Variations in the Hypothalamic-Pituitary-Adrenal Response to Stress during the Estrous Cycle in the Rat

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ABSTRACT

To investigate the role of gonadal steroids in the hypothalamic-pituitary-adrenal (HPA) response to stress, we studied adrenocorticotrophin (ACTH) and corticosterone (B) responses to 20 min restraint stress in cycling female rats, and in ovariectomized (OVX) rats replaced with physiological levels of estradiol (E2) and progesterone (P). In cycling rats, we found significantly higher peak ACTH ($p < 0.01$) and B ($p < 0.05$) responses to stress during proestrous compared to the estrous and diestrous phases. No differences were found in either basal ACTH and B levels across the cycle phases. In a seperate study, OVX rats were maintained on low, physiological levels of E2 and P with silastic implants for three days, and injected either with oil (O'), 10 ug of E2 (E') 24 h before stress testing, or with E2 and 500 ug P 24 h and 4 h, respectively, prior to stress (EP'). These treatments mimicked endogenous profiles of E2 and P occuring during diestrous, proestrous, and late proestrous-early estrous phases, respectively. In response to stress, ACTH levels were higher ($p < 0.01$) in the E' group compared to the EP' and O' groups. Although the peak B response was similar in all groups, the E' and EP' groups secreted more B following the termination of stress than did the O' group. Within the 20 min stress period, ACTH levels in the E' group were significantly ($p < 0.05$) higher at 5, 10, and 15 min after the onset of stress, compared to the EP' and O' groups. Plasma B levels were significantly higher in the E' group at 5 and 10 min ($p < 0.05$ and $p < 0.01$, respectively) compared to the EP' and O' group. ß-endorphin-like immuno-reactive responses to restraint stress were also significantly higher in the E' group compared to the EP' ($p < 0.05$) and O' ($p < 0.01$) groups. There was no effect of E2 on ACTH clearance. These results indicate that the HPA axis in the female rat is most

sensitive to stress during proestrous. Such enhanced HPA responses to stress are limited to the early portion of proestrous, as progesterone appears to inhibit the facilitatory effects of estrogen on ACTH release during stress. Taken together, these results suggest an ovarian influence on both activatonal and inhibitory components of HPA activity.

Nous avons investigué le rôle des stéroides ovariens dans la réponse de l'axe hypothalamo-hypophyso-surrénalien (HPA) aux stress. Pour ceci, la réponse de l'ACTH et de la corticostérone (B) à un stress d'immobilization de 20 min a été examiné chez des rattes femelles intacte et chez des rattes ovariectomisées (OVX) et traitées avec des taux physiologiques de l'estradiol (E2) et de la progestérone (P). Chez les animaux intactes, une augmentation significative du niveau maximal de la libération d'ACTH ($p < 0.01$) et de B ($p < 0.05$) en réponse au stress a été observée pendant la période " proestrous " par rapport aux périodes d' " estrous " et de " diestrous ". Aucunes différences entre les taux de base de l'ACTH et de B ont été notées au cours du cycle ovarien. Dans une autre étude, des rattes OVX ont été traitées avec des taux physiologiques d'E2 et de P pendant 3 jours en utilisant les implantes silastiques et injectées avec soit de l'huile (O'), 10 µg d'E2 (E') 24 h avant le stress, ou avec de l'E2 et 500 µg de P 24 h et 4 h avant le stress (EP'), respectivement. Ces traitments imitent les profiles endogènes d'E2 et de P des périodes d' " estrous ", de " diestrous ", de " proestrous " et de " late proestrous ", respectivement. En réponse au stress, les niveaux d'ACTH étaient élevés ($p < 0.01$) dans le groupe E' par rapport aux groupes EP' et O'. Quoique le taux maximal de B soit semblable parmis tous les groupes, les groupes E' et EP' ont libérés plus de B après la fin du stress que les animaux O'. Pendant les premiers 20 min de la

période de stress, les taux d'ACTH du groupe E' étaient élevés ($P < 0.05$) à 5, 10, et 15 min après le début du stress par rapport à ceux des groupes EP' et O'. La réponse au stress d'immobilisation du taux de la ß-endorphine immunoreactive était significativement plus élevée dans le group E' par rapport aux groups EP' ($p < 0.05$) et O' ($p < 0.01$). L'E2 n'avait pas d'effet sur le " clearance " d'ACTH. Ces résultats indiquent que chez la ratte femelle l'axe HPA est plus sensible au stress pendant la période " pro-estrous ". De telles augmentations de la réponse de l'axe HPA au stress sont limitées à la première période du " proestrous ", puisque la progestérone semble inhiber les effets facilitateurs de l'estrogène sur la libération de l'ACTH pendant le stress. En conclusion, ces résultats suggèrent que les ovaries exercent une influence sur les aspects activateurs et inhibiteurs de l'axe HPA.

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INTRODUCTION

Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-pituitary-adrenal (HPA) axis is the principle endocrine axis regulating the secretion of glucocorticoids (GCs). Primary control of GC secretion is mediated by the hypothalamic peptide, corticotropin-releasing hormone (CRH). CRH is synthesized in the parvocellular neurons of the paraventricular nucleus (pvcPVN) located in the hypothalamus, which project to the median eminence (ME). These neurosecretory projections terminate in the zona externa of the ME in close association with the capillary network of the pituitary portal system (Merchenthaler et al., 1983). CRH released into portal circulation stimulates the synthesis and release of proopiomelanocortin (POMC) - derived peptides from anterior pituitary corticotrophs (De Souza and Van Loon, 1989). Thus, adrenocorticotrophin (ACTH), ß-lipotrophin (ß-LPH), and ß-endorphin (ß-END) released in response to CRH, are derived from the same parent POMC molecule (Lundblad and Roberts, 1988; Eipper and Mains, 1980). Although CRH is extensively distributed throughout the hypothalamus and the brain (Palkovits et al., 1985), the PVN is the only source of CRH in the ME (Palkovits, 1987a). ACTH released into the systemic circulation causes the synthesis and secretion of the corticosteroids from cells of the zona fasciculata of the adrenal cortex, of which corticosterone (B) is the major GC in the rat, and cortisol (F) in man and other primates (Baxter and Tyrrell, 1987).

Other potent hypothalamic ACTH co-secretagogues including, oxytocin (OT) and arginine vasopressin (AVP), enhance corticotroph sensitivity to CRH (Aizawa et al.,

1982). The source of AVP in the ME originates from the pvcPVN and is colocalized with CRH (Gibbs, 1986). Although the majority of OT derived from the magnocellular neurons of the supraoptic nuclei (SON) project to the posterior pituitary, a portion of these neurons also project to the ME. Oxytocinergic neurons of the magnocellular division of the PVN may also send projections to the PVN-CRH pathway (Swanson and Sawchenko, 1983). *In vitro*, OT and AVP have been shown to potentiate the stimulation of ACTH synthesis and release by CRH (Watanabe and Orth, 1988; Plotsky et al., 1985; Vale et al., 1983). However, OT or AVP alone are comparatively weak stimulants of ACTH (Vale et al., 1983). Thus, OT and AVP affect the release of ACTH both directly and by increasing corticotroph sensitivity to CRH.

Central catecholamines (CAs), epinephrine (EPI) and norepinephrine (NE) are major regulators of HPA activity. Ascending brainstem CA projections via the ventral noradrenergic bundle directly innervate the pvcPVN and the ME in close proximity to CRH secreting nerve terminals (Palkovits, 1987a). In addition, there is evidence for CA release into the portal circulation and direct stimulation or potentiation of ACTH release. Intracerebroventricular (icv) administration of CAs has generally shown that NE and EPI have both stimulatory and inhibitory effects on CRH release (Plotsky et al., 1989), and NE regulation of CRH release is clearly dose-dependent. Plotsky (1987a) showed that icv doses of NE up to 5 nM facilitated immunoreactive (ir) CRH release into the portal circulation, while higher doses attenuated irCRH. This biphasic NE effect on CRH release was then shown to be mediated via the stimulatory α1- and inhibitory ß-adrenergic receptors (Plotsky, 1987a; Plotsky, 1987b).

A central role for serotonin (5-hydroxytryptamine; 5-HT) in regulating HPA activity has been suggested (Antoni, 1986). Immunohistochemical studies have shown that ascending 5-HT neurons originating from midbrain and dorsal raphe nuclei, directly

innervate the PVN (Sawchenko et al., 1983). *In vitro*, 5-HT can stimulate CRH release from perfused hypothalami (Nakagami et al., 1986). In addition, 5-HT can directly stimulate corticotroph ACTH release *in vitro*, suggestive of a direct role at the level of the anterior pituitary (Negro-Vilar et al., 1987).

The PVN also receives input from circumventricular organs, the subfornical organ and the organon vasculosum laminae terminalis (Palkovits, 1987a; Palkovits, 1987b, Lind et al., 1985). These specialized sites bordering the brain ventricles, possess highly vascularized capillary fenestrations that allow the infiltration of blood-borne factors. Thus, HPA activity is governed by diverse input to the PVN, potentially regulating CRH synthesis, CRH release into portal circulation, and corticotroph sensitivity to CRH.

Basal HPA activity. Basal HPA activity in the rat follows a circadian rhythm evident in circulating levels of both ACTH and B. Basal HPA activity is in phase with both light-dark and feeding cycles, as ACTH and B levels are relatively low during the AM (lights on) and high during the PM (lights out) (Dallman et al., 1987). Hypothalamic content of CRH reflects this circadian periodicity, as it is increased during the PM (Hroshige et al., 1973a). CRH content in the ME during the PM is about twice the AM levels, 9.0 and 4.1 pg, respectively (Assenmacher et al., 1987). This rhythm, is generated by stimulatory input from the suprachiasmatic nuclei (SCN) to the PVN during the PM. Lesions of the SCN abolish the circadian rhythm of ACTH and B, inhibiting basal release of ACTH and B in the PM, but not in the AM phase of the cycle (Dallman et al., 1987). Lesions of the hypothalamus which prevent corticotroph exposure to CRH, disrupt the circadian rhythm of ACTH, but do not decrease plasma ACTH levels below that observed during the AM (Dallman et al., 1987). These data suggest that the HPA axis is under brain drive during the PM, while the axis acts somewhat independently of

the CNS during the AM phase. Daily AM - PM fluctuations in plasma concentrations of B (2 to 20 μg/dL) show a comparatively wider variation than ACTH (50 to 75 pg/ml) (Kaneko et al., 1980) This has been attributed to enhanced adrenocortical sensitivity to ACTH in the PM.

The observed diurnal rhythm in HPA activity is regulated by GC negative feedback. The GCs act at target sites within the HPA axis, ultimately regulating the synthesis and release of CRH and ACTH. Exogenous B administration decreases CRH and ACTH release, while adrenalectomy (ADX) results in the hypersecretion of CRH and ACTH (Dallman et al., 1972) The GC receptor antagonist RU486 causes an increase in ACTH levels during the PM (above its circadian maximum), but not during the AM (Dallman et al., 1987). Likewise, inhibiting adrenal synthesis of B with cyanoketone results in elevated plasma ACTH levels, but only in the PM phase of the cycle (Dallman et al., 1987). Both these treatments effectively decrease GC feedback, suggesting that basal HPA activity is inhibited by B only during the PM. During the PM, ADX rats require a higher dose of B to normalize ACTH secretion. This apparent shift in HPA sensitivity reflects the enhanced brain drive during the PM. Thus, during the AM phase of the cycle, very low doses of exogenous B can effectively inhibit ACTH secretion in ADX animals (Akana et al., 1988). Taken together, the circadian rhythm of basal HPA activity is the result of cyclical changes in brain drive and the circulating level of B.

