

Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences

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The means by which stress influences reproduction is not clearly understood, but may involve a number of endocrine, paracrine and neural systems. Stress impacts on the reproductive axis at the hypothalamus (to affect GnRH secretion) and the pituitary gland (to affect gonadotrophin secretion), with direct effects on the gonads being of less importance. Different stressors have different effects and there are differences in response to short- and long-term stress. Many short-term stresses fail to affect reproduction and there are reports of stimulatory effects of some 'stressors'. There are species differences in the way that specific stressors affect reproduction. Sex differences in the effects of a particular stressor have been delineated and these may relate to effects of stress at different levels of the hypothalamo-pituitary axis. The significance of stress-induced secretion of cortisol varies with species. In some instances, there appears to be little impact of short-term increases in cortisol concentrations and protracted increases in plasma concentration seem to be required before any deleterious effect on reproduction is apparent. Issues of sex, sex steroid status, type of stressor and duration of stress need to be considered to improve understanding of this issue.

Despite extensive research, the means by which stress impacts on reproduction is not clearly understood. The initial challenge is to define stress and, in this review, a definition that has widespread acceptance is adopted. Stress is a disruption to homeostasis (Rivier and Rivest, 1991). A series of complex adaptive responses are stimulated by stress and, when these are inadequate, excessive or prolonged physiological systems, such as reproduction, are perturbed. The effects of stress on reproduction in mammals have been documented extensively in rodents (Rivier and Rivest, 1991), but these studies are not always pertinent to other species. There are species differences in responses to stress and in the neuroendocrine regulation of reproduction, which require consideration (Chatterton, 1990). The present review focuses on the effects of stress on reproduction in non-rodent mammals, in which most research has been done in domestic ungulates and monkeys.

Physiological responses to stress

Stimuli that challenge homeostasis are commonly called stressors. Usually, a stressor activates the hypothalamo-pituitary-adrenal axis and the sympathoadrenal system. Stimulation of the hypothalamo-pituitary-adrenal axis is characterized by activation of corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP) neurones in the paraventricular nucleus, and secretion of these neuropeptides into the hypophyseal portal system to stimulate the corticotrophs of the anterior pituitary gland. The corticotrophs produce a variety of peptides derived from pro-opiomelanocortin, including adrenocorticotrophic hormone (ACTH), β -endorphin and α -melanocyte-stimulating hormone, all of which are released in response to stress (Engler *et al.*, 1989). In terms of

the response to stress, the physiological significance of the release of β -endorphin and α -melanocyte-stimulating hormone is not clear, but ACTH acts on the cortex of the adrenal glands to stimulate the synthesis and secretion of glucocorticoids. By way of a classical feedback loop, the glucocorticoids exert negative feedback actions on the hypothalamo-pituitary unit to regulate the secretion of CRF, AVP and ACTH (Plotsky *et al.*, 1989). The sympathoadrenal system consists of the sympathetic nervous system and the adrenal medulla. Activation of the sympathoadrenal system evokes the release of noradrenaline from postganglionic nerve terminals, while preganglionic innervation of the adrenal medulla results in increased secretion of catecholamines, principally adrenaline, into the bloodstream (Goldstein, 1987). The glucocorticoids and catecholamines act widely to alleviate the effects of stress.

Many central pathways are also involved in the response to stress, but these are beyond the scope of this review and have been reviewed comprehensively for rodents (for example, by Plotsky *et al.*, 1989). The central pathways that are activated during stress have not been determined in non-rodent mammals.

Effects of stress on reproduction

Systems activated by stress can influence reproduction at the hypothalamus, pituitary gland or gonads. However, the major impact is thought to be within the brain or at the pituitary gland (Moberg, 1987; Brann and Mahesh, 1991; Rivier and Rivest, 1991). Measurement of plasma concentrations of the gonadotrophins provides a good indication of the effects at these higher levels, since the pulsatile secretion of LH is a reflection of the secretion of GnRH from the hypothalamus (Clarke and Cummins, 1982; Tilbrook and Clarke, 1995). Both

