Stress
The International Journal on the Biology of Stress

ISSN: 1025-3890 (Print) 1607-8888 (Online) Journal homepage: informahealthcare.com/journals/ists20

The Influence of 17β-oestradiol on Corticotrophin-releasing Hormone Induced Suppression of Luteinising Hormone Pulses and the Role of CRH in Hypoglycaemic Stress-induced Suppression of Pulsatile LH Secretion in the Female Rat

P.S. Cates, X.F. Li & K.T. O'Byrne

To cite this article: P.S. Cates, X.F. Li & K.T. O'Byrne (2004) The Influence of 17β-oestradiol on Corticotrophin-releasing Hormone Induced Suppression of Luteinising Hormone Pulses and the Role of CRH in Hypoglycaemic Stress-induced Suppression of Pulsatile LH Secretion in the Female Rat, Stress, 7:2, 113-118, DOI: 10.1080/1025389042000218988

To link to this article: https://doi.org/10.1080/1025389042000218988

Published online: 07 Jul 2009.

Stress, June 2004 Vol. 7 (2), pp. 113–118

Taylor & Francis
healthsciences

The Influence of 17β-oestradiol on Corticotrophin-releasing Hormone Induced Suppression of Luteinising Hormone Pulses and the Role of CRH in Hypoglycaemic Stress-induced Suppression of Pulsatile LH Secretion in the Female Rat

P.S. CATES, X.F. LI and K.T. O'BYRNE*

Centre for Reproduction, Endocrinology and Diabetes, 2.36D New Hunt's House, Guy's Campus, GKT School of Biomedical Sciences, King's College London, London, SE1 1UL, UK

(Received 20 January 2004; Revised 5 March 2004; In final form 8 March 2004)

Corticotrophin-releasing hormone (CRH) released during stress has been implicated in the disruption of the reproductive neuroendocrine axis, and 17β-oestradiol (E_2) has been shown to enhance stress-induced suppression of pulsatile gonadotrophin-releasing hormone (GnRH) and luteinising hormone (LH) release. The aims of the present study were to examine the role of CRH in hypoglycaemic stress-induced suppression of LH pulses, and to investigate the influence of E_2 on the inhibitory effect of CRH on pulsatile LH secretion in the female rat. Suppression of LH pulses by insulin-induced hypoglycaemic (IIH) stress was completely prevented by intracerebroventricular (icv) administration of a CRH antagonist. Central administration of CRH (5 μg) resulted in an interruption of LH pulses in E_2 treated animals, but had little or no effect in the absence of this gonadal steroid. These results provide evidence of a pivotal role for CRH in mediating the suppressive effect of IIH stress on pulsatile LH secretion in the female rat, and highlight a sensitising role for E_2 in CRH-induced suppression of LH pulses.

Keywords: CRH; CRH antagonist; Hypoglycaemia; LH pulses; Oestradiol; Stress

INTRODUCTION

Stressful stimuli activate the hypothalamic–pituitary–adrenocortical (HPA) axis and disrupt the hypothalamic–pituitary–gonadal (HPG) axis, specifically pulsatile gonadotrophin-releasing hormone (GnRH) and luteinising hormone (LH) secretion. Corticotrophin-releasing hormone (CRH), a central component of the HPA axis, has been shown to have a direct inhibitory effect on the frequency of the hypothalamic GnRH pulse generator in the rhesus monkey (Olster and Ferin, 1987; Williams *et al.*, 1990). The effect of CRH on pulsatile LH secretion is less clear in other species. In contrast to the response to CRH observed in the rhesus monkey, Naylor and colleagues (1990) demonstrated a stimulation of LH pulse frequency by CRH in the ovariectomised (OVX) sheep, whilst Caraty and colleagues (1997) only observed this stimulatory effect in the presence of gonadal steroids; in the absence of steroid replacement no response to CRH was seen in the ewe. While there are no data on the effect of CRH on hypothalamic GnRH pulse generator frequency or LH pulse frequency in the rat, CRH clearly decreases *mean* circulating levels of LH in this species (Ono *et al.*, 1984; Rivier and Vale, 1984; Petraglia *et al.*, 1987).

The suppression of the GnRH pulse generator by a variety of stressful stimuli can be blocked by CRH antagonists. For example, in the rat, CRH antagonists prevent the suppression of LH release in response to foot shock (Rivier *et al.*, 1986) and 2-deoxyglucose-induced glucoprivation (Tsukahara *et al.*, 1999). While insulin-induced hypoglycaemic (IIH) stress is a potent suppressor of pulsatile LH secretion in the rat (Goubillon and Thalabard, 1996; Cagampang *et al.*, 1997; Li *et al.*, 2003; Li *et al.*, 2004), monkey (Chen *et al.*, 1992; 1996) and sheep (Clarke *et al.*, 1990; Adam and Findlay, 1998), the role of CRH in this response remains to be firmly established. CRH antagonists prevent IIH stress-induced suppression of the GnRH pulse generator in the monkey (Chen *et al.*, 1996; Van Vugt *et al.*, 1997), are without

*Corresponding author. Tel.: +44-207-848-6286. Fax: +44-207-848-6220. E-mail: kevin.o'byrne@kcl.ac.uk

ISSN 1025-3890 print/ISSN 1607-8888 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/1025389042000218988

effect in the sheep (Clarke *et al.*, 1990), and remain to be examined in the rat.