HPA Activity During Stress. During stress, CRH content in the median eminence is elevated, as well as portal concentrations of CRH, AVP, and OT (Murakami et al., 1989; Plotsky, 1987b; Plotsky and Vale, 1984). The level of HPA activity achieved during stress, is regulated by two important variables: the magnitude of the stimulus, and the level of the GC response or negative feedback signal. Pituitary and adrenal responses

to a variety of stressors have been shown to be related to the stimulus (stress) intensity For example, in the dog, the magnitude of ACTH and F responses to insulin induced hypoglycemia, and hemorrhage-induced hypovolemia and hypotension is proportional to the intensity of stimulus (Keller-Wood and Dallman, 1984). However, in response to hypoglycemia, the maximum F response occurs within the lower 1/3 of the physiological range of the ACTH response. Thus, Keller-Wood and Dallman (1984) suggest that while measuring GC response is a good index of HPA activation under mild stress, measuring ACTH is a more sensitive index at high stimulus intensities. Importantly, both the ACTH and GC response to stress should be studied under conditions where variations in HPA sensitivity to stress is suspect. Furthermore, these parameters also provide information regarding GC-mediated feedback dynamics.

Hemorrhage has been shown to stimulate an increase in ACTH secretagogue release into the pituitary portal system, as determined by portal cannula sampling. Measured in cats, arterial withdrawal of different blood volumes caused a graded increase in CRH, AVP, and EPI concentrations in the portal circulation (Plotsky, 1987b). Again here the magnitude of ACTH secretagogue responses to stress are also determined by the level of stimulus intensity. However, the release of these portal factors dissociate in response to different types of stressors (Plotsky, 1987b; Gibbs, 1986; Gibbs, 1984). For example, systemic increases in ACTH levels in response to nitroprusside-induced hypotension were only attributed to increases in portal levels of CRH, as no change was observed for AVP or EPI. In response to insulin-induced hypoglycemia, systemic levels of ACTH increased in the presence of increased portal levels of AVP, despite stable levels of portal CRH (Plotsky, 1987b). The nature of this response parallels *in vitro* studies. Increasing amounts of AVP applied to cultured pituitaries produces a graded response in ACTH release in the presence of uniform concentrations of CRH (Vale et al , 1983).

However, the potency of AVP is attenuated in the absence of CRH. Stress-induced secretion of ACTH in rats is inhibited by intravenous administration of antisera to CRH (Linton et al., 1985; Rivier et al., 1982), AVP (Linton et al., 1985), or OT (Gibbs, 1985). Thus, ACTH co-secretagogues can themselves regulate corticotroph release of ACTH, but require the obligatory presence of CRH. *In vivo*, the resultant ACTH response to stress is dictated by the combination of portal secretagogues that are released. The release of ACTH secretagogues dissociate in response to different types of stress in the rat (Gibbs, 1984). Thus, portal OT concentrations were increased in rats subjected to restraint or ether stress, but not cold, while AVP levels were increased by ether. The dissociation of ACTH secretagogues, in response to different types of stressors, illustrates the complexity of integration that occurs at the level of the hypothalamic-hypophysial complex coordinating pituitary release of ACTH. In light of the differences in CRH, OT, and AVP release, this also suggests that integration of the stress response also occurs by differential input to the PVN and SON.

Negative Feedback

The GCs are major regulators of the HPA axis. Removal of GCs by ADX, causes a detectable increase in CRH and AVP mRNA in the pvcPVN, using complementary cDNA probes (Dallman et al., 1987). ADX also increases the content of CRH and AVP in the ME and in the hypophysial-portal plasma, measured by immunohistochemical staining (Plotsky, 1987b). GC treatment also causes a decrease in POMC mRNA (Lundblad and Roberts, 1988) Plasma levels of ACTH, following ADX, are elevated well above those observed in intact animals. GC treatment prevents these effects in ADX rats. For example, exogenous administration of B or the synthetic GC dexamethasone

(DEX) have been shown to decrease portal concentrations of CRH and AVP as well as decrease plasma levels of ACTH (Plotsky, 1987b).

Temporal studies have correlated the elevated plasma levels of ACTH in response to ADX with the increases in anterior pituitary POMC gene expression and content (Birnberg et al., 1983). Shortly after ADX, plasma concentrations of ACTH transiently increase as ACTH content in the pituitary is depleted. This is followed by a gradual increase in pituitary POMC mRNA and an increase in plasma ACTH and pituitary content. DEX treatment decreases plasma ACTH and transiently decreases pituitary content, because it inhibits ACTH release more rapidly than synthesis (Lundblad and Roberts, 1988).

Infusion of DEX attenuates ACTH responses to CRH and other ACTH co-secretagogues in cultured pituitaries (Keller-Wood and Dallman, 1984). In AtT20 cells, a clonal mouse corticotroph tumor cell line, treatment with synthetic or natural GCs causes a specific decrease in the level of POMC mRNA. ADX rats with hypothalamic lesions, thus severing CRH input to the anterior pituitary, show exaggerated ACTH responses to CRH infusions compared to sham lesioned non-ADX animals (Dallman et al., 1987). Taken together, the HPA axis is negatively regulated by its endpoint, the GCs. GC-mediated negative feedback is a dominant characteristic of the system, observed by the ADX-induced effects on forward components of the axis.

Types of Negative Feedback: Fast Feed Back. Fast feedback, was first postulated by Dallman and Yates (1969) and is defined as a transient, rapidly occuring form of inhibition that is determined by the rate of increase of plasma B. Continuous infusion of B blocks the corticosteroid response to histamine stress applied 5 min, but not 15 after the start of infusion (Keller-Wood and Dallman, 1984). Thus, fast feedback occurs

during the period of increasing plasma B concentrations. This fast component of feedback is rate sensitive and the minimal rate of increase in B required for the rapid inhibition of ACTH is 1.3 μg/dL · min (Jones et al., 1972).

GC-mediated fast feedback limits the peak as well as the duration of the ACTH response. ADX rats replaced with low (3-6 μg/dL) plasma levels levels of B, exhibited ACTH hypersecretion in the period immediately following an acute stress (Akana et al., 1988) compared to sham-ADX controls. Thus, lacking a fast feedback signal, these animals show an exaggerated ACTH response. De Souza and Van Loon (1989) have also shown that fast feedback serves to limit the duration of ACTH and ß-END/ßLPH responses to stress. Animals who were exposed to 2 min of restraint stress repeated 5 min after the onset of an earlier identical stress (during which time the levels of B are rapidly rising), showed a faster rate of decline of plasma ACTH and ß-END following the second stressor. In addition, the ACTH response to the second stressor was potentiated (Van Loon and De Souza, 1987). This suggests that during the second response, facilitation and fast feedback inhibition are both evident. In this case, only when stress is terminated, and the facilitatory input has been removed, does enhanced feedback become operational (observed as a more rapid of decline in ACTH post-stress). Thus, GC fast feedback serves to limit the HPA response to acute stress, such that the magnitude of the ACTH response is determined by the balance between the stimulatory and the rate-sensitive inhibitory inputs.

Fast feedback inhibition has been demonstrated *in vitro* at the level of the anterior pituitary. ACTH secretion from AtT20 cells stimulated by AVP is inhibited by a 30 sec infusion of DEX (Keller-Wood and Dallman, 1984). DEX or B rapidly inhibits pituitary secretion of ACTH in response to hypothalamic extracts, but does not affect ACTH content. GC-mediated fast feedback does not require protein synthesis, as inhibition of

ACTH secretion is not affected by cycloheximide pretreatment in these preparations. Similar to *in vitro* studies in the pituitary, fast feedback in the hypothalamus is mediated by inhibitory effects on secretion. *In vitro*, it has been shown that B rapidly inhibits the release of CRH and also increases hypothalamic content of CRH (Jones et al. 1979). *In vivo*, exogenous administration of DEX rapidly decreases the portal levels of CRH and AVP released in response to stress (Plotsky, 1987b; Plotsky and Vale, 1984). Thus, GC fast feedback inhibition of ACTH occurs by the inhibition of the release of hypothalamic releasing factors and the pituitary release of ACTH. Considering these rapid effects on secretion, GC fast feedback is likely not mediated via a genomic mechanism. There is evidence for a membrane site of action, as B *in vitro* can rapidly inhibit Ca^{2+} induction of hypothalamic CRH release (Jones et al., 1979). Moreover, GC receptors have been characterized in hippocampal and hypothalamic membrane preparations that could possibly mediate rapidly occurring steroid effects (Towle and Sze, 1983).

Delayed Feedback. Dallman and Yates (1969) showed that infusion of B into rats 120 min before stress inhibited the endogenous B response, but not in rats infused 45 or 10 min before. *In vitro*, DEX causes an immediate, but transient inhibition of acetylcholine-induced CRH release. This inhibition wanes, but then reappears after 100 min (Jones et al., 1982). Hypothalami removed from rats injected with B 4 h earlier, showed a reduced CRH response to acetylcholine or 5-HT stimulation (Hillhouse and Jones 1976). These findings indicate the existence of a delayed form of GC feedback by B that requires a greater length of time to develop.

Abe and Critchlow (1980) showed that a bolus injection or a 20 min infusion of B failed to inhibit the endogenous B response to ether stress applied 60 min later. In

contrast, infusing the same dose over a 40 or 60 min period was effective in inhibiting the stress response. This indicates that the delayed feedback of HPA activity is sensitive to the integrated level of B. In other words, delayed feedback inhibition is not necessarily proportional to the level of B achieved during stress, but is proportional to the duration over which elevated levels of B respond. However, these studies fail to address the fact that the inhibitory feedback signal also resides at the level of the GC receptor (see below). Indeed, individual differences in the HPA response to stress occur, despite comparable levels of B achieved during stress.

ADX rats replaced with B in their drinking water (B-water rats) exhibited a normal circadian rhythm in circulating levels of ACTH and B, generated by drinking more water during the dark phase of the cycle (Jacobson et al., 1988). These B-water rats also showed an ACTH response to acute stress comparable to sham ADX rats, in terms of amplitude and duration. Thus, B-water rats, lacking a B response to stress similar to rats replaced with low constant levels of B (Akana et al., 1988), are able to rapidly terminate the ACTH response. It is probable that the elevated levels of B occuring during the PM, provided a latent inhibitory influence on the ACTH response to the relatively mild stressor the following morning.

A pituitary site for delayed feedback has been demonstrated *in vitro* (Arimura et al., 1969, Sayers and Portanova, 1974). Dispersed pituitary cells incubated with GCs for 4 h inhibits CRH-induced release of ACTH (Sayers and Portanova, 1974), but not the synthesis of ACTH (Arimura et al., 1969). This inhibition requires DNA-dependent RNA synthesis (transcription) and protein synthesis (translation), as preincubation with actinomycin D or cycloheximide, respectively, block GC-mediated inhibition of stimulated ACTH release (Arimura et al., 1969).

Local injections or implants of synthetic or endogenous GCs into the hypothalamus decrease pituitary and plasma concentrations of ACTH (Chowers et al., 1967). Hypothalamic tissue taken from rats with surgically isolated hypothalami treated with B 4 h earlier, show an inhibited response to acetylcholine-induced CRH release without a change in tissue content (Jones et al., 1979). This suggests that delayed feedback effects are mediated by the inhibition of CRH secretion. Thus, delayed feedback is temporally associated with a genomic mechanism of action, resulting in the inhibition of release of CRH in the hypothalamus, and the release of ACTH from the anterior pituitary.

GC Receptors

GCs circulate in the blood bound to corticosterone-binding globulin (CBG) and in the free form. Since blood flow in the brain is extremely fast, the rate of dissociation from CBG is limited. Thus, cellular uptake of B in the brain is proportional to the amount of circulating levels of unbound B (Partridge et al., 1983). At the cellular level GCs, like all steroids, are taken-up by passive diffusion. The interaction of the GC with its specific cytoplasmic receptor protein, activates (or transforms) the receptor, resulting in a stabilized receptor-steroid complex (Luttge et al., 1984). This complex is then able to translocate into the nucleus, and alter gene transcription. Thus, unlike the estrogen receptor which is located in the nucleus, the GC must first bind a soluble cytoplasmic protein receptor before entering the nucleus. The GC hormone receptor signal is defined by the amount of receptor that is translocated into the nucleus (and associated with chromatin) (Spelsberg et al., 1989). A prerequisite for a cellular GC response is the presence of the GC receptor. Furthermore, the level of the cellular response (sensitivity) is determined by the relative affinity of the the receptor for the steroid as well as the

receptor density.

