus GnRH at + 10.5 h and + 13 h	18.9 ± 0.9*	5
Cyclic, 24 h after prostaglandin synchronization		
Oestradiol alone	20.5 ± 5.3	7
Oestradiol plus 3 × GnRH (at + 8 h, + 10 h and + 12 h)	24.3 ± 5.9	8
Oestradiol plus 3 × GnRH plus transport from + 7.5 h to + 15 h	23.8 ± 3.7	8
Oestradiol plus 0.8 mg ACTH at 0 h and + 7.5 h plus 3 × GnRH	28 and 18**	6

Data from Smart *et al.* (1994) and C. Guy, J. B. Phogat, R. F. Smith and H. Dobson (unpublished).
*Different from other means for ewes of the same reproductive status ($P < 0.05$).
**Four of the ewes in this group did not have an LH surge.

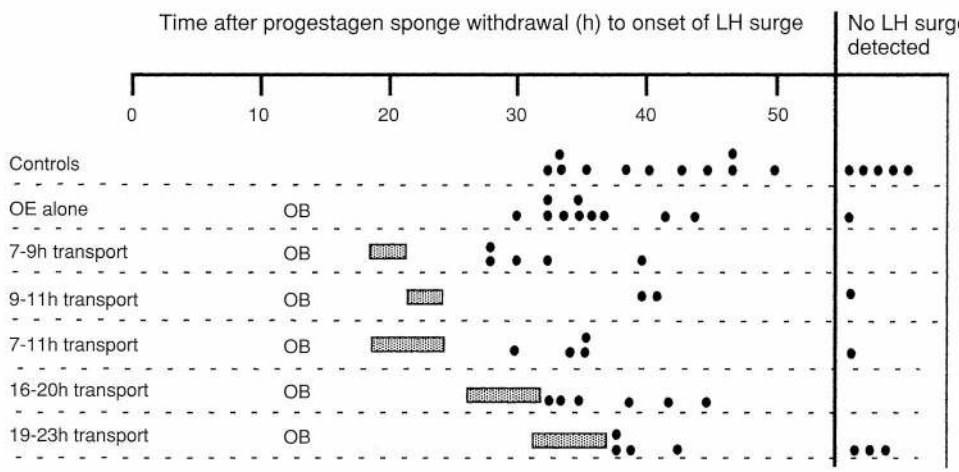

Fig. 2. Time of onset (●) of the LH surge after progestagen sponge withdrawal in individual ewes in the breeding season that were treated with oestradiol benzoate (50 μg) at times indicated by OB, and subjected to 2 or 4 h transport as shown by the horizontal bar (▭). Data from Dobson and Nanda (1992).

Restraint

Restraint, of varying severity and usually accompanied by isolation from other animals of the same species, has been used by several groups as a psychological stressor. The occurrence of spontaneous LH surges was prevented in two out of seven heifers restrained for 15 min twice a day for 3.5 days during the follicular phase (Stoebel and Moberg, 1982a). There was no delay in the onset of the LH surge when ewes were restrained after administration of oestradiol during anoestrus; however, if the ewes had

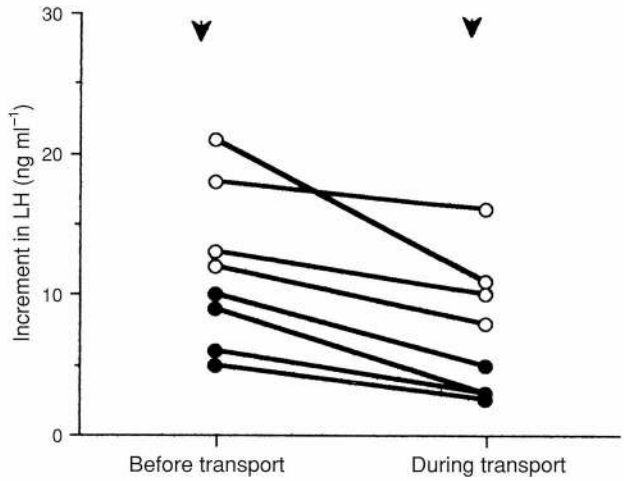

Fig. 3. Maximum increment in LH concentrations in individual cows treated with 20 (●) or 40 (○) μg GnRH (arrows) either one day before or 15 min after the start of transport. Data from Dobson (1987).