Most studies have provided evidence that the presence of the gonadal steroid 17β-oestradiol (E_2) potentiates the suppression of LH pulses in response to a number of perturbations in a variety of species. For example, the disruption of pulsatile LH secretion in response to fasting (Cagampang *et al.*, 1990; 1991) or 2-deoxyglucose-induced glucoprivation (Nagatani *et al.*, 1996) in the rat, and to IIH stress in the rhesus monkey (Chen *et al.*, 1992), rat (Cagampang *et al.*, 1997; Li *et al.*, 2003), and sheep (Adam and Findlay, 1998) is more severe in intact or OVX animals which have received E_2 replacement than in OVX animals without steroid treatment. The mechanism by which E_2 exerts this sensitising influence on stress-induced suppression of LH pulses has yet to be fully elucidated. There is evidence, however, that circulating gonadal steroids may influence the expression of CRH since its mRNA levels in the hypothalamic paraventricular nucleus (PVN) are elevated on the afternoon of proestrus (Bohler *et al.*, 1990). More recently, we have shown that E_2 *per se* increased CRH mRNA expression in the PVN and preoptic area in the OVX rat (Li *et al.*, 2003), and Van Vugt and colleagues (1997) have shown a similar response in the PVN of the monkey; this could result in an increased readily releasable pool of CRH. Although basal non-stress levels of CRH receptor mRNA are low in the PVN, stress or elevated levels of central CRH, achieved by intracerebroventricular (icv) administration of CRH, result in a marked increase in the immediate early gene, c-fos, and in CRH receptor density in the PVN (Mansi *et al.*, 1996). Since stress-induced increases in CRH receptor expression occur primarily in CRH neurones of the PVN, this may represent a positive ultra-short feedback regulation of CRH on the CRH neuronal system (Mansi *et al.*, 1996; Imaki *et al.*, 2001). Furthermore, stress-induced increases in CRH receptor expression are elevated during proestrus, which might suggest a modulatory role for E_2 (Nappi and Rivest, 1995).

The aims of the present study were to test the hypotheses that CRH mediates hypoglycaemic stress-induced suppression of pulsatile LH secretion, and that E_2 enhances the sensitivity of the hypothalamic GnRH pulse generator to the inhibitory influence of CRH in the rat.

MATERIALS AND METHODS

Animals and Surgical Procedures

Adult female Wistar rats (250–300 g) obtained from Tuck Suppliers, Ltd (Battlesbridge, UK) were housed under controlled conditions (14 h light/10 h dark; lights on at 07:00 h; temperature at 22 ± 2°C) and provided with food and water *ad libitum*. All animal procedures were undertaken in accordance with the United Kingdom Home Office regulations. All surgical procedures were carried out under ketamine anaesthesia (100 mg/kg ip; Pharmacia and Upjohn Ltd, Crawley, UK) and Rompun (10 mg/kg ip; Bayer, Leverkusen, Germany). Rats were bilaterally OVX and immediately implanted subcutaneously with a Silastic capsule (ID 1.57 mm; OD 3.18 mm; Sanitech, Havant, UK) filled to a length of 25 mm with E_2 (Sigma Chemicals Ltd, Pool, UK) dissolved at a concentration of 20 μg/ml arachis oil (Sigma Chemicals Ltd). Non-E_2 treated rats were implanted with capsules containing arachis oil only. The concentration of E_2 in the capsules produced levels of E_2 in the circulation to within the range observed during the diestrous phase of the oestrous cycle (38.8 ± 1.2 pg/ml) (Cagampang *et al.*, 1991). At the same time as ovariectomy, all rats were implanted with an icv guide cannula (22 gauge; Plastics One, Virginia, USA) positioned into the left lateral ventricle; the co-ordinates for implantation being 1.5 mm lateral and 0.6 mm posterior to Bregma, and 4.5 mm below the surface of the dura (Paxinos and Watson, 1986). The guide cannula was secured using dental cement (Dental Filling Ltd, Swindon, UK), and fitted with a dummy cannula (Plastics One) to maintain patency. Ten days later the rats were fitted with one or two indwelling cardiac catheters introduced via the jugular veins. The catheters were exteriorised at the back of the head and secured to a cranial attachment: the rats were fitted with a 30 cm long metal spring tether (Instec Laboratories Inc., Boulder, Colorado, USA). The distal end of the tether was attached to a fluid swivel (Instec Laboratories Inc.), which allowed the rat freedom to move around the enclosure. Rats were allowed to recover for 3 days before experimentation commenced.