Mapping studies using radioactive forms of synthetic and endogenous GCs, demonstrated an extensive distribution of the GC receptor within the CNS (McEwen et al., 1968). *In vivo* uptake studies, with peripheral injections of either ^{3}H-CORT or -DEX, showed that systemically circulating GCs are accessible to the brain, but also demonstrated differential uptake of these steroids. ^{3}H-B uptake showed a particular prevalence in neurons of the hippocampus and the septum, compared to the hypothalamus, cortex, and pituitary, while ^{3}H-DEX showed a relatively even distribution throughout the brain (Veldhius HD et al., 1982). Uptake of ^{3}H-B was blocked by pretreatment with (cold) aldosterone (mineralcorticoid; ALDO), but not with DEX. Furthermore, ^{3}H-ALDO binding sites showed a similar pattern of distribution to ^{3}H-B binding in the brain (De Kloet et al., 1975). Thus, it was concluded that a heterologous population of GC receptors existed in the brain, preferentially distributed in different target sites.

With the advance of *in vitro* binding techniques, the GC receptors were identified as two receptor subtypes based on their relative binding affinities for ALDO and DEX . The ALDO or mineralcorticoid receptor was named as type I, and the DEX receptor as type II. The type I receptor in the brain was found to be physiochemically similar to peripheral ALDO receptors of the kidney (Krozowski and Funder, 1983), and distinct from type II (Coirini et al., 1983). Type II receptors found in the brain appear to be identical to peripheral type II receptors. The type I receptor displays a higher affinity for ALDO (Kd ~ 1 nM) than the type II receptor ($Kd \geq 20$ nM) (Emadian et al., 1986; Reul and De Kloet, 1985). These receptor subtypes are no longer distinguished by DEX, as it has recently been shown that both type I and type II receptors display affinity for DEX (Kd 1- 3 nM, and 1 nM, respectively) (Luttge et al., 1989; Luttge and Emadian, 1988).

Newly developed selective GC analogs RU 26752 and RU 28362, have permitted study of each receptor separately (Lowy, 1989; Luttge et al.,1989). The type II receptor displays a very high affinity for RU 28362 (Kd $\leq$ 1 nM), greater than its affinity for B. RU 26752 selectively binds to the type I receptor, which displays high affinity (Kd ~1 nM) for the ligand.

These receptor subtypes bind B with different affinity, which can be explained in terms of their physiochemical differences. Pharmacologically, type I receptors show a greater affinity for B than type II receptors (with dissociation constants (Kd) of ~ 0.5 nM, and ~ 3.0 nM, respectively) (Reul and De Kloet, 1985). It should be noted that the earlier *in vivo* autoradiographic experiments with ^{3}H-B were performed using tracer level (~ 1 μg) injections of the radiolabelled steroids in ADX rats (McEwen et al., 1968). Under these low steroid levels, binding would likely occur only to type I sites. Thus, the selective *in vivo* uptake of ^{3}H-B in the hippocampus and septum is probably explained by the exclusive presence of high affinity type I receptors in these regions. This issue has been resolved recently in studies using a monoclonal antibody against the type II receptor (Fuxe, et al., 1985), and *in vivo* autoradiography (Reul and De Kloet, 1986) and *in vitro* cytosol binding assays using selective ligands (RU 28362, RU 26752) (Lowy, 1989; Luttge et al.,1989; Reul and De Kloet, 1986). Use of these ligands in mapping studies, have shown that type I receptors are restricted to the septal-hippocampal complex, while the distribution of type II receptors is quite extensive. The highest levels of type II receptors are found in the pituitary, lateral septum, hippocampus, paraventricular nucleus, and cortical and thalamic regions (Lowy, 1989; Luttge et al.,1989; Reul and De Kloet, 1986).

The difference between GC receptor subtypes in binding affinity for B, although less than one order of magnitude, has important physiological consequences. Under basal

and stressed conditions, the hippocampal GC receptors show distinct patterns of occupation that are likely related to their affinity for B (Meaney et al., 1988; Reul and De Kloet, 1985). Under low basal B conditions, type I receptors were shown to be almost completely occupied (~90 %), whereas type II receptors only slightly occupied (< 15%). However, in response to stress, occupation of type II receptors is markedly increased (> 75%) (Meaney et al., 1988; Reul and De Kloet, 1985). Thus, it is probable that type I and type II receptors mediate GC feedback under conditions when levels of B are elevated, and type I under low B levels.

Dallman et al., (1989) have shown evidence suggesting that the diurnal rhythm in ACTH is mediated via the occupancy of type I and type II receptors. In the ADX rat under B replacement, the plasma concentration of free B required to normalize ACTH levels, was found to be ~ 0.7 nM in the AM and ~ 4.0 nM in the PM. These plasma concentrations approximate the Kd values of B for the type I and type II receptors, respectively (Reul and De Kloet, 1985). During the PM when the HPA axis is under stimulatory drive, higher levels of B are required to limit ACTH secretion, perhaps involving the occupancy of both type I and type II receptors. During the AM, low levels of circulating B can effectively maintain HPA activity by occupancy of the high affinity type I receptor. In addition, type I receptors also respond to diurnal changes in circulating levels of B (Reul et al., 1987). Type I receptor binding capacity is increased during the PM, when plasma B levels are higher, affording an increase in the type I receptor signal.

Central Feedback Regulation

The *in vitro* studies detailed above, have pointed to the pituitary and hypothalamus as target regions mediating GC inhibition of HPA activity. However, *in vivo* studies suggest that feedback occurs above the level of the pituitary. Rats with medial basal hypothalamic lesions, thus severing hypothalamic input to the pituitary, do not show rate sensitive feedback over ACTH secretion when treated with exogenous CRH (Abe and Critchlow, 1977). In response to infusion of B, inhibition of ACTH secretion in these animals only occurs when plasma levels of B reach a plateau. This suggests that in the intact animal, fast feedback occurs above the level of the pituitary. Local injections or implants of synthetic or endogenous GCs into the hypothalamus decrease pituitary and plasma concentrations of ACTH (Chowers et al., 1967), but not when implanted into the anterior pituitary (Smelik and Sawyer, 1962).

This apparent lack of sensitivity in the pituitary is explained by the presence of high levels of cytosolic transcortin receptors. These receptors have a high affinity for B, but unlike the GC receptors, do not translocate into the nucleus, effectively decreasing the nuclear signal of B (Sakly and Koch, 1983a). Neonatal rats (2-10 days) exhibit an enhanced sensitivity to exogenous B inhibition of ACTH release in response to ether stress, and this has been attributed to a transient decrease in the expression of pituitary transcortin receptors (Walker et al., 1986; Sakly and Koch, 1983b).

Rapid Feedback persists in rats with surgically isolated hypothalami (Abe and Critchlow, 1977), suggesting that fast feedback can occur directly at the level of the hypothalamus. However, in rats whose brain CAs were depleted by icv injection of 6-hydroxydopamine (destroying noradrenergic and dopaminergic nerve terminals), fast,

rate sensitive feedback inhibition was eliminated (Kaneko and Hiroshige, 1987). Furthermore, central administration of *p*- chlorophenylalanine (which inhibits 5-HT synthesis), was shown to block delayed feedback following stress (Kaneko and Hiroshige, 1987). Thus, a fast and delayed feedback component is located, in part, centrally and at the level of the hypothalamus.

The hippocampus has been implicated as a major anatomical substrate mediating GC feedback inhibition. Hippocampectomy (HPX) results in elevated basal levels of ACTH and B, and this effect is abolished by ADX (Feldman and Conforti, 1980). Interestingly, systemic administration of DEX is less effective in attenuating elevated levels of B in HPX animals compared to sham-lesioned controls (Wilson et al., 1980). This suggest that the hippocampus is an important central site mediating GC feedback. Electrical stimulation of the hippocampus inhibits the ACTH response to stress (Feldman and Conforti, 1980). The hippocampus has the richest population of GC receptors in the brain (Reul and De Kloet, 1986). GC receptors in the hippocampus appear to be preferentially sensitive to regulation by circulating levels of B. Thus, chronic stress or replacement with high doses of GCs, selectively down-regulates type II GC receptor expression in the hippocampus, with no substantial reductions in the hypothalamus and pituitary (Sapolsky et al., 1984a). Central administration of GC specific type I and II receptor antagonists RU28318 or RU38486, respectively, block GC mediated negative feedback of HPA activity during stress (Ratka et al., 1989). Thus, it is thought that the elevated plasma levels of B in response to stress, inhibit HPA activity via occupancy of hippocampal GC receptors.

In response to 1 h of immobilization stress, young and aged rats showed similar peak plasma levels of B, sampled immediately following stress (Sapolsky et al. 1984b) However, post-stress B levels remained elevated up to 4 h in the aged rat, while plasma

B levels in the young rat declined to basal values by 2 h following the termination of stress. This dissociation in B profiles between the young and aged animals post-stress is associated with age-related reductions in type I and type II GC receptors specific to the hippocampus (Sapolsky et al., 1984a; Meaney et al., 1988). The fact that the hormone receptor signal (stress-induced occupancy and translocation of hippocampal GC receptors), is maintained for about 4 h post-stress, is consistent with a role for the hippocampus in mediating delayed feedback (Meaney et al. 1988). The inhibitory signal resides in the circulating levels of B and at the the receptor level. Thus, aged rats in response to stress, with a reduced capacity for type II binding, show decreased delayed feedback sensitivity to a comparable B response occurring in young animals (Meaney et al., 1989).

The mechanism by which the hippocampus mediates GC negative feedback is by regulating the release of hypothalamic ACTH secretagogues. Fornix lesions which remove inhibitory hippocampal input to the hypothalamus, have been shown to produce a hypersecretion of CRH, AVP, and OT levels in the pituitary portal system (Sapolsky et al. 1989). However, after fornix transection, in response to hypovolemic stress, injection of high amounts of exogenous B could only inhibit portal levels of AVP. This suggests that the GC mediated inhibitory signal in the hippocampus only serves to limit the CRH and OT responses to stress. The amount of GC receptor occupancy (negative feedback signal) in various brain sites has been well correlated with the inhibition of ACTH secretagogues (Sapolsky et el., 1990). The inhibition of portal concentrations of CRH in response to stress were best predicted by the occupancy of hippocampal type I and type II receptor occupancy; stress-induced OT secretion with hippocampal and hypothalamic type II occupancy; and stress-induced AVP secretion with hippocampal type II occupancy. Thus, it appears that CRH and OT are negatively regulated by

hippocampal input. Stress-induced inhibition of AVP is probably regulated by the hippocampus and some other central site, given its sensitivity to B inhibition in fornix-lesioned animals. The hippocampus represents one critical site where GCs can centrally mediate negative feedback, as GC receptors are also distributed in other brain areas. For example, GC receptors are localized in the amygdala, and in various mesencephalic nuclei (Sapolsky et al. 1990). Conflicting data exists regarding an amygdaloid role in regulating HPA activity, given the hetorogeneity of adrenalcortical responses to microstimulation of various nuclei within the amygdaloid complex (Dunn and Whitener, 1986). However, amygdalectomy results in an inhibition of ACTH released in response to various stimuli (Feldman and Conforti, 1981), and decreased ME CRH content (Beaulieu et al., 1989), suggesting a stimulatory role for the amygdala in regulating HPA activity. High densities of GC receptors are found in the frontal cortex (Meaney and Aitken, 1985). Rats with frontal cortex lesions show higher plasma ACTH and B levels in response to immobilization stress (Diorio and Meaney, 1990). These observations suggest that various GC target sites in the CNS play a predominant role in GC negative feedback.