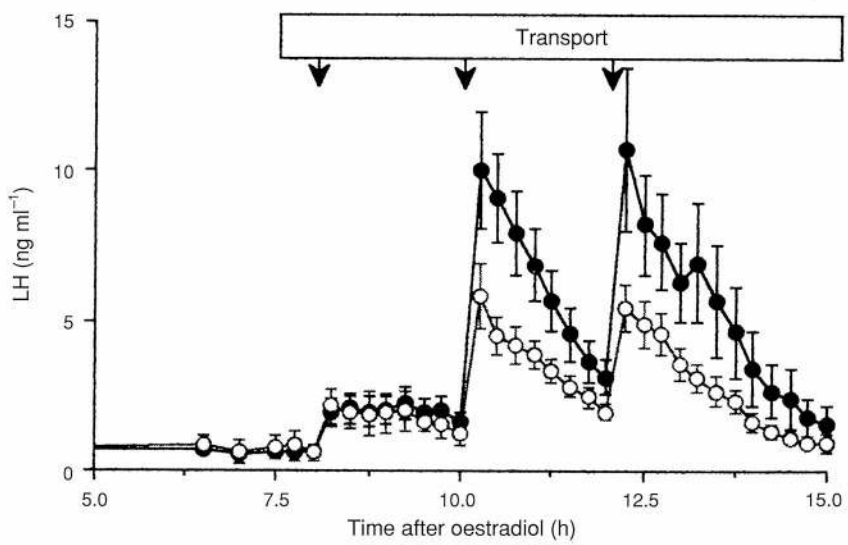

Fig. 4. Concentration of LH (± SEM) in ewes in the breeding season treated with oestradiol benzoate (50 μg) 24 h after prostaglandin synchronization with (○; $n = 8$) or without (●; $n = 8$) transport (duration indicated by the horizontal bar □) and 500 ng GnRH administered at times shown by the arrows. In control ewes not given GnRH, the concentration of LH did not exceed 1.5 ng ml^{-1}.

already released LH in response to additional treatment with two 500 ng doses of GnRH during restraint, the LH surge was delayed by 3 h (Table 1). In ewes and monkeys, restraint decreased the amplitude and frequency of LH secretion indicating effects at the hypothalamus (Rasmussen and Malven, 1983; Norman and Smith, 1992). There are similar observations in ovariectomized rats, and effects in this species were reversed by intracerebroventricular (i.c.v.) administration of a CRH antagonist (Rivier and Rivest, 1991). In an experiment with oestradiol-treated anoestrous ewes, we were unable to show a reduction in the LH response to 500 ng GnRH at 0.5 and 3.0 h after the start of restraint and isolation

(C. Guy, R. F. Smith and H. Dobson, unpublished) but 11 out of 16 rams had a lowered response to one injection of 10 μg GnRH after 3–6 h restraint (Matteri *et al.*, 1984).

Foot-shocks

Intermittent electrical foot-shocks for 9 h on each day after days 14–16 of the cycle resulted in the absence of a spontaneous preovulatory LH surge in two of ten ewes (Przekop *et al.*, 1984). Such treatment for 20 min h^{-1} for 3 consecutive days in anoestrus resulted in increased concentrations of GnRH in the median eminence and preoptic areas of the hypothalamus presumably as a result of inhibition of GnRH release (Przekop *et al.*, 1988). In adult castrated male rats, intermittent foot-shocks decreased peripheral LH concentrations even after adrenalectomy; this effect was reversed by i.c.v. administration of a CRH antagonist (Rivier and Rivest, 1991).

Hypoglycaemia

Hypoglycaemia induced by acute administration of insulin caused a simultaneous decrease in GnRH and LH secretion in testosterone-treated rams (A. Caraty, personal communication). Similarly, insulin reduced the frequency and amplitude of pulsatile LH secretion in long-term ovariectomized ewes; the effect was reversed by prior and simultaneous glucose administration (substantiating a neuroglycopneic effect) as well as by naloxone treatment, the latter suggesting opioid mediation during this particular stressor (Clarke *et al.*, 1990).

Infection

Infectious agents are often reputed to cause a reduction in reproductive efficiency in farm animals. Treatment of heifers with *Escherichia coli* toxin lowered the concentration of LH in the early follicular phase and inhibited the release of the preovulatory LH surge (Peter *et al.*, 1990). There is considerable evidence from work in monkeys and rats that implicates interruption of GnRH secretion by neuronal components of the immune response, for example interleukin 1, by CRH-mediated mechanisms (Ferin, 1993; Rivest and Rivier, 1993).