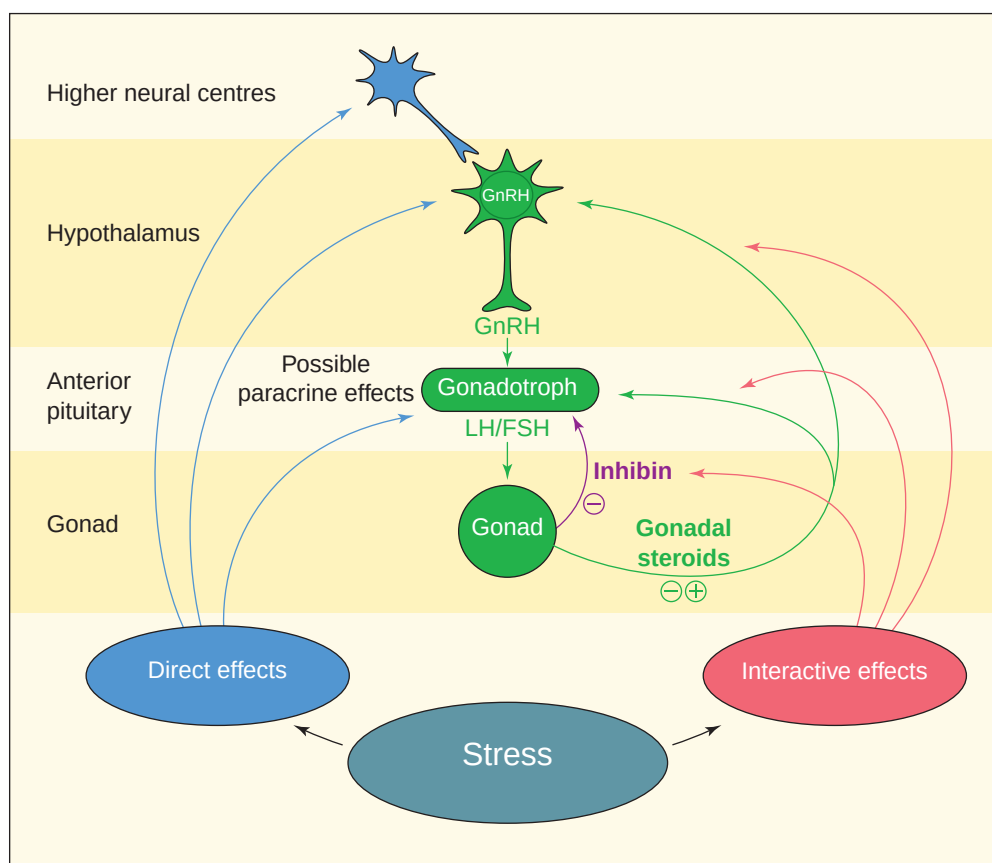


Fig. 1. Sites within the hypothalamo–pituitary gonadal axis at which stress may act to influence the secretion of the gonadotrophins. Activation of stress pathways may directly affect the activity of GnRH neurones within the hypothalamus or higher neural centres that project to GnRH neurones and, therefore, the synthesis or secretion of GnRH into the hypophyseal portal blood. It is also possible that stress directly influences the responsiveness of gonadotroph cells in the anterior pituitary gland to the actions of GnRH. A further potential action of stress is to alter the feedback actions of sex steroids in the hypothalamus or pituitary and of inhibin in the anterior pituitary gland (interactive effects).

the secretion and actions of GnRH are influenced by the feedback actions of gonadal sex steroids and inhibin. Thus, stress may affect the secretion of the gonadotrophins through mechanisms that modify the synthesis or the secretion of GnRH, the responsiveness of the gonadotrophs to the actions of GnRH or the feedback actions of gonadal hormones (Fig. 1). There is evidence for both hypothalamic and pituitary actions of stress but little attention has been paid to the effects of stress on the feedback actions of gonadal hormones. No studies have been performed in any species to determine whether stress influences the feedback actions of inhibin, despite this glycoprotein being a major feedback regulator of the secretion of FSH in both males (Tilbrook and Clarke, 1995) and females (Clarke *et al.*, 1986).

Effects of different stressors on reproduction

In a range of non-rodent mammals, physical stressors (for example, transport, shearing, restraint, foot-shocks and exercise), metabolic stressors (for example, insulin-induced hypoglycaemia), immunological stressors (for example, infection

and administration of cytokines or endotoxins), cardiovascular stressors (for example, administration of nitroprusside), and psychological stressors (for example, isolation, social interactions, human–animal interactions and mental arithmetic tasks) have all been studied at various times in efforts to establish how stress can affect reproduction. Comprehensive reviews on domestic species (Moberg, 1987) and primates (Ferin, 1999) show that the universal response is activation of the hypothalamo–pituitary–adrenal axis, and this can be associated with reduced LH secretion. A range of environmental factors, such as nutrition, can also influence reproduction, but these special conditions are beyond the scope of this review.

Prolonged or chronic stress results in suppressed gonadotrophin secretion and inhibition of reproduction but, when the duration of the stress response is transient or acute, the effects are less clear. Variable effects of acute stress have been found in rodents, with reports of no effect, stimulatory and inhibitory effects on reproduction (Brann and Mahesh, 1991); variable effects have also been recorded in non-rodent mammals. Deleterious effects have been reported, for example basal LH secretion was decreased in rams that were restrained for 3 h