The Hypothalamic-Pituitary-Gonadal Axis

The reproductive cycle in the rat otherwise known as the estrous cycle, lasts 4 to 5 days. The estrous cycle in unmated rats was initially observed by cyclic changes in female sexual behavior and vaginal cytology. These changes have been shown to be caused by fluctuations in the release of ovarian hormones, because ovariectomy (OVX) prevents sexual behavior and fluctuations in vaginal cytology and uterine growth. Vaginal cornification, uterine and ovarian follicular growth, and sexual receptivity, all have a cycle of activity with a duration of 4 or 5 days. For reproduction to be successful

in the female rat, sexual behavior and ovulation must be synchronized given its short gametic cycle. The endocrine system that maintains control over reproductive function is known as the hypothalamic-pituitary-gonadal (HPG) axis. The HPG axis is sensitive to the well-being of the animal, hence in response to various physiological and environmental stressors, sexual receptivity and ovulation will not occur. Thus, the HPG axis serves to regulate reproductive function to conditions favorable for optimal breeding. Given the role of the HPA axis during stress, therefore, it is possible that the HPG axis may in turn regulate the HPA response to stress. What follows is a basic description of the HPG axis, towards underscoring the relationship between these two endocrine systems.

Estrous Cycle

Each day of the estrous cycle (in the unmated rat) is characterized by fluctuations in the release of hypothalamic-pituitary factors and ovarian hormones (Feder, 1981). During diestrous I (Di I) systemic levels of estrogen, prolactin (PRL), and the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are low (Kalra and Kalra, 1974). Plasma progesterone levels are slightly elevated, produced by corpora lutea cells of the ovary. These glandular structures develop from differentiated granulosa cells following the expulsion of the mature oocyte during ovulation (Erickson 1981).

During diestrous II (Di II), PRL and gonadotropin levels are low. However, FSH and LH begin to act on the ovary, stimulating follicular growth and secretion of estrogen. LH is believed to also have a lytic effect on the corpora lutea, resulting in a decrease in progesterone levels in the peripheral circulation and in the ovarian vein (Smith et al.,

1975). It has been shown that progesterone secretion from the corpora lutea acts locally to limit follicular secretion of estrogen. Injecting progesterone into animals on Di II, thus preventing a fall in progesterone circulating levels, delays the onset of ovulation (Kalra and Kalra, 1974), while administration of progesterone antisera advances the onset of ovulation (Kaneko et al., 1986). In the Long-Evans rat the extended life of the corpora luteum is observed by higher levels of progesterone during Di II. This accounts for the generation of an additional diestrous phase (Di III) in the Long-Evans rat which cycles for 5, rather than 4 days.

During early proestrous (0900 - 1400 h) plasma gonadotrophins and PRL levels remain low, but plasma estrogen levels have already reached their peak. By 1600 - 1730 h plasma levels of FSH, LH, and PRL have peaked, followed by a sharp increase in FSH and progesterone levels (Smith et al., 1975). During this time estrogen levels fall, as ovarian follicles switch from estrogen producing cells to progesterone producing cells. Depriving the rat of estrogen during the morning of proestrous, by OVX or with antisera to estrogen, does not prevent the LH surge occurring during the PM (Kaneko et al., 1986). However, OVX at this time does prevent the display of lordosis behavior (Parsons, et al., 1980). Since estrogen levels are maximal throughout early proestrous, and progesterone only during the PM, this suggests that the LH surge is estrogen-dependent while the onset of sexual behavior is dependent on the presence of both hormones. Pituitary luteinizing hormone-releasing hormone (LHRH) receptors become elevated as early as Di II, and remain so until the time of the LH surge concomitant with fluctuations in plasma estrogen levels (Clayton et al., 1980). The appearance and disappearance of lordosis have been shown to follow estrogen-induced increases in progesterone receptors in areas of the brain which mediate sexual behavior, the mediobasal hypothalamus (MBH) and the preoptic area (POA) (Parsons et al., 1980).

Intracranial implantation studies have identified the ventromedial hypothalamic nucleus (VMN) as the most effective site for estrogen regulation of female sexual behavior (Davis et al., 1982). Taken together, it appears the threshold for estrogen induction of the gonadotropin surge is comparatively lower than the induction of sexual behavior. The onset of sexual behavior requires both the induction of progesterone receptor synthesis and the necessary occupation of these receptors by progesterone.

Increased LHRH activity in the MBH has been shown 2- 3 h before peak plasma levels of LH occur (Levine and Ramirez, 1982). It has been suggested that this early increase in MBH activity is necessary to increase pituitary expression of LHRH receptors, as LHRH has been shown to up-regulate its own receptor (Clayton et al., 1980). The increased levels of PRL, LH and FSH during proestrous are required for the selection of a dominant preovulatory follicle destined to ovulate (Erickson, 1981). Progesterone released during proestrous has been shown to assist follicular rupture occurring the following day (Shaikh and Shaikh, 1975). During estrous, ovulation occurs, and LH, PRL, estrogen, and progesterone levels are low (Brown-Grant and Naftolin, 1972). FSH levels remain elevated until 1300 - 1400 h, causing corpus luteum formation and initiating the next cycle of follicular growth (Feder, 1981).

Organization

Most of the experimental evidence to date indicates that the LHRH decapeptide is the principle hypothalamic regulator involved in the activation of pituitary gonadotroph release of LH and FSH (Chappel et al., 1983). In the rat, mapping studies localizing LHRH by bioassays and radioimmunoassays (RIA), coupled with lesion studies, showed that LHRH is localized in various hypothalamic regions, differentially

contributing to the regulation of tonic and preovulatory release of pituitary LH (Kalra and Kalra, 1983). The concentration of LHRH is highest in the median eminence (ME), preoptic area-suprachiasmatic area (POA-SCN), and the anterior hypothalamic area (AHA). Thus, the bulk of LHRH cell bodies reside outside the MBH. Lesion studies have shown that the preovulatory LH surge is regulated by LHRH perikarya restricted to the POA-SCN (Kalra and Kalra, 1983). Rush et al. (1980) showed that rostral deafferentation (lesioning anterior input) of the MBH at 1200 h on proestrous, blocked the proestrous LH surge and elevations in plasma FSH during proestrous. Thus, it appears that most of the LHRH-hypophysiotropic complex is involved in the preovulatory LH surge and secretion of FSH.

It has been difficult to locate the origin of LHRH found in the ME, as lesions in many individual nuclei of the hypothalamus cause a decrease in LHRH activity in the ME. Immunohistochemical tracings have pointed to LHRH perikarya located in the SCN, AHA, and ventral aspects of the medial preoptic-area (MPOA) projecting to the ME (Witkin et al., 1982; Ibata et al., 1979; Schneider et al., 1969). The pattern of LHRH content in these discrete hypothalamic areas have been shown to parallel the changes in ME LHRH content occurring during proestrous. Thus, it has been concluded that the hypothalamic LHRH system functions as an integrated unit regulating the release of LHRH from the ME (Wise et al., 1981). The extensive distribution of LHRH in the hypothalamus suggests that LHRH can probably affect other hypophysiotropic factors.

Hypothalamic LHRH fibers terminating in the external layer of the ME release LHRH into the portal system to stimulate pituitary gonadotroph secretion of LH and FSH. In general, the pattern of LH secretion follows that of LHRH secretion. Thus, the LH surge occurring during proestrous has been shown to be the result of increased LHRH activity in the ME (Levine and Ramirez, 1982). Gonadotroph dependency of

hypothalamic LHRH activity is shown by the fact that MBH lesions block the proestrous surge of LH and elevation of FSH occurring during the PM, and ovulation the following day. Interestingly, MBH lesions at 2000 h on proestrous do not prevent further elevations of FSH and ovulation during estrous (Rush et al., 1980). Thus, following the proestrous increase in hypothalamic LHRH activity and the LH surge, the gonadotroph is able to selectively release FSH independent of the hypothalamus.

Morphologically, the growing follicles can be divided into five classes: primary, secondary, tertiary, graafian, and atretic. Primordial follicular differentiation occurs under intraovarian regulation independent of the pituitary, while the survival and development of a selected tertiary follicle is entirely dependent on the presence of LH and FSH (Erickson, 1981). Follicular selection involves the differentiation of specialized follicular granulosa and theca interna cells. A follicle destined to ovulate involves the selective induction of FSH receptors in granulosa cells within the developing follicle. At this time the granulosa cells also acquire intracellular receptors for estrogen, progesterone, testosterone, and glucocorticoids. Granulosa cells are now sensitive to follicular estrogens which cause a further induction of FSH receptors. Enhanced sensitivity to circulating levels of FSH increases granulosa aromatase activity, thus acquiring the ability to convert androgens to estrogen. The androgen precursors of estrogen (androstenedione) synthesized in the theca interna cells diffuse into the granulosa cells where they are converted to estrogen. FSH also stimulates the induction of granulosa LH and PRL membrane receptors, central to the mechanism of ovulation.

By early proestrous granulosa activity is maximal, observed by the increased plasma levels of estrogen. Following the LH surge, LH mediates the conversion of thecal synthesis of androgens to progesterone resulting in a reduction of granulosa

synthesis of estrogen and a rapid increase in plasma progesterone levels (Erickson, 1981). During estrous, the oocyte is expelled and the follicle undergoes luteinization, forming the corpus luteum.

Gonadal Feedback Regulation

The pattern of gonadal steroid levels during the estrous cycle is a function of changes in ovarian sensitivity to the gonadotropins occuring over the course of follicular development. Moreover, the estrous cycle is dictated by local effects of estrogen and progesterone on the ovary and at the level of the pituitary and hypothalamus to mediate cyclic fluctuations in gonadotropin release. Thus, the gonadal steroids mediate short and long feedback regulation of the HPG axis. Given the cyclic nature of gonadotropin release, it appears that this feedback regulation is both stimulatory and inhibitory in nature.

Removal of endogenous gonadal steroids by OVX causes a tonic increase in LH release. Goodman (1978) has shown that replacement with diestrous levels of either estrogen or progesterone alone does not prevent the post-OVX rise in plasma LH levels. However, in the presence of diestrous levels of both steroids tonic LH secretion was inhibited. Goodman (1978) also showed that in the OVX rat in the presence of low progesterone levels, only high (preovulatory) levels of estrogen could induce an LH surge. During diestrous LH activity is inhibited by negative feedback action of both gonadal steroids working in concert. High levels of estrogen are required during proestrous to induce the LH surge, as it appears that progesterone raises the threshold for estrogen-induced LH activity.

The site of stimulatory estrogen action on LH activity appears to be at the level of

the pituitary. Increases in anterior pituitary LHRH receptors occurs coincident with rising levels of estrogen during the cycle (Clayton et al., 1980). In addition, exogenous administration of estrogen in OVX animals has been shown to enhance pituitary response to LHRH (Ferin et al., 1969). This estrogen effect could also occur at the level of the hypothalamus, since OVX during early proestrous prevents the preovulatory rise in MBH LHRH activity (Levine, 1982). Interestingly, there also appears to be an inhibitory component of estrogen on pituitary sensitivity to LHRH. Pituitary LH release in response to exogenous LHRH is enhanced following OVX in rats at 1300 h on proestrous (Turgeon and Barraclough, 1977). Thus, estrogen might have transient effects on LH release during proestrous. This possibly explains why some studies have shown that chronic estrogen implants in the MBH and chronically elevated serum estrogen levels suppress LH (Goodman and Knobil, 1981). However, since acute OVX also removes progesterone, it is arguable in this case that the enhanced pituitary response to LHRH occurs via the removal of inhibitory progesterone affects.