Hormonal Mediators of the Stress Response

Cortisol

In cattle, infusion of cortisol for 90 h during the follicular phase prevented the preovulatory LH surge in six of eight heifers (Stoebel and Moberg, 1982b; Li and Wagner, 1983a). However, basal LH concentration was not affected (Stoebel and Moberg, 1982b), nor was the LH response to GnRH *in vivo* (cited by Moberg *et al.*, 1981), although these latter observations have been disputed (Li and Wagner, 1983b; Dobson *et al.*, 1987). In bulls, dexamethasone reduced LH and testosterone concentrations, and lowered the LH response to GnRH (Chantaraprateep and Thibier, 1979). Perifusion with medium containing cortisol *in vitro* did not affect GnRH secretion from bovine median eminence tissue (Katawe *et al.*, 1993), or basal LH secretion from bovine pituitary cell cultures (Padmanabhan *et al.*, 1983). Using a perifusion system, Katawe *et al.* (1993) could not demonstrate an acute effect of treatment with cortisol for 4 h on LH released by exposure to 8 nmol GnRH l^{-1} for 20 min; however, pituitary cells cultured for 5 days and incubated with cortisol for 6–24 h secreted less LH after stimulation with 0.85 nmol GnRH l^{-1} (Li and Wagner, 1983b; Padmanabhan *et al.*, 1983). Significantly, if the pituitary cells were already primed with GnRH, the suppressive effects of cortisol were reversed (Padmanabhan *et al.*, 1983).

In sheep, neither cortisol nor the synthetic glucocorticoid dexamethasone affected an oestradiol-induced LH surge in anoestrous ewes (Moberg *et al.*, 1981; Phillips and Clarke, 1990), although tonic LH secretion was reduced by infusion of cortisol for 10 h in ewes ovariectomized for more than 2 months

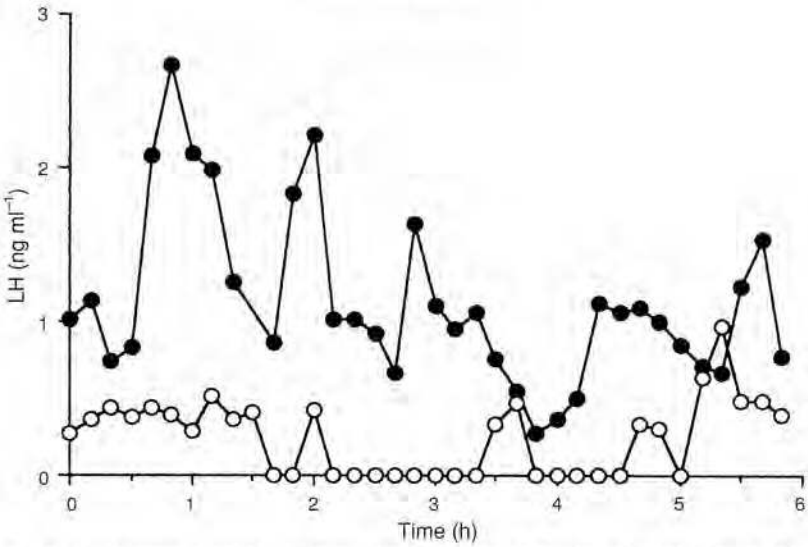

Fig. 5. Concentration of LH in a representative heifer on day 19 of a control cycle (●) and on day 19 of a cycle in which treatment with 1 mg ACTH every 12 h for 7 days commenced on day 15 (○).

(Porter *et al.*, 1990). Long-acting dexamethasone did not affect the negative feedback effect of chronic oestradiol treatment in ovariectomized ewes (Phillips and Clarke, 1990). Treatment of intact or adrenalectomized rams with cortisol did not alter the LH response to the high dose of 100 μg GnRH (Fuquay and Moberg, 1983), nor did dexamethasone affect the LH response to 1 μg GnRH given to ewes in anoestrus or in the breeding season (Phillips and Clarke, 1990). However, infusion of cortisol to long-term ovariectomized ewes reduced the LH response to 500 ng GnRH (Porter *et al.*, 1990). Much of the confusion about the effects of cortisol on GnRH-induced LH release in ruminants is probably due to dose-dependent effects of GnRH. In rats, large doses of dexamethasone affect GnRH-induced LH release in castrated male rats (Rivier and Rivest, 1991).

