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Corticotropin-releasing hormone in nonhuman primates

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TABLE OF CONTENTS

1. ABSTRACT

Understanding the many roles that corticotropin-releasing hormone (CRH) plays in facilitating the ordinary and extraordinary events that an individual faces during a lifetime is a complex task, and yet this knowledge is fundamental to understanding our own behaviour and physiology. During the past 25 years, the study of CRH in nonhuman primates, our closest genetic relatives, has grown rapidly. The intention of this review is to provide a broad overview of the research areas in which CRH has been investigated in monkeys and apes. The review begins with a detailed description of what we know about CRH, CRH receptors, and their distribution in the brain and periphery. The narrative then follows the life cycle, from the role of CRH in fertility, pregnancy and parturition, to the shaping of behaviour and neural processes by stressful experiences early in life. CRH is also examined in the context of its other regulatory roles, including appetitive behaviour and immune responses. Finally, the review examines the insights that nonhuman primate research offers us as to how CRH helps to shape our behaviour, whether it be our ability to socialize with our peers or to be a good parent.

2. INTRODUCTION

Corticotropin-releasing hormone (CRH), also known as corticotropin-releasing factor (CRF), has been the subject of both inferential and direct studies since its existence was first deduced in 1955 (1, 2). Since the isolation and identification of CRH in 1981 (3), and the development of selective pharmacological CRH antagonists, the amount of research in the field has grown exponentially. As the physiological and behavioural functions of CRH have become better characterized, interest from the scientific community has grown due to the perception that CRH agonists and antagonists may have therapeutic utility in a variety of clinical applications. Major technological advances have included the development of sensitive assays for the measurement of CRH and other peptide and steroid hormones, the pioneering work that enabled direct sampling of CRH from the hypophyseal-portal system, modeling of the structure of CRH receptors and their critical binding domains, and breeding of mutant mouse strains with alterations in CRH receptors. These advances have helped to elucidate the complex role of CRH in health and disease.

The purpose of this review is to describe the current state of knowledge of CRH as has been derived from studies conducted in nonhuman primates. This detailed overview will attempt to incorporate studies that have had a significant impact in our understanding of the *in vivo* functions of CRH. As will be seen, CRH has an important role in a wide variety of physiological systems, both as a hormone and as a neurotransmitter. Ultimately, many of these physiological effects are translated into behaviour. With the research community and pharmaceutical industry both investigating CRH-related compounds for the treatment of depression, anxiety and substance abuse, behavioural outcomes are especially relevant. This review will therefore highlight behavioural endpoints from CRH research in non-human primates.

3. BASIC ENDOCRINOLOGY OF CRH IN NONHUMAN PRIMATES

CRH has a variety of effects when released in response to any stressor that challenges an organism's ability to maintain homeostasis. Once such a stimulus is perceived, neural projections from different locations in the CNS are activated to increase CRH secretion. Hypothalamic CRH is released from parvicellular neurons located in the paraventricular nucleus. These cells have projections to the median eminence of the hypothalamus, and release CRH into blood vessels of the hypophyseal-portal system. In this way, CRH is transported along the infundibulum which joins the ventral hypothalamus to the pituitary gland. Once CRH reaches the anterior lobe of the pituitary gland, it binds with CRH receptors located on corticotropic cells of the anterior pituitary. These bound receptors stimulate the secretion of adrenocorticotropic hormone (ACTH) into the circulation, an important function of which is to increase the secretion of glucocorticoids (consisting primarily of cortisol in larger mammals) when ACTH reaches the adrenal cortex. The activation of the HPA axis is readily measured as pituitary (ACTH) and adrenal (cortisol) hormones, both of which are present in venous blood. Activation of the HPA axis and the subsequent release of ACTH and cortisol result in a range of physiological responses designed primarily to restore homeostasis to an organism in which normal vegetative and reproductive functions have been temporarily suspended. CRH also mediates a variety of other actions, some immediately evident while others are expressed more in the long-term. These include adaptive behavioural changes that occur in response to the repeated application of a stressor.

The earliest nonhuman primate studies of CRH commenced in 1982, shortly after the identification of ovine CRH (oCRH) (3). One of the first *in vivo* studies of CRH in non-human primates was to evaluate the effect of synthetic oCRH administration on cortisol secretion (4). The study was done in four cynomolgus monkeys (*Macaca fascicularis*) that had their pituitary stalks surgically transsected, thereby blocking the effects of endogenous CRH on pituitary ACTH release. Ten doses of oCRH (0 – 40 micrograms/kg) were intravenously administered in a randomised order to chair-restrained monkeys and venous blood was sampled during the two hours following oCRH injection. Cortisol levels rose within 15 min of the oCRH injection, peaking at later times following the larger doses. The smallest bolus dose that produced a significant rise in cortisol was 0.5 micrograms/kg oCRH. The larger oCRH doses (1.0 - 40 micrograms/kg) also increased plasma growth hormone (GH) and prolactin levels 15-30 min after oCRH injection (4), providing an early demonstration of the integrated nature of endocrine physiology. In similar studies conducted in humans, i.v. CRH produced increases in cortisol secretion at similar doses to those reported in non-human primates, with the lowest effective dose in both species being 0.1 micrograms/kg (5).

The study done by Schulte and colleagues (4) also investigated the plasma half-life and metabolic clearance rate of oCRH in cynomolgus monkeys (*Macaca fascicularis*). Young adult male monkeys, each with a transsected pituitary stalk, had intravenous and lumbar catheters inserted under ketamine anaesthesia on the day of testing. ^{125}I-labeled oCRH was infused in either a pulsatile manner or as a continuous infusion. The plasma half-life of oCRH was biphasic and of relatively long duration compared with other hypothalamic hormones (17.1 ± 0.5 min in the short phase, and 198 ± 0.3 min in the long phase) (6). This is similar to what has since been reported following continuous infusion studies in humans (5). However, as CRH is normally released in relatively minute quantities into the hypophyseal-portal system and is rapidly diluted in the systemic circulatory system, the pharmacokinetics of CRH in the periphery may be of limited physiological significance. It was also noted that the volume of distribution in the monkey was small, similar to the volume of the circulation. This led to speculation that CRH was extensively plasma-bound, which was subsequently verified with the identification of CRH binding protein (described below). Another important observation was that no ^{125}I-labeled oCRH was measured in CSF during the ^{125}I-labeled CRH venous infusions, implying that <1% of oCRH penetrated the blood-brain-barrier (6).

Another significant development during the early 80's was the identification and sequencing of human (7) and rat CRH (8) which were found to be identical and came to be referred to as h/rCRH. A study conducted in rhesus monkeys (*Macaca mulatta*) compared the pituitary-adrenal effects, pharmacokinetics and cardiovascular activity of oCRH and r/hCRH (9). Doses of h/rCRH (0, 0.1, 1, 10, and 100 micrograms/kg) and oCRH (1 micrograms/kg) were administered intravenously to eight chair-restrained monkeys. The ACTH and cortisol responses to 1 ug/kg oCRH and h/rCRH were very similar (see Figure 1) and the pituitary-adrenal response to h/rCRH was dose-dependent. However, the time courses of ACTH and cortisol following administration of h/rCRH were generally brief and monophasic, whereas the response to oCRH in cynomolgus monkeys was more prolonged and sometimes biphasic (4). This was reflected in a greater metabolic clearance rate of h/rCRH (10.5 ml/kg.min) than for oCRH (3.4 mg/kg.min) following a 1 micrograms/kg dose despite both oCRH and h/rCRH having a similar volume of distribution following a

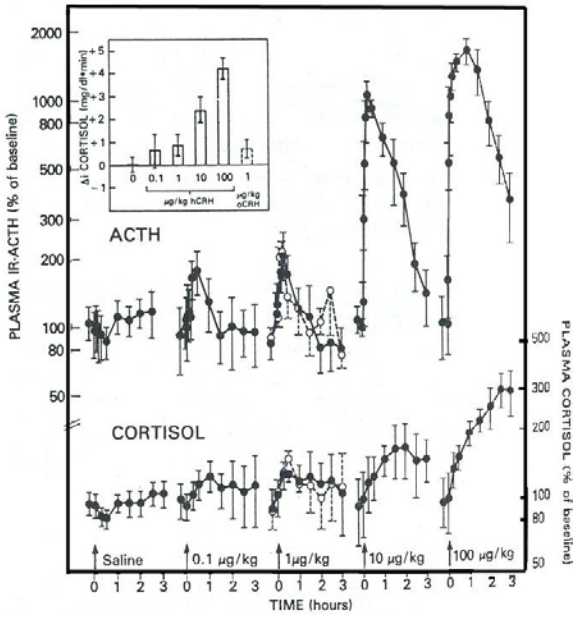

Figure 1. Responses of plasma immunoreactive ACTH and cortisol to graded doses of r/hCRH (●) and to a single dose (1 µg/kg) of oCRH (○), administered as i.v. bolus injections in rhesus monkeys. Inset, Integrated plasma cortisol responses (areas under the curve). From T. H. Schurmeyer, P. W. Gold & W. T. Gallucci: Effects of pharmacokinetic properties of the rat/human corticotropin-releasing factor in rhesus monkeys. Reproduced with permission from Endocrinology (9).

bolus injection (9). Dose-dependent increases in heart rate and decreases in mean arterial blood pressure were measured following 1.0, 10 and 100 micrograms/kg h/rCRH. These changes were likely due to the rapid fall in peripheral vascular resistance that has been reported in rhesus monkeys following administration of h/rCRH (10).

An investigation into the circumstances underlying the pulsatile nature of ACTH release was undertaken by Mershon and colleagues (1992), using perifused hypothalami of rhesus and cynomolgus monkeys. The hypothalami were divided into halves and the medium with which each half hypothalamus was perfused was collected every 10 min. Both gonadotropin-releasing hormone (GnRH) and CRH were found to be released in a pulsatile manner, with pulse intervals averaging 65 and 90 min respectively. The presence of regular pulses of both hormones suggests that the pulse generator lies within the hypothalamus. The addition of the synthetic glucocorticoid agonist, dexamethasone, did not affect the pulsatility of CRH release, suggesting that a site other than the hypothalamus is responsible for the negative feedback of glucocorticoids on HPA axis activity (11).

3.1 CRH and CRH receptors – Location in the CNS

Corticotropin releasing hormone (CRH) is a 41-amino acid straight-chain polypeptide. After the original isolation of CRH from ovine hypothalamus (3), it was rapidly established that CRH was not expressed in the hypothalamus simply to regulate HPA axis activity. Rather the expression of CRH and its high-affinity binding sites is widely distributed throughout the CNS and periphery, mediating a variety of endocrine and neurotransmitter functions.

Perhaps the first immunohistochemical identification of CRH-containing nerve fibres in the hypothalamus was reported in the Japanese macaque (*Macaca fuscata*) in 1982. Immunoreactive cell fibres were visualized in the capillary loops of the hypophyseal portal vessels in the external layers of the median eminence (12). This study was followed shortly after by a more precise localization of CRH immunoreactive cells in the hypothalamus, which identified the supraoptic and paraventricular nuclei of the hypothalamus as the primary locations in the Japanese macaque, cat, dog and pig (13). There is also evidence of extensive CRH immunoreactivity in the cells and fibres of the olfactory tract of both squirrel and cynomolgus monkeys, which suggests that CRH is a neurotransmitter or neuromodulator in the processing of primate olfactory information (14).

The location of CRH immunoreactive (CRH-IR) cell bodies and fibres in the hypothalamus of the squirrel monkey (*Saimiri sciureus*) has also been reported (15). CRH-IR cell bodies were visualised in the magnocellular region of the paraventricular nucleus (PVN), and in the supraoptic nucleus (SON), co-localised with arginine vasopressin (AVP) immunoreactive cells. This is in contrast to the rat, which shows little CRH-IR in the SON. In addition, the intermingling of CRH- and AVP-immunoreactive cell bodies seen in the squirrel monkey is dissimilar to the rat, in which most CRH and AVP-IR cells are segregated (16). Most of the CRH-IR fibres leaving the PVN travel to the median eminence, with a small number continuing along the infundibulum to end in the posterior lobe of the pituitary gland, from which AVP is secreted (15). The functional significance of this was demonstrated in a study that identified synergism between CRH and AVP in stimulating the release of ACTH in the Japanese macaque (*Macaca fuscata*) (17).

CRH receptors were identified and characterized in the marmoset (*Callithrix jacchus jacchus*), cynomolgus monkey (*Macaca fascicularis*) and human pituitary in a study by Millan and colleagues (1987). In all three primate species, specific CRH binding was most apparent in the intermediate lobe, with similar or less binding being found in the anterior pituitary lobe. The contrast between CRH binding in the intermediate and anterior lobes was greatest in the marmoset. There was no CRH-binding in the posterior lobe (neurohypophysis). The intensity of CRH receptor binding in the intermediate lobe is an indication of the importance of CRH in mediating the release of pro-opiomelanocortin (POMC)-derived peptides other than ACTH (e.g. β-endorphin). The concentrations of CRH required for the release of ACTH from monkey pituitary cells were similar to the binding affinity that CRH has for its receptor, suggesting that the actions of CRH on corticotropes is mediated through these receptors. Cyclic-AMP appears to be the second messenger involved in the release of ACTH following CRH receptor occupation.

When cultured cynomolgus monkey pituitary cells were incubated with arginine vasopressin (AVP) or norepinephrine (NE), the release of ACTH was small compared with the ACTH released in response to CRH. However the combination of CRH with either AVP or NE potentiated the effects of CRH on ACTH release (18). Although there are similarities between these three primate species, the marmoset and other New World monkeys distinguish themselves by having primary end-organ resistance to glucocorticoids, a genetic defect that results in very high plasma levels of glucocorticoids, ACTH and β-endorphin (19). This may help to explain the low binding density of CRH observed in the marmoset anterior pituitary, a possible consequence of the high circulating levels of pituitary and adrenal hormones.

Immunohistochemical visualization of CRH has also been undertaken in the olivocerebellar system in two monkey species, squirrel monkeys (*Saimiri sciureus*) and cynomolgus monkeys (*Macaca fascicularis*). The prominent staining of regions of the cerebellum and the inferior olivary nucleus that were reported (20) were later shown to be regions expressing mRNA for the CRH-R1 receptor subtype (21). CRH mRNA has also been reported in the inferior olives of baboons (*Papio ursinus*) (22), and in squirrel monkey amygdala by Bassett and Foote (1988). In the latter study, the main CRH-containing areas, identified using a polyclonal antisera raised against the human form of CRH, were the basal and lateral nuclei (CRH-positive cells) and the lateral, central and cortical nuclei (CRH-positive fibres) (23). This is distinct from the rat, in which the highest concentration of CRH-staining neurons is found in the central nucleus of the amygdala (e.g. (24)).

In summary, CRH mRNA immunoreactivity has been found throughout the CNS, in both hypothalamic and extrahypothalamic structures. In 1995, a second CRH-like peptide, urocortin, was identified (25). This 40 amino acid peptide is structurally similar to carp urotensin I, with 63% homology, as well as having 45% homology with CRH. The distribution of urocortin immunoreactive cells and fibres has been characterized in the brain and spinal cord of the capuchin monkey (*Cebus apella*). Similar to what has been consistently reported in non-primate species, the dominant site in the CNS for urocortin expression is the Edinger Westphal nucleus in the brainstem (26) as well as in lamina IX of the spinal cord. Urocortin-labeled axons and terminals have also been reported in the medial preoptic area and paraventricular nucleus of the hypothalamus, several brainstem nuclei, including the spinal trigeminal nucleus, the flocculus of the cerebellum and laminae VII and X of the spinal cord. No urocortin-IR was measured in the pituitary gland. The distribution of urocortin immunoreactivity suggests that urocortin plays a role in the control of endocrine, sensorimotor and autonomic function in primates (26).

3.2. CRH and CRH receptors – Location in the periphery

CRH-IR in sites outside the central nervous system was examined at different stages in the life cycle in the baboon (*Papio ursinus*) by Dotzler and colleagues (2003). The distribution of CRH in the pituitary, adrenal, lung, liver, and kidney at different stages of foetal development, in the newborn, juvenile, and adult was characterized in tissue homogenates using radioimmunoassay. Each of these tissues contained CRH, but for most tissues the quantity of CRH differed according to the developmental stage. For instance, CRH was at its highest concentrations in the lung and adrenal during foetal development, which is consistent with its role in lung and adrenal development prior to birth. CRH levels in the pituitary were the highest of all the tissues and remained at a consistently high level throughout the life span of the baboon. It was not ascertained in this study whether CRH was synthesized in these tissues or stored in them, as CRH mRNA concentrations were not measured (27). The results of the Dotzler study were consistent with CRH-IR measurements made in adult human tissue samples (28), apart from the detection of CRH in the baboon kidney which was not found in human kidney. Similar to what was reported in baboon, the expression of CRH-R1 transcript has also been reported in cultured kidney cells (COS-7 cells) from the African green monkey (*Aethiops sabeusa*) (29). CRH-IR has also been detected in the pancreas of major vertebrate classes including primates (rhesus monkey) (30).