Progesterone has a biphasic stimulatory-inhibitory effect on the release of gonadotropins. Mann and Barraclough (1973) have shown that progesterone given 24 h after the administration of estrogen, potentiates estrogen's induction of the LH surge in OVX animals. This effect is temporally associated with the administration of progesterone, as progesterone also synchronizes the onset of the LH surge occuring during proestrous. Estrogen alone can produce a rise in LH following OVX on proestrous. However, in animals that are both OVX and ADX, estrogen requires the presence of progesterone to increase plasma LH levels (Mann and Barraclough, 1973). Thus, it has been suggested that the source of the initial rise of progesterone during proestrous originates from the adrenal gland. It has also been shown that 48 and 72 h following the administration of progesterone in estrogen-primed animals, LH levels have

been shown to decrease and then increase, respectively, in response to progesterone (Caligaris et al., 1971). This effect does not occur in OVX animals without estrogen replacement. Taken together, this requirement for estrogen priming suggests that the potentiated and biphasic LH responses to progesterone are mediated by estrogen-induction of progesterone receptors. Moreover, as progesterone receptor induction occurs in both the pituitary and hypothalamus (Calderon et al., 1987), this suggests that progesterone-mediated affects (stimulatory and inhibitory) on LH activity may occur at both these sites.

Progesterone given acutely increases the pituitary LH response to exogenous LHRH (Martin et al., 1974). Conversely, implantation of progesterone in the POA-AHA blocked the delayed LH surge occuring in pentobarbital treated proestrous rats and also decreased pituitary response to LHRH (Banks and Freeman, 1980). Therefore, it appears that estrogen and progesterone have transient affects on HPG activity. During diestrous both gonadal steroids serve to limit LH activity. During proestrous, estrogen and progesterone have acute stimulatory feedback effects on LH release. Following the preovulatory LH surge, progesterone (and probably estrogen as well) serve to inhibit both hypothalamic LHRH and pituitary LH release. Since these progesterone effects are only observed following estrogen priming, progesterone probably acts in a delayed fashion to block the positive feedback effects of estrogen. Furthermore, the mode of action of these hormones on HPG activity illustrate gonadal stimulatory and inhibitory feedback effects at the level of the hypothalamus and pituitary.

Gonadal Receptors

The highest level of estrogen receptors are found in the pituitary (Attardi, 1981) and in the hypothalamus within the MBH, including the POA, superchiasmatic preoptic nucleus (SCPOA), arcuate-median eminence (ARC-ME), VMN, and the medial amygdaloid nucleus (MA) (Rainbow et al., 1982). The distribution of estrogen-inducible progesterone receptors is more discrete, as estrogen treatment induced the highest levels of progesterone receptors in the pituitary and within theVMN and POA (Parsons et al., 1982). The distribution of estrogen and estrogen-inducible progesterone receptors in the hypothalamus is consistent with the gonadal steroid feedback affects on gonadotropin release and sexual behavior (Davis et al.; 1982, MacLusky and McEwen, 1978). Thus, estrogen and progesterone operate within a defined region of the hypothalamus underlying the sequential changes in behavioural and gonadotropic responses during proestrous. Furthermore, the estrogen-progesterone receptor interactions in the pituitary clearly support the view that gonadal regulation of LH release occurs at the level of the gonadotroph.

The pattern of LH release and onset of sexual behavior during proestrous show a critical temporal reliance for the occupancy of estrogen and progesterone receptors. The ability of nafoxidine to block estrogen induction as well as estrogen-progesterone facilitation of LH surges has been associated with the antiestrogen's ability to prevent nuclear binding of the estrogen receptor complex (Attardi and Palumbo, 1981). Furthermore, nafoxidine blocks estrogen-induced increases in progesterone receptors in the POA and pituitary (Attardi and Palumbo, 1981). Thus, estrogen priming and positive feedback actions on LH release is dictated by nuclear estrogen receptor occupancy in the

POA of the hypothalamus and in the pituitary. Interestingly, progesterone has been shown to suppress nuclear accumulation of the estrogen receptor in the pituitary, but not in the hypothalamus (Calderon et al., 1987). This suggests that the principle inhibitory site of progesterone action on LH release occurs in the pituitary.

HPA-HPG Interactions

The relationship between the hypothalamic-pituitary-adrenal (HPA) and -gonadal (HPG) axis has been firmly established. Stress inhibits reproductive function, and this effect has been observed as a decrease in gonadotrophin secretion (Kamel and Kubajak; 1987, Rivier and Vale, 1984), as well as an inhibition of sexual behavior (Armstrong, 1986; Plas-Roser and Aron, 1981). The degree to which the HPG and HPA axis are associated is apparent whenever abnormalities occur. Thus for example, women with hypothalamic amenorrhea, which is associated with estrogen deficiency, show elevated basal F secretion as well as blunted responses to CRH (Biller et al., 1990). Likewise, female rats lacking circadian B rhythms, show irregular ovulatory cycles (Ramaley, 1975).

Gonadal steroids in turn, are known to affect HPA function and HPA activity under both basal and stressful conditions. E2 receptors are localized in brain regions that mediate HPA function (Palkovits, 1987; Rainbow et al., 1982) and E2 is known to affect many elements of the HPA axis, including, neural input to CRH cells of the hypothalamo-hypophysial system, the synthesis and release of CRH (Haas and George, 1989), OT, and AVP (Van Tol et al.; 1988, Greer et al., 1986), corticotroph ACTH synthesis and secretion (Kitay, 1963), and glucocorticoid metabolism (Grant et al.,

1965).Female rats typically show greater adrenocorticotrophin (ACTH) and corticosterone (B) responses to stress (Le Mevel et al.; 1979, Lescoat et al., 1970), and also secrete higher basal levels of B (Critchlow et al., 1963). The sex difference during stress is abolished by OVX and is reinstated by estradiol (E2) administration (Le Mevel et al., 1978). Although female rats secrete higher basal levels of B, circulating levels of CBG are also elevated, thus reducing exposure to free, bioactive B to levels comparable to those in males (Gala and Westphal, 1965).

Basal levels of ACTH and B have been observed to increase about the time of proestrous in the rat (Critchlow et al., 1963). In women during the menstrual cycle, ACTH levels rise towards the end of the follicular phase (Genazzani et al., 1975). Although one study showed a phase difference in basal F levels (Genazzani et al., 1975), it was later confirmed that basal levels of F do not differ as a function of menstrual cycle phase (Aedo et al.; 1981a, Aedo et al; 1981b, Durber and Lawson, 1976) It is during ovulation when E2 levels are highest in the rat, and in women during ovulation and the mid-luteal phase. During proestrous in the rat, higher B levels occur in response to stress than during diestrous and estrous (Pollard et al.; 1975, Dean et al., 1969). F, as well as catecholaminergic responses to stress also vary as a function of menstrual phase (Collins et al; 1985, Hastrup and Light; 1984, Marinari et al, 1976). Moreover, post-DEX F values are higher in subjects tested during the middle two weeks of the menstrual cycle (about the time of ovulation), compared to the other weeks of the cycle (Roy-Byrne et al., 1986).

HPA activity appears to be influenced by the gonadal axis, and clearly suggest that the level of HPA activity is temporally related to fluctuations in gonadal steroids. The results of these studies suggest that both activational and inhibitory control over HPA function varies over the menstrual cycle. Furthermore, this suggests that the gonadal

steroids can regulate both HPA sensitivity and GC-mediated negative feedback, central to the stress response. To further examine the relationship between HPA activity and gonadal steroids during the estrous cycle, we have examined the plasma ACTH and B responses to 20 minutes of restraint stress during different phases of the cycle. We have also studied the stress response in OVX, steroid-treated rats, to identify more clearly the excitatory and inhibitory affects of estrogen and progesterone on HPA activity.

MATERIALS AND METHODS

The animals used were female Long-Evans, hooded rats (Charles River Canada, St. Constant, Que.) weighing approximately 225-250 grams at testing. Only those animals exhibiting normal estrous cyclicity, verified by vaginal smears, were used in the study. Animals were singly housed with food and drinking water available *ad libitum*, room temperature maintained at 20-22 C and a 12:12 h light schedule (lights on from 0800 2000 h). Unless otherwise stated, blood was collected from indwelling right jugular vein silastic cannula (Dow Corning, Midland, MI, .025 in I.D., .047 in O.D.) exiting from the back of the neck, and replaced with an equal volume of normal saline via the same route. During surgery, rats were anesthetized using Metofane (Methoxyflurane; Pitman-Moore Inc., Washington Crossing, NJ). No changes in estrous cyclicity were observed following surgery, as cyclic variations in vaginal cytology remained normal. Blood samples were collected in iced tubes containing EDTA and Trasylol (Aprotonin; Miles Canada Inc.), centrifuged at 3000 X g for 10 min, after which plasma was stored in separate aliquots at - 80 C until assayed.

Plasma B was measured by RIA (Krey et al., 1975) with a highly specific B antiserum (B3-163, Endocrine Sciences, Tarzana, CA) and (^{3}H) B as tracer (105 Ci/mmol; New England Nuclear, Boston MA. The antiserum cross-reacts slightly with desoxycorticosterone (~ 4%), but not with aldosterone, cortisol, and progesterone (< 1%). Plasma ACTH was measured using an ACTH antibody (IgG, Nashville TN) and (^{125}I) ACTH (Incstar, Stillwater, MI). The ACTH antibody cross-reacts 100 % with ACTH 1-39 and ACTH 1-24, but does not cross-react with ß-endorphin, α- and ß-MSH, α- and ß-lipotrophin (all < 1 %). The intraassay coefficients of variation for the plasma

B and ACTH assays were 8.9 % and 6.0 %, respectively, whereas the interassay coefficients of variation were 10.9 % for both assays.

ß-endorphin-like immunoreactivity (ß-LIR), was determined by RIA, using an antiserum specific for the C-terminus of ß-endorphin (ß-END) and (^{125}I) ß-END as tracer. The ß-END antibody (Iny et al., 1987) cross-reacts almost 100 % with bovine ß-lipotrophin (ß-LPH) and α-N-acetylated ß-END, 70 % with bovine ß-END$_{1-27}$, but does not cross-react with ACTH, α-MSH, ß-MSH, nor with bovine ß-LPH fragments 61-65, 62-67, and 80-84. Thus, the ß-LIR measured includes recognition of POMC, ß-LPH, both the α-N-acetylated and non-acetylated forms of ß-END fragments 1-31 and 1-27, but no recognition for ß-END fragments 1-16 and 1-17. The intra- and interassay coefficients of variation were 9.0 and 10.0 %, respectively.

Plasma estradiol (E2) was measured using the RIA kit of ICN-Biochemicals (Carson, CA) with (^{125}I) estradiol 17ß as tracer. The E2 antibody cross-reacts 100 % with estradiol-17ß, 20 % with estrone, 1.5 % with estriol, and 0.7 % with estradiol-17α. The E2 antibody does not cross-react with progesterone, testosterone, the mineralcorticoids, nor with the glucocorticoids (all < 0.01 %). The intra- and interassay coefficients of variation were 7.2 and 8.9 %, respectively.

Plasma progesterone (P) was measured using the RIA kit of Diagnostic Products Corporation (Los Angeles, CA) with (^{125}I)Progesterone as tracer. The P antibody cross-reacts 100 % with progesterone, 2.4 % with 11-deoxycortisol, 2.0 % with 20α-dihydroprogesterone, 1.7 % with 11-deoxycorticosterone, and 0.4 % with corticosterone, but does not cross-react with cortisol, estradiol, testosterone, and pregnenolone (all below detection). The intra- and interassay coefficients of variation were 4.5 and 10.8 %, respectively.

Plasma ACTH and B levels were assayed from 0.3 mls of blood sampled from

individual rats at 1000 and 2200 h, during different phases of the estrous cycle. Sampling at 2200 h was performed under diffuse and distant lighting conditions directed away from the animals quarters. ACTH and B levels were measured in response to 20 min of restraint stress, during one phase of the cycle. Rats were singly housed throughout the study, intubated with jugular cannula and stressed 48 h later. In all cases restraint stress was performed between 1200 and 1300 h. Pre-stress blood samples were taken from rats, within 30 s of removal from the cage, then immediately placed in restrainers for a period of 20 min, after which blood was sampled at 0, 30, 60, 120, and 180 min post-stress. Estrous cycles were verified, for one full cycle, before and after the stress study.