(Matteri *et al.*, 1984), and in ovariectomized ewes confined for 3–4 h (Rasmussen and Malven, 1983). Similar effects were found in gonadectomized rams and ewes during 4 h of isolation and restraint (Tilbrook *et al.*, 1999), in sheep after injection of insulin (Clarke *et al.*, 1990) or endotoxin (Battaglia *et al.*, 1998), in ovariectomized rhesus monkeys after injection of insulin (Chen *et al.*, 1992) and in male (Norman and Smith, 1992) and female (Norman *et al.*, 1994) rhesus monkeys after 6 h of restraint. A number of studies have also investigated the effects of repeated acute stress on LH secretion. In dairy heifers, application of an acute stress treatment (electric shock, confinement, restraint and rotation) twice a day during the follicular phase of the oestrous cycle prevented the preovulatory LH surge in two out of seven animals (Stoebel and Moberg, 1982). Laparoscopy of anoestrous ewes every 4 h over 48 h suppressed the preovulatory LH surge and ovulation induced by the introduction of rams (Martin *et al.*, 1981) while, in ewes subjected to 6 h of wetting stress each day from day 13 of the oestrous cycle, the LH surge was delayed (Doney *et al.*, 1976). Confinement suppressed the secretion of LH in ewes, but repetition on each of 3 consecutive days resulted in acclimatization of the animals to the stressor, with LH secretion no longer affected (Rasmussen and Malven, 1983). Repeated introduction of gilts to boars (Turner *et al.*, 1998a) or repeated application of electric shock to gilts (Turner *et al.*, 1998b) did not impair reproduction despite these treatments repeatedly increasing plasma concentrations of cortisol.

There are also examples of stimulatory effects of acute stress or short-term administration of stress hormones on reproduction. In bulls, there was a brief increase before a decrease in the plasma testosterone concentrations after dexamethasone, (Thibier and Rolland, 1976). In boars, intracarotid infusion of cortisol for 1 h enhanced the release of LH in response to exogenous GnRH (Liptrap and Raeside, 1983), and infusion of cortisol increased the basal secretion of LH in prepubertal gilts (Pearce *et al.*, 1988). Incubation of dispersed pig pituitary cells with cortisol increased the basal secretion of LH (Li, 1987), indicating a direct effect of the glucocorticoid on the gonadotroph in this species, although this effect could have been due to the production of paracrine factors in the pituitary gland (Schwartz and Cherney, 1992). However, there was no induction of LH pulses or ovulation in anoestrous ewes immediately after an injection of ACTH or dexamethasone, or exposure to a barking dog (I. J. Clarke and B. W. Doughton, unpublished). Although the repeated introduction of gilts to boars for the detection of oestrus increased cortisol concentrations and enhanced ovulation rate, it seems likely that the enhancement of ovulation rate was due to the sexual stimulus provided by the boars rather than stimulatory actions of stress pathways (Turner *et al.*, 1998a). The transport of gilts stimulates the attainment of puberty (Hughes, 1982). In sheep also, transport of ewes during the non-breeding, but not the breeding, season was reported to cause ovulation, but this was usually not accompanied by behavioural oestrus and did not initiate a series of oestrous cycles (Braden and Moule, 1964). Transport of cows was also found to stimulate ovulation but not oestrus (Lamond, 1962). Nonetheless, most studies with sheep and cows have found inhibitory effects of transport stress on reproduction (for review, see Moberg, 1987). Thus, while chronic stress consistently inhibits reproduction, the reported effects of acute

or repeated acute stress are more variable. Different stressors may stimulate different systems, some being inhibitory and some stimulatory with respect to reproduction. At least some of the variable effects of acute stress may also be explained by differences between the sexes and the influence of sex steroids.

Importance of sex differences in relation to the effect of stress on reproduction

There are pronounced differences between males and females in their responses to stress. These differences have been most clearly demonstrated in the case of the hypothalamo–pituitary adrenal axis and it has been proposed that sex differences in response to stress are due principally to the influence of the sex steroids (for example, see Handa *et al.*, 1994). Since males and females respond differently to stress and these differences are due, at least in part, to the actions of sex steroids, the mechanisms by which stress impacts on reproduction may also differ between the sexes and may be influenced by the predominance of secretion of particular sex steroids at particular times. Indeed, there are clearly differences between rams and ewes in the mechanisms by which stress suppresses the secretion of LH, and sex steroids influence these mechanisms (Tilbrook *et al.*, 1999). When gonadectomized male and female sheep were treated with different combinations of sex steroids and subjected to 4 h of isolation and restraint, the secretion of LH was decreased in all animals but the mechanisms for these effects appeared to be different between the sexes and with respect to sex steroid status (Fig. 2). These differences provide some clue to the mechanism of the stress effect. Reduction in LH pulse frequency most likely indicates an effect on the secretion of GnRH. Alteration in LH pulse amplitude could be the result of central effects to reduce GnRH pulse amplitude or a direct pituitary effect on the gonadotroph, and these could be measured by direct portal sampling or GnRH challenge, respectively. It can be concluded that the mechanisms by which isolation and restraint suppressed LH secretion in males treated with testosterone, and in ewes treated with progesterone, involved a reduction in the secretion of GnRH. In ewes treated with oestradiol, GnRH secretion was inhibited but a reduction in pituitary responsiveness to GnRH may also have occurred. In ewes treated with oestradiol and progesterone, the reduction in LH secretion due to the stressor may have been due to actions at either or both sites (Fig. 2). The LH response to an injection of GnRH was reduced in gonadectomized rams but was not affected in gonadectomized ewes subjected to isolation and restraint (Tilbrook *et al.*, 1999), indicating a sex difference in the mechanism by which the stressor elicited an effect on the reproductive axis. It remains to be determined whether sex steroids influence the response to GnRH during the imposition of the stress. These findings indicate that stress impacts on reproduction by mechanisms that differ between males and females and with different backgrounds of sex steroids. Further work, especially with direct portal sampling of GnRH concentrations, is required to dissect the hypothalamic versus the pituitary effects of these types of stress.