Adrenocorticotrophic hormone

In cows, administration of ACTH in spontaneous follicular phases inhibits the appearance of a spontaneous LH surge, but it has yet to be resolved whether this is a direct effect of ACTH on the hypothalamus–pituitary–gonad axis or an indirect effect via the stimulation of adrenal progesterone secretion (Liptrap and McNally, 1976; Stoebel and Moberg, 1982b; Li and Wagner, 1983b; Fig. 1). Baseline LH values were also suppressed by ACTH in the follicular phase as well as during the postpartum period (Dunlap *et al.*, 1981; Stoebel and Moberg, 1982b; Li and Wagner, 1983b). More recent work has confirmed that the effect in the follicular phase is reflected in fewer LH pulses (Fig. 5; K. Noble, R. F. Smith and H. Dobson, unpublished) as in ovariectomized adrenalectomized rats (Rivier and Rivest, 1991). Infusion of ACTH for 5–9 days suppressed LH release in response to GnRH *in vivo* (Li and Wagner, 1983b), but LH basal secretion *in vitro* was not affected, nor was that stimulated by a single exposure to GnRH (Padmanabhan *et al.*, 1983).

In ewes, either in anoestrus or early in the postpartum period, ACTH inhibited or delayed oestradiol-induced LH surges if administered immediately before the expected onset (Dobson *et al.*, 1988; Table 1). The LH response to GnRH was suppressed in intact or adrenalectomized rams and intact anoestrous ewes, and the greatest effect was 3–6 h rather than 0.5 h after ACTH treatment (Fig. 6; Fuquay and Moberg, 1983; Matteri *et al.*, 1984; Dobson *et al.*, 1988; Mohamed *et al.*, 1988). This delay of effect was in contrast to that seen during hypothalamus–pituitary–adrenal stimulation by transport

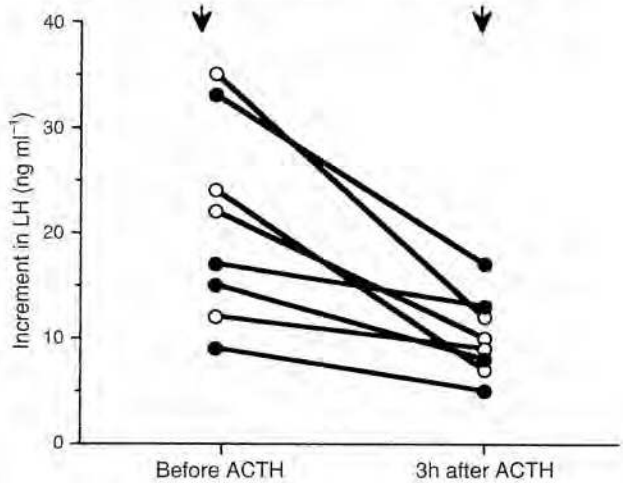

Fig. 6. Maximum increment in LH concentrations in individual anoestrous ewes treated with 500 ng GnRH (arrows) one week before and 3 h after 1 mg (●) or 0.5 mg (○) ACTH treatment. Data redrawn from Dobson *et al.* (1988).

(Dobson, 1987); the discrepancy might be due to immediate suppressive effects exerted by adrenaline secretion from the adrenal medulla (Parrott *et al.*, 1994). Perifusion of either male or female pituitary fragments *in vitro* confirmed the suppressive effects of ACTH on GnRH-induced LH release and emphasized effects mediated by the self-priming nature of GnRH action (Matteri *et al.*, 1986; Smart, 1994; Fig. 7). This effect was observed in all perifused pituitaries, irrespective of prior treatment with oestradiol *in vivo* (D. Smart, personal communication), suggesting that the effect might not be mediated by affecting oestradiol-sensitizing mechanisms at the pituitary.

Corticotrophin releasing hormone and arginine vasopressin

In sheep, the situation is confusing. Administration of CRH (10 μg) i.c.v. with or without simultaneous AVP (10 μg) to ovariectomized ewes was reported to have no effect on peripheral LH profiles (Clarke *et al.*, 1990), whereas similar doses of CRH i.c.v. increased LH pulse frequency presumably via the hypothalamus but this was not altered by prior i.c.v. treatment with naloxone (Naylor *et al.*, 1990). In contrast, prior exposure of perifused pituitary tissue to CRH resulted in a suppression of GnRH-induced LH release; however, this could have been mediated by release of ACTH *in vitro* (Fig. 7; Smart, 1994).