3.3. CRH receptor subtypes and CRH-binding protein (CRH-BP)

CRH receptors belong to the family of seven-transmembrane G-protein coupled receptors, and are positively linked to adenylate cyclase. Two different CRH receptors (R1 and R2) have been cloned in humans (31), and both receptor subtypes have distinct distributions in the CNS and periphery. In rhesus monkeys (*Macaca mulatta*), CRH-R1 mRNA in brain was found primarily in the pituitary, cerebral cortex (prefrontal, striate, and cingulate cortices), hippocampus (dentate gyrus, presubiculum, and entorhinal cortex), claustrum, amygdala (central, medial, lateral, paralaminar, accessory basal and basal nuclei), hypothalamus (SON, PVN, mamillary bodies, ventromedial nucleus), thalamus (medial dorsal and thalamic PV nuclei, lateral geniculate body), brainstem (locus coeruleus, nucleus of the solitary tract), inferior olivary nucleus) and cerebellum (granular layer (see Table 1 in (21)). A 2004 study conducted in rhesus monkeys determined the distribution of CRH-R1 receptors in primate brain using immunohistochemistry (32). Anti-CRH-R1 immuno-reactivity was concentrated in the pituitary, cerebellum, and areas of the brainstem that are associated with sensorimotor processing. CRH-R1-positive cell bodies and dendrites were also located in the cerebral cortex, basal forebrain, some parts of the basal ganglia and in the thalamus. However, the antiserum used in this study was unable to confirm the presence of CRH-R1 in hippocampus, amygdala, or prefrontal, cingulate or entorhinal cortices as had been previously identified in rhesus monkey brain (21), due possibly to slight differences in the isoform of the receptor in these tissues and the specificity of the antiserum.

CRH-R2 receptors share approximately 70% homology with CRH-R1 receptors, and exist in four known

isoforms: CRH-R2alpha (rat: (33)), CRH-R2alpha-tr (rat: (34)), CRH-R2β (rat and mouse: (35)), and CRH-R2delta (human: (36)). CRH-R2alpha receptors are expressed in the lateral septum, as well as the paraventricular and ventral medial nuclei of the hypothalamus (33). In the rat, the CRH-R2alpha-tr receptor subtype, a shortened form of CRH-R2alpha, has a somewhat different distribution in brain (34). The CRH-R2β receptor is located more peripherally in heart and skeletal muscle, in cerebral arterioles and in choroid plexus in rodent brain (35). Finally, mRNA for the CRH-R2delta receptor-subtype has been identified in human amygdala and hippocampus but possibly does not appear to exist in the rat (36). Sanchez and colleagues (1999) have described the distribution of CRH-R2 receptor binding sites in rhesus monkey (*Macaca mulatta*) brain. The binding pattern was distinct from the distribution described for CRH-R1 (above), with relatively scant R2-subtype binding in the pituitary, cerebral cortex, hippocampus, amygdala, thalamus, and cerebellum. However, there was prominent CRH-R2 binding in the hypothalamus (SON, PVN, mamillary bodies and VMH), choroid plexus, lateral septal nucleus, nucleus of the stria terminalis, and in some brainstem nuclei (prepositus and raphe) (21).

CRH binds with high affinity to CRH-R1 receptors, but may have a lower affinity for CRH-R2 receptors. However, the CRH-like peptide, urocortin, does have high affinity for the CRH-R2 receptor subtype, which suggests that urocortin may be the endogenous ligand for this receptor. Both CRH and urocortin have a higher affinity for CRH-binding protein (CRH-BP) than for CRH receptors (25). CRH-BP, a 37-kDa protein comprised of 322 amino acids, has been identified in the plasma of pregnant humans, monkeys and apes (37). CRH-BP is expressed in the brain and pituitary gland of several species including rat and human (38) and is thought to regulate the HPA and extrahypothalamic actions of CRH and urocortin. CRH-BP may play a particularly important role in regulating the actions of placental CRH secreted during primate pregnancy (discussed below).

4. EFFECTS OF CRH ON FERTILITY

4.1. CRH and sex steroids

The presence of gonadal sex steroids has been shown to have a modulatory effect on the secretion of anterior pituitary hormones, as indicated by the sex differences measured in prolactin (PRL), thyroid stimulating hormone (TSH), growth hormone (GH) and luteinizing hormone (LH) secretion in humans (e.g. (39)). Pituitary secretion of ACTH in the cynomolgus monkey (*Macaca fascicularis*) has been examined to determine the effects of sex and sex steroid environment (40). A combination of hypothalamic-releasing peptides, including oCRH, was administered i.v. to male and female cynomolgus monkeys. Female monkeys were divided into three groups; cycling normally, or treated with either orally administered contraceptive steroids or intravaginally administered contraceptive steroids. The ACTH response measured after the administration of the hypothalamic-releasing peptide cocktail was greater in the females receiving intravaginal contraceptive hormones than in the male or other female groups, whereas the subsequent cortisol response was greater in the oral contraceptive-treated females than in the males. Basal cortisol levels in the oral contraceptive group were elevated relative to the other groups, which implied that sex steroids may have increased pituitary-adrenal responsiveness to the effects of CRH (40). However, caution is needed in the interpretation of these results since the blood samples were obtained following capture and subsequent sedation with ketamine; the effects of these procedures on the outcome measures were not evaluated.

A study that has implicated the HPA axis in the ovulatory mid-cycle LH surge examined changes in the hypothalamic and anterior pituitary content of CRH, ACTH, and beta-endorphin, as well as plasma concentrations of ACTH, beta-endorphin and cortisol, during the 17beta estradiol benzoate-induced LH surge in ovariectomized cynomolgus monkeys (41). Initially, the 17beta estradiol benzoate injection suppressed LH secretion through a negative feedback mechanism. However after 36 h, there was a switch to positive feedback that caused a large increase in LH secretion that peaked at 60 h. During the inhibition and subsequent surge in LH levels, there was a fall in the hypothalamic content of beta-endorphin and CRH with a corresponding increase in plasma levels of cortisol, ACTH and beta-endorphin at 30 and 48 h. This demonstrated a possible activation of the HPA axis during the negative and positive feedback stages of the 17beta estradiol benzoate-induced LH surge in ovariectomized cynomolgus monkeys (41). The reduction in hypothalamic beta-endorphin content at 30 h (and the corresponding increase in plasma beta-endorphin levels) is consistent with the inhibitory action of beta-endorphin on LH in ovariectomized rhesus monkeys (42). The recovery of pituitary CRH content at 72 h to levels exceeding those measured at baseline was correlated with the post-surge decrease in LH levels, consistent with the inhibitory effects of CRH infusion on LH pulsatility in ovariectomized rhesus monkeys (43).

The effects of ovarian steroids on mRNA levels of both CRH and AVP in the PVN and SON of the monkey hypothalamus have been investigated (44). Ovariectomized rhesus monkeys (*Macaca mulatta*) with exogenous sex steroid-simulated menstrual cycle phases were used and *in situ* hybridization histochemistry was done to label CRH and AVP mRNA in the hypothalamus. CRH mRNA levels in the PVN were higher during the simulated follicular phase than during either the simulated luteal phase or in the absence of sex steroid treatment. AVP mRNA levels were unaffected by sex steroid condition. The change in CRH mRNA levels is consistent with the observation that restraint stress-induced activation of the HPA axis in association with decreases in LH secretion are observed during the follicular (oestrogen-dominated) but not the luteal phases of the menstrual cycle (45). It has been suggested that oestrogen stimulates CRH gene transcription, increasing CRH synthesis and release either basally (44) or in response to a stressor such as the restraint stress (45), although Roy and colleagues (1999)

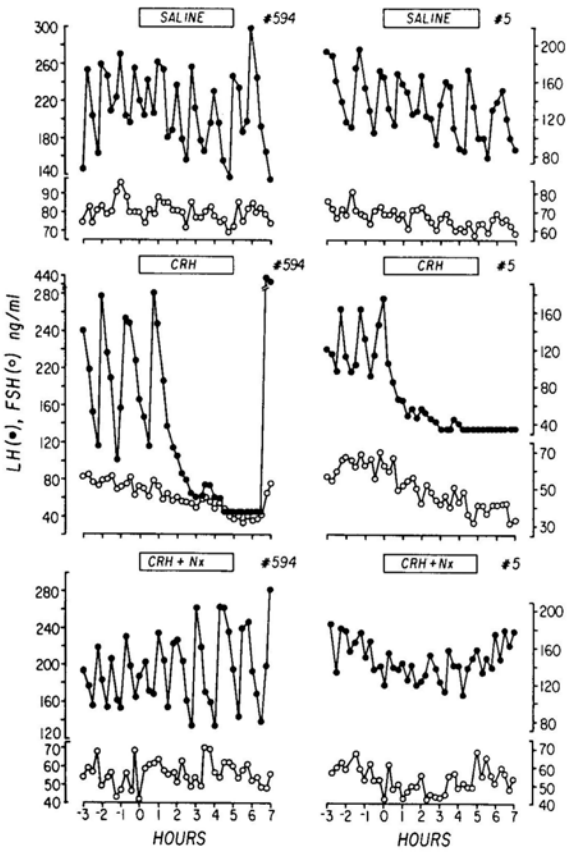

Figure 2. The effects of a 5 h infusion of physiological saline, CRH, and CRH plus naloxone (Nx) in two ovariectomized rhesus monkeys. From P. R. Gindoff & M. Ferin: Endogenous opioid peptides modulate the effect of corticotropin-releasing factor on gonadotropin release in the primate. Reproduced with permission from Endocrinology (42).

did not report pituitary-adrenal hormone concentrations in plasma (44).

A more recent study, published in 2003, examined the effects of estradiol and tamoxifen, an oestrogen receptor antagonist, on CRH-stimulated ACTH and cortisol secretion (46). Ovariectomized rhesus monkeys were treated with placebo, estradiol, tamoxifen, or with both estradiol and tamoxifen. Estradiol treatment facilitated basal and CRH-stimulated ACTH and cortisol secretion. Tamoxifen administered with estradiol antagonized both of these effects and also attenuated basal and CRH-stimulated ACTH and cortisol levels so that they were lower than even the placebo group; the tamoxifen-only group gave the same results. These data illustrate the importance of the neuromodulatory effects of estradiol in HPA axis function (46).

4.2. CRH and the effects of stress on fertility

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are described as gonadotropins because they stimulate the gonads - the testes in males, and the ovaries in females – resulting in the secretion of sex steroids. Both hormones are secreted from gonadotrophs in the anterior pituitary. Although neither hormone is necessary for life, both are essential for reproduction. In females, FSH stimulates the maturation of ovarian follicles, while ovulation is induced by a large burst of LH secretion known as the preovulatory LH surge. Stress is known to inhibit gonadotropin secretion in both rodents and primates (47).

The effects of exogenous oCRH and h/rCRH on gonadotropin release have been studied in ovariectomized rhesus monkeys (43). CRH was administered either as a single injection or as an infusion and LH, FSH and cortisol secretion were measured. Intravenous administration of either oCRH or h/rCRH rapidly inhibited LH and FSH secretion, implicating CRH as an inhibitory modulator of gonadotropin release (43). The mechanism for this effect was investigated in a subsequent study by Gindoff and Ferin (1987), who examined the role of endogenous opioid peptides in modulating the inhibitory effects of CRH on gonadotropic hormone release from the pituitary. Ovariectomized monkeys were sedated with ketamine and chair-restrained on the evening prior to testing. A bolus injection of h/rCRH was administered at the start of the observation period followed by a five hour infusion of h/rCRH. This was done in the presence or absence of pre-treatment with the opioid antagonist, naloxone, an infusion of which commenced 5-10 min prior to the h/rCRH infusion. Naloxone pre-treatment prevented the inhibitory effect of CRH on LH and FSH secretion, suggesting that endogenous opioid peptides such as beta-endorphin, released from the pituitary gland along with ACTH and other POMC-derived hormones, mediate the inhibitory effect of CRH on gonadotropin release (Figure 2) (42). To further investigate the role of endogenous opioids in the modulation of LH and FSH secretion, ovariectomized rhesus monkeys were pre-treated with a five day infusion of the synthetic glucocorticoid, dexamethasone (48). Dexamethasone treatment suppresses subsequent HPA axis activation via negative feedback inhibition, attenuating the release of CRH, ACTH, endogenous opioids, and cortisol. The dexamethasone infusion completely blocked the inhibitory effects of CRH on LH and FSH secretion. However treatment with the opioid agonist, morphine, administered as an i.v. bolus injection, restored the inhibitory effect of CRH on gonadotropin release. Co-administration of exogenous gonadotropin-releasing hormone (GnRH) reversed the inhibitory effect of morphine, restoring LH secretion to normal levels in monkeys treated with dexamethasone, morphine and CRH (48). There were several important conclusions drawn from this study. Negative feedback by glucocorticoids such as dexamethasone appears to inhibit the release of endogenous opioids as well as ACTH, rendering them insensitive to the stimulatory effects of subsequently administered CRH. This study also suggests that the effects of endogenous opioids on gonadotropin release may involve the centrally mediated inhibition of GnRH release, since the inhibitory effect of morphine on LH secretion was reversed by the administration of exogenous GnRH (48). This is consistent with an earlier study by Dubey and Plant (1985), in which it was reported that gonadotropin release

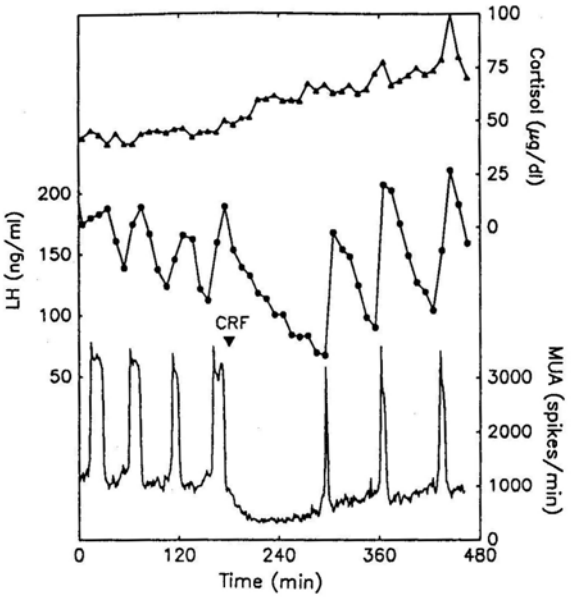

Figure 3. Effects of a single i.v. injection of CRH (200 μg, at arrow) on hypothalamic multiunit activity (MUA) as well as on serum levels of LH and cortisol in an ovariectomized rhesus monkey. From C. L. Williams, M. Nishihara, J.-C. Thalabard, P. M. Grosser, J. Hotchkiss & E. Knobil: Corticotropin-releasing factor and gonadotropin-releasing hormone pulse generator activity in the rhesus monkey. Electrophysiological studies. Reproduced with permission from Neuroendocrinology (50).

in orchidectomized rhesus monkeys could be inhibited by chronic treatment with exogenous hydrocortisone and temporarily restored following i.v. administration of GnRH (49).

A different experimental approach that was used to identify the neural location of the CRH-related inhibition of GnRH pulse generator was to measure neuronal firing rates, recorded as 'volleys' of increased multiunit activity (MUA). Surgical implantation of bilateral electrodes in the mediobasal hypothalamus of ovariectomized rhesus monkeys provided immediate feedback concerning the generation of GnRH pulses, as the increases in MUA were temporally correlated with increases in plasma LH concentrations (50). Williams and colleagues found that the administration of CRH resulted in a decrease in the frequency and duration of MUA, which corresponded to an inhibition of LH secretion (Figure 3). This effect was not blocked by pre-treatment with metyrapone, a glucocorticoid synthesis inhibitor, which suggested that increased glucocorticoid levels do not contribute to the inhibition of MUA and LH secretion under these circumstances. Pretreatment with the opioid antagonist naloxone prior to the CRH infusion blocked the inhibitory effect of CRH on MUA volley frequency but not the inhibitory effect of CRH on volley duration, implying that these two attributes of the GnRH pulse generator are independently regulated. However the physiological significance of this is unknown since there was no statistically significant relationship between volley duration and LH pulse characteristics (50), although refer also to (51).

Other intermediate neurotransmitters have also been investigated. In an attempt to determine whether CRH may have been producing its effect on LH secretion via a dopaminergic mechanism, Thind and Goldsmith (1989) examined the hypothalamic tissue of infant cynomolgus monkeys (*Macaca fascicularis*). After immunostaining for tyrosine hydroxylase (TH, the rate-limiting step in the synthesis of catecholamines; used to identify DA neurons) and CRH, they were able to demonstrate that CRH neurons were indeed presynaptic to DA (TH-stained) neurons in the paraventricular nucleus (PVN) of the hypothalamus (52). Another neurotransmitter, *N*-methyl-D,L,-aspartate (NMDA), has been shown to stimulate the release of gonadotropins in the adult female rhesus monkey following its i.v. administration (53). Conversely, in ovariectomized rhesus monkeys, NMDA inhibits LH secretion and pulse amplitude (54). Prior administration of a single i.c.v. dose of CRH antiserum blocked the inhibitory effect of NMDA on LH, suggesting that CRH may play a role in this effect (54). This also suggests that gonadotropin release is heavily influenced by the presence of sex steroids in female rhesus monkeys.