Sex comparisons of the effects of stress on reproduction have not been made in other species, although various observations in female rhesus monkeys indicate that the ovaries, and particularly oestradiol secretion, influence the effects of stress on LH

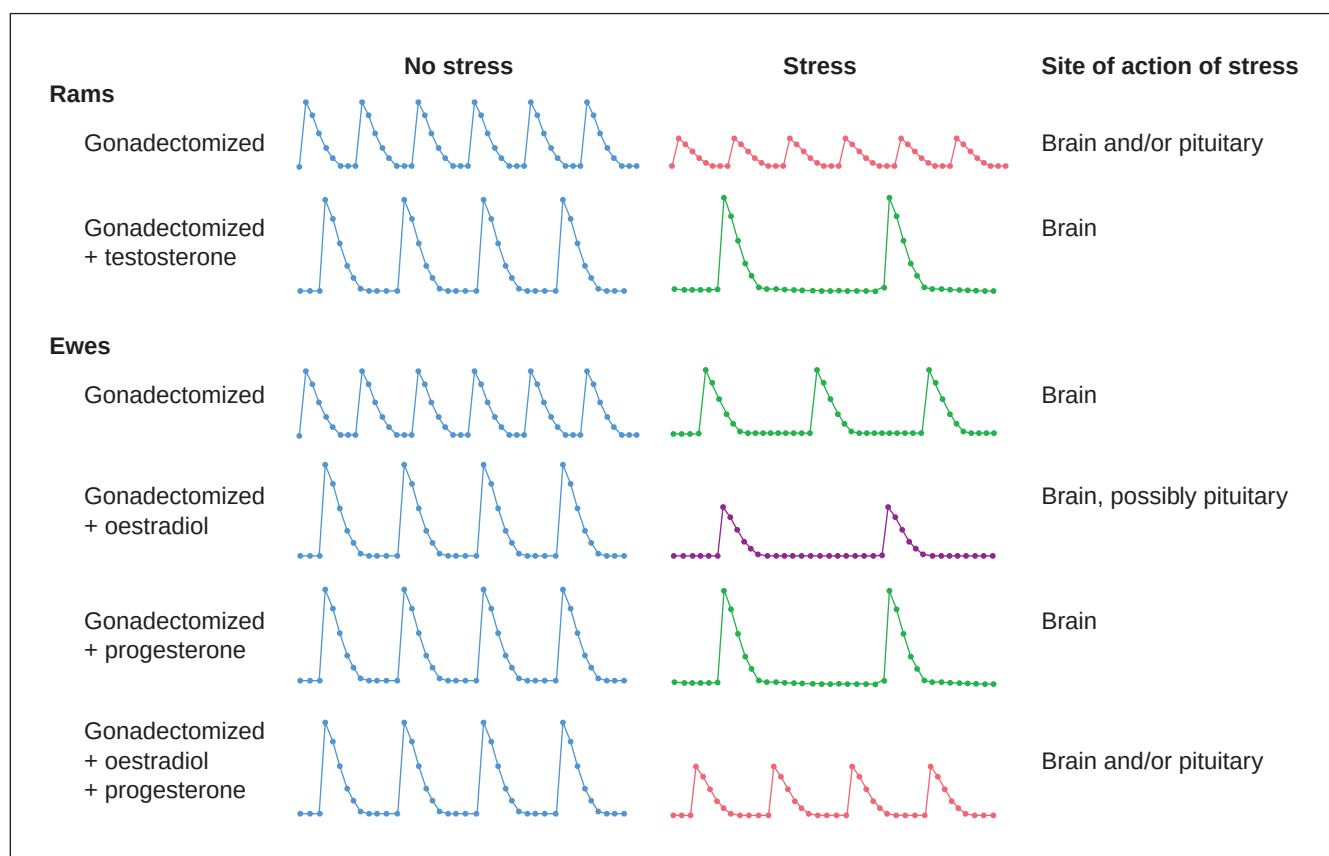


Fig. 2. Schematic representation of the effects of 4 h of isolation and restraint stress on the frequency and amplitude of LH pulses in gonadectomized sheep treated with different combinations of sex steroids (Tilbrook *et al.*, 1999). There were differences between sexes and different steroid treatments in the extent to which stress affected the frequency and amplitude of the LH pulses, and possible sites of action of stress to inhibit the secretion of LH are suggested. LH pulses are shown for each group before (blue pulses; no stress) and during isolation and restraint (stress). Changes in the amplitude (red) or the frequency and amplitude (purple) of LH pulses indicate that the stress invokes actions that affect the activity of GnRH neurones in the brain or the responsiveness of the pituitary to the actions of GnRH, whereas changes in the frequency without changes in the amplitude of LH pulses (green) are indicative of central actions of stress to inhibit the activity of GnRH neurones.