Effect of a CRH Antagonist on Hypoglycaemic Stress-induced Inhibition of LH Pulses

Fifteen OVX rats were implanted with E_2 capsules, an icv cannula and two cardiac catheters. Rats were fasted overnight. The following morning an icv injection cannula (Plastics One) with extension tubing, preloaded with CRH antagonist ([D-Phe^{12}, NleLE^{21,38}, C$^{\alpha}$MeLeu^{37}]$_{r/h}$ CRF$_{(12-41)}$; Rivier and Vale, The Salk Institute, California, USA; Hernandez *et al.*, 1993) dissolved in artificial cerebrospinal fluid (aCSF) or aCSF alone, was inserted into the guide cannula. The distal end of the tubing was extended outside the rat's cage to allow remote infusion without disturbing the animal during the experiment. Rats were then attached via one of the two cardiac catheters to a computer-controlled automated blood sampling system, which allows for the intermittent withdrawal of small blood samples (25 μl) without disturbing the rat (Cagampang *et al.*, 1997). Once connected the animals were left undisturbed for 1 h before blood sampling commences. Experimentation commenced between 09:00 and 11:00 h when blood samples were taken every 5 min for 6.5 h, for measurement of LH. Following removal of each 25 μl blood sample, an equal volume of heparinized saline (10 U/ml normal saline; CP Pharmaceuticals Ltd, Wrexham, UK) was automatically infused into

the animal to maintain patency of the catheter and blood volume. After 2 h 20 min of blood sampling, 100 μg CRH antagonist in 5 μl aCSF was administered over 5 min via icv injection. Ten minutes later, a single iv injection of 0.5 U/kg insulin (Nordisk Wellcome Human Insulin, Crawley, UK) in 0.2 ml saline was given. Blood glucose was monitored throughout the experiment and for this purpose blood samples (0.05–0.1 ml) were manually collected through the second cardiac catheter every 30 min before insulin administration and every 5 min for 35 min after insulin treatment, followed by 10 min intervals for the next 20 min. The frequency of sampling for blood glucose was then reduced to 20–60 min intervals for the remainder of the experiment. Blood glucose concentrations were measured using a Reflolux S blood glucose monitor (Boehringer Mannheim, Mannheim, Germany). Six rats were treated with this regime. Control rats were fasted overnight but received either CRH antagonist icv and saline iv ($n = 5$), or aCSF icv and insulin iv ($n = 5$).

Effect of Central CRH Administration on Pulsatile LH Secretion

OVX Wistar rats were fitted with an icv cannula and one indwelling cardiac catheter. Of these, 10 received E_2 filled capsules, while 10 received oil-filled capsules. Rats were connected to the pre-loaded icv injection cannula, and the automated blood sampling system via the indwelling cardiac catheter as described above. Blood sampling commenced between 09:00 and 11:00 h when 25 μl blood samples were taken every 5 min for 6.5 h for measurement of LH. After 2.5 h of blood sampling, CRH (5 μg in 5 μl aCSF) (Sigma Chemicals Ltd) was infused into the lateral ventricle over 5 min. Control rats received 5 μl aCSF icv.

LH Radioimmunoassay

A double antibody radioimmunoassay supplied by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), was used to determine LH concentration in the 25 μl whole blood samples. The sensitivity of the assay was 0.093 ng/ml. The intra-assay variation was 5.8% and the inter-assay variation was 5%.

Statistical Analysis

Detection of LH pulses was established by use of the algorithm ULTRA (Van Cauter, 1988). Two intra-assay coefficients of variation of the assay were used as the reference threshold for the pulse detection. The inhibitory effect of hypoglycaemic stress on LH pulses was calculated by comparing the mean LH pulse interval before insulin with the first LH pulse interval after administration and expressed as "prolongation of LH pulse interval" as the percentage by which the mean first interval exceeds the pre-treatment control value. The decrease in blood glucose concentrations in response to insulin was determined by comparing the mean glucose level before insulin injection with the mean blood glucose concentration during the 45-min period after insulin injection. The effect of CRH on pulsatile LH secretion, with or without E_2 replacement, was calculated by comparing the mean LH pulse interval before and after administration of CRH and expressed as "prolongation of LH pulse interval" as a percentage of the pre-treatment control value. The significance of the effect of CRH, E_2 or insulin was assessed using ANOVA followed by a Dunnett's test. A $p < 0.05$ was considered statistically significant.

RESULTS

The Effect of a CRH Antagonist on the Inhibition of Pulsatile LH Secretion in Response to IIH Stress

Administration of 0.5 U/kg insulin iv resulted in a significant decrease in blood glucose in both CRH antagonist treated animals and in those animals which received 5 μl aCSF icv (61 ± 3.4% and 66.5 ± 4.1%, mean ± SEM; $p < 0.05$, CRH antagonist and aCSF treated animals, respectively). The LH pulse interval in the pre-treatment control period was 21.3 ± 1.7 min and 22.4 ± 2.1 min (mean ± SEM) for aCSF vehicle and CRH antagonist treated rats, respectively. Hypoglycaemia resulted in a significant interruption of LH pulses in the aCSF treated animals ($p < 0.05$; Figs. 1A and 2) as previously demonstrated (Cagampang *et al.*, 1997; Li *et al.*, 2003; 2004). Despite the rats exhibiting a comparable decrease in blood glucose after insulin and icv vehicle or CRH, administration of 100 μg CRH antagonist icv ten minutes prior to the injection of insulin completely prevented the interruption of pulsatile LH release in response to this stress ($p < 0.05$; Figs. 1B and 2). The administration of CRH antagonist icv with saline iv to control rats did not affect either blood glucose or LH pulse interval (Figs. 1C and 2).