The role of the adrenal glands in the inhibitory effect of CRH on gonadotropin release has also been investigated in the rhesus monkey (*Macaca mulatta*) (55). Ovariectomized rhesus monkeys that also underwent adrenalectomy were maintained with hydrocortisone replacement therapy, with different doses of hydrocortisone (0, 5 and 10 mg daily) substituted on a sub-chronic basis (3-7 days) in order to evaluate the importance of glucocorticoid secretion in the inhibitory effects of CRH on LH and FSH secretion. The infusion of CRH inhibited gonadotropin release in the 0 and 5 mg hydrocortisone monkeys, indicating that this inhibitory effect does not require the presence of adrenal steroids (55) or, as previously reported, ACTH (56). However the highest dose, 10 mg hydrocortisone, blocked the inhibitory effect of CRH on FSH and LH, suggesting that this dose was sufficient to exert negative feedback inhibition similar to what has been reported in dexamethasone-treated monkeys (48). It was suggested that this apparently protective effect of glucocorticoids on gonadotropin release may be peculiar to situations involving chronic activation of the HPA axis. This is consistent with the lack of glucocorticoid involvement in the inhibition of gonadotropins observed when glucocorticoid synthesis was pharmacologically inhibited with metyrapone (57) or in the presence of acute CRH infusions in adrenal-intact monkeys (42, 47).

It is noteworthy that the effects reported here are apparently unique to ovariectomized rhesus monkeys. It appears that one effect of ovariectomy is to introduce a resistance of the GnRH pulse generator to the inhibitory effects of stress. LH pulses are not as easily measured in chair-restrained intact females, probably due to the effects of negative feedback from ovarian steroids, but also due to the inhibitory effects of stress on gonadotropin release,

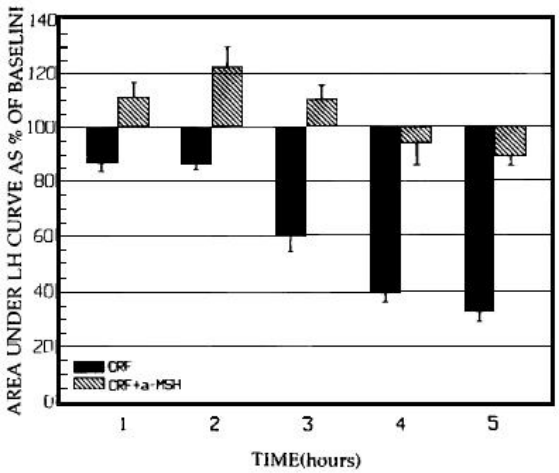

Figure 4. Effect of an infusion of CRH compared to CRH plus alpha-MSH on LH secretion in four ovariectomized monkeys. Shown are mean ±SEM hourly areas under the LH curve (expressed as a percentage of the 3-h morning control baseline) during the 5-h i.c.v. infusion of CRH alone (15 μg/h) or in combination with alpha-MSH (60 μg/h). The inhibition of LH by CRH was prevented by alpha-MSH. From E. Shalts, Y.-J. Feng, M. Ferin & S. L. Wardlaw: (alpha)-Melanocyte-stimulating hormone antagonizes the neuroendocrine effects of corticotropin-releasing factor and interleukin-1(alpha) in the primate. Reproduced with permission from Endocrinology (62).

evident even in monkeys that are apparently habituated to chair restraint (58, 59). In the study by Van Vugt and colleagues in intact female rhesus monkeys (1983), LH pulses in unrestrained or ketamine-sedated monkeys were increased two to three-fold following the administration of the opioid antagonist naloxone; however naloxone had no effect on LH pulses in chair-restrained monkeys. In addition, the insulin stress-induced inhibition of LH in intact females was not reliably blocked by pre-treatment with naloxone (58). The effectiveness of naloxone has been shown to vary across the menstrual cycle, being most effective during the luteal phase when endogenous opioid peptide secretion is most active. Thus a role for the endogenous opioids as a mediator of stress-induced gonadotropin inhibition is not ruled out, but may depend on the conditions under which LH inhibition is observed (58).

One of the experimental constraints when studying the CRH-induced inhibition of LH in rhesus monkeys is the requirement that monkeys be chair-restrained, often overnight, prior to testing. This is likely to result in stress-induced and activation of the HPA axis, even in monkeys that are behaviourally habituated to this procedure. A study that addressed this limitation used unrestrained intact female monkeys and measured the effect of an i.v. CRH infusion on LH secretion using remote blood sampling (60). Cortisol levels measured prior to CRH administration were reportedly less than half of the baseline levels measured in chair-restrained monkeys. The administration of CRH to monkeys during the follicular and luteal phases of the estrus cycle did not affect the LH secretory pattern. Similar to conclusions from the earlier study in intact male monkeys by Norman (1993) described below (61), CRH alone did not appear to substitute for the physiological effects of a stressor, nor was CRH sufficient to inhibit LH secretion in intact female monkeys (60).

Following the interest in the regulatory role of endogenous opioids such as beta-endorphin, another pro-opiomelanocortin (POMC)-derived peptide, alpha-melanocyte stimulating hormone (alpha-MSH) was investigated to determine its role in mediating the CRH-induced inhibition of gonadotropin release (62). In an earlier study in the rat, alpha-MSH reportedly reversed both the beta-endorphin-induced decrease in LH as well as the increase in prolactin (PRL) secretion (63). When i.v. CRH and i.c.v. alpha-MSH infusions were co-administered to chair-restrained ovariectomized rhesus monkeys, alpha-MSH blocked the inhibitory effect of CRH on LH pulse frequency and the total amount of LH secreted (estimated by area under the curve). However, alpha-MSH did not affect basal LH secretion measured during saline infusion (Figure 4). Neither did alpha-MSH treatment affect the increase in cortisol following CRH infusion (62). The authors concluded that alpha-MSH antagonized the inhibitory effect of beta-endorphin on LH secretion. Since alpha-MSH and beta-endorphin are both derived from the same pre-cursor (POMC) yet have opposing effects, this suggests that their release may differ with respect to time and/or neural location.

Arginine vasopressin (AVP), like CRH, stimulates the release of ACTH from the anterior pituitary. Intracerebroventricular administration of either AVP or CRH inhibits the release of LH in ovariectomized rhesus monkeys (43). Similar to the studies using CRH, the effects of AVP on LH secretion were blocked by prior administration of naloxone, indicating that this inhibitory effect is mediated by endogenous opioids. However, i.c.v. administration of a CRH antagonist blocked the effect of AVP on LH, implying that endogenous CRH receptor activity is required for this effect of exogenous AVP to occur (64).

As mentioned previously (58), the secretion of LH may also be inhibited by insulin-induced hypoglycaemia, a physiological stressor that results in activation of the HPA axis (65). A study to investigate the roles of CRH and AVP in the inhibition of LH secretion during hypoglycaemia was carried out in ovariectomized rhesus monkeys. The monkeys, in which mediobasal hypothalamic recording electrodes had been surgically placed in order to record increases in multiunit activity (MUA – described above (50)), were given an i.c.v. injection of a CRH antagonist ([D-Phe^{12},Nle^{21,38},CalphaMeLeu^{37}]rat CRH-(12-41))or AVP antagonist ([deamino-Pen^{1},*O*-Me-Tyr^{2},Arg^{8}]vasopressin) prior to insulin administration (51). The centrally administered AVP antagonist did not block the insulin-induced hypoglycaemia inhibition of GnRH release, whereas the centrally administered CRH antagonist delayed the interruption to the MUA volleys caused by insulin administration. However, CRH antagonist administration did not prevent the decrease in the duration of the MUA

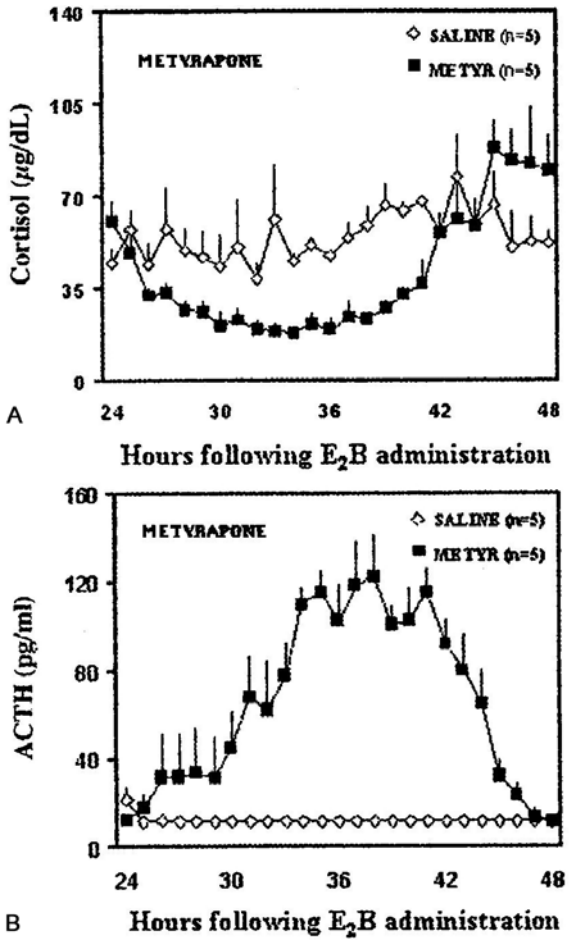

Figure 5. Effect of metyrapone (METYR) on circulating cortisol and ACTH concentrations. Mean responses (±SEM) in five ovariectomized rhesus monkeys given a 10-h infusion of either saline or metyrapone (5 mg/kg/h) starting at t=24 h are shown. A) Cortisol decreased following metyrapone, with levels remaining below baseline and saline-treated controls for 18 h ($P < 0.001$). B) Administration of metyrapone significantly increased ACTH ($P<0.001$) relative to baseline and saline-treated controls from 30 to 43 h following E_2B administration. From M. E. Lujan, P. J. MacTavish, A. A. Krzemien, M. W. Bradstock & D. A. Van Vugt: Estrogen-induced gonadotropin surge in rhesus monkeys is not inhibited by cortisol synthesis inhibition or hypoglycemia. Reproduced with permission from Endocrine, Humana Press (66).

volleys, the decrease in LH levels, or the increase in cortisol levels, implying that CRH was unlikely to be the sole mediator of the hypoglycaemia-induced inhibition of GnRH pulse generator activity. An alternative explanation that was suggested is that insulin-induced hypoglycaemia may have non-specific inhibitory effects on neuronal activity as indicated by the marked somnolence seen in the monkeys, as well as decreases that were measured in baseline hypothalamic electrical activity (51). In another study using ovariectomized rhesus monkeys, insulin treatment resulted in an increase in ACTH and cortisol secretion and a decrease in LH secretion that was not prevented by naloxone pre-treatment. However both the inhibition of LH and the stimulation of ACTH were prevented by prior treatment with the benzodiazepine, alprazolam. Alprazolam did not affect the fall in glucose levels following the administration of insulin. These results indicate that the effects of hypoglycaemia on LH secretion are not mediated via endogenous opioids, although a role for CRH in this effect was not conclusively demonstrated (65).

A more recent study (2002) evaluated the effects of hypoglycaemic stress on the LH and FSH surges that precede ovulation (66). In this study, the HPA axis was stimulated using either insulin-induced hypoglycaemia or by inhibiting cortisol synthesis using metyrapone. These treatments were administered 24 h after a challenge with estradiol benzoate (which results in an LH surge beginning 36 h after injection; Figure 5). Hypoglycaemic stress did not affect the LH and FSH surges. However, metyrapone treatment actually advanced the onset of the LH and FSH surges compared with the saline (unstressed) controls (Figure 6), perhaps due to the increased progesterone secretion measured in the metyrapone-treated animals (66).

Individual differences with respect to stress sensitivity during the menstrual cycle have been linked to endogenous serotonin activity. In a study using normally cycling cynomolgus monkeys, the monkeys' resistance to the effects of stress on fertility was categorized by using changes in their menstrual cycles in response to a regimen of mild psychological stress (cage relocation), mild diet stress (a 20% reduction in caloric intake) and moderate exercise (running on a treadmill at 80% of an individual's maximum speed). The monkeys were classified as highly stress resistant (HSR; maintained normal cycling during two stress cycles), moderately stress resistant (MSR; one normal cycle and one anovulatory cycle) and stress sensitive (SS; two anovulatory cycles). After a stress-free cycle, the serotonergic activity level for each monkey was determined prior to day 5 of the follicular phase of their menstrual cycle. Animals were examined under anaesthesia, which was maintained using i.v. propofol. Fenfluramine, a serotonin releasing agent, was administered and blood was sampled for the measurement of prolactin (an estimate of serotonergic activity) and cortisol. The HSR group had significantly higher prolactin levels than the other groups, and the SS group had significantly higher cortisol levels following fenfluramine challenge. Each group showed the same cortisol response following a CRH challenge. These results suggest that animals which were sensitive to the disruptive effects of stress on the menstrual cycle (MSR and SS groups) had lower functional capacity in their serotonin systems, as their prolactin response to fenfluramine challenge was lower than for the HSR group. The larger cortisol response to fenfluramine challenge may indicate that the HPA axis in the SS group was super-sensitive to serotonergic stimulation, since the cortisol responses of all groups to CRH challenge were similar (67).

The effects of CRH and glucocorticoids on male fertility have also been studied in nonhuman primates. A

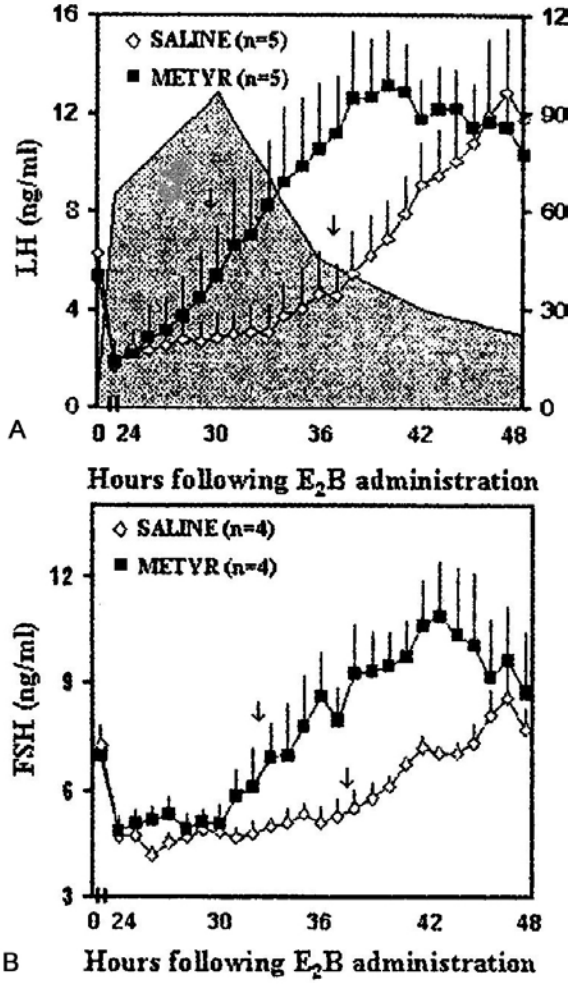

Figure 6. Effect of metyrapone (METYR) treatment on the E_2B-induced LH and FSH surge. A) The mean LH response (±SEM) in five ovariectomized rhesus monkeys given an oestrogen challenge at t=0 h and infused with saline or metyrapone (5 mg/kg/h) from 24 to 34 h is shown. The plasma estradiol levels achieved in control animals following administration of estradiol benzoate are represented by the shaded area. LH surges were detected in all monkeys under both experimental conditions. Metyrapone treatment advanced the LH surge by 7.4 ± 0.4 h ($P < 0.001$). B) Data from four monkeys demonstrating FSH surges under both experimental conditions (one monkey did not exhibit an FSH surge). Metyrapone treatment advanced the FSH surge by 4.8 ± 1.4 h ($P = 0.04$) and time to peak FSH levels by 6.5 ± 1.8 h ($P = 0.03$). The average time of surge onset is denoted by the arrows. From M. E. Lujan, P. J. MacTavish, A. A. Krzemien, M. W. Bradstock & D. A. Van Vugt: Estrogen-induced gonadotropin surge in rhesus monkeys is not inhibited by cortisol synthesis inhibition or hypoglycemia. Reproduced with permission from Endocrine, Humana Press (66).

suppressive effect of chronic glucocorticoid administration on gonadotropin release has been observed in orchidectomized rhesus monkeys, as reported in a study done by Dubey and colleagues (1985). Following 3-4 weeks of hydrocortisone injections, the LH and FSH secretory patterns showed signs of inhibition, demonstrating an inhibitory effect of chronic glucocorticoid treatment on gonadotropin release (49). In free-ranging wild male baboons, it was noted that the process of capture and tranquilization suppressed LH secretion and produced a corresponding increase in the sensitivity of the testes to LH. A subsequent study demonstrated pharmacologically that this effect of stress on LH was not mediated by glucocorticoids or catecholamines in male baboons. However, as with ovariectomized female rhesus monkeys (42), the inhibitory effect of acute stress (or CRH administration in the case of the female monkeys) on LH secretion in male baboons was blocked by prior treatment with naloxone (47). Restraint stress has been shown to suppress LH and testosterone secretion in intact male rhesus monkeys (68). Simulating this stress using an infusion of CRH in unrestrained intact male rhesus monkeys did not result in a decrease in LH release, and in some cases may have stimulated LH secretion. CRH appeared to reduce the sensitivity of the testes to LH, and testosterone levels fell during the CRH infusion despite the lack of inhibition of LH. The administration of i.v. CRH did not simulate the physiological experience of stress on LH, implying that CRH is not the sole agent responsible for the inhibition of LH during stress in intact male rhesus monkeys (61).