secretion. In female rhesus monkeys that were restrained in a chair for sampling, LH pulses (Dierschke *et al.*, 1970) and hypothalamic multiunit electrical activity (Wilson *et al.*, 1984) were detected in ovariectomized but not intact animals. Insulin-induced hypoglycaemia inhibited hypothalamic multiunit electrical activity to a greater extent in intact than in ovariectomized animals and this reduced responsiveness in the ovariectomized females was reversed when they were treated with oestradiol (Chen *et al.*, 1992). Moreover, when female rhesus monkeys were subjected to 6 h of chair restraint, plasma LH concentrations were suppressed during the follicular phase but not during the luteal phase of the menstrual cycle (Norman *et al.*, 1994). Sex steroids also influence the effect of immune stress on gonadotrophin secretion in female rhesus monkeys. While it was found that intracerebroventricular infusion of the cytokine interleukin-1 α into ovariectomized rhesus monkeys increased the secretion of cortisol and inhibited the secretion of LH and FSH (Feng *et al.*, 1991), treatment with oestradiol to provide concentrations of oestrogen typical of the late follicular phase allowed intracerebroventricular infusion of interleukin-1 α to

stimulate LH secretion, even though cortisol secretion also increased (Xiao *et al.*, 1994). Subsequently, it was shown that the stage of the menstrual cycle influenced these effects of interleukin-1 α . When intact female rhesus monkeys were treated with interleukin-1 α during the early follicular phase (when oestradiol concentrations are low), there was no acute change in LH, although the duration of the follicular phase was prolonged. Treatment during the mid-follicular phase, when oestradiol concentrations were higher, increased LH and FSH secretion (Xiao *et al.*, 1996). Furthermore, the i.v. administration of the bacterial endotoxin, lipopolysaccharide (LPS), twice a day for 5 days to rhesus monkeys during the early-mid follicular phase activated the hypothalamo-pituitary-adrenal axis and resulted in an increase in serum concentrations of LH and FSH, and either a reduction or no change in oestradiol concentrations, although there was an increase in the duration of the follicular phase (Xiao *et al.*, 1998). In contrast, activation of the hypothalamo-pituitary-adrenal axis by administration of LPS to rhesus monkeys during the luteal phase of the menstrual cycle decreased LH, FSH and progesterone concentrations

without an effect on the duration of the follicular or luteal phases or on the preovulatory oestradiol peak value (Xiao *et al.*, 1999). There have been no direct sex comparisons with respect to the effects of stress on gonadotrophin secretion in monkeys.

Role of glucocorticoids in mediating the effects of stress on reproduction

Any of the components of the stress pathways has the potential to inhibit reproduction. In the brain, these components include neurone pathways that are activated during stress and synapse with GnRH neurones or interneurons that project to GnRH neurones, and circulating hormones released during stress that act in the central nervous system. Such inputs have been documented extensively in rodents (Clarke, 1996) and many of these are components of the neurone circuits that are activated during stress. Nonetheless, it is not yet clear which neural inputs relay the stress effects on GnRH neurones. At the anterior pituitary gland, the responsiveness of gonadotrophs to GnRH and the feedback actions of gonadal steroids may be affected by neuropeptides released into the hypophysial portal system during stress, by paracrine interactions within the pituitary or by factors released into the peripheral circulation during stress. The mediators of the effects of stress on the hypothalamo-pituitary unit may include glucocorticoids, CRF, AVP, endogenous opioid peptides, catecholamines and 5-hydroxytryptamine. In the present review, only the increase in plasma glucocorticoid concentrations is considered.

The association between stress, increased secretion of glucocorticoids and inhibition of reproduction has led to the general argument that glucocorticoids are paramount in mediating the inhibitory effects of stress on reproduction. In support of this notion, various studies have shown that administration of natural or synthetic glucocorticoids can inhibit the secretion of the gonadotrophins in sheep (Juniewicz *et al.*, 1987), pigs (Turner *et al.*, 1999a,b), cattle (Thibier and Rolland, 1976), rhesus monkeys (Dubey and Plant, 1985) and humans (Sakatos *et al.*, 1993). Nevertheless, increased secretion of glucocorticoids is not always associated with decreased secretion of the gonadotrophins, particularly in cases of acute stress. Furthermore, there may be species differences in the extent to which glucocorticoids inhibit the secretion of LH and FSH. Finally, sex and sex steroid status may compound the effects of glucocorticoids on GnRH and gonadotrophin secretion.