The Effect of CRH on Pulsatile LH Secretion in the Presence and Absence of E_2

The LH pulse interval in the pre-treatment control period was not significantly different between the aCSF vehicle and CRH treated rats with a group mean ± SEM of 22.5 ± 1.6 min. The icv administration of 5 μg CRH to OVX rats with E_2 replacement resulted in a significant prolongation of the LH pulse interval immediately following injection ($p < 0.05$; $n = 7$; see Figs. 3A and 4). In contrast, the same dose of CRH was without significant effect on pulsatile LH secretion in non-E_2 treated rats (Fig. 4), although several rats showed a tendency for an increase in LH pulse interval (Fig. 3B). Artificial CSF (5 μl) was without effect on pulsatile LH secretion in E_2 and non-E_2 treated rats (Figs. 3C and 4).

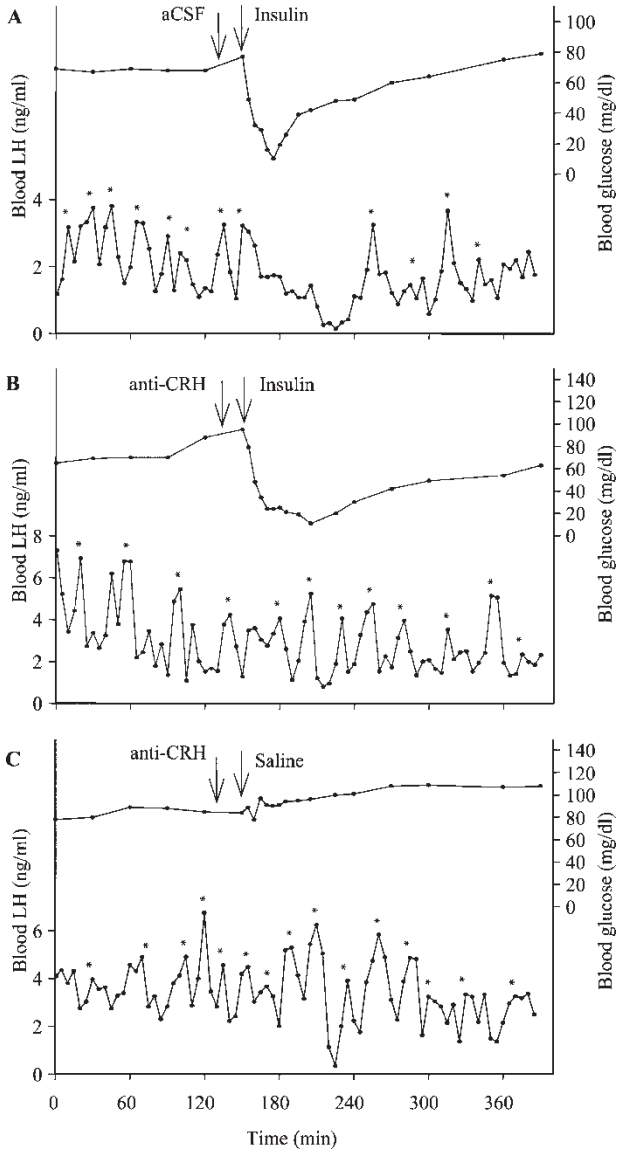

FIGURE 1 Representative examples depicting the effect of insulin-induced hypoglycaemia (0.5 U insulin/kg iv) on pulsatile LH secretion, measured as blood LH concentration, in OVX E_2 treated rats administered aCSF (5 μl) (A), or CRH antagonist (anti-CRH; 100 μg in aCSF) (B) by icv injection 10 min before insulin administration. (C) Representative example of an OVX E_2 treated rat administered CRH antagonist (100 μg, icv) 10 min before saline iv. Note that the CRH antagonist had no effect on LH pulses, but it was able to prevent the decrease in LH pulse frequency in response to hypoglycaemia. *LH pulse.