5. EFFECTS OF CRH IN PREGNANCY AND PARTURITION

The HPA axis makes an important contribution to the timing of important stages in the growth and development of the foetus in a number of primate species. The activity of the maternal HPA axis is influenced by several factors, the first of which is the development of the placenta which serves as an endocrine organ during pregnancy. CRH is secreted by the placenta, as demonstrated by the presence of CRH mRNA in human, marmoset (37), gorilla and rhesus monkey placental tissue (69). *In situ* hybridization and immunocytochemistry have been used to identify the syncytiotrophoblast cells in rhesus monkey placenta as the source of CRH mRNA and CRH itself. However there was no evidence for CRH receptor mRNA in placenta, suggesting that the receptor targets of placental CRH are located in other tissues (70) such as the myometrium (muscular uterine wall) (71). It is noteworthy that although CRH is expressed only in the placentas of primates (69), CRH is not found in the maternal plasma of prosimians (37, 69). This suggests that CRH plays a role in the pregnancies of humans, apes and New and Old World monkeys but not in prosimians.

The plasma concentration of corticotrophin-releasing hormone (CRH) secreted by the placenta progressively rises during the primate pregnancy, although there is some variability betweens species. In humans, plasma CRH levels are not detectable until 20 weeks' gestation, with a precipitous rise in CRH beginning much later, at about six weeks prior to parturition (72). Similar to the human, the gorilla and chimpanzee both show an

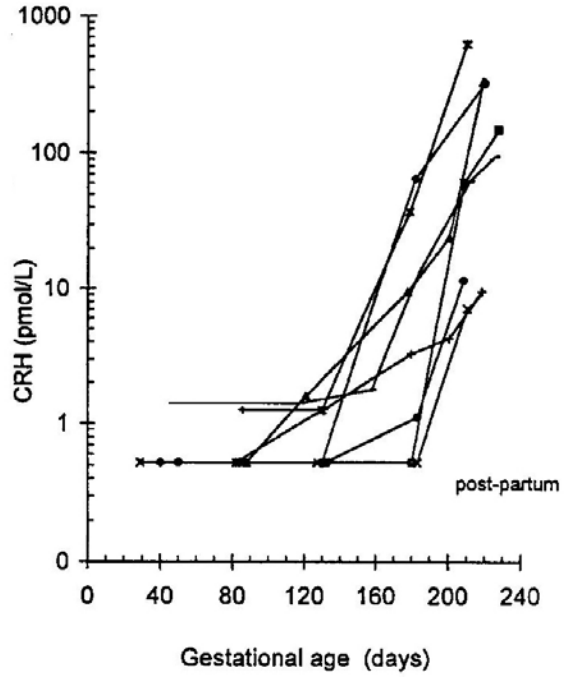

Figure 7. Plasma CRH-IR concentrations in individual pregnant and post-partum chimpanzees. Individual animals are represented by different symbols, with the lines joining the concentrations at various gestational ages of the same animal. From R. Smith, J. E. Wickings, M. E. Bowman, A. Belleoud, G. Dubreuil, J. J. Davies & G. Madsen: Corticotropin-releasing hormone in chimpanzee and gorilla pregnancies. Reproduced with permission from J. Clin. Endocrinol. Metab, The Endocrine Society (73).

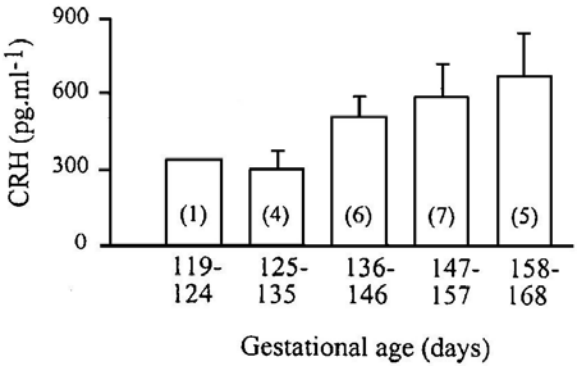

Figure 8. Maternal plasma CRH concentrations (mean ± SEM) in pregnant rhesus monkeys in late gestation. The number of different animals for which samples were averaged in each group is shown in parenthesis. From D. A. Giussani, J. A. Winter, S. L. Jenkins, J. D. Tame, L. M. Abrams, Y. Ding X.- & P. W. Nathanielsz: Changes in foetal plasma corticotropin-releasing hormone during androstenedione-induced labor in the rhesus monkey: Lack of an effect on the foetal hypothalamo-pituitary-adrenal axis. Reproduced with permission from Endocrinology, The Endocrine Society (74).

exponential rise in maternal plasma CRH (Figure 7) (73). The rhesus monkey (*Macaca mulatta*) shows a more gradual increase in maternal plasma CRH levels during the 30 days prior to parturition (Figure 8) (74). This placental release of CRH in these primate species results in an increase in cortisol secretion from the maternal adrenal glands as term approaches. Although cortisol inhibits CRH release from the maternal hypothalamus, it stimulates the expression of the CRH gene in the placenta to further increase CRH secretion.

In contrast with human, gorilla, chimpanzee and rhesus monkey, CRH was detectable in the maternal plasma of the baboon (*Papio hamadryas*) as early as 30 days of gestation (term is 184 days). The levels of CRH continued to rise until day 44 and remained elevated until term, returning to undetectable non-pregnant levels within 24 h of parturition (75, 76). However the pregnant baboon does not have the elevated maternal HPA axis activity that might be expected in response to the increase in plasma CRH levels (77). When CRH stimulation tests were performed in pregnant female baboons, the maternal ACTH and cortisol responses were blunted relative to levels measured in non-pregnant females, indicating that the presence of placental CRH attenuates maternal pituitary-adrenal axis function (77). When another corticotropin-releasing agent, arginine vasopressin (AVP), was administered, pregnant baboons showed a larger ACTH and similar or larger cortisol response to AVP than did non-pregnant females. This heightened sensitivity to AVP was shown to develop progressively through the pregnancy, returning to normal levels soon after parturition, as measured in a single baboon (78). These studies suggest that the pituitary-adrenal axis of the pregnant baboon is less sensitive to CRH and more sensitive to AVP when circulating levels of placental CRH are elevated. Finally, the baboon does not appear to have a high affinity CRH-binding protein (CRH-BP) to regulate the bioactivity of placental CRH (also described below), suggesting important differences between the baboon and other primates in their mechanism of labour (76).

The responsiveness of the foetal HPA axis has also been studied. Mid-gestation baboon foetuses were tested *in utero* by the administration of a bolus injection of saline or oCRH via the foetal carotid artery. Foetal blood samples were obtained for the measurement of ACTH. Foetal ACTH remained constant following saline injection, but increased significantly following oCRH injection. However, following injection into the foetal antecubital vein (which circulates foetal blood to the placenta), oCRH did not increase ACTH levels, indicating that the increase in ACTH was due to the action of oCRH on the foetal pituitary gland rather than on the placenta (79). A 2003 study in preterm baboons that was conducted at 125 days' gestation examined the effects of ACTH and CRH administration on cortisol levels (80). ACTH infusion did not result in a significant rise in cortisol in the preterm baboons until 11 days after caesarean delivery. Administration of CRH produced significant increases in cortisol levels at 5 and 11 days after preterm birth, with much larger cortisol responses measured after CRH

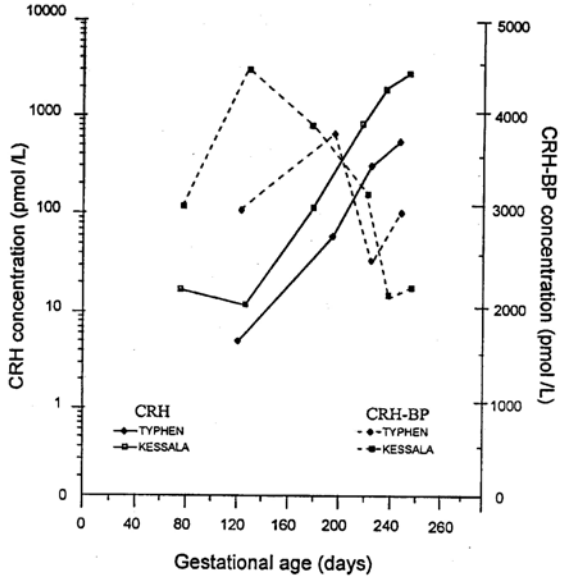

Figure 9. Plasma CRH-IR and CRH-BP concentrations in individual pregnant gorillas. Individual animals are represented by different symbols, with the lines joining the concentrations at various gestational ages of the same animal. From R. Smith, J. E. Wickings, M. E. Bowman, A. Belleoud, G. Dubreuil, J. J. Davies & G. Madsen: Corticotropin-releasing hormone in chimpanzee and gorilla pregnancies. Reproduced with permission from J. Clin. Endocrinol. Metab.,The Endocrine Society (73).

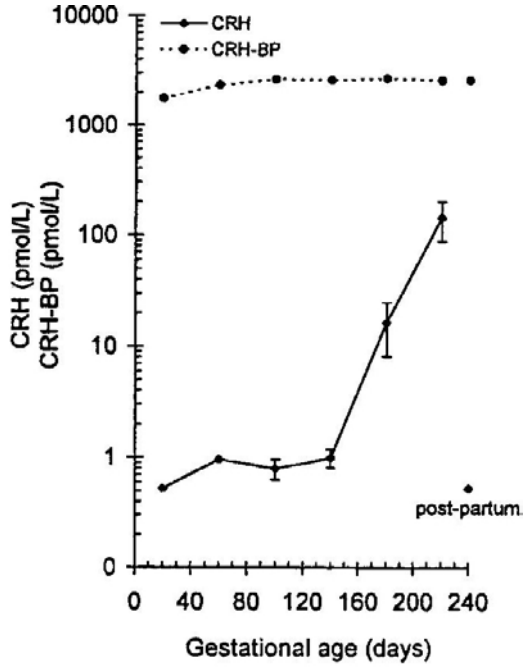

Figure 10. Mean plasma CRH and CRH-BP concentrations in pregnant and postpartum chimpanzees. From R. Smith, J. E. Wickings, M. E. Bowman, A. Belleoud, G. Dubreuil, J. J. Davies & G. Madsen: Corticotropin-releasing hormone in chimpanzee and gorilla pregnancies. Reproduced with permission from J. Clin. Endocrinol. Metab.,The Endocrine Society (73).

administration on day 12. This study demonstrates an apparent immaturity of the pituitary and adrenal glands in the preterm baboon that persists for several days following birth, underscoring the importance of the glucocorticoid therapies that are used to reduce the incidence of complications of preterm birth such as lung immaturity (80).

Prior to parturition, stimulation of the maternal HPA axis by placental CRH may be prevented by the presence of circulating CRH-BP. CRH-BP is present in excess and is capable of taking up a vast amount of CRH, thereby reducing its bioactivity (81). CRH-BP has been identified in human, gorilla (*Gorilla gorilla)* and chimpanzee (*Pan troglodytes*) (73), orang-utan (*Pongo pygmaeus*), gibbon (*Hyloblates lar*), squirrel monkey (*Saimiri boliviensis*), marmoset (*Callithrix jacchus*), and rhesus monkey maternal plasma (*Macaca mulatta*) (37). However, CRH-BP was absent in the baboon (*Papio hamadryas*) (76), spider monkey (*Ateles*), mandrill (*Mandrill sphinx*) and lemur (*Lemur catta* and *Eulemur macaco*) (37). During pregnancy, CRH-BP mRNA is expressed in the placenta, decidua, myometrium, and foetal membranes (82). During the latter stages of pregnancy in the rhesus monkey (83), the gorilla (Figure 9), and the human, the concentration of CRH-BP gradually decreases, which leads to an increase in the amount of bioactive CRH which occupies myometrial smooth muscle CRH receptors to facilitate uterine contractility (73). This inverse relationship between CRH-BP and CRH levels was not observed in the chimpanzee (Figure 10) (73).

The relationship between CRH and CRH-BP is thought to be critical in both normal and abnormal parturition. An abnormal ratio of CRH to CRH-BP has been linked with preterm labour and preeclampsia, both conditions being characterized by high circulating CRH levels and low CRH-BP levels (84), as well as with delayed onset to labour in which these ratios are reversed (81). The former changes may represent an adaptive response to external environmental stimuli that signal a need to hasten the maturation process of the foetus but may also threaten its survival.

It has been shown that the continuous i.v. infusion of testosterone precursor, androstenedione, can be administered to pregnant rhesus monkeys to initiate labour two to four days after the infusion is initiated (85). Androstenedione-induced labour has been shown to be accompanied by increases in placental CRH mRNA and CRH protein, similar to the concentrations measured at spontaneous term birth (70). The foetal plasma levels of CRH, ACTH and cortisol in umbilical cord blood have also been examined in groups of anaesthetized rhesus monkeys that were in spontaneous labour, androstenedione-induced labour or had not yet commenced labour (74). Foetal CRH was elevated to a similar extent in both spontaneous and induced labour relative to the non-labour group. Foetal ACTH and cortisol were only elevated in the spontaneous labour group. This study demonstrated that both spontaneous and induced labours trigger an increase in

foetal CRH levels, but that only spontaneous labour triggers the foetal HPA axis (74).

In the developing primate foetus, the adrenal gland is deficient in the enzyme DELTA^5-3*beta* hydroxysteroid dehydrogenase (3*beta*-HSD) that is required for cortisol production. In its absence, the primary product of adrenal steroid synthesis is DELTA^5-3*beta* hydroxysteroid dehydroepiandrosterone sulphate (DHEA-S), the precursor to the placental synthesis of oestrogen. An increase in foetal ACTH has been shown to increase the expression of 3*beta*-HSD which then stimulates cortisol synthesis. In a study to investigate the role of melatonin in foetal cortisol production, pregnant capuchin monkeys (*Cebus paella or Cebus albifrons*) were exposed to constant light during the last trimester of their pregnancy to suppress melatonin production. Half of the constant light animals were supplemented with oral melatonin daily. The constant light suppressed maternal melatonin levels, and cortisol levels of the infants whose mothers weren't supplemented with melatonin were doubled relative to the foetuses of both supplemented and normal light/dark cycle mothers, suggesting an inhibitory role for melatonin in foetal cortisol production (86). In support of this finding, the same study also reported that melatonin inhibited the effects of ACTH on 3*beta*-HSD expression and thereby on cortisol production in the foetal capuchin monkey adrenal gland *in vitro* (86).

The importance of prostaglandins in the initiation and propagation of labour in nonhuman primates has been reviewed (87). When prostaglandin synthesis is inhibited, labour is delayed in humans (88) as well as in rhesus monkeys (89). It has been demonstrated in humans that cortisol, CRH, and interleukin-1β each increase prostaglandin levels by up-regulating prostaglandin H synthase (PGHS-2) and down-regulating prostaglandin dehydrogenase (PGDH) in foetal membranes (90). PGHS-2 can be induced by infection, glucocorticoids and growth factors (as reviewed in (90)). An examination of CRH, glucocorticoid receptor (GR), PGHS and PGDH immunoreactivity in rhesus monkey foetal membranes and decidua was undertaken following the initiation of spontaneous labour, infection-induced preterm labour and in gestational age-matched controls (91). CRH, GR, PGHS and PGDH-IR were all detected in rhesus monkey foetal membranes and decidua, with immunostaining for PGDH (which inactivates prostaglandins) being somewhat reduced during spontaneous labour. Interuterine infection-induced labour resulted in a marked loss of cells that were immunoreactive for PGDH. Both of these changes in PGDH immunostaining would result in a net increase in prostaglandins, providing further support for the roles of CRH, glucocorticoids and prostaglandins in the labour process (91).