Although some studies have found inhibitory effects of glucocorticoids on reproduction in sheep, most have indicated that glucocorticoids are not the predominant mediators of stress-induced suppression of LH secretion. Treatment of ovariectomized ewes with 1–2 mg dexamethasone once a day for 14 days did not decrease the secretion of LH or inhibit the LH response to GnRH (Phillips and Clarke, 1990), neither did cortisol nor dexamethasone treatment affect the generation of the LH surge in ewes (Phillips and Clarke, 1990). In rams, an infusion of 2–4 mg cortisol min⁻¹ for 9 h did not affect the LH response to GnRH (Fuquay and Moberg, 1983). However, the infusion of castrated rams with a combination of cortisol and oestradiol decreased the frequency of LH pulses (Daley *et al.*, 1999). Infusion of oestradiol into castrated rams also increased the tissue concentrations of GnRH receptor and mRNA encoding GnRH receptor, and cortisol infusion reduced these responses

(Adams *et al.*, 1999; Daley *et al.*, 1999). The authors of these studies concluded that cortisol is capable of enhancing the negative feedback effects of oestradiol and reducing the stimulation of GnRH receptor expression by oestrogen. Nevertheless, their results also indicate that cortisol alone is not the major mediator of these effects. Infusion of cortisol alone into castrated rams for 7 days did not affect the frequency or amplitude of LH pulses, and pulse frequency was reduced only when oestradiol was also present (Daley *et al.*, 1999). Furthermore, the suppression of the oestrogen-dependent increase in GnRH receptor gene expression by stress-like concentrations of cortisol was seen to be transient in nature (Adams *et al.*, 1999). These studies highlight the importance of the interaction between cortisol and oestradiol. Cortisol infusion had no effect on responsiveness to GnRH or the extent of oestradiol-induced augmentation of responsiveness to GnRH in castrated rams (Daley *et al.*, 1999), indicating a sex difference in its effect. Furthermore, LH secretion is not always inhibited when cortisol secretion is increased by stress (isolation and restraint) in gonadectomized rams and ewes treated with various combinations of sex steroids (Tilbrook *et al.*, 1999).

Studies in female pigs indicate that plasma cortisol concentrations need to be high for a prolonged period to affect reproduction and, even then, there is considerable variation among individuals in the effects of high concentrations of cortisol. Application for 4–15 days of acute stressors that activate the hypothalamo-pituitary-adrenal axis in pigs did not impair reproduction (Turner *et al.*, 1998a,b). Consistent with this finding, repeated (twice a day) i.v. injection of cortisol had no effect on LH secretion in ovariectomized pigs, even in the presence of oestradiol (Turner *et al.*, 1999a). There was no effect of i.v. cortisol treatment on the generation of the LH surge, oestrus or ovulation in gilts (Turner *et al.*, 1999b). In contrast, sustained high cortisol concentrations achieved through i.m. injections of cortisol twice a day impaired the secretion of LH in ovariectomized pigs in the absence, but not the presence, of oestradiol (Turner *et al.*, 1999a). In intact pigs, chronic cortisol treatment impaired the expression of oestrus, the generation of the LH surge and ovulation (Turner *et al.*, 1999b).

In monkeys, as in pigs, plasma glucocorticoid concentrations appear to need to be high for an extended period to affect reproduction. For instance, Dubey and Plant (1985) found that treatment of gonadectomized male rhesus monkeys with daily i.m. injections of 10–20 mg cortisol kg⁻¹ body weight caused circulating LH and FSH concentrations to decrease, but these animals were treated for 62 days and the decrease in the secretion of the gonadotrophins did not occur until 21–28 days after initiation of the treatment. A single i.m. injection of ACTH did not affect serum concentrations of LH in male rhesus monkeys but, after 5 days of i.m. ACTH or cortisol injections, basal LH concentrations were suppressed (Hayashi and Moberg, 1987), indicating that increased concentrations of hormones are necessary to elicit inhibitory effects on gonadotrophin secretion. Constant infusion of glucocorticoids into female cynomolgous monkeys for 60 days suppressed plasma progesterone but had no apparent effect on ovulation (Chatterton, 1990). That glucocorticoids are not the primary mediators of short-term stress on reproduction in monkeys has been confirmed in studies in which circulating cortisol is reduced by treatment with metyrapone, an inhibitor of adrenal steroid