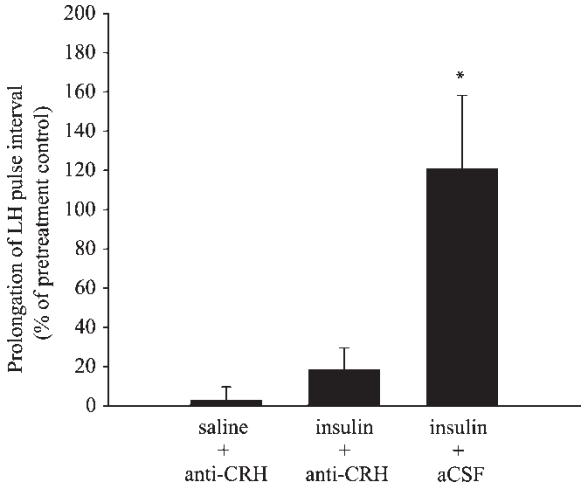

FIGURE 2 Summary of the effects of IIH stress (0.5 U insulin/kg iv) on pulsatile LH secretion in OVX E_2 treated rats administered CRH antagonist (anti-CRH; 100 μg by icv injection; $n = 6$) or aCSF (5 μl, icv; $n = 5$) 10 min prior to insulin injection. Further control rats received saline iv 10 min after an icv injection of CRH antagonist ($n = 5$). *$p < 0.05$ when compared to pre-treatment values.

DISCUSSION

The ability of centrally administered CRH to inhibit pulsatile LH release in the present study extends previous investigations demonstrating a suppressive effect of CRH on *mean* LH levels in the rat (Ono *et al.*, 1984; Rivier and Vale, 1984; Petraglia *et al.*, 1987; Almeida *et al.*, 1988; Ortega *et al.*, 1994), and concurs with a similar suppressive effect of CRH on the hypothalamic GnRH pulse generator in the monkey (Olster and Ferin, 1987; Williams *et al.*, 1990). The finding that central administration of a CRH antagonist prior to the induction of hypoglycaemic stress completely prevented the suppression of pulsatile LH secretion demonstrates a pivotal role for CRH in mediating this response in the rat. Indeed, this is in agreement with similar findings in the rhesus monkey (Chen *et al.*, 1996; Van Vugt *et al.*, 1997), but at variance with findings in the sheep (Clarke *et al.*, 1990). Since c-fos expression, a marker of neuronal activation, is increased in CRH neurons of the PVN in response to various stressors, including IIH stress (Brown and Sawchenko, 1997), and the latter is known to inhibit LH pulses (Clarke *et al.*, 1990; Chen *et al.*, 1992; 1996; Goubillon and Thalabard, 1996; Cagampang *et al.*, 1997; Van Vugt *et al.*, 1997; Adam and Findlay, 1998; Li *et al.*, 2003; 2004), it is tempting to speculate that the CRH neurons of the PVN are involved in mediating the suppressive effect of IIH stress on the hypothalamic GnRH pulse generator. Furthermore, central administration of CRH induces c-fos expression in CRH neurons of the PVN (Parkes *et al.*, 1993).

It is important to note that in the current study icv administration of CRH (5 μg) inhibited LH pulses in OVX E_2-treated animals, but was without significant effect on pulsatile LH release in OVX non-E_2 replaced animals. Nevertheless, a larger dose of CRH (10 μg) did inhibit LH pulses in OVX non-E_2 treated rats (Unpublished observation, Cates and O'Byrne). Interestingly, Rivier and Vale (1984) did not observe a sensitising effect of E_2 on CRH-induced suppression of LH in the OVX rat. However, it has been reported that stress has a more profound suppressive effect on LH release in the presence of gonadal steroids. For example, IIH stress has a more profound inhibitory effect on pulsatile LH secretion in rats (Cagampang *et al.*, 1997; Li *et al.*, 2003), monkeys (Chen *et al.*, 1992) and sheep (Adam and Findlay, 1998) that are either gonadally intact or OVX with E_2 replacement, compared to non-E_2 replaced OVX animals.