In studies with OVX steroid-treated rats, animals were OVX and implanted subcutaneously (sc) with one silastic capsule (.062 in I.D., .125 in O.D.) containing E2 dissolved in peanut oil (Sigma, St. Louis, MO, ß- Estradiol 3- Benzoate, 30 µg/ml; 10 mm/100 g b.w.), and another capsule (.132 in I.D., .183 in O.D.) containing crystalline progesterone (Sigma, 4- Pregnene - 3, 20, dione, 10 mm/animal). This treatment provides physiological levels of E2 and P in the range observed during diestrous (Brandi et al., 1990). Forty-eight h after receiving steroid implants, rats were then intubated with jugular catheters. Twenty-four h later, rats received either a 0.2 ml injection of vehicle (peanut oil) subcutaneously (O' group), or an injection of 10 µg of E2 (E' group). Twenty h later both the O' group and the E' group received an injection of vehicle, with a portion of the E' group receiving an injection of 500 µg of P (EP' group). Four h later all groups were stressed and sampled as described above. These groups, O', E', and EP', represent diestrous, early-proestrous, and late-proestrous-early estrous phases of the cycle, as they mimic endogenous E2 and P cycle profiles in terms of amplitude and acute exposure time, see below (and Brandi et al., 1990). In a separate study we

measured the ACTH and B response of the treatment groups at certain points within the 20 min stress period. Blood samples (0.3 mls) were taken immediately after the rat was placed in the restrainer (time 0), and at 5, 10, 15, 20 min during the 20 min of restraint stress. Using the same treatment as above, we also measured the ß-END/ ß-LPH responses to stress in E', EP', and O' animals with blood samples obtained by decapitation within 30 s of removal from the cage, or immediately after 10 min exposure to restraint stress. It is at this time-point where ß-END/ ß-LPH levels are known to peak in response to restraint stress (De Souza and Van Loon, 1989).

Clearance studies were performed using the O' and E' treated animals to determine if E2 had acute effects on ACTH metabolism. Animals were injected with 250 µg of DEX 4 h prior to the study in order to block endogenous ACTH release. Animals were then injected intravenously with 0.2 mls normal saline containing 0.5 µg ACTH1-39 and (^{125}I) ACTH1-39 (0.2 µCi). The 0.5 µg dose of ACTH was chosen to mimic the plasma levels achieved during stress. Blood was sampled at 0.25, 0.5, 1, 2, 3, 5.5, 8, 10.5, and 13 min after ACTH administration (see De Souza and Van Loon, 1989). Plasma (0.1 ml) was extracted on C18 columns (Waters Associates, Milford MA) to remove radiolabeled metabolites of (^{125}I) ACTH. The columns were then washed with 2 mls 60 % Acetonitrile in 0.1 % Trifluoroacetic acid (TFA), and 5 mls 0.1 % TFA, eluted with 3 mls 60 % acetonitrile in 0.1 % TFA, and counted to measure levels of radiolabelled ACTH.

The data were analyzed using an analysis of variance with Tukey post-hoc tests performed when appropriate.

RESULTS

Basal And Stress ACTH And B Levels In Cycling Rats

Analysis of basal HPA function during different phases of the estrous cycle showed a significant ($p < 0.0001$) circadian variation in both ACTH and B (Table 1). However, there were no differences in plasma ACTH and B levels as a function of estrous cycle phase. Likewise, pre-stress levels of ACTH and B did not differ across cycle phase (Figs. 1 and 2). Animals in proestrous showed significantly higher levels of ACTH ($P < 0.01$) during stress (measured at 0 min following 20 min of restraint stress; see Fig. 1) than animals in estrous or diestrous. At 30 and 60 min post-stress, no significant differences in ACTH levels were found as a function of estrous phase. Likewise, animals in proestrous showed significantly higher B responses ($p < 0.05$) during stress (Fig. 2) than animals in estrous or diestrous. At 30 min post-stress, animals in either proestrous and estrous had significantly higher levels of B ($p < 0.05$ and $p < 0.01$, respectively) than animals in diestrous. At 60 min post-stress, all groups showed comparable levels in B. Together these findings indicate that ACTH and B responses to stress are enhanced during proestrous, with no effect on basal secretion.

Table 1. Mean (± SEM) plasma ACTH (pg/ml) and Corticosterone (μg/dL) secretion under basal conditions in animals during proestrous (PRO), estrous (EST), and diestrous (DI) (n = 9, 8, 16/group, respectively). AM; 2 h after lights on, PM; 2 h after lights off.

	PM	AM
ACTH		
PRO	20.3 ± 2.4	29.5 ± 2.9[a]
EST	16.9 ± 3.1	27.9 ± 2.0[a]
DI	20.2 ± 2.2	33.8 ± 3.6[a]
Corticosterone		
PRO	11.3 ± 1.5	24.5 ± 2.1[a]
EST	13.7 ± 2.9	27.7 ± 1.8[a]
DI	9.3 ± 1.5	26.5 ± 4.1[a]

[a] indicates value that is significantly ($p < 0.05$) different from AM value.

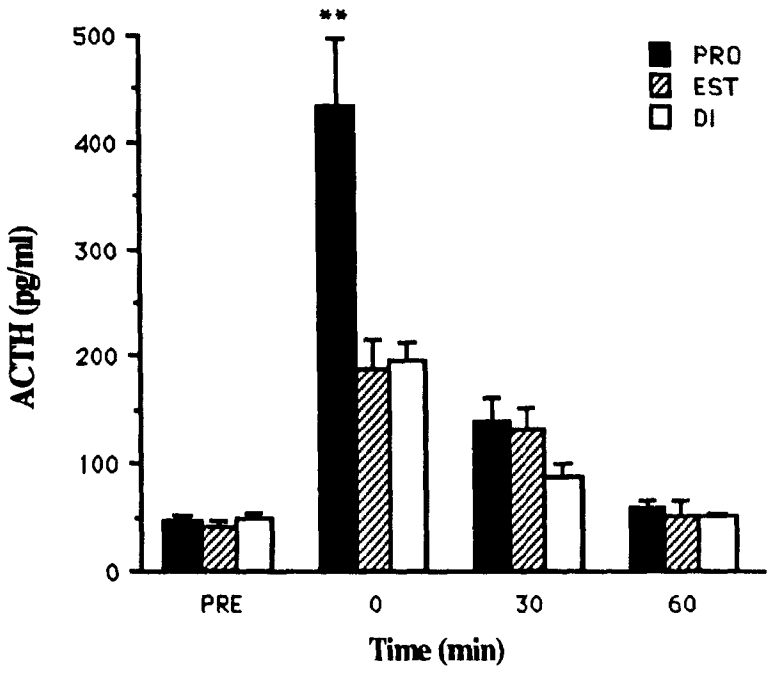

Fig. 1. Mean (± SEM) ACTH values (pg/ml) prior to (PRE) and following the termination of 20 min restraint stress in animals during proestrous (PRO), estrous (EST), and diestrous (DI) (n = 13, 12, and 32 animals/phase, respectively). **, $P < 0.01$ vs EST and DI

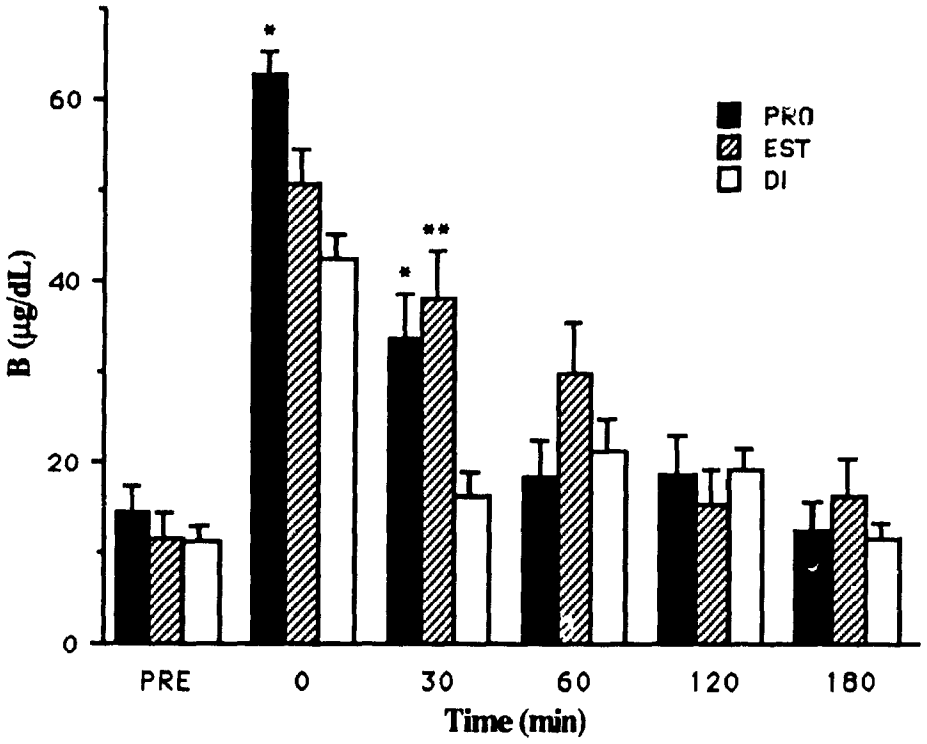

Fig. 2. Mean (+ SEM) corticosterone values (µg/dL) prior to (PRE) and following the termination of 20 min restraint stress in animals during proestrous (PRO), estrous (EST), and diestrous (DI) (n = 13, 12, and 32 animals/phase, respectively). *, $P < 0.05$ *vs* EST and DI; **, $P < 0.01$ *vs* DI

HPA Response To Stress In OVX, Steroid -Treated Rats

Steroid-treated rats showed the following E2 (pg/ml) and P (ng/ml) levels (n = 5/group): O', 71.4 $\pm$ 1.6 and 4.4 $\pm$ 0.4, respectively; E', 510.7 $\pm$ 38.9 and 5.1 $\pm$ 0.6, respectively; EP', 526.7 $\pm$ 95.1 and 28.9 $\pm$ 1.1, respectively. These values are comparable to endogenous levels of estrogen and progesterone, observed during the estrous cycle of the rat (Brandi et al., 1990).

The ACTH response to stress was significantly ($p < 0.05$) higher in the E' animals (at 0 min following stress), compared to the O' and EP' treated animals (Fig. 3). By 30 min post-stress ACTH levels were comparable in all groups. Although the B responses at 0 min were similar across groups, B levels remained significantly ($p < 0.05$) higher in the E' and EP' compared to the O' animals up to 120 min post-stress (Fig. 4). No differences were found in pre-stress levels of ACTH and B as a function of E2 and P treatment. E' animals stressed 48 h following a 10 µg dose of E2, showed ACTH responses comparable to O' treated animals (Fig. 5). The 24 h and 48 h ACTH data obtained from separate RIAs, are expressed as a percentage of the O' response.

Within the 20 min stress period, plasma ACTH levels peaked after 10 min in all groups (Fig. 6). ACTH levels were consistently higher in the E' group throughout the stress period, significantly higher ($p < 0.05$) than the O' and EP' groups at 5, 10, and 15 min. Area under the curve analysis indicated significantly ($p < 0.01$) higher ACTH levels during stress in E' animals compared to the other groups; 283.4 $\pm$ 33.5, 173.0 $\pm$ 13.6, and 178.8 $\pm$ 17.7 pg/ml/min; E', EP', and O', respectively. Likewise, B levels during stress were significantly higher in the E' group at 5 ($p < 0.05$) and 10 min ($p < 0.01$) following the start of stress (Fig. 7).