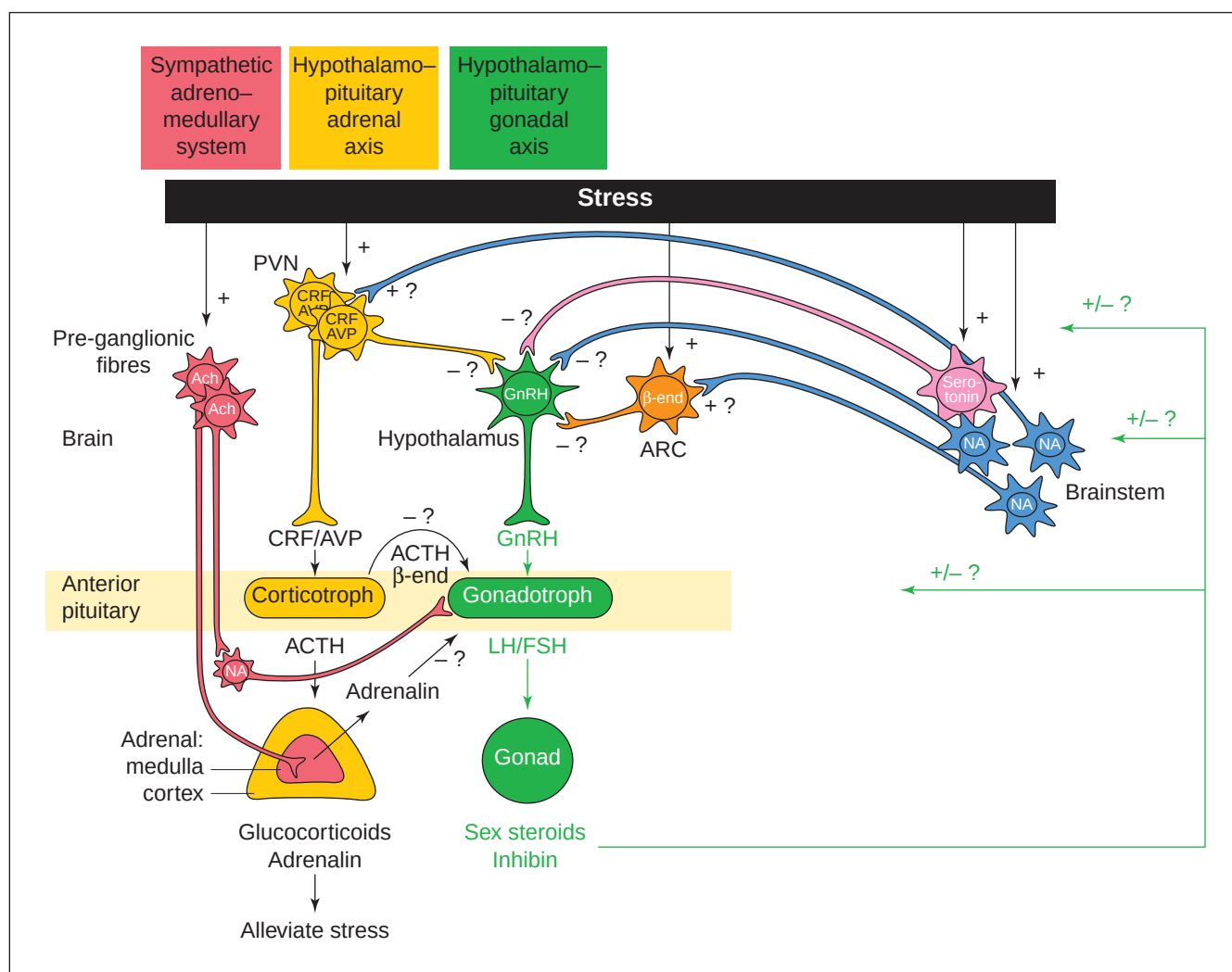


Fig. 3. Schematic representation of some of the mechanisms by which stress may impair the secretion of the gonadotrophins. In the brain, GnRH neurones (green) in the hypothalamus may be influenced by neuronal pathways that are activated during stress and project, either directly or via other pathways, to the cell bodies, dendrites, axons or terminals of GnRH neurones. While many of these pathways have been identified for rodents, they have not been determined for non-rodent mammals. The most likely central pathways are shown and include noradrenergic (NA) pathways from the brainstem (blue), β -endorphinergic (β -end) neurones located in the arcuate nucleus (ARC: orange), corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP) neurones located in the paraventricular nucleus (PVN: yellow) and serotonergic neurones from the raphe nucleus of the brainstem (pink). For simplicity, not all possible interactions are indicated. Moreover, the gonadotroph cells of the anterior pituitary gland (green) may be affected by release of these factors into the hypophysial portal blood. Evidence indicates that the responsiveness of the anterior pituitary gland to GnRH will also be affected by adrenocorticotrophic hormone (ACTH), but it is unknown whether this is a paracrine action or whether circulating ACTH acts on the anterior pituitary gland. Secretion of catecholamines from the sympathoadrenal system (red) may also play a role in influencing the responsiveness of the pituitary to GnRH. The anterior pituitary may receive innervation from the sympathetic nervous system or be acted upon by adrenaline released from the adrenal medulla. The extent to which these pathways are activated by stress and affect the secretion and actions of GnRH are likely to be influenced by sex and sex steroids. Finally, glucocorticoids are unlikely to be major mediators of the affects of acute stress on reproduction.

synthesis. For example, in ovariectomized monkeys treated with metyrapone, insulin-induced hypoglycaemia suppressed LH secretion (Van Vugt *et al.*, 1997). Indeed, most evidence from studies in ovariectomized rhesus monkeys indicates that acute decreases in the secretion of LH are more likely to be due to central actions of CRF, AVP or other endogenous factors (for example, opioid peptides) than to the glucocorticoids.