CRH and CRH receptors are present in human lung cancer cell lines, suggesting a role for CRH in lung development. The importance of glucocorticoids in foetal lung development has been clearly established. For example, a non-human primate study showed that glucocorticoid treatment during gestation accelerated lung development in foetal rhesus monkeys prior to preterm delivery (92). The role of CRH in lung development has also been investigated using baboon foetal lung explants. CRH-R1 receptor mRNA was detected in 30% of animals, starting at mid-gestation. The presence of other CRH receptor subtypes was not measured. The addition of CRH triggered an increase in mesenchymal cell proliferation in the baboon foetal lung explants, and was most potent in lung tissue obtained at mid-gestation, indicating that CRH as well as glucocorticoids play a role in lung development (93).

6. CRH AND EARLY LIFE STRESSORS

The prenatal administration of dexamethasone has been shown to result in dose-dependent pyramidal cell death in the CA regions and loss of granular fibres in the dentate gyrus of the immature foetal rhesus monkey (*Macaca mulatta*) hippocampus (94). This suggests a role of CRH in neurological development. The gestational age at which the exposure to high levels of CRH occurs appears to be an important determinant in whether preterm infants have significant neurological defects. This has been demonstrated in a study in which a mild psychological stressor was applied to pregnant rhesus monkeys (*Macaca mulatta*) during early and mid-late gestation (95). Infant monkeys of mothers that had been stressed early in gestation weighed less and had lower scores on measures of attention and neuromotor maturity than the late stress and non-stress groups; both stress conditions resulted in developmental deficits that weren't measured in the offspring of the control (maternally unstressed) animals.

Maternal separation and isolation are the most commonly investigated early life stressors in mammalian studies. The effect of exogenous CRH administration on the behavioural changes associated with maternal separation has been measured in infant rhesus monkeys (96). When h/rCRH (0, 0.5, 1.0, and 10 micrograms) was administered i.c.v. to infant monkeys immediately before they underwent maternal separation lasting one hour, there were no significant changes in distress vocalizations although there was a slight decrease in activity levels after treatment with 10 μg CRH (97). This CRH dose also had a number of physiological effects, decreasing body temperature, marginally reducing mean arterial blood pressure and significantly increasing plasma concentrations of ACTH and cortisol. In contrast to the effects of i.c.v. CRH administration, i.v. administration of CRH to infant rhesus monkeys did not produce any significant behavioural, physiological or endocrine changes (96). The results for i.c.v. CRH contrasted with to the behavioural changes observed in maternally-separated infant rhesus monkeys following anxiolytic treatment with the benzodiazepine, diazepam. Diazepam treatment resulted in decreased distress vocalizations, decreased secretion of ACTH and cortisol and increased activity levels. However, the effects of i.c.v. CRH treatment bore a greater similarity to the behavioural changes observed following anxiogenic treatment with the inverse benzodiazepine receptor agonist, β-carboline, which produced a significant increase in distress vocalizations and a decrease in activity levels (97).

Unlike the effects of i.c.v. CRH in unrestrained adult male monkeys (98), the administration of i.c.v. CRH to the infant monkeys did not produce huddling or lying down behaviour.

Differences in rearing experiences may lead to changes in brain, behaviour, and physiology in later life. In a study of early social deprivation, infant rhesus monkeys were either raised with their mother (first 6 months) and then gang-caged with peers, or separated from the mother at 3 days, raised in isolation until one month of age, and then singly housed within auditory and visual contact of other monkeys (99). The aim of the study was to investigate whether differences in early social environment led to differences in the regulation of neuroendocrine and autonomic responses by the hypothalamus. When the monkeys were 1.5 to 2.5 years of age, their hypothalami were sectioned and examined for differences in tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of catecholamines) and CRH-immunoreactivity. No differences were found in the distribution of TH-IR neurons, dendritic processes, fibres or terminals in the paraventricular and arcuate nuclei for social versus socially deprived animals. Similarly, no differences in CRH-IR were seen in the PVN of the social and socially deprived animals. The study concluded that hypothalamic CRH and catecholamine immunoreactivity were unaffected by early social deprivation (99). However, nursery rearing has been reported to change CRH receptor density in the basilateral nucleus of the amygdala relative to what is measured in mother-reared monkeys (100). This may explain some of the differences in HPA axis activity that have been measured in monkeys from different rearing conditions as these are likely to be influenced by neural projections from extrahypothalamic brain structures such as the amygdala. This is also consistent with the rearing-related changes in the CRH concentration measured in CSF that have been reported (described below (101)), as the CRH in CSF is likely to be extrahypothalamic in origin.

A study that examined the sensitivity of the HPA axis to negative feedback by glucocorticoids was conducted in adult squirrel monkeys (*Saimiri sciureus*) that had experienced either intermittent separation stress during their first 13-21 weeks of life, or had been raised by mothers under low or high demand food foraging conditions for the first 10-21 weeks of life (102). Monkeys were tested between 3.5 and 6 years of age with an injection of cortisol or saline 60 min prior to an i.v. injection of oCRH. Their sensitivity to negative feedback inhibition by exogenous cortisol was determined by measuring their ACTH response to the subsequent CRH injection. The ACTH response varied between the pre-natal stressor groups, with the group that had experienced intermittent separation being more sensitive to the inhibitory effects of glucocorticoids; the HPA axes of female monkeys were more sensitive to glucocorticoids than were those of males. There were no significant differences between the high and low demand foraging groups (102). These results suggest that not all types of early life stress affect endocrine mechanisms during development.

With respect to the extrahypothalamic CRH secretion of monkeys that had experienced early life stress, this was addressed in a study conducted in young adult bonnet macaques (*Macaca radiata*) each of which had been raised by its mother under one of three different foraging conditions (101). These three conditions were; consistently low demand, consistently high demand and variable (unpredictable) demand. The foraging conditions were applied from about 17 weeks of age for a period of 12 weeks [Footnote]. This study was designed to investigate the relationship between early-life foraging conditions (and its effects on the quality of maternal care) and CRH and cortisol levels in CSF in the monkeys as juveniles (2 years of age). Both the high and low foraging condition groups were similar, with lower CSF levels of CRH than the variable foraging demand group. Conversely, the cortisol levels in CSF were lowest in the variable foraging demand group. No measurements of HPA axis activity were done. These results indicate that a substantial period of unpredictable food availability early in life has long-term consequence on the endocrine system with respect to the basal release of stress hormones (101). This conclusion was supported by the findings of a follow-up study that was carried out when the monkeys were approximately 4.5 years of age (103). The CRH levels in CSF were still significantly higher in the variable demand foraging group compared with an *ad-libitum* feeding group, with significant positive correlations between CRH levels measured as juveniles and as young adults (103). Elevations in the CSF levels of CRH have also been reported in post-traumatic stress disorder in humans (104). Another study of the effects of the variable demand foraging condition as an early life stressor reported that bonnet macaques raised under the variable demand foraging condition showed hyperactive dopaminergic responses and hypoactive serotonergic responses (105). Both of these neurotransmitters have been reported to function abnormally in depressive and anxiety disorders. This model may be useful in the study of disorders affecting humans that have their origins in early life stress.

A 2002 study by the same research group (105) examined how the timing of early life stress affects subsequent neurological systems and behaviour (106). In this instance, the infant bonnet macaques (*Macaca radiata*) were introduced to the variable demand foraging condition at approximately 18 weeks of age. The variable demand foraging condition was initiated 8-10 weeks later than in the earlier study, and lasted for an additional 4 weeks. The control group had *ad libitum* access to food. CSF was sampled at between 2 and 2.5 years of age and analysed for CRH and the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA), the dopamine metabolite homovanillic acid (HVA) and the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG). The variable demand foraging condition monkeys had higher 5-HIAA and lower CRH levels in CSF relative to the *ad libitum* fed group, and there was a significant negative correlation between the levels of 5-HIAA and CRH. The lower CRH levels reported in this study (106) also contrast with the higher CRH levels reported in monkeys that had experienced the variable demand foraging condition at a younger age (101,

103). The behavioural responses of the two groups to a threatening object (a technician in a mask revealed for 30 s to group-housed animals), were categorized either as changes in gregariousness (sum of negative behaviours subtracted from the sum of positive behaviours) or as changes in hierarchical behaviour (initiation or receipt of threat, mounting, location displacement or aggressive chasing or biting). Baseline behaviour was more hierarchical in animals from the variable demand foraging condition, and positive correlations between CRH levels in CSF and changes in gregariousness were reported for the control animals only in the mask stress condition (106). The main finding from these studies was that a difference in timing of the same stressful event may have opposing effects on CRH levels in CSF, indicating age-dependent variability in the way that neuroadaptive responses to the same type of stress are expressed.

The quality of maternal care has also been demonstrated to affect physical growth, behavioural development and HPA axis sensitivity in the marmoset (*Callithrix jacchus*). Negative parenting behaviour was associated with smaller body size and lower weight at 10 and 20 weeks as well as abnormal social behaviour in the offspring. Their cortisol responses following administration of oCRH were blunted relative to the cortisol levels of monkeys that had been the recipients of positive rearing behaviours (107). The poorer growth outcomes may be related to the inhibition of growth hormone release under conditions of chronic HPA axis activation, such as reported in a study of bonnet macaques (*Macaca radiata*) raised under the variable demand foraging condition (which was likely to have resulted in a lower standard of maternal care). The mean growth hormone response following a clonidine (alpha-$_2$ adrenergic agonist) challenge was inversely proportional to the levels of CRH measured in CSF, suggesting that higher concentrations of extrahypothalamic CRH may suppress growth hormone secretion (108).

A recent report (2005) has taken a different approach to the study of adverse early rearing conditions by examining the effects of a more 'naturalistic' infant-maltreatment scenario – physical abuse by rhesus macaque mothers that were themselves physically abused as infants (109). Pregnant abusive mothers that had shown physically abusive behaviour towards their previous offspring, as well as being known or suspected to have been themselves physically abused as infants, were used in this study. Maternal CSF samples were obtained 4-8 weeks prior to parturition and again 3-4 weeks following parturition. Behavioural observation commenced 8 weeks prior to the estimated parturition date and continued weekly until the fourth post-partum week. Maternal CSF was analysed for CRH and serotonin (5-HIAA), dopamine (HVA) and norepinephrine metabolites (MHPG). The CSF concentrations of both CRH and 5-HIAA were significantly higher in the abusive mothers than in the matched control mothers. Although the CSF concentrations of each of the monoamine metabolites were positively correlated with each other; none were correlated with CSF levels of CRH. This contrasts with similar measures that were made in monkeys that experienced early life stress due to variable demand foraging conditions, in which negative correlations were measured between CSF concentrations of CRH and 5-HIAA and HVA (106, 110). Higher concentrations of monoamine metabolites were also correlated with anti-social behaviour, including maternal aggression and infant rejection (109). This study suggests the co-involvement of CRH and the monoamine neurotransmitters in the sequelae and propagation of intergenerational infant abuse in the rhesus monkey.

7. OTHER REGULATORY ROLES OF CRH

7.1. CRH and appetitive behaviour

Both physical and psychological stressors have been shown to affect feeding behaviour in humans as well as in animals. The effect of the stress on ingestive behaviour is dependent on the type of stressor that is used and, particularly in the case of humans, on individual differences. For instance, one study found that 44% of people reported that they increased eating in response to stress, while 48% decreased eating. Pathological over-eating (bulimia) and under-eating (anorexia) may each be precipitated or accompanied by stress (reviewed in (111)).

With respect to the effects of CRH on schedule-maintained behaviour, squirrel monkeys (*Macaca fascicularis*) that were trained on either a food-maintained or shock avoidance schedule were given a range of i.c.v. oCRH doses prior to operant testing. oCRH treatment had little or no effect on responding in either task across this dose range, apart from an initial increase in food-maintained responding at a low dose; this effect diminished with repeated testing and tolerance to this effect of CRH was assumed to have developed (112). Another study that examined the effects of i.c.v. CRH on food-maintained behaviour reached a rather different conclusion. This study was conducted in adult male rhesus monkeys (*Macaca mulatta*) and despite using a similar range of CRH doses as the previous study, a dose and time-dependent decrease in food-maintained behaviour was reported (113). This effect was not thought to be due to a perception of satiety, since administration of a CRH antagonist did not increase food-maintained responding. Rather, the pattern of responding resembled the effect of an aversive stimulus as demonstrated by a persistent reduction in food-maintained responding that lasted for up to 30 days, as well as reduced food consumption in the home cage during the first one to two weeks following as few as two successive administrations of i.c.v. 10 µg/kg CRH (Figure 11). During this time the monkeys' weight decreased by approximately 10% and they refused even preferred foods when they were offered during the first days after CRH administration was discontinued (113). This raises the possibility that the over activity of the HPA axis observed in eating disorders such as anorexia nervosa may contribute to a conditioned aversion to food. This effect of CRH was reportedly selective for food-maintained responding, since i.c.v. CRH injection of the same range of doses did not affect responding to terminate the delivery of a mild electric shock (Figure 12) (114). The levels of catecholamine metabolites (HVA, 5-HIAA) were elevated in CSF during

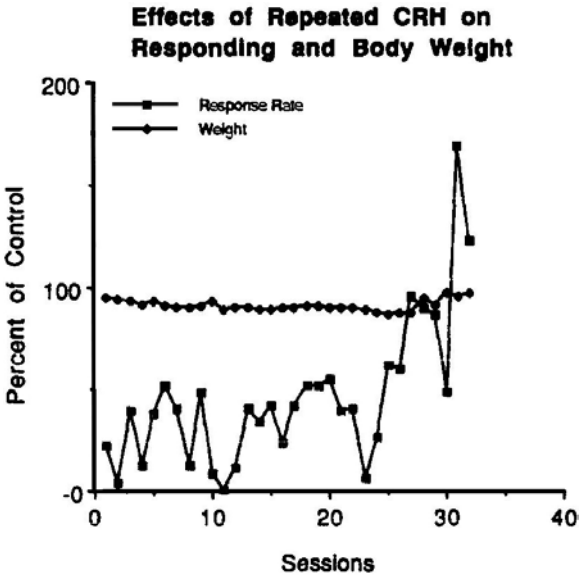

Figure 11. The effects of several repeated daily administrations of 10 µg/kg i.c.v CRH on responding and weight over the next 30 sessions in three monkeys responding under the FR30 TO schedule. Abscissa: Daily sessions. Ordinate: Response rate (□) and body weight (●) as percent of control. From J. R. Glowa & P. W. Gold, Corticotropin releasing hormone produces profound anorexigenic effects in the rhesus monkey. Reproduced with permission from Neuropeptides, Elsevier (113).

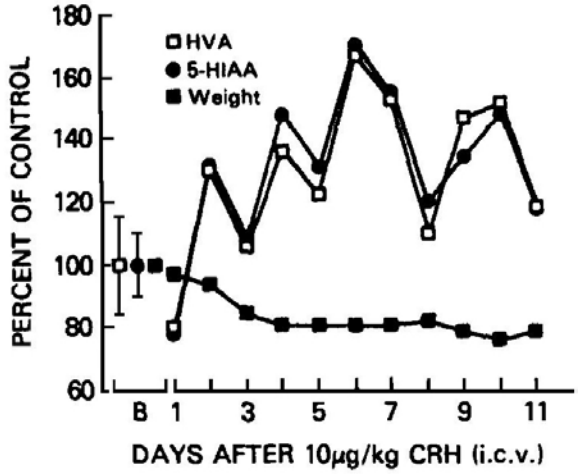

Figure 12. The effect of oCRH, over the 10 days subsequent to a single 10 µg/kg i.c.v. dose, on CSF levels of HVA, 5-HIAA and body weight in a rhesus monkey, as compared to pre-injection means (10 preceding days) of the same measures. From J. R. Glowa, J. E. Barrett, J. Russell & P. W. Gold, Effects of corticotropin releasing hormone on appetitive behaviors. Reproduced with permission from Peptides, Elsevier (115).

the days that i.c.v. CRH treatment attenuated eating behaviour; this effect on the dopamine and serotonin metabolites was not replicated when an equivalent amount of food was withheld (115).

The effects of corticotropins such as CRH and urocortin (which have similar receptor binding characteristics) on ingestive behaviours were studied more recently (2002) in the adult male baboon (*Papio hamadryas*) (116). The baboons were fitted with i.c.v. infusion devices and housed in metabolic cages for the duration of the study. CRH or urocortin was infused at a steady rate for 7 days at a time while food and water intake were recorded. During the time that CRH or urocortin were infused, food intake was reduced relative to the baseline week prior to the infusion. There was a rapid return of food intake to baseline levels once CRH treatment ceased. However, although the reduction in eating during the urocortin infusion was similar to the reduction observed with CRH, the intake of food during the week after cessation of the urocortin infusion remained below baseline levels. Water intake was not affected by either treatment although salt intake decreased on day three of the infusion of CRH (116). These results demonstrated that both CRH and urocortin are anorexigenic in baboons, similar to what has been reported for CRH in rhesus monkeys (113).

The role of CRH in the regulation of food intake may be modulated in part by circulating levels of leptin. Leptin is a peptide hormone that is secreted by adipocytes. An increase in circulating leptin levels modulates energy balance by attenuating food intake and increasing energy expenditure. Leptin receptors are located in the arcuate and paraventricular nuclei of the hypothalamus, and leptin has been shown to increase CRH content in the hypothalamus in the rat (117). In a study of the effects of leptin on HPA axis activity, the ACTH and cortisol responses to both a predictable and an unpredictable stressor, as well as to a dexamethasone suppression-CRH stimulation test, were measured in ovariectomized rhesus monkeys in the presence and absence of estradiol replacement (118). Leptin was administered via a constant subcutaneous infusion; estradiol injections simulated plasma estradiol levels measured during the early follicular phase. The study found that leptin attenuated the pituitary-adrenal response to an unpredictable stressor and enhanced the negative feedback effects of dexamethasone, as shown by an attenuated ACTH and cortisol response to CRH administration. Basal cortisol levels were unaffected by the leptin infusion. Leptin therefore appears to have an important homeostatic role, helping to regulate eating as well as having some control of HPA axis function (118).

7.2. CRH and the cardiovascular system

Corticotropin-releasing hormone has the unusual distinction of mediating opposing effects on mean arterial blood pressure depending on the route of administration that is used. One of the first studies of the hypotensive effects of CRH was reported in 1982, following the i.v. administration of oCRH to rhesus monkeys (*Macaca mulatta*). Mean arterial pressure decreased dose-dependently in two ketamine-anaesthetized monkeys, but in a third, a moderate dose of oCRH produced a profound and long-lasting hypotension (119, 120). Intracerebro-ventricular (i.c.v.) administration of oCRH to rhesus monkeys is reported to cause slight increases in mean arterial pressure in chair-restrained rhesus monkeys which

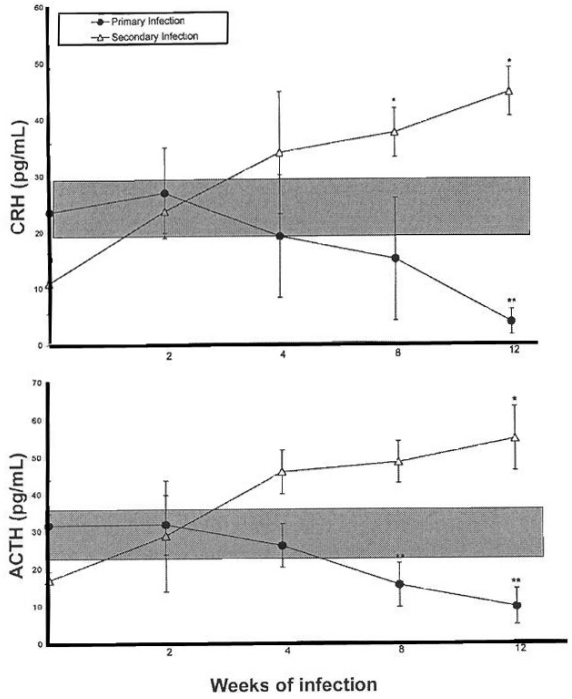

Figure 13. CRH and ACTH serum levels in baboons during primary (n=7) and secondary (n=9) infections with Schistosoma mansoni (mean ± SD). Shaded areas represent ranges of normal, pre-infection hormone levels in nine baboons. *P < 0.05, primary infections vs. secondary infection group; **P < 0.05, primary infection group vs. control group. From J. Morales-Montor, E. Newhouse, F. Mohamed, A. Baghdadi & R. T. Damian: Altered levels of hypothalamic-pituitary-adrenocortical axis hormones in baboons and mice during the course of infection with Schistosoma mansoni. Reproduced with permission from J. Infect. Dis. Society of America. Data are presented with permission from the University of Chicago Press (127).

may or may not be statistically or clinically significant (98, 121). The hemodynamic effects of h/rCRH were measured in the cynomolgus monkey (*Macaca fascicularis*) under ketamine and halothane anaesthesia. The administration of i.v. h/rCRH led to a drop in peripheral vascular resistance and mean systemic blood pressure, with vasodilation being measured in the large veins as well as the cutaneous capillary network, the latter producing the facial and upper body flush (10) that is sometimes reported in humans (122). It was noted that the rapidity of the change in blood pressure following CRH administration meant that it was unlikely to have been mediated via changes in HPA axis activity; this provided early evidence of the existence of peripheral CRH receptors (10).

7.3. CRH and infection/immune response

Cytokines, small proteins released from cells to trigger inflammatory responses as well as to respond to infection, have also been shown to stimulate endocrine pathways, demonstrating the interrelatedness of the immune and endocrine systems. Cytokine proteins may include interleukins and cell signal molecules such as tumour necrosis factor and the interferons. The candidates thought to have primary responsibility for this immune-endocrine interaction are CRH and the cytokine, interleukin-1alpha (IL-1alpha). IL-1alpha is one of the principle mediators of the immunological and pathological responses to stress and infection. Stimulatory effects of IL-1alpha on CRH release from perfused hypothalami of ovariectomized rhesus monkeys (Macaca mulatta) have been reported; treatment with IL-1alpha increased CRH secretion from whole hypothalamus as well as from the isolated median eminence (123). In addition, IL-1alpha was administered via i.c.v. infusion to chaired monkeys from which CSF was sampled. The concentration of CRH measured in CSF rose significantly during subsequent hours, indicating that IL-1alpha stimulates the release of CRH from both hypothalamic and extrahypothalamic brain regions (123). Acute activation of the HPA axis by IL-1alpha results in inhibition of LH secretion in ovariectomized rhesus monkeys, an effect that is reversed by prior treatment with a CRH antagonist (124). However there is evidence that this effect is modulated by oestradiol. When IL-1alpha was administered to ovariectomized rhesus monkeys treated with oestradiol to produce concentrations similar to those measured during the late follicular phase of the estrus cycle, IL-1alpha produced a stimulatory effect on LH. This effect was blocked by prior i.c.v. treatment with a CRH antagonist, and also by a progesterone receptor antagonist (125). As discussed earlier, the inhibition of gonadotropin release does not require pituitary-adrenal axis activation following either acute stress or CRH infusion in the ovariectomized monkey. However in the presence of sex steroids, IL-1alpha (which increases CRH secretion) produced the opposite effect on LH secretion, which demonstrates the importance of the sex steroid environment (e.g. (126).

The role of CRH and the pituitary-adrenal axis has been investigated under conditions in which infection has been introduced. Baboons (*Papiocynocephalus anubis*) that had been experimentally infected with schistosomiasis (*Schistosoma mansoni*), a common blood fluke parasite, were examined with respect to the course and severity of both a primary and a secondary infection. Plasma levels of CRH, ACTH, cortisol and dehydroepiandrosterone-sulfate (DHEA-S) were measured (127). DHEA-S is an adrenal steroid with weak androgenic properties, the levels of which have been associated with disease susceptibility (128). During primary infection with schistosomiasis, CRH, ACTH, cortisol and DHEA-S levels in serum gradually declined, with their levels inversely related to the severity of infection. However, upon secondary infection (after animals had made a complete or near-complete recovery from their primary infection), the serum levels of CRH, ACTH (Figure 13) and DHEA-S gradually increased and the symptoms of infection were negatively correlated once again (Figure 14) (127). The reduction in cortisol during primary infection, and the consequent loss of cortisol's anti-inflammatory effects, is to the advantage of the parasitic host. The mechanism through which this is achieved is not presently known. The response of CRH, ACTH, cortisol and DHEA-S during schistosomiasis infection therefore seems predictive of the degree to which the immune system is successfully responding to this pathogen.

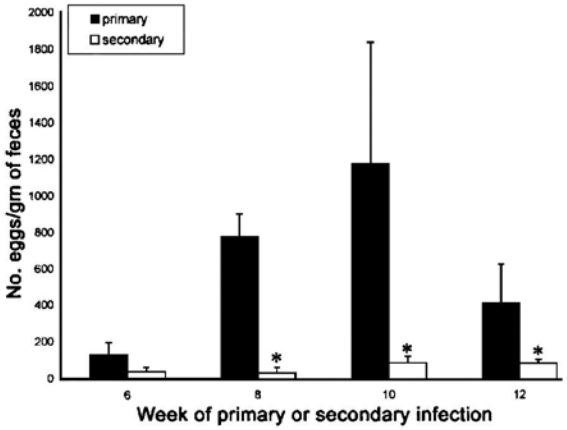

Figure 14. Faecal egg counts in baboons with primary and secondary infection. Data are mean ± SD eggs per gram of faeces. *P < 0.05, secondary infection vs. primary infection. From J. Morales-Montor, E. Newhouse, F. Mohamed, A. Baghdadi & R. T. Damian: Altered levels of hypothalamic-pituitary-adrenocortical axis hormones in baboons and mice during the course of infection with Schistosoma mansoni. Reproduced with permission from J. Infect. Dis., Infectious Diseases Society of America. Data are presented with permission from the University of Chicago Press (127).

8. ROLE OF CRH IN BEHAVIOUR

CRH and urocortin (an endogenous mammalian CRH-like peptide that also has high affinity for the CRH-R2 receptor subtype) both elicit behavioural effects when administered into the CNS. Central routes of administration are necessary as neither peptide is likely to have significant access to the CNS following parenteral administration due to their limited ability to cross the blood-brain-barrier (e.g. (6)). As a general rule, administration of CRH increases the level of arousal in unstressed animals, and enhances the stress-related behaviour under conditions of pre-existing or co-administered stress. This is consistent with CRH having an anxiogenic or 'stress-like' effect on behaviour. Urocortin shares some of the behavioural effects of CRH and these are likely to be mediated via activation of CRH-R1 receptors. However urocortin has the distinction of being a more potent suppressor of food intake (129). This has led to speculation that the anorexigenic effects of stress may be mediated via CRH-R2 receptors.

8.1. CRH: Effects on overt (fearful) behaviour

When i.c.v. oCRH (0, 20 and 180 micrograms) was administered to adult male chair-trained rhesus monkeys (*Macaca mulatta*) via an indwelling lateral ventricular cannula, significant increases in ACTH and cortisol were measured and there was a small increase in mean arterial blood pressure (98). CRH increased behavioural arousal, with individual monkeys showing different responses that included increased vocalization, head-shaking, struggling and environmental exploration. Unchaired (unrestrained) monkeys that received i.c.v. CRH in their home cages showed a different profile of behaviours that included huddling and lying down behaviours (180 μg CRH). The behavioural changes were not statistically significant, possibly due to the individual variability in behavioural responses and to the small number of subjects; however the probable influence of the testing environment on the behavioural response to exogenous CRH is noteworthy. The likelihood of CRH having either a sedative effect or a hypotensive effect was ruled out in the latter study as monkeys remained responsive to environmental stimuli. The behavioural effects of i.c.v. CRH and its antagonist, alpha-helical CRH, were reported in 1989 in the male squirrel monkey (*Saimiri sciureus*) (130). Monkeys were chair-restrained for i.c.v. administration of placebo or CRH (0.1, 1.0, or 10 μg), with or without co-administration of alpha-helical CRH, and then placed in a test cage where their behaviour was observed. CRH (10 μg dose) increased locomotor activity, a departure from the findings of Kalin and colleagues (1983a) described above (98), which may represent differences among species or CRH doses. This effect was blocked by co-administration of the CRH antagonist. Vigilance checking however was increased by treatment with the CRH antagonist, with or without co-administration of CRH, as well as by the low dose of CRH (0.1 μg). The authors reported a biphasic behavioural response, with low doses of CRH appearing to trigger exploratory and aggressive behaviour, whilst larger doses were associated with escape (increased locomotion) and withdrawal behaviour (130).

Kalin and colleagues published a similar study (1983b) in which they administered oCRH to monkeys, but this time via the intravenous route. When CRH (0, 10 and 125 micrograms/kg) was administered to six chair-restrained male rhesus monkeys, plasma levels of ACTH and cortisol increased, and statistically significant behavioural changes occurred that were similar to those observed following i.c.v. administration of CRH (increased struggling, environmental exploration). To evaluate the influence of test environment on the effects of i.v. CRH, a subset of four monkeys received i.v. CRH in their home cages. Significant increases in vocalization, lying down, and threatening behaviour were seen at both doses, while the low dose only was associated with increased ingestive and self-directed behaviours. Only the high dose resulted in significant decreases in environmental exploration, grooming and huddling (120). Blood pressure decreases were also measured in three monkeys following i.v. CRH administration under ketamine anaesthesia (Kalin et al. 1983b). Although the behavioural changes following i.v. CRH were similar to those observed following i.c.v. CRH, the direction of the change in blood pressure was opposite to the slight increase that was measured following i.c.v. CRH. Decreases in mean arterial blood pressure following i.v. CRH have also been reported in man (131).

The activation of the HPA axis in rhesus monkeys following i.c.v. and i.v. administration of CRH was also examined in a study by Rock and colleagues (1984) (132). Ovine CRH was administered via either an indwelling fourth ventricle cannula or intravenous catheter to three chaired monkeys using a range of doses (0, 0.001, 0.01. 0.1 and 1.0 micrograms/kg) and venous blood was

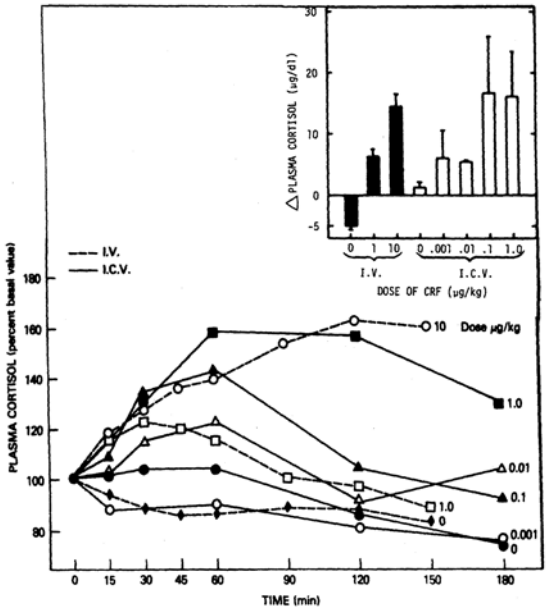

Figure 15. Plasma cortisol responses to graded doses of CRH administered i.v. and i.c.v. Cortisol responses are expressed as percent basal value. Insert: A comparison of plasma cortisol dose-responses to i.v. and i.c.v. doses of CRH. Cortisol responses are expressed as the difference of the peak value and the basal values. From G. P. Chrousos, J. R. Calabrese & P. Avgerinos: Corticotropin releasing factor: Basic studies and clinical applications. Reproduced with permission from Prog. Neuro-Psychopharmacol. Biol. Psychiatry, Elsevier (5).

Figure 16. The behavioural effects of i.c.v. CRH in an adult male rhesus monkey when housed in its normal social group. The animal has withdrawn from its peers and is exhibiting huddling/wall-facing behaviour, one of the depressive-like behaviours induced by i.c.v. CRH in socially housed monkeys. The animal is wearing a brown jacket that houses the pump used to infuse the CRH. From E. M. Strome, G. H. T. Wheler, J. D. Higley, D. L. Loriaux, S. J. Suomi & D. J. Doudet: Intracerebroventricular corticotropin-releasing factor increases limbic glucose metabolism and has social context-dependent behavioral effects in nonhuman primates. Reproduced with permission from Proc. Natl. Acad. Sci. USA (133).

sampled during the subsequent three hours. CRH administered into the fourth ventricle (0.1 and 1.0 micrograms/kg) produced significant increases in plasma cortisol levels within 30 min. The i.v. CRH doses required to stimulate cortisol secretion appeared to be at least 10-fold larger, suggesting that CRH was more potent when administered via the i.c.v. route (Figure 15). Since the plasma concentration of CRH following i.c.v. CRH administration was determined to be insufficient to activate the pituitary-adrenal axis via the peripheral circulation, is was concluded that CRH reached the pituitary gland via the CSF (5,132). A similar study conducted in three chair-restrained rhesus monkeys, using larger doses of CRH (10, 20 and 60 micrograms/kg) delivered to the fourth ventricle, also demonstrated a dose-dependent increase in cortisol secretion (121). In addition to cortisol, catecholamine concentrations and cardiovascular changes were also measured. Although the largest dose of CRH (60 micrograms/kg) increased epinephrine levels, there were no changes in norepinephrine. Mean arterial pressure showed small, non-significant increases following CRH administration; there were no changes in heart rate. Although the cardiovascular results were similar to the earlier study by Kalin et al. (1983a), the small number of monkeys that were tested meant that the study had little statistical power. Unlike earlier findings (98), changes in overt behaviour were only observed in one monkey, and only after it received the largest dose that was tested; this monkey showed agitation, vocalization and struggling behaviour for several hours until being sedated with diazepam. The authors concluded that CRH was unlikely to activate the sympathetic nervous system via the same pathway that the pituitary-adrenal axis is activated since increases in plasma catecholamine levels were not consistently measured following CRH doses that produced HPA axis activation (121).

The effects of a 40 min i.c.v. infusion of CRH or saline was recently reported (2002) in group-housed rhesus monkeys (*Macaca mulatta*) that were tested either with their social group or in isolation. CRH was found to produce behavioural changes that varied according to the social context (133). The CRH-treated monkeys placed in social isolation showed the same anxiety-like and depressive-like behaviours as did the placebo control group. However, the CRH-treated monkeys that were tested in their group cage showed significant increases in anxiety and depressive-like behaviours in contrast with placebo treatment (Figure 16). It is noteworthy that there were no differences in the extent of anxiety or depressive-like behaviours of CRH-treated monkeys in the two testing environments; the difference was due to the effects of the two testing environments on the placebo-control group, as the placebo-treated monkeys showed a similar increase in anxious and depressed-type behaviours only when they were socially isolated. The effect of CRH treatment was to reproduce behaviours observed during social isolation, but in the animals' home group cage. Regional brain activity following the i.c.v. infusion of CRH in anaesthetized monkeys was also measured in this study using positron emission tomography (PET). Glucose metabolic rates increased in the hippocampus, amygdala and

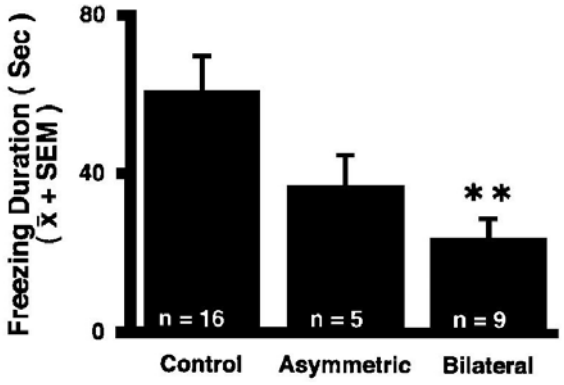

Figure 17. Effect of lesions of the central nucleus of the amygdala on freezing duration in the human intruder paradigm. The data were transformed during analysis to achieve normality ($F = 4.330$; df 2, 27; $P = 0.052$). Duncan post hoc test: *$P < 0.05$, bilateral group vs. control group; **$P < 0.01$, control vs. other groups. From N. H. Kalin, S. E. Shelton & R. J. Davidson: The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. Reproduced with permission from J. Neurosci., The Society for Neuroscience (135).

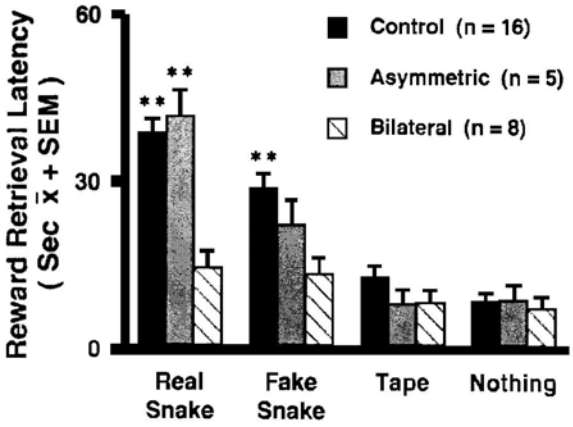

Figure 18. Effects of lesions of the central nucleus of the amygdala on snake fear as determined by the monkeys' latency to reach for a treat in the presence of snake stimuli compared with a neutral stimulus and no stimulus. The object by lesion interaction was significant ($F = 2.88$; df 6, 78; $P < 0.02$). Duncan post hoc test: **$P < 0.01$ differs from bilateral lesion group. From N. H. Kalin, S. E. Shelton & R. J. Davidson: The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. Reproduced with permission from J. Neurosci., The Society for Neuroscience (135).

pituitary/infundibulum areas following i.c.v. CRH infusion relative to placebo infusion (133), suggesting receptor activation in brain areas that correspond to those identified as having CRH immunoreactivity (21). There were also decreases in glucose metabolic rates in the parietal and left central cortices. Although the behavioural and PET data are unable to be correlated due to methodological differences, these data indicate that the brain areas activated by i.c.v. CRH correspond with brain areas that modulate emotional behaviour, anxiety and HPA axis activation (133).

Since there is little evidence that changes in the concentrations of CRH measured in CSF are correlated with hypothalamic concentrations of CRH (as reflected by changes in HPA axis activity), therefore the levels of CRH in CSF are thought to reflect extrahypothalamic CRH activity. One study that suggested that such a correlation may exist proposed this on the basis of innate differences between rhesus monkey temperament and frontal lobe electrical activity (134). It had been reported previously that monkeys with extreme right frontal asymmetries in their electrical brain activity also showed fearful/anxious behaviour traits and have high plasma cortisol levels (135). Plasma and CSF samples were obtained from rhesus monkeys (*Macaca mulatta*) that were identified at 13 months of age as having extreme left or right frontal lobe electrical activity. Samples were collected from 4 until 52 months of age during brief ketamine anaesthesia. There were systematic differences in the levels of CRH measured in CSF between the extreme left and right frontal lobe activity groups, and these differences remained stable across the sampling period (134). Monkeys from the extreme right frontal lobe activity group that also had higher CSF CRH levels were also shown to have high basal cortisol levels from 14 to 40 months of age (135). These results indicate that hypothalamic and extrahypothalamic CRH secretion may be correlated under some conditions, and are also consistent with the observation that i.c.v. administration of CRH may increase fearful behaviour in rhesus monkeys (98).

The role of the amygdala in mediating the behavioural and neuroendocrine responses to stressful stimuli has been evaluated in rhesus monkeys in which the central nucleus of the amygdala had been bilaterally or asymmetrically destroyed by excitotoxic lesioning (136). The monkeys' behavioural responses to two stressors were evaluated. The stressors were a human intruder who was either making eye contact or presenting her profile to each monkey, and a live snake randomly presented in a clear box on the top of which was placed a favoured food. The monkeys' plasma hormone responses were also assessed before and after 30 min of restraint and confinement. The group with bilaterally lesioned central nuclei showed significantly less fear (freezing duration) in the intruder paradigm than did the asymmetric lesion or control groups (Figure 17). The bilateral lesion group also showed a shorter latency to reward retrieval in the snake fear test than did the asymmetric lesion or control groups (Figure 18). However, both the bilateral and asymmetrical lesion groups had similarly small basal and restraint-test ACTH responses than did the control group (Figure 19), although cortisol levels did not differ between groups. The levels of CRH in CSF were also significantly lower in the bilateral lesion group than in the asymmetric lesion or control groups (Figure 19) (136). Thus the absence of the central nucleus appeared to attenuate the behavioural fear responses and extrahypothalamic release of CRH, whereas it's partial or complete absence attenuated restraint-stress

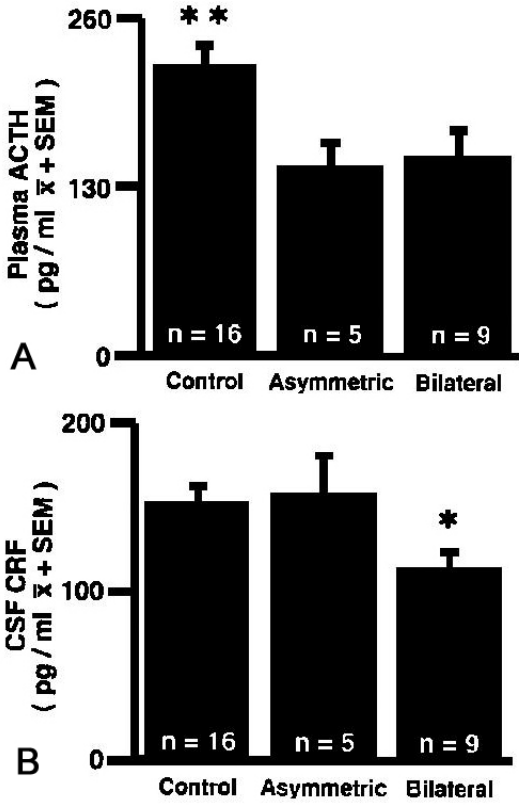

Figure 19. Effects of lesions of the central nucleus of the amygdala on (A) plasma ACTH concentrations ($F = 3.629$; df 2, 27; $P < 0.04$) and (B) CSF CRH concentrations ($F = 3.30$; df 2, 27; $P = 0.052$). Duncan post hoc test: $*P < 0.05$, bilateral group vs. control group; $**P < 0.01$, control vs. other groups. From N. H. Kalin, S. E. Shelton & R. J. Davidson: The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. Reproduced with permission from J. Neurosci., The Society for Neuroscience (135).

induced ACTH secretion. These data suggest a role for the amygdala, particularly the central nucleus, in mediating the behavioural and endocrine responses to stressors that provoke fear and/or anxiety.

8.2. CRH and drugs of dependence-related behaviour

The role of stress in addictive substance-related behaviour has featured prominently in studies published during the last decade. The majority of these studies have been conducted in the rat, generally focusing on the three phases of drug-related behaviour – the acquisition, maintenance and reinstatement of drug self-administration. Three research groups in particular have developed a remarkably consistent model of the role of the HPA axis in drug-related behaviour in the rat (137-139).

Among the first of the papers to examine the effect of cocaine on ACTH and cortisol release in nonhuman primates were two studies in which intravenous bolus injections of cocaine were administered to chair-restrained intact male and ovariectomized female rhesus monkeys. Cocaine had stimulatory effects on ACTH and cortisol in some of the male monkeys, with the degree of stimulation appearing to be inversely related to the baseline level of HPA axis activity (140). However the same doses of cocaine had no effect on ACTH and cortisol levels in ovariectomized rhesus monkeys. This was despite the fact that i.v. CRH produced significant increases in both ACTH and cortisol in these monkeys (141). A likely explanation for cocaine's lack of effect in ovariectomized monkeys was the absence of circulating sex steroids, particularly since cocaine was later shown to stimulate the HPA axis in intact female rhesus monkeys (142).

Several studies have examined the effects of intravenously self-administered cocaine on ACTH and cortisol secretion in rhesus monkeys. In monkeys that were trained to press a lever in order to receive i.v. injections of cocaine, cocaine dose-dependently increased ACTH and cortisol levels over a range of self-administered doses (143). Pharmacological antagonism using the cortisol synthesis inhibitors ketaconazole and etomidate, resulted in attenuation of the cortisol responses to cocaine whilst exaggerating the ACTH secretory response to self-administration of the largest cocaine dose that was tested (144). Administration of the potent glucocorticoid agonist, dexamethasone, on the day prior to testing, resulted in basal ACTH and cortisol levels that were at or below the limits of detection; this HPA axis suppression was not overcome by the stimulatory effects of self-administered cocaine (Figure 20). Finally, i.v. pre-treatment with the peptide CRH antagonist, astressin, resulted in a dose-dependent decrease in cocaine-stimulated ACTH and cortisol secretion, probably via antagonism of pituitary CRH receptors as astressin is unlikely to cross the blood brain barrier to block CRH receptors in the CNS (Figure 21). Despite each of these substantial pharmacological manipulations of the HPA axis, the behavioural response to cocaine (as measured by the rate of lever pressing and the number of injections of cocaine that were earned) was unaffected by these changes in HPA axis sensitivity to the stimulatory effects of cocaine (144). Thus there was an absence of evidence of a role for HPA axis hormones in the reinforcing effects of cocaine in the rhesus monkey, since profound changes in the cortisol and ACTH responses to cocaine did not affect drug-maintained behaviour.

Later studies have examined the effects of other classes of abused drugs on the adrenal-pituitary axis and found that several drug classes produced a dose-dependent attenuation of basal HPA axis activity when intravenously self-administered by rhesus monkeys. Drugs that suppressed basal ACTH and cortisol secretion were fentanyl (a mu-opioid receptor agonist), ketamine (a NMDA receptor antagonist), and ethanol. By contrast, midazolam (a benzodiazepine) and methohexital (a barbiturate) did not have a consistent effect on basal ACTH and cortisol secretion (145, 146). When the HPA axis stimulatory effects of self-administered cocaine and bupropion (unpublished data) are included, there appears to be a lack of consistency as to how drugs that are reinforcing, and hence have abuse potential, affect the function of the HPA axis in the rhesus monkey. Rather,

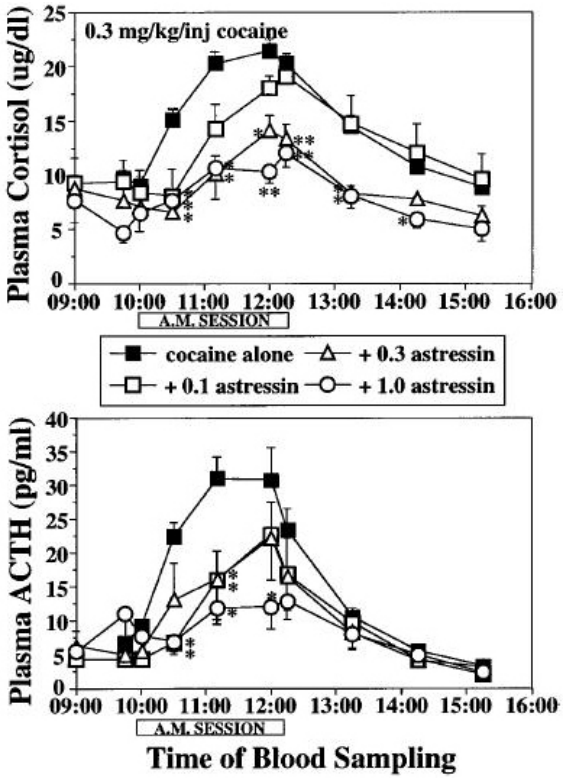

Figure 20. Plasma ACTH and cortisol levels were measured in samples obtained before, during, and after self-administration of 0.3 mg/kg/inj i.v. cocaine on an FR 30 TO 10-min schedule of cocaine delivery. Pre-treatment with 0.5 mg/kg dexamethasone (n=3) abolished the cortisol and ACTH response to self-administered cocaine. ***P < 0.001, dexamethasone vs. no pre-treatment on days 1 and 2; *P < 0.05 on day 2. All cortisol concentrations measured on day 1 differed from the no pre-treatment condition (P < 0.001). From J. H. Broadbear, G. Winger & J. H. Woods: Cocaine-reinforced responding in rhesus monkeys: Pharmacological attenuation of the hypothalamic-pituitary-adrenal axis response. Reproduced with permission from J. Pharmacol. Exp. Ther., American Society for Pharmacology and Experimental Therapeutics (144).

these observations highlight the multiplicity of neurotransmitter pathways through which HPA axis activity can be modulated.

In humans, the stress of acute drug withdrawal may contribute to the maintenance of drug-maintained behaviour. Acute drug withdrawal may lead to dysphoria, anxiety, depression and irritability, all of which have been reported during cocaine withdrawal in an outpatient setting (147). In addition, dysregulation of the pituitary-adrenal axis in former drug addicts may continue beyond the acute withdrawal stage (148) and exposure to stressors has been linked to relapse in former alcoholics (149). In a recent paper that investigated the role of stress in relapse to cocaine-seeking behaviour, squirrel monkeys (*Saimiri sciureus*) were trained to self-administer i.v. injections of cocaine (150). After 4-8 weeks of stable responding, the behaviour was extinguished by substituting saline for the cocaine until responding was less than 10% of what had been previously measured for cocaine. Reinstatement tests involved the administration of priming injections of CRH, ACTH, cortisol or cocaine prior to a self-administration session for which only saline was made available. The CRH antagonist, CP-154,526, was also administered prior to CRH or cocaine priming injections to determine its effect on reinstatement behaviour. Although the CRH, ACTH, and cortisol treatments were sufficient to increase salivary cortisol levels, none of these treatments resulted in an increase in drug-appropriate behaviour. The cocaine priming injection did reinstate drug-appropriate behaviour, and this was not attenuated by prior treatment with the CRH antagonist. The authors concluded that HPA axis activation was not necessary for reinstatement of cocaine-seeking behaviour in the squirrel monkey (150).

8.3. CRH and stress (depression)

Studies in humans published during the 1980's reported that there were abnormal concentrations of CRH in cerebrospinal fluid (CSF) in diseases such as depression (151) and anorexia nervosa (152), in which levels of CRH in CSF appeared to be elevated, and amyotrophic lateral sclerosis (153) and Alzheimer's disease (154) in which levels of CRH in CSF appeared low. However the relationship between levels of CRH in CSF and hypothalamic CRH was uncertain. Did extrahypothalamic CRH secretion follow the same circadian pattern of diurnal fluctuations as hypothalamic CRH? In order to measure this, CSF was continuously sampled from the cisterna magna of conscious, partially restrained adult male rhesus monkeys (*Macaca mulatta*) over a 48 h period. The CRH and cortisol concentrations that were measured in CSF were found to be in direct temporal opposition to plasma levels of cortisol in terms their peaks and nadirs (155). These results suggest that the circadian pattern of CRH from extra-hypothalamic sites in the CNS is distinct from and opposite to the release of hypothalamic CRH which controls the diurnal rhythm of basal pituitary-adrenal axis function. In the same study, Kalin and colleagues also reported that none of several interventions that stimulated the HPA axis resulted in an increase in the level of CRH measured in CSF. These interventions included cholinergic stimulation with physostigmine, administration of the glucocorticoid synthesis inhibitor, metyrapone, and the introduction of environmental stress by placing each monkey into a novel confinement cage. Although each of these conditions caused substantial increases in pituitary-adrenal activation, none of these conditions produced any change in the levels of CRH measured in CSF (155). The finding that levels of CRH in CSF are unaffected by factors that stimulate hypothalamic CRH secretion and are instead a consequence of unrelated extra-hypothalamic release of CRH has implications with respect to the CNS origins of the abnormal CRH levels measured in CSF.

An examination of the relationship between sex, social status and the HPA axis response to socially stressful situations was examined in 1996 in the marmoset (*Callithrix jacchus*) by Johnson and colleagues (156). One of their findings was that the high basal cortisol levels

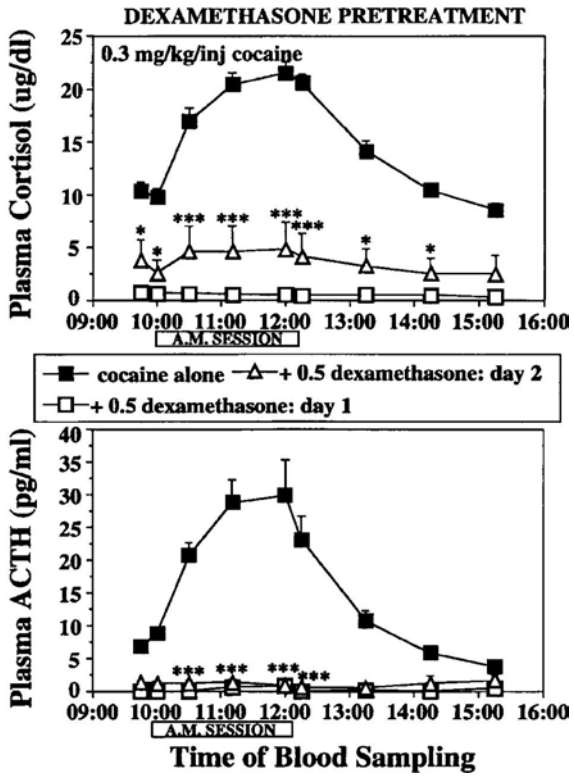

Figure 21. Astressin (n = 3 or 4) dose-dependently attenuated the cortisol and ACTH responses to cocaine. *P < 0.05, astressin vs. no pre-treatment. **P < 0.05, 0.3 and 1.0 mg/kg astressin vs. no pre-treatment and 0.1 mg/kg astressin. Other details as for Figure 20. From J. H. Broadbear, G. Winger & J. H. Woods: Cocaine-reinforced responding in rhesus monkeys: Pharmacological attenuation of the hypothalamic-pituitary-adrenal axis response. Reproduced with permission from J. Pharmacol., The American Society for Pharmacology and Experimental Therapeutics (144).

measured in socially isolated females, as well as in males paired with females, were predictive of subsequent low social status when animals were group-housed. Once a group hierarchy was established, basal cortisol levels normalized and were no longer predictive of social position. In unstressed animals, females had higher basal cortisol levels than males, as well as a blunted cortisol response following the administration of oCRH. During social isolation, both male and female marmosets showed an increased tendency to 'escape' from dexamethasone suppression, as cortisol levels recovered more quickly from the negative feedback stimulus of dexamethasone, a potent glucocorticoid. Socially isolated females also showed a blunted HPA response to oCRH and both male and female monkeys lost weight. These results were replicated in animals housed in an unstable peer group. In the stable peer group, the lowest ranking female in each group was the only monkey to show any change in basal cortisol levels relative to their levels measured in an unstressed state. In these females, cortisol levels were lower, and these monkeys tended to adopt submissive postures and lose weight. This animal model offers interesting endocrine and behavioural correlations with human disorders such as depression and anorexia nervosa, and demonstrates a possible role for social stress in these disorders (156). A naturalistic study with a similar objective, the examination of the relationship between social rank and HPA axis activity, was conducted in two troops of wild olive baboons (*Papiocynocephalus anubis*) that had been monitored during a long-term study. It has been a consistent finding that subordinate male troop members are hypercortisolaemic and resistant to dexamethasone, but at the same time show normal adrenal responsiveness to ACTH. These monkeys do however show less pituitary sensitivity to CRH administration, even when glucocorticoid negative feedback has been blocked by metyrapone treatment. This indicates that the HPA over activity measured in male baboons that are experiencing chronic social stress originates at the level of the CNS (157).

A similar study conducted in squirrel monkeys (*Saimiri sciureus*) showed that when monkeys were isolated from their social group, there were significant increases in ACTH and cortisol levels (158). Monkeys with larger elevations in cortisol levels (which could last for several days) showed a greater inhibition of ACTH release during subsequent observations. Blockade of this increase in cortisol using the glucocorticoid synthesis inhibitor, metyrapone, resulted in a greater elevation in ACTH levels that persisted until the effects of metyrapone dissipated (after about two days), making it likely that negative glucocorticoid feedback is important for both of these effects. Similar to what has been reported in the rhesus monkey (*Macaca mulatta*) (155), there was no correlation between stress-induced increases in HPA axis activity and CRH levels measured in CSF. The authors suggested that the hypercortisolaemia that was evident in the socially isolated monkeys may have been due to a heightened sensitivity of the adrenal cortex to ACTH; this may explain the high sustained levels of cortisol in the face of falling ACTH levels (158). A comparable situation has been reported in healthy humans, whose cortisol secretion remained hyper-responsive to ACTH for several days following an infusion of exogenous ACTH (159).

In order to investigate the effects of a social stressor on HPA responsiveness in monkeys of different ages, a study was conducted in young (7 - 8 y) and older (15 – 27 y) female rhesus monkeys to assess the effects of social isolation on basal HPA axis activity as well as pituitary-adrenal responsiveness to the glucocorticoid agonist, dexamethasone, and to exogenous CRH administration (160). These randomly selected female monkeys were housed together in groups ranging from 80-100 monkeys. During the study, the young and older monkeys were separated from their group for a seven day period and relocated to either the indoor part of their enclosure where visual, acoustic and tactile contact with other monkeys in their group was retained (control condition) or to a cage in a remote building that was isolated from other monkeys. The main effect of age was reflected in their basal ACTH and cortisol concentrations in

plasma, with the young monkeys in the control condition having higher levels in the morning and lower levels in the evening than the older monkeys, which showed less evidence of diurnal variation in ACTH and cortisol secretion. The basal levels of ACTH and cortisol for the socially isolated young and older female monkeys were within the range defined by the control condition, indicating that the socially stressed animals had a blunted circadian rhythm. The ACTH and cortisol responses to CRH were affected by the isolation stressor but not by monkey age. The ACTH and cortisol responses to CRH for the socially isolated monkeys were blunted relative to the monkeys in the control condition. In summary, young and older monkeys showed similar HPA responses to CRH stimulation, but the older monkeys showed evidence of impaired glucocorticoid negative feedback in basal and dexamethasone-suppressed pituitary-adrenal activity (160), a conclusion that is supported in a review of older rhesus monkeys' HPA axis function (161). However, a contrasting finding was that older female rhesus monkeys had larger cortisol responses following the exogenous administration of bovine CRH than did the younger monkeys (162). Except that bovine CRH was used in the latter study rather than the more commonly used ovine or human/rat CRH (species of origin of CRH was not specified in (160)), these studies were methodologically similar.

In one of the earliest studies of its kind (1985), CRH was administered to three drug-free patients, each of whom had been diagnosed with depression (5). This study found that although the ACTH response to exogenous CRH appeared blunted, cortisol levels remained within the normal range. This suggested that in cases of HPA axis hyperactivity that have been associated with some types of depression, CRH hypersecretion may lead to a desensitization of corticotropes in combination with an increased sensitivity of the adrenal gland to ACTH. When hypercortisolaemia was simulated in chair-restrained rhesus monkeys using a continuous infusion of dexamethasone (1 μg/kg/h) over 48 h, there was a time-dependent suppression of both ACTH and cortisol, which at 46 h was approximately 75% of control for ACTH, and 35% of control for cortisol. When a h/rCRH challenge (5 μg/kg bolus injection) was administered to measure pituitary-adrenal responsiveness, there were no differences in the peak responses and duration of ACTH and cortisol when measured before or after dexamethasone infusion, demonstrating that under these circumstances, normal pituitary-adrenal responsiveness to CRH remained despite the attenuated basal concentrations of both hormones (163).

An interesting link between vulnerability to stress-induced disruption of the menstrual cycle and characteristics of the serotonin system was reported in cynomolgus monkeys in a study described earlier in this review (67). The outcome, that the stress-sensitive animals showed a smaller prolactin response and a larger cortisol response following a challenge with the serotonin releasing agent, fenfluramine, despite their having normal cortisol responses to CRH challenge, implied that their serotonin systems had less functional capacity but that their HPA axes were hypersensitive to serotonergic stimulation (67). This is consistent with the HPA axis hyperactivity that is noted in some mood disorders such as anxiety and depression, as well as with evidence for the clinical utility of serotonin-releasing agents or agonists in the treatment of these disorders.

8.4. Effects of CRH antagonism on behaviour

The development of CRH-selective antagonists has been particularly vital for confirming that the various behavioural changes associated with CRH administration are mediated via CRH receptors. A recent study by Ayala and colleagues (2004) examined chronically administered antalarmin, a non-peptide CRH-R1 selective antagonist (164). Preadolescent male rhesus monkeys' behavioural and endocrine responses to social separation were evaluated during the third and fourth weeks of oral antalarmin (20 mg/kg per day) or placebo treatment. Antalarmin treatment had no effect on the ACTH, cortisol, epinephrine or norepinephrine levels that were measured in plasma following acute or chronic stress, nor did antalarmin affect any of the behavioural measures apart from environmental exploration, which was reported more frequently in animals treated with antalarmin. The lack of efficacy of antalarmin in this study may have been due to its variable oral bioavailability, as measured in venous blood, as well as to the lack of antalarmin detected in CSF (164). In a similar study, Habib and colleagues (2000) also administered oral doses of antalarmin (0, 5, 10, 20, and 40 mg/kg) to male rhesus monkeys but on an acute basis prior to exposure to a social stressor (165). The stressor in this case was an 'intruder paradigm', which consisted of a 30 min exposure to another male monkey separated from the test monkey by a transparent barrier. In the dose-response study, 20 mg/kg antalarmin was the only dose to significantly attenuate the ACTH response to the social stressor; the increase in cortisol was not significantly attenuated by antalarmin. Several behaviours were affected by acute pre-treatment with antalarmin prior to the social stressor; the anxiety ratings decreased, and behaviours associated with low levels of stress such as masturbation and environmental exploration were seen to increase. It is notable that antalarmin treatment resulted in a significant decrease in the concentration of CRH in CSF following the social stressor, although the reason for this is unclear (165). Antalarmin was one of the first non-peptide CRH antagonists to be made in sufficient quantities for testing in non-human primates. Antalarmin has been reported to have several surprising properties that seem theoretically unlikely for a CRH-R1-selective antagonist. For instance, the intravenous administration of antalarmin (10 mg/kg) to rhesus monkeys resulted in profound sedation and behavioural unresponsiveness that lasted 30-45 min. This was accompanied by an *increase* in ACTH and cortisol secretion (Figure 22); these behavioural and endocrine effects diminished with subsequent antalarmin treatments (166). This striking behavioural sedation provided the impetus for examining antalarmin in a drug self-administration paradigm in rhesus monkeys. A range of doses of antalarmin was made available to rhesus monkeys that had been trained to self-administer the barbiturate, methohexital. Antalarmin maintained responding relative

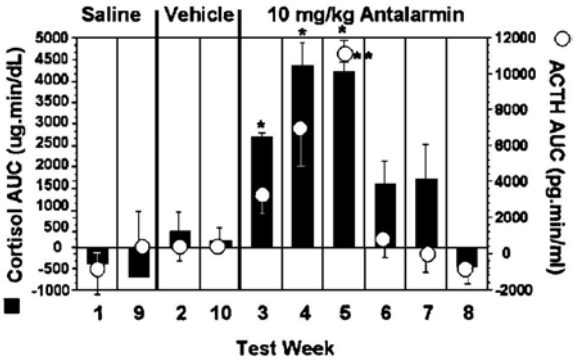

Figure 22. Comparison of the effects of repeated administration of saline, vehicle and 10 mg/kg antalarmin on ACTH and cortisol secretion when tested at weekly intervals (n=3). Neither saline nor vehicle had any effect on ACTH and cortisol release. I.v. administration of 10 mg/kg antalarmin resulted in an acute stimulation of ACTH (**P < 0.05; week 3) and cortisol *P < 0.05; weeks 1, 2, and 3) that diminished with repeated testing. The stimulation of stress hormone secretion was accompanied by behavioural sedation and a lack of responsiveness to environmental stimuli, the extent and duration of which also diminished upon repeated exposure to antalarmin. From J. H. Broadbear, G. Winger, J. E. Rivier, K. C. Rice & J. H. Woods: Corticotropin-releasing hormone antagonists, astressin B and antalarmin: Differing profiles of activity in rhesus monkeys. Reproduced with permission from Neuropsychopharmacology, Nature Publishing Group (166).

to saline for two doses (0.1 and 0.3 mg/kg/inj); however the number of injections that was earned decreased with repeated exposure to these doses (167). With respect to its CRH antagonism, a lower dose of antalarmin that did not itself produce behavioural or pituitary-adrenal changes (3.2 mg/kg), attenuated the ACTH but not the cortisol response to i.v. CRH, implying that cortisol release was not mediated solely by ACTH. By contrast, prior treatment with the peptide CRH antagonist, astressin B, attenuated ACTH and cortisol responses to i.v. CRH as would be expected, without producing any accompanying behavioural or pituitary-adrenal stimulatory effects of its own (166). It is possible that the pituitary-adrenal response is mediated via both CRH-R1 and R2 receptor subtypes, as suggested by the visualization of both subtypes (R2 in particular) in the rhesus monkey brain (21). If this is the case, it makes some sense that astressin B, which has similar affinity for both R1 and R2-type CRH receptors, would be more effective at blocking the stimulatory effects of CRH on ACTH and cortisol as compared with antalarmin which is reportedly selective for the CRH-R1 subtype.

Collectively, these data imply that antalarmin may be exerting effects that are not solely CRH-R1 mediated following intravenous administration. As of yet no other nonhuman primate studies have examined the behavioural (centrally mediated) effects of other systemically active CRH antagonists in studies that might confirm or clarify the results published to date with antalarmin. One recent candidate molecule, the non-peptidic lipophilic compound R121919, appears to fit the expected profile of a centrally active CRH-R1 antagonist. R121919 attenuated the pituitary-adrenal response to CRH and also attenuated the anxiety responses in a range of stress tests in rats (168) and appears to have antidepressant effects in humans without blocking the pituitary-adrenal response to exogenous CRH administration (169). However at this time there have been no studies published in nonhuman primates.

9. PERSPECTIVE

This review addresses many of the important roles that CRH has in the mediation of functions that are critical to survival and reproductive success, as demonstrated in a wide variety of monkey and ape species. The examination of CRH in the nonhuman primate provides valuable knowledge that broadens our basic understanding of the interactions between CRH, physiology and behaviour. Nonhuman primate research also adds to knowledge of the human condition, both its normal and abnormal states. The shared genetic and developmental heritage of humans and nonhuman primates makes these studies especially relevant with respect to addressing questions that are of interest to humans but are unable to be directly asked of humans.

10. ACKNOWLEDGEMENTS

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Footnote: There is some confusion regarding the methodology, as the original 1996 paper (101) stated that the various food foraging conditions were initiated when the infant monkeys were approximately 17 weeks of age. This description was revised in a subsequent paper published in 2001 (103) to state that the food foraging conditions were initiated when the infants were 8-10 weeks of age.

Glossary of terms: ACTH - adrenocorticotropic hormone (corticotropin), AVP - arginine vasopressin, CNS - central nervous system, CRH - corticotropin-releasing hormone (also called corticotropin releasing factor, or CRF), CSF - cerebral spinal fluid, FSH - follicle-stimulating hormone, GH - growth hormone, GnRH - gonadotropin-releasing hormone, HPA - hypothalamic-pituitary-adrenal, i.c.v. - intracerebroventricular, i.v. - intravenous, LH- luteinizing hormone, MUA - multi-unit activity, PVN - paraventricular nucleus, SON - supraoptic nucleus

Key words: Nonhuman Primate, Monkey, Ape, Corticotropin-Releasing Hormone, Behaviour, Fertility, Parturition, Stress, Endocrinology, Appetite, Cortisol, Glucocorticoids, Adrenocorticotropin, Review

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