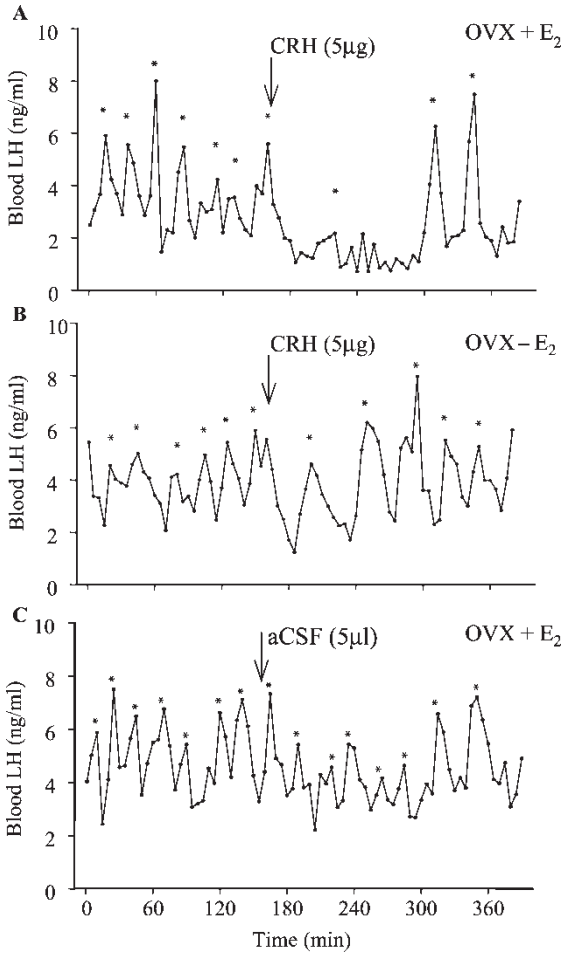

FIGURE 3 The effect of CRH on pulsatile LH secretion. Representative examples illustrating the effect of icv administration of CRH (5 μg in 5 μl aCSF) on pulsatile LH secretion, measured as blood LH concentration, in an OVX E_2 treated (A) and OVX non-E_2 treated rats (B). Note that the suppressive effect of CRH on pulsatile LH secretion was only significant in the OVX E_2 treated rats. There was no effect of aCSF (5 μl) on LH pulse frequency in the OVX E_2 treated rats (C). *LH pulses.

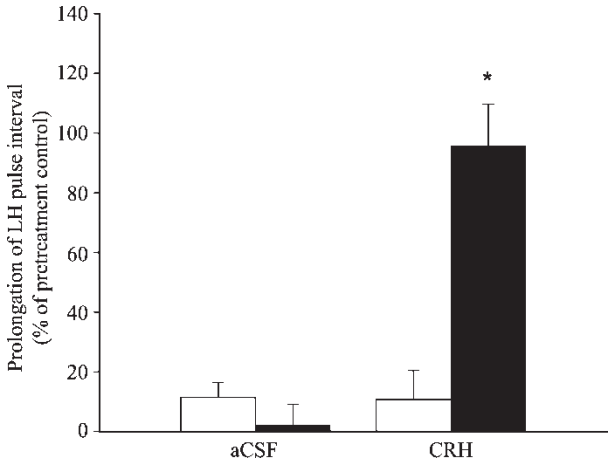

FIGURE 4 Summary of the effect of icv administration of 5 μg CRH or 5 μl aCSF on pulsatile LH secretion in OVX rats (open bars) and in OVX E_2 treated rats (black bars). Note that administration of 5 μg CRH (icv) was only effective in prolonging the LH inter-pulse interval in OVX E_2 treated rats. *$p < 0.05$ compared with rats infused with aCSF with or without E_2, and to rats administered with 5 μg CRH without E_2 treatment. $n = 5–7$ per group.

It remains to be fully established how E_2 acts to enhance the suppressive effect of CRH and stress on GnRH and LH release. E_2 may act to enhance the synthesis and/or efficacy of CRH in suppressing the GnRH pulse generator. It has been reported that CRH type 1 receptor mRNA expression in the PVN is modulated in a positive manner by exogenous CRH (Mansi *et al.*, 1996; Bittencourt and Sawchenko, 2000). It has also been demonstrated that in response to immobilisation stress, CRH receptor mRNA expression is markedly increased in the PVN (Nappi and Rivest, 1995; Imaki *et al.*, 2001). Moreover, this increase is significantly higher on the morning of proestrus than in the afternoon of proestrus, or on dioestrus, suggesting an influence of E_2 on the synthesis of CRH receptors in the PVN in response to stress (Nappi and Rivest, 1995). Given these findings, E_2 may exert its sensitising effect on both stress- and CRH-induced suppression of LH pulses by enhancing the number of CRH receptors. Furthermore, E_2 has been shown to significantly increase basal levels of CRH mRNA in the PVN and POA of rats (Li *et al.*, 2003), and the PVN of rhesus monkeys (Roy *et al.*, 1999), which could result in a greater readily releasable pool of CRH in response to stress. Indeed, Buckingham (1982) has clearly demonstrated that E_2 enhances stimulus evoked hypothalamic CRH release. Given that the CRH gene contains an oestrogen response element (Vamvakopoulos and Chrousos, 1993) and that oestrogen receptor β, and to a much lesser extent oestrogen receptor α, is expressed in the parvocellular division of the PVN (LaFlamme *et al.*, 1998), a direct action of E_2 on the CRH neurones is possible. Since CRH neurones have been demonstrated to synapse directly onto GnRH neurones (MacLusky *et al.*, 1988), any E_2-induced enhancement of CRH synthesis might result in a greater suppression of GnRH directly by CRH.

In summary, the results of the present study provide evidence of a pivotal role for CRH in mediating the suppressive effect of IIH stress on pulsatile LH release in the female rat. In addition, E_2 profoundly enhanced CRH-induced suppression of LH pulses. Thus, increased responsiveness to CRH may underlie, in part, the sensitising action of E_2 on CRH- and stress-induced suppression of hypothalamic GnRH pulse generator frequency.

Acknowledgements

The authors wish to thank the National Institutes of Diabetes, Digestive and Kidney Diseases (NIDDK) for providing the LH radioimmunoassay kit. This work was supported by The Wellcome Trust. PSC was a recipient of a Medical Research Council Ph.D. Studentship.

References

Adam, C.L. and Findlay, P.A. (1998) Inhibition of luteinizing hormone secretion and expression of c-fos and corticotrophin-releasing factor genes in the paraventricular nucleus during insulin-induced hypoglycaemia in sheep, *J. Neuroendocrinol.* **10**, 777–783.