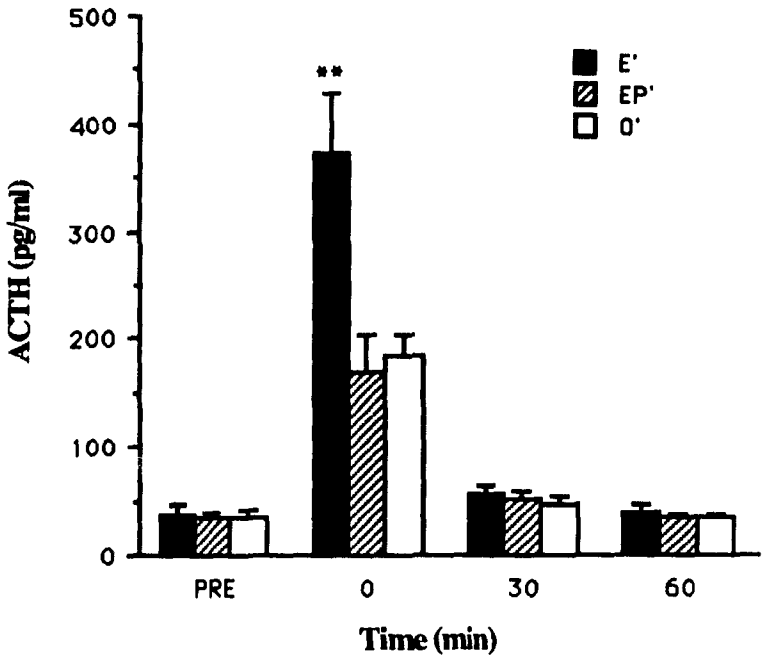

Fig. 3. Mean (± SEM) ACTH values (pg/ml) prior to (PRE) and following the termination of 20 min restraint stress in E', EP', and O' treated animals (n = 6, 7, and 10 animals, respectively). **, $P < 0.01$ vs EP' and O'

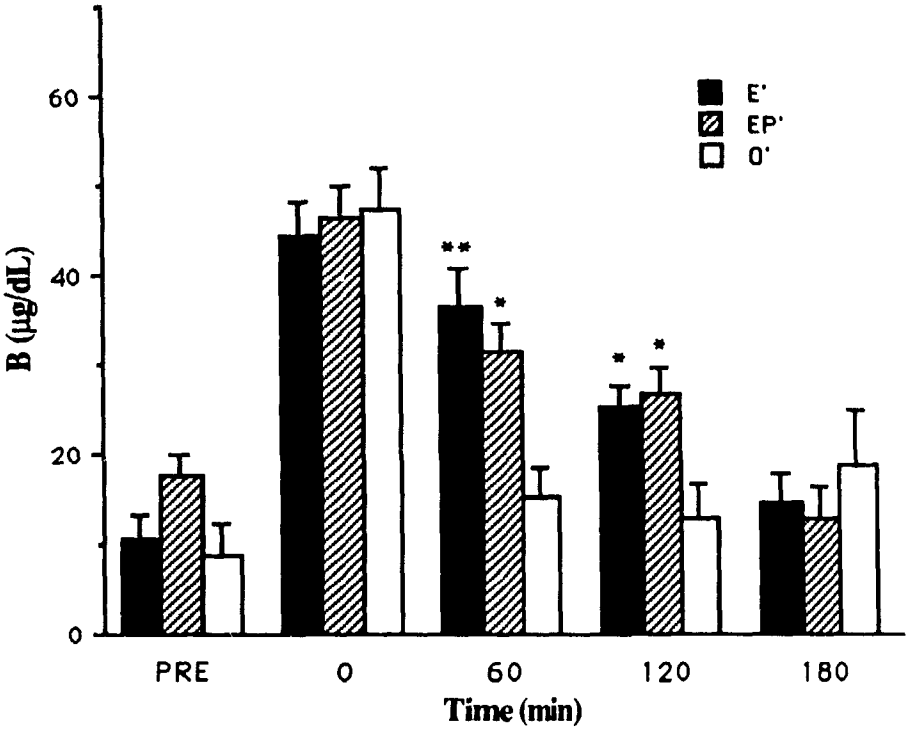

Fig. 4. Mean (+ SEM) corticosterone values (µg/dL) prior to (PRE) and following the termination of 20 min restraint stress in E', EP', and O' treated animals (n = 6, 7, and 10 animals, respectively). **, $P < 0.01$ *vs* O'; *, $P < 0.05$ *vs* O'

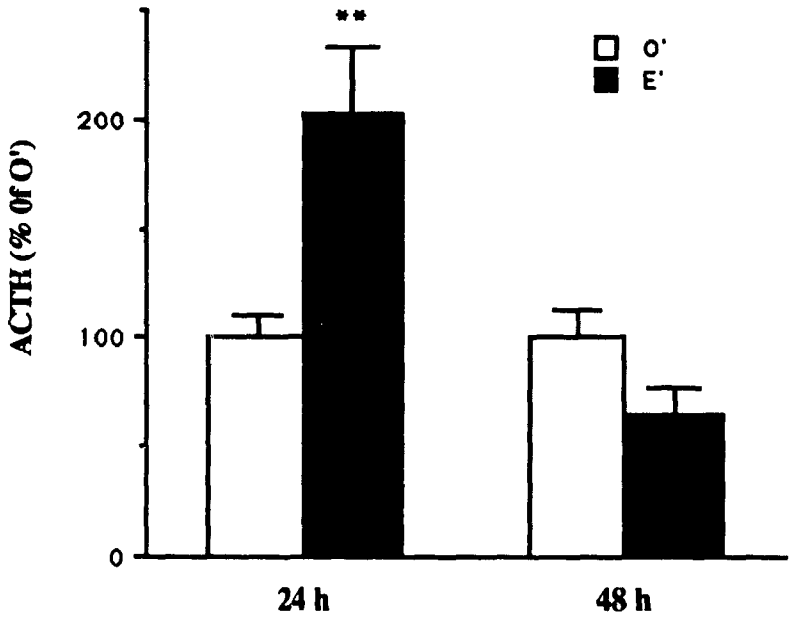

Fig. 5. Mean (± SEM) ACTH values (pg/ml) in E' treated animals, 24 or 48 h after E2 (10 μg/animal) injection, following termination of 20 min restraint stress (n = 7, 5, respectively). Values are expressed as a percentage of O' response. **, $P < 0.01$ *vs* O'

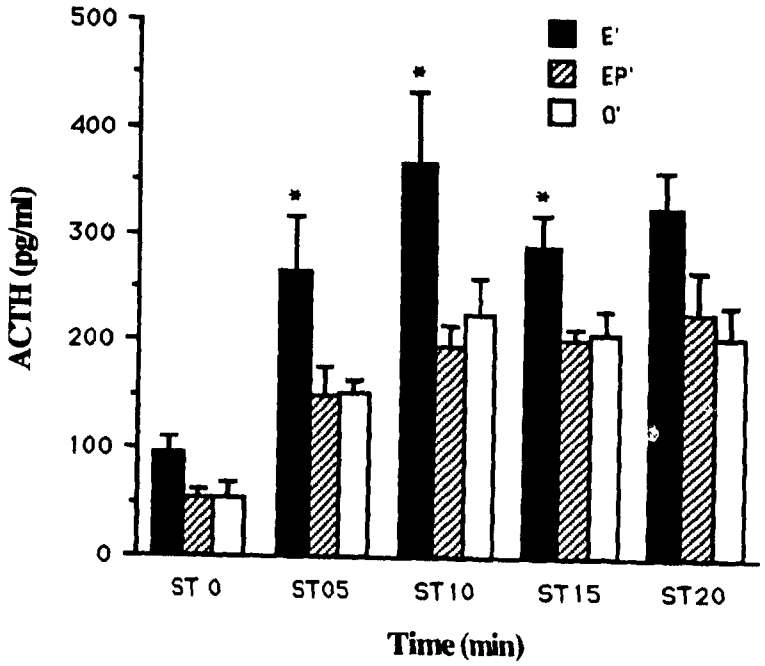

Fig. 6. Mean (± SEM) ACTH values (pg/ml) in E', EP', and O' treated animals during stress (n = 6, 9, and 9 animals/group). *, $P < 0.05$ *vs* EP' and O'

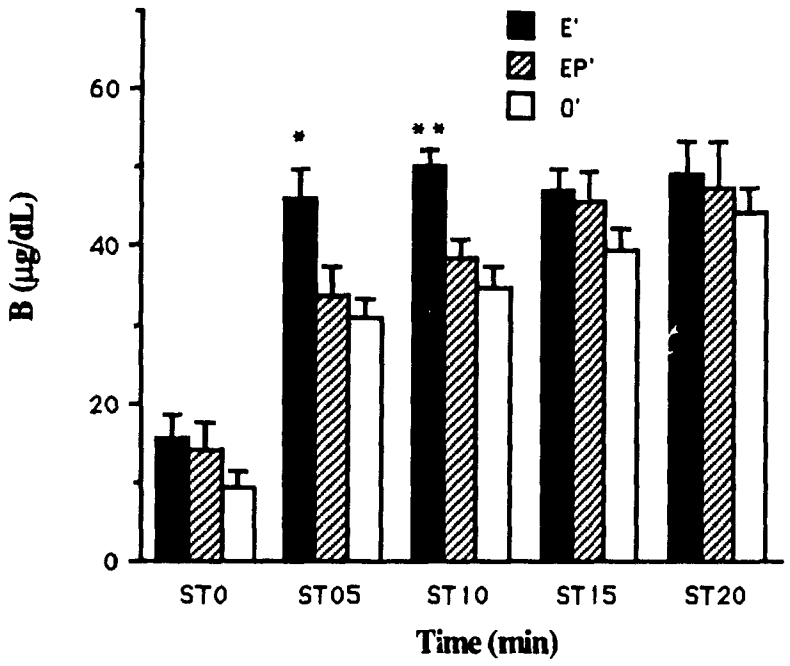

Fig. 7. Mean (± SEM) corticosterone values (µg/dL) in E', EP', and O' treated animals during stress (n = 6, 9, and 9 animals/group). *, $P < 0.05$ *vs* EP' and O'; **, $P < 0.01$ *vs* EP' and O'

Plasma ß-END/ ß-LPH levels following 10 min of restraint stress were significantly higher in the E' animals as compared to the EP' ($p < 0.05$) and O' ($P < 0.01$) treated animals (Table 2). No significant difference was found in ß-END/ ß-LPH levels under basal conditions.

ACTH Clearance

Measurement of (^{125}I) ACTH clearance indicated comparable rates of decline in ACTH between the E' and O' treated animals (Fig. 8). The observed half-life for ACTH of approximately 5 min is similar to that observed by De Souza and Van Loon (1989). This suggests that the differences in plasma levels of ACTH observed between groups under stress conditions can not be explained as a function of E2 on clearance rate.

Table 2. Mean (± SEM) plasma ß-endorphin/ ß-lipotropin secretion (pg/ml) under basal conditions and after 10 min of restraint stress in E', EP', and O' treated animals (n = 5, and 9/group during basal, and stress conditions, respectively).

Group	Basal	Stress
E'	40.4 ± 15.7	144.9 ± 17.4[ab]
EP'	38.4 ± 9.7	96.8 ± 14.4[a]
O'	31.1 ± 4.7	76.6 ± 10.3[a]

[a] indicates value significantly ($p < 0.05$) different from basal value.
[b] differs significantly *vs* O' ($p < 0.01$) and EP' ($p < 0.05$).