Despite the association of chronically increased plasma concentrations of cortisol with depressed reproduction in humans (Loriaux and Nieman, 1990), not all studies have found that administration of glucocorticoids suppresses the secretion of gonadotrophins. Infusion of cortisol in six men and four women for over 24 h did not affect the mean or pulsatile LH, FSH or α -subunit secretion, and it has been suggested that

higher concentrations of cortisol or more prolonged treatment might be necessary to obtain an effect, or that other mediators of the response to stress inhibit the gonadal axis (Samuels *et al.*, 1994). Unfortunately, Samuels *et al.* (1994) did not conduct a sex comparison. Although administration of CRF every 90 min over 8 h to women of reproductive age increased concentrations of cortisol, there was no effect of this treatment on the concentrations of LH or FSH (Thomas *et al.*, 1991). Studies in humans are limited by the pharmacological doses of glucocorticoids permitted and the restrictions placed on experimental manipulations.

Various experimental approaches have been undertaken to determine the extent to which glucocorticoids act at the hypothalamus and anterior pituitary gland to inhibit the secretion of gonadotrophins. Glucocorticoids were found to decrease the transcriptional activity of the GnRH promoter in GT1 hypothalamic cell lines (Chandran *et al.*, 1994) and, in rats, glucocorticoid receptors have been identified in hypothalamic GnRH neurones (Ahima and Harlan, 1992) and on pituitary gonadotrophs (Kononen *et al.*, 1992). Experimental evidence supports the notion that glucocorticoids act at either of these sites to affect LH secretion in rodents. Glucocorticoid binding was found in the hypothalamus of pigs (Stith and Weingarten, 1978) but the phenotype of the cells containing the receptors was not determined. In rams treated with dexamethasone for 5 days (Juniewicz *et al.*, 1987) and gonadectomized male rhesus monkeys treated with cortisol for 62 days (Dubey and Plant, 1985), LH secretion was decreased but there was no inhibition of the LH response to GnRH, which led to the conclusion that glucocorticoids act at the hypothalamus. The frequency of LH pulses decreased but there was no change in the amplitude of LH pulses in women administered supraphysiological amounts of cortisol for up to 10 days (Saketos *et al.*, 1993), which also implies a hypothalamic site of action. In ovariectomized gilts, administration of cortisol decreased the serum concentrations of LH and the LH pulse frequency whereas, in gilts that had undergone pituitary-stalk transection and were administered pulses of GnRH every 45 min, cortisol did not affect the secretory pattern of LH (Estienne *et al.*, 1991). Collectively, these results indicate that, in gilts, cortisol acts at the hypothalamus and not the pituitary gland. In agreement with this conclusion, the LH response to exogenous GnRH in female pigs was not affected by treatments with cortisol that resulted in repeated acute or sustained increases in plasma cortisol (Turner *et al.*, 1999a,b). Unfortunately, there have been no studies in which GnRH has been measured in hypophyseal portal blood in animals treated with glucocorticoids, which are necessary to firmly establish a hypothalamic site of action. Pituitary sites of action of glucocorticoids have been suggested on the basis of a reduced LH response to GnRH in cows (Li and Wagner, 1983), bulls (Chantaraprateep and Thibier, 1978), pigs (Pearce *et al.*, 1988) and women (Melis *et al.*, 1987). Direct pituitary action of glucocorticoids on gonadotrophin secretion is also inferred from studies *in vitro* that used tissue from pigs (Li, 1994).

Conclusion

When stress is prolonged, it is likely that the secretion of the gonadotrophins will be suppressed and reproduction will be inhibited. However, for acute stress or repeated acute stress,

which are probably the most commonly experienced forms of stress in most animals including humans, it is unclear whether, or how, reproduction will be affected. It is apparent from various studies that glucocorticoids can inhibit gonadotrophin secretion in some circumstances. Suppression of reproduction is more likely under conditions of chronic stress and may involve action at the hypothalamus or pituitary. Furthermore, there are likely to be species differences in the effect of glucocorticoids on gonadotrophin secretion, and the presence of sex steroids and the sex of an individual are likely to be factors. A fresh approach is required to understand the mechanisms by which chronic and acute stress influence reproduction. This approach must consider the stress pathways that are activated by particular stressors and determine how these pathways affect the secretion and actions of GnRH (Fig. 3). There is also a need to know how stress influences the feedback actions of gonadal steroids and inhibin. Finally, future research on the effects of stress on reproduction must consider sex differences.

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