Almeida, O.F.X., Nikolarakis, K.E. and Herz, A. (1988) Evidence for the involvement of endogenous opioids in the inhibition of luteinizing

hormone by corticotropin-releasing factor, *Endocrinology* **122**, 1034–1041.
Bittencourt, J.C. and Sawchenko, P.E. (2000) Do centrally administered neuropeptides access cognate receptors? An analysis in the central corticotropin-releasing factor system, *J. Neurosci.* **20**, 1142–1156.
Bohler, H.C., Jr., Zoeller, R.T., King, J.C., Rubin, B.S., Weber, R. and Merriam, G.R. (1990) Corticotropin releasing hormone mRNA is elevated on the afternoon of proestrus in the parvocellular paraventricular nuclei of the female rat, *Mol. Brain Res.* **8**, 259–262.
Brown, E.R. and Sawchenko, P.E. (1997) Hypophysiotropic CRF neurons display a sustained immediate-early gene response to chronic stress but not to adrenalectomy, *J. Neuroendocrinol.* **4**, 307–316.
Buckingham, J.C. (1982) Effects of adrenocortical and gonadal steroids on the secretion *in vitro* of corticotrophin and its hypothalamic releasing factor, *J. Endocrinol.* **93**, 123–132.
Cagampang, F.R.A., Maeda, K-I., Yokoyama, A. and Ôta, K. (1990) Effect of food deprivation on the pulsatile LH release in the cycling and ovariectomized female rat, *Horm. Metab. Res.* **22**, 269–272.
Cagampang, F.R.A., Maeda, K-I., Tsukamura, H., Ohkura, S. and Ôta, K. (1991) Involvement of ovarian steroids and endogenous opioids in the fasting-induced suppression of pulsatile LH release in ovariectomized rats, *J. Endocrinol.* **129**, 321–328.
Cagampang, F.R.A., Cates, P.S., Sandhu, S., Strutton, P.H., McGarvey, C., Coen, C.W. and O'Byrne, K.T. (1997) Hypoglycaemia-induced inhibition of pulsatile luteinizing hormone secretion in female rats: role of oestradiol, endogenous opioids and the adrenal medulla, *J. Neuroendocrinol.* **9**, 867–872.
Caraty, A., Miller, D.W., Delaleu, B. and Martin, G.B. (1997) Stimulation of LH secretion in sheep by central administration of corticotrophin-releasing hormone, *J. Reprod. Fertil.* **111**, 249–257.
Chen, M-D., O'Byrne, K.T., Chiappini, S.E., Hotchkiss, J. and Knobil, E. (1992) Hypoglycemic 'stress' and gonadotropin-releasing hormone pulse generator activity in the rhesus monkey: role of the ovary, *Neuroendocrinology* **56**, 666–673.
Chen, M-D., Ördög, T., O'Byrne, K.T., Goldsmith, J.R., Connaughton, M.A. and Knobil, E. (1996) The insulin hypoglycemia-induced inhibition of gonadotropin-releasing hormone pulse generator activity in the rhesus monkey: roles of vasopressin and corticotropin-releasing factor, *Endocrinology* **137**, 2012–2021.
Clarke, I.J., Horton, R.J.E. and Doughton, B.W. (1990) Investigation of the mechanism by which insulin-induced hypoglycemia decreases luteinizing hormone secretion in ovariectomized ewes, *Endocrinology* **127**, 1470–1476.
Goubillon, M-L. and Thalabard, J-C. (1996) Insulin-induced hypoglycemia decreases luteinizing hormone secretion in the castrated male rat: involvement of opiate peptides, *Neuroendocrinology* **64**, 49–56.
Hernandez, J.F., Kornreich, W., Rivier, C., Miranda, A., Yamamoto, G., Andrews, J., Tache, Y., Vale, W. and Rivier, J. (1993) Synthesis and relative potencies of new constrained CRF antagonists, *J. Med. Chem.* **36**, 2860–2867.
Imaki, T., Katsumata, H., Miyata, M., Naruse, M., Imaki, J. and Minami, S. (2001) Expression of corticotropin-releasing hormone type 1 receptor in paraventricular nucleus after acute stress, *Neuroendocrinology* **73**, 293–301.
LaFlamme, N., Nappi, R.E., Drolet, G., Labrie, C. and Rivest, S. (1998) Expression and neuropeptidergic characterization of estrogen receptors (ERα and ERβ) throughout the rat brain: anatomical evidence of distinct roles of each subtype, *J. Neurobiol.* **36**, 357–378.
Li, X.F., Mitchell, J.C., Wood, S., Coen, C.W., Lightman, S.L. and O'Byrne, K.T. (2003) The effect of oestradiol and progesterone on hypoglycaemic stress-induced suppression of pulsatile luteinizing hormone release and on corticotrophin-releasing hormone mRNA expression in the rat, *J. Neuroendocrinol.* **15**, 1–9.
Li, X.F., Bowe, J.E., Mitchell, J.C., Brain, S.D., Lightman, S.L. and O'Byrne, K.T. (2004) Stress-induced suppression of the gonadotropin-releasing hormone pulse generator in the female rat: a novel neural action for calcitonin gene-related peptide, *Endocrinology* **145**, 1556–1563.