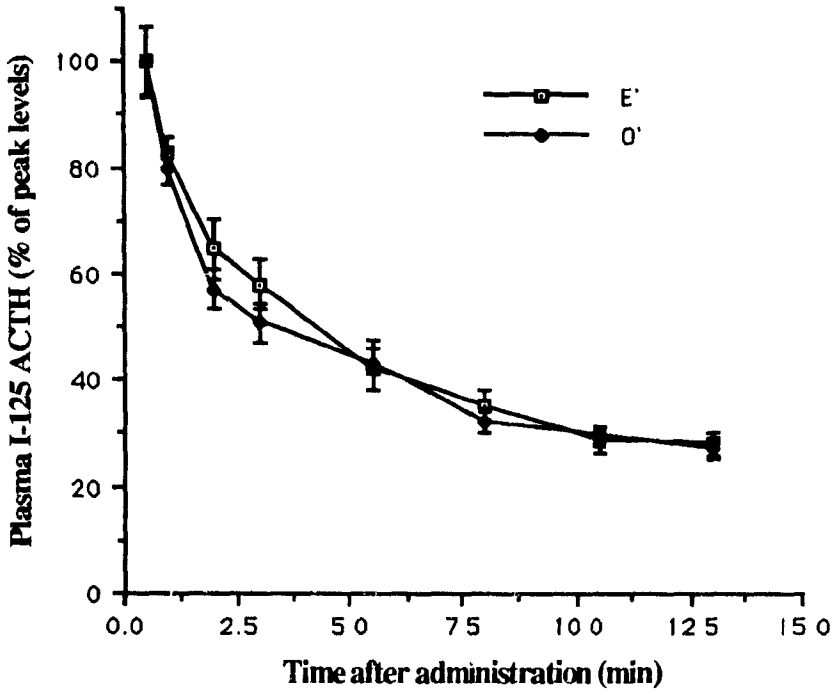

Fig. 8. Clearance of (^{125}I) ACTH $_{1\text{-}39}$ from plasma after its iv administration in OVX rats replaced with low levels of E2 and P (O'), or with high levels of E2 and low levels of P (E'), n = 7/group

DISCUSSION

Stress-induced plasma ACTH and B levels were markedly higher during the proestrous phase of the estrous cycle in the rat (Figs. 1, 2). An estrogen treatment that mimicked the ovarian status occurring during proestrous similarly enhanced ACTH, ß-END/ß-LPH, and B responses to stress (see Figs. 3, 4, and Table 2). In contrast, acute (4 h) exposure to progesterone 20 h following estrogen administration, mimicking the late proestrous/early estrous phases, appears to inhibit the estrogen-enhanced release of POMC-derived peptides during stress (Table 2, Figs. 1 - 4).

The stimulatory effect of estrogen on ACTH responses to stress seems to be transient. Animals treated with 10 µg E2 showed enhanced ACTH responses to stress 24 h, but not 48 h following treatment: Plasma ACTH levels during stress in animals treated with E2 48 h prior to testing were comparable to those in O' treated animals (Fig. 5). Thus, enhanced ACTH response to stress during the cycle seems to be restricted to the early portion of proestrous, although we did not test cycling animals during the evening of proestrous, where both estrogen and progesterone levels are high. The period of enhanced ACTH responses to stress might then be limited by both the waning of the estrogen effect and the inhibitory influence of elevated progesterone levels. Taken together, these results reflect the dynamic changes in HPA response to stress over the estrous cycle and suggest that these changes are associated with variations in circulating estrogen and progesterone levels.

During stress, E' and proestrous animals secreted substantially higher levels of ACTH than the other animals, but by 30 min post-stress, ACTH levels were comparable in all groups. These data suggest that estrogen might also enhance post-stress, negative-feedback regulation over ACTH release. Alternatively, the rapid decline in post-stress

ACTH levels in proestrous and E' animals might reflect increased negative-feedback associated with higher GC levels. Indeed, plasma B levels in these animals were significantly elevated not only during stress, but up to 30 and 120 minutes, respectively, following the termination of the stressor. These are not mutually exclusive possibilities, and we are currently examining the effects of gonadal steroids on HPA negative-feedback sensitivity.

Estrogen affects the HPA axis at multiple sites. Estrogen stimulates steroidogenesis in adrenal slices (Kitay, 1963a), perhaps explaining the dissociation, observed here, between ACTH and B levels in the post-stress period (see Figs. 1 - 4). *In vivo*, OVX decreases pituitary synthesis and release of ACTH as well as adrenal synthesis of B (Coyne and Kitay, 1969; Kitay, 1963b). These effects are reversible by estrogen replacement, consistent with increased ACTH release and adrenal secretion of B (Kitay, 1963b). In contrast, corticotrophin releasing activity in median eminence extracts is not affected by OVX, but chronic administration of high levels of estrogen in OVX rats decreases releasing activity (Kitay, 1963a) and hypothalamic content of CRH (Haas, 1989). Likewise, chronic estrogen treatment decreases the adrenal secretory capacity of B. However, the ACTH response of pituitaries incubated with uniform CRH stimulation is depressed in OVX animals, and this effect is partially reversible by estrogen administration (Kitay, 1963a). Taken together, these somewhat conflicting data show that gonadal steroids can regulate several aspects of HPA function. However, these data emerge from experiments using chronic estrogen treatments and OVX animals without replacement. The pertinence of these findings in understanding the effects of dynamic variations in estrogen levels on HPA responsivity to stress is not clear.

Gonadal steroids regulate synthesis and release of ACTH secretagogues, in acute gonadal steroid manipulations and over the course of the estrous cycle. OT and AVP

gene expression and content, in the supraoptic and paraventricular nuclei, vary during the estrous cycle (Van Tol et al., 1988) . During proestrous, the AVP/ OT ratio is significantly higher, while OT mRNA is highest in the supraoptic nuclei during estrous (Van Tol et al., 1988; Greer et al., 1986). In addition, peripheral estrogen implants acutely increase OT receptors in ventromedial hypothalamic nuclei (Johnson et al., 1989). As OT and AVP secretion differ in response to various stressors (Gibbs, 1986; Gibbs, 1984), this suggests that estrogen effects on ACTH secretion may be stress specific. Sex differences exist in circadian periodicity of CRH (Hiroshige et al., 1973), and hypothalamic CRH content also varies during the estrous cycle (Hiroshige and Wada-Okada,1973), highest during proestrous and diestrous. It is not known, however, whether estrogen can affect CRH synthesis in the PVN. However, there is evidence for a gonadal influence on PVN activity. Extracellular recordings demonstrate an increase in PVN unit firing rates during proestrous and estrous, while in OVX rats, firing rates are increased after estrogen priming and subsequently depressed 4 h after progesterone administration (Negoro et al., 1973a). Likewise,the percentage of PVN units responding to foot pinching are the highest during proestrous, enhanced after estrogen replacement, and depressed after progesterone treatment in OVX rats (Negoro et al., 1973b). Together this demonstrates that estrogen and progesterone specifically influence PVN activity in response to stress, in a manner consistent with our results for ACTH.

Estrogen also regulates central catecholamine (CA) systems, which are a major modulator of HPA activity (reviewed in Plotsky et al., 1989). During proestrous, CA turnover rapidly increases prior to ovulation (Rance et al., 1981a; Rance et al., 1981b). CA effects on CRH synthesis and release are dose dependent, as ICV administration of NE at low doses enhances CRH release (at α_1 adrenergic receptors), while at high doses release is inhibited (at ß receptors) (Plotsky et al., 1989; Plotsky, 1987b). Interestingly,

estrogen appears to up-regulate α_1-adrenergic and down regulate ß-adrenergic receptors (Condon et al., 1989; Weiland et al., 1989). Thus, it is possible that during early proestrous, as NE levels are rising, CRH release is enhanced as a function of increased α_1- (stimulatory) and decreased ß- (inhibitory) adrenergic receptor density, leading to an increase in ACTH synthesis.

Progesterone appears to limit enhanced ACTH responses to the early portion of proestrous. Progesterone has been shown to inhibit CRH-induced release of ACTH from cultured pituitaries (Buckingham, 1982), and to inhibit hypothalamic CRH and pituitary ACTH release *in vitro* (Buckingham, 1982; Jones and Hillhouse, 1976). *In vivo*, administration of progesterone decreases ACTH release in the presence of low levels of B, and increases ACTH release during stress (Keller-Wood et al., 1988). Progesterone displays GC receptor agonist activity, as it down-regulates GC receptors in the hippocampus (Sarrieau et al., 1987), and induces muscle glutamine synthetase activity via a GC receptor (Max et al., 1987). Considering the fact that the relevant progesterone effect here occurs against a background of low (pre-stress) B levels , progesterone's affinity for the GC receptor could account for its antagonist effects on ACTH release, explaining the inhibition of ACTH released during stress in estrous and EP' animals (see Figs. 1 - 4, 6, 7).

Central 5-HT and catecholamine (CA) systems have been implicated in mediating delayed- and fast- feedback mechanisms occuring during stress (Kaneko and Hiroshige, 1978). Hypothalamic 5-HT content varies over the estrous cycle (Rozsahegyi et al., 1973), and estrogen has been known to cause an acute biphasic up- and down- regulation of 5-HT receptors (Biegon and McEwen, 1982). Likewise during early proestrous, estrogen increases NA and DA turnover rate in various hypothalamic nuclei including the

suprachiasmatic nucleus and the median eminence (Rance et al., 1981; Wise et al., 1981). Both these sites mediate CRH activity during basal and stress conditions (Assenmacher et al., 1987). Furthermore 5-HT receptors are co-expressed in many brain regions with the GC receptor (Assenmacher et al., 1987). This suggests that estrogen can influence GC feedback via its affects on 5-HT transmission. These findings provide further possible mechanisms whereby estrogen and progesterone might regulate both stimulatory and inhibitory components of the HPA axis.

Our results suggest that ACTH responses to stress vary over the estrous cycle, and it is possible that basal ACTH responses obtained by decapitation reflect differences in stress-related HPA responses, rather than basal activity. Enhanced basal HPA activity has been reported to occur during proestrous in various rat strains, sampled by decapitation (Buckingham et al., 1978; Raps et al., 1970; Critchlow et al., 1963). Wistar rats, however, have not been shown to exhibit any significant differences in basal HPA activity across the estrous cycle (Hiroshige et al ., 1973). There were no differences in our basal samples, obtained via jugular catheter, as a function of the estrous cycle or in response to hormonal manipulations. Our data suggest that acute gonadal steroid regulation of HPA function is evident only during stress.

HPG and HPA function are dynamic over the estrous cycle. During proestrous both systems undergo radical changes in activity. A role for progesterone during the estrous cycle is to synchronize estrogen effects on the gonadotrophin cascade (Attardi, 1984; Goodman, 1978; Caligaris et al., 1971). Likewise, progesterone has the same role in estrogen's induction of HPA activity. It is intriguing that we did not observe changes in basal HPA activity in intact cycling rats, nor in OVX- estrogen and progesterone treated animals. Our data suggests therefore, that high physiological levels of estrogen increase synthesis of a separate pool of ACTH (and perhaps CRH), only released in response to

stress. It seems that enhanced basal HPA activity would oppose the gonadotrophic cascade occuring during proestrous, as GCs are known to inhibit LHRH release. Although it has been shown that ovulation is not inhibited by ADX (Peppler and Jacobs, 1976), the fact that low levels of GC agonists enhance LH and FSH release (Brann et al., 1990), suggests that the HPA axis may serve a permissive role in maintaining the metabolic needs of ovulation.

Our data suggest that estrogen has a preparatory role, in terms of gauging the HPA axis in anticipation of stress. It has been repeatedly shown that stress inhibits reproductive function, but GC administration prior to, and up to 5 h after estrogen treatment in OVX rats, does not inhibit gonadotrophin release the following day (Baldwin, 1979). Increased HPA sensitivity to stress on the day of proestrous, is one mechanism whereby environmental conditions unfavorable to reproduction could signal an inhibition of HPG function. The functional significance of such HPA hyperresponsiveness to stress during the proestrous phase would then be adaptive given the energetic costs of reproduction and short gametic cycle of the female rat (Bronson, 1987). Viewed in the context of natural populations, this allows the rat alternative strategies to reproduction in response to unpredictable events occuring during optimal breeding conditions.

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