In monkeys, neither corticoid nor ACTH treatment affects LH secretion; however, peripheral administration of CRH to intact or ovariectomized monkeys inhibits LH pulse frequency, suggesting a hypothalamic site of action (Ferin, 1993) confirmed by CRH-induced inhibition of GnRH secretion from hypothalamic tissue *in vitro* (Williams *et al.*, 1990).

In rats, there is evidence both *in vivo* and *in vitro* for a suppressive effect of CRH on GnRH (and hence LH) secretion mediated at the hypothalamus by opioidergic and catecholaminergic pathways (Rivier and Rivest, 1991).

Conclusion

We were led to our hypothesis through a consideration of the widespread evidence, often contradictory both within and between species, concerning the influence of a variety of stressors or hypothalamus–

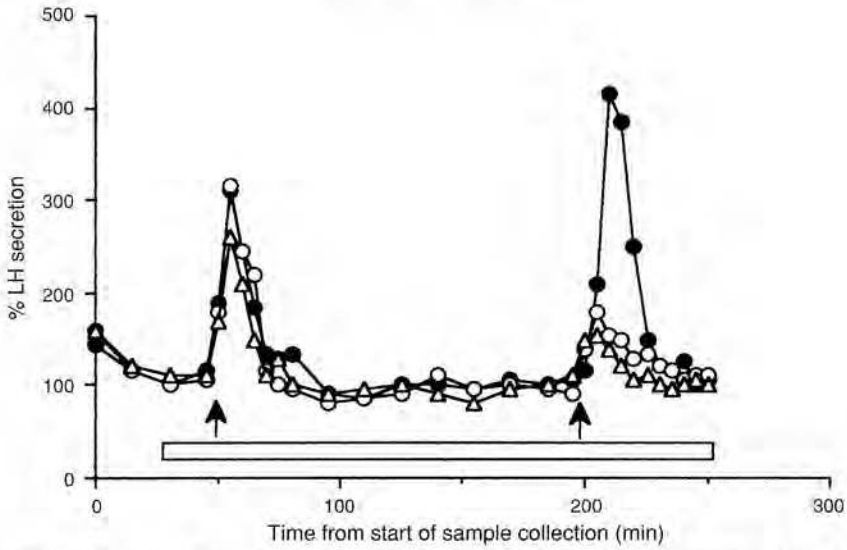

Fig. 7. Percentage increase in LH concentrations (in relation to pretreatment values) in ovine pituitary *in vitro* perifusion eluates after two 2 min exposures (arrows) to 10^{-9} mol GnRH l^{-1} at intervals of 2.5 h in the absence of other additions to the medium (●), or in the presence of 10^{-7} mol ACTH l^{-1} (○) or 2.5×10^{-8} mol CRH l^{-1} (△) for duration indicated by the horizontal bar (□).

pituitary–adrenal hormones on hypothalamic–pituitary function. After reinterpretation of some of the data, we present a unified framework for further investigation.

In rats and monkeys, there is good evidence that, in situations of increased CRH synthesis and secretion, the release of GnRH is inhibited. Synapses between CRH and GnRH neurones have been identified in rats (MacLusky *et al.*, 1988) and, although such neuronal links remain to be described in sheep and cattle, it is clear that the role of CRH via catecholaminergic and opioidergic mechanisms must be clarified in ruminants. The doses of exogenous CRH used so far in sheep may exert so strong a downregulation on CRH neurones that GnRH neurones are released from suppression and hence LH secretion is increased; alternatively, CRH may act as a neurotransmitter to release GnRH in this species.

Stressors or exposure to different components of the hypothalamus–pituitary–adrenal axis have a maximum suppressive effect on gonadotrophin secretion when the hypothalamus–pituitary–gonad axis is either under compromise, as in the early postpartum period (Alam and Dobson, 1987; Dobson and Alam, 1987), or in situations in which stimulation of the hypothalamus–pituitary is maximal, such as immediately before the LH surge or in the absence of steroid suppression, either *in vitro* or in gonadectomized animals. Components of the hypothalamus–pituitary–adrenal axis may inhibit GnRH self-priming by disrupting (i) GnRH receptor regeneration as self-priming involves increased numbers of GnRH receptors (Khalid *et al.*, 1991), (ii) post-receptor signal transduction necessary for LH secretion (Clayton, 1989), or (iii) provision of sufficient LH in a releasable form (Hoff *et al.*, 1977). Each of these aspects requires investigation.

Finally, it remains to be determined which site of action is of paramount importance, the hypothalamus or the pituitary.

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