MacLusky, N.J., Naftolin, F. and Leranth, C. (1988) Immunocytochemical evidence for direct synaptic connections between corticotrophin-releasing factor (CRF) and gonadotrophin-releasing hormone (GnRH)-containing neurons in the preoptic area of the rat, *Brain Res.* **439**, 391–395.
Mansi, J.A., Rivest, S. and Drolet, G. (1996) Regulation of corticotropin-releasing factor type 1 (CRF1) receptor messenger ribonucleic acid in the paraventricular nucleus of rat hypothalamus by exogenous CRF, *Endocrinology* **137**, 4619–4629.
Nagatani, S., Bucholtz, D.C., Murahashi, K., Estacio, M.A.C., Tsukamura, H., Foster, D.L. and Maeda, K-I. (1996) Reduction of glucose availability suppresses pulsatile luteinizing hormone release in female and male rats, *Endocrinology* **137**, 1166–1170.
Nappi, R.E. and Rivest, S. (1995) Ovulatory cycle influences the stimulatory effect of stress on the expression of corticotropin-releasing factor receptor messenger ribonucleic acid in the paraventricular nucleus of the female rat hypothalamus, *Endocrinology* **136**, 4073–4083.
Naylor, A.M., Porter, D.W. and Lincoln, D.W. (1990) Central administration of corticotrophin-releasing factor in the sheep: effects on secretion of gonadotrophins, prolactin and cortisol, *J. Endocrinol.* **124**, 117–125.
Olster, D.H. and Ferin, M. (1987) Corticotropin-releasing hormone inhibits gonadotropin secretion in the ovariectomized rhesus monkey, *J. Clin. Endocrinol. Metab.* **65**, 262–267.
Ono, N., Lumpkin, M.D., Samson, W.K., McDonald, J.K. and McCann, S.M. (1984) Intrahypothalamic action of corticotrophin-releasing factor (CRF) to inhibit growth hormone and LH release in the rat, *Life Sci.* **35**, 1117–1123.
Ortega, E., Ruiz, E., Rodriguez, E. and Frias, J. (1994) Effect of corticotropin releasing factor (CRF) in the median eminence on gonadotropins in ovariectomized rats with or without steroid priming: dose-response study, *Neurochem. Res.* **19**, 1225–1230.
Parkes, D., Rivest, S., Lee, S., Rivier, C. and Vale, W. (1993) Corticotropin-releasing factor activates c-fos, NGFI-B, and corticotropin-releasing factor gene expression within the paraventricular nucleus of the rat hypothalamus, *Mol. Endocrinol.* **7**, 1357–1367.
Paxinos, G. and Watson, C. (1986) The Rat Brain in Stereotaxic Coordinates, 2nd ed. (Academic Press, London).
Petraglia, F., Sutton, S., Vale, W. and Plotsky, P. (1987) Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophysial-portal circulation, *Endocrinology* **120**, 1083–1088.
Rivier, C. and Vale, W. (1984) Influence of corticotropin-releasing factor on reproductive functions in the rat, *Endocrinology* **114**, 914–921.
Rivier, C., Rivier, J. and Vale, W. (1986) Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor, *Science* **231**, 607–609.
Roy, B.N., Reid, R.L. and Van Vugt, D.A. (1999) The effects of estrogen and progesterone on corticotropin-releasing hormone and arginine vasopressin messenger ribonucleic acid levels in the paraventricular nucleus and supraoptic nucleus of the rhesus monkey, *Endocrinology* **140**, 2191–2198.
Tsukahara, S., Tsukamura, H., Foster, D.L. and Maeda, K.I. (1999) Effect of corticotropin-releasing hormone antagonist on oestrogen-dependent glucoprivic suppression of luteinizing hormone secretion in female rats, *J. Neuroendocrinol.* **11**, 101–105.
Vamvakopoulos, N.C. and Chrousos, G.P. (1993) Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression. Potential implications for the sexual dimorphism of stress response and immune/inflammatory reaction, *J. Clin. Investig.* **92**, 1896–1902.
Van Cauter, E. (1988) Estimating false-positive and false-negative errors in analyses of hormonal pulsatility, *Am. J. Physiol.* **254**, E786–E794.
Van Vugt, D.A., Washburn, D.L.S., Farley, A.E. and Reid, R.L. (1997) Hypoglycemia-induced inhibition of LH and stimulation of ACTH secretion in the rhesus monkey is blocked by alprazolam, *Neuroendocrinology* **65**, 344–352.
Williams, C.L., Nishihara, M., Thalabard, J-C., Grosser, P.M., Hotchkiss, J. and Knobil, E. (1990) Corticotropin-releasing factor and gonadotropin-releasing hormone pulse generator activity in the rhesus monkey. Electrophysiological studies, *Neuroendocrinology* **52**, 